VARIABILIDAD GENÉTICA Y ESTRÉS PSICOSOCIAL PREVIO COMO DETERMINANTES PROSPECTIVOS DE DEPRESIÓN

Análisis longitudinal del Estudio PREDICT-Gene

Esther Molina Rivas

Instituto de Neurociencias "Federico Olóriz"
Universidad de Granada

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INSTITUTO DE NEUROCIENCIAS "FEDERICO OLÓRIZ"

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TESIS DOCTORAL

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ESTHER MOLINA RIVAS

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Análisis longitudinal del Estudio PREDICT-Gene

Memoria presentada por

Esther Molina Rivas

para optar al grado de Doctor por la Universidad de Granada

Dirigida por los Doctores

Blanca Gutiérrez Martínez

Jorge A. Cervilla Ballesteros

Junio de 2009

BLANCA GUTIÉRREZ MARTÍNEZ, Profesora Contratada Doctora de la Universidad de Granada y JORGE A. CERVILLA BALLESTEROS, Profesor Titular de la Universidad de Granada

CERTIFICAN:

Que el trabajo que presenta para aspirar al Grado de Doctor D ª. ESTHER MOLINA RIVAS, titulado: **VARIABILIDAD GENÉTICA Y ESTRÉS PSICOSOCIAL PREVIO COMO DETERMINANTES PROSPECTIVOS DE DEPRESIÓN: Análisis longitudinal del Estudio PREDICT-Gene**, se ha realizado bajo su dirección, y reúne los requisitos académicos, formales y de calidad necesarios como para que pueda ser defendido públicamente ante la Comisión que se constituya al efecto.

Granada, 1 de Junio de 2009

Blanca Gutiérrez Martínez Jorge Cervilla Ballesteros

A Blas

A mi madre

A mi abuelo

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Índice

I. INTRODUCCIÓN

I. Introducción

1. CONCEPTO DE DEPRESIÓN

Todos conocemos por propia experiencia el significado de la palabra tristeza. Es un sentimiento que hemos experimentado en algún momento de nuestra vida, con mayor o menor intensidad, y que aparece en respuesta a muy diversas situaciones. La pérdida de un ser querido, el padecimiento de una larga enfermedad o el fracaso de una relación de pareja pueden ser, entre otras, las causas de ese estado de ánimo apenado y afligido. Este sentimiento es transitorio y suele tener una intensidad y duración acordes con el hecho que lo ha desencadenado. Tras un periodo de tiempo más o menos largo, la persona acepta esa nueva situación, se repone y reorganiza su nueva vida, desapareciendo así este sentimiento.

Sin embargo, algunas personas experimentan una tristeza patológica, que no remite cuando la causa que la produce desaparece y en la que no existe un motivo fácilmente identificable, o cuando lo hay, tal tristeza es desproporcionada al hecho desencadenante, por lo que es difícil para el paciente explicar su estado y es, en general, difícilmente comprendido por los que están a su alrededor. Esta tristeza se mezcla con pensamientos negativos y sentimientos de inferioridad y de culpa, que en ocasiones pueden llegar a ser delirantes, y que generan en el paciente un ánimo aún más deprimido, y lo hacen entrar en un oscuro agujero sin salida.

Podemos definir el trastorno depresivo mayor basándonos en los criterios recogidos en el Manual Diagnóstico y Estadístico de Enfermedades Mentales, DSM-IV-TR (*American Psychiatry Association, 2000)*. El DSM-IV-TR define la depresión mayor como un trastorno afectivo cuyos síntomas nucleares son un estado de ánimo anormalmente bajo (hipotimia), fatigabilidad y pérdida de interés y de la capacidad de obtener placer por las cosas (anhedonia). Estos

síntomas suelen ir, además, acompañados de cambios en distintas funciones biológicas y cognitivas, como alteraciones del sueño, disminución del apetito, enlentecimiento motor, estreñimiento, disminución de la líbido, sensación de pérdida de energía, disminución de la capacidad para pensar, concentrarse o tomar decisiones, sentimientos de infravaloración y culpa o pensamientos de muerte y desesperanza.

2. EPIDEMIOLOGÍA DE LA DEPRESIÓN

Los trastornos del humor, y en especial la depresión mayor, se encuentran entre las enfermedades más frecuentes, recurrentes y discapacitantes en países desarrollados. La Organización Mundial de la Salud anticipa que para el año 2020, el trastorno depresivo será la segunda causa de discapacidad, medida en DALYs (Dissability Adjusted Living Years), en el ranking global de enfermedades, sólo superada por las enfermedades cardiovasculares *(Murray y Lopez 1996)*.

La edad media de inicio de esta enfermedad se sitúa entre los 18 y 44 años, aunque puede aparecer en cualquier etapa de la vida. La incidencia de este trastorno en la población es de 200 nuevos casos por cada 10000 individuos-año *(Murphy et al. 2002)*. La probabilidad que tiene una persona de desarrollar un trastorno depresivo en algún momento de su vida (prevalencia vida) es del 10-20% en la población general *(Kato, 2007; Shigemura and Nomura, 2007*). Concretamente, se calcula en torno a un 10% en los hombres y un 20-25% en las mujeres *(Angst 1999; Cervilla 2005)*.

La prevalencia, incidencia y riesgo mórbido de esta enfermedad son mayores en mujeres que en hombres**,** con una relación de 2:1 *(Piccinelli and Wilkinson, 2000; Kuehner, 2003),* tanto en población general como en muestras clínicas, independientemente del lugar, del método de evaluación y del sistema diagnóstico utilizados *(Bebbington, 1996)*. Sin embargo, si consideramos sólo los

trastornos depresivos graves, la prevalencia vida es de un 5%, desapareciendo en ese caso las diferencias de género. Asimismo, hay autores que muestran que las tasas de prevalencia son similares entre hombres y mujeres cuando se trata de un trastorno depresivo no asociado con ansiedad o síntomas somáticos (*Silverstein, 1999)*.

Esa mayor prevalencia de depresión en mujeres que en hombres podría quizá ser debida a una mayor exposición y sensibilidad de la mujer al abuso sexual, o a una mayor sensibilidad a experiencias adversas en la infancia *(Weiss et al., 1999)*, a limitaciones sociales o a roles impuestos (cuidado de los hijos, realización de las tareas del hogar, etc.), o a la existencia de normas culturales discriminantes *(Piccinelli and Wilkinson, 2000)* y no tanto a factores genéticos *(McGuffin et al., 1996; Kendler and Prescott, 1999; Sullivan et al., 2000)* y biológicos (*Weiss et.al. 1999*), o a un deficiente apoyo social *(Kuehner 2003; Piccinelli and Wilkinson 2000).*

Otros autores han sugerido que esas diferencias podrían ser el resultado de meros artefactos, como la mayor probabilidad de usar los servicios médicos y psiquiátricos o de pedir ayuda por parte de las mujeres, o el hecho de que el sistema de clasificación actual favorece la identificación de síntomas más "femeninos" de la depresión como la hipotimia o la anhedonia, sobre los "masculinos", que suelen consistir en más irritabilidad, consumo y abuso de sustancias tóxicas y personalidad antisocial (*Kuehner 2003*).

Los trastornos depresivos presentan una alta recurrencia. Así, al menos el 50% de los individuos que han sufrido un primer episodio depresivo presentarán al menos otro a lo largo de su vida, y esta probabilidad aumenta conforme lo hace el número de episodios previos de depresión, de modo que aproximadamente el 80% de aquellos individuos con una historia de al menos dos episodios depresivos previos, sufrirán algún otro a lo largo de su vida *(Angst, 1986).*

El trastorno depresivo podría considerarse una enfermedad cotidiana, ya que aproximadamente entre uno y dos de cada cinco pacientes que acuden a los servicios de Atención Primaria cumple criterios diagnósticos de depresión (*Bellón et al 2008*). Es fundamental, por tanto, que el médico de familia sepa identificar y tratar estos casos, pero sobre todo, tal y como se ha comentado con anterioridad, que tenga a su disposición las herramientas necesarias para prevenirlos.

3. DEFINICIÓN CLÍNICA

Ante la imposibilidad de usar validadores externos para el diagnóstico de los trastornos depresivos (como marcadores biológicos, bioquímicos o de morfología cerebral), el diagnóstico de depresión debe hacerse utilizando criterios psicopatológicos y clínicos (*Peralta y Cuesta, 2002*).

Así, para el diagnóstico de los distintos trastornos mentales en general, y de la depresión en particular, en la práctica clínica se utilizan dos clasificaciones categoriales: el DSM-IV-TR (*Diagnostic and Statistical Manual of Mental Disorders, APA, 2002*) de la American Psychiatric Association, y la CIE-10 (Clasificación Internacional de Enfermedades de la OMS, décima edición) de la Organización Mundial de la Salud. Al tratarse de clasificaciones arbitrarias, aunque consensuadas por la comunidad psiquiátrica internacional, no están exentas de problemas de validez y fiabilidad y han de ser revisadas periódicamente.

En las Tablas 1 y 2 se muestran los criterios diagnósticos necesarios para un diagnóstico de depresión mayor recogidos en las clasificaciones DSM-IV y CIE-10, respectivamente.

TABLA 1: Diagnóstico de depresión según criterios DSM-IV.

Ha presentado el paciente cinco (o más) de los siguientes síntomas durante un período de 2 semanas, que representan un cambio respecto al funcionamiento previo? Al menos uno de los síntomas ha de ser:
1.- Estado de ánimo deprimido o
2.- Pérdida de interés o de la capacidad para disfrutar.
No incluir síntomas que son claramente debidos a una enfermedad médica o de las ideas delirantes o alucinaciones no congruentes con el estado de ánimo.

1.- Estado de ánimo depresivo la mayor parte del día, casi cada día según lo indica el propio sujeto (por ejemplo, se siente triste o vacío) o la observación realizada por otros (por ejemplo, llanto). En los niños y adolescentes el estado de ánimo puede ser irritable.

2.- Disminución acusada del interés o de la capacidad para disfrutar en todas o casi todas las actividades del día, casi todos los días (según refiere el propio sujeto y observan los demás).

3.- Pérdida importante de peso sin hacer régimen, o aumento de peso (por ejemplo, un cambio de más del 5% del peso corporal en un mes), o pérdida o aumento del apetito casi cada día.
Nota: en niños hay que valorar el fracaso en lograr los aumentos de peso esperables.

4.- Insomnio o hipersomnia casi cada día.

5.- Agitación o enlentecimiento psicomotor casi cada día (observable por los demás, no meras sensaciones subjetivas de inquietud o de estar enlentecido).

6.- Fatiga o pérdida de energía casi cada día.

7.- Sentimientos de inutilidad o de culpa excesivos e inapropiados (que pueden ser delirantes) casi cada día (no simples autorreproches o culpabilidad por el hecho de estar enfermo).

8.- Disminución de la capacidad para pensar o concentrarse, o indecisión, casi cada día (sea una atribución subjetiva o una observación ajena).

9.- Pensamientos recurrentes de muerte (no sólo temor a la muerte), ideación suicida recurrente sin un plan específico o una tentativa de suicidio o un plan específico para suicidarse.

Además debe ser verdadero lo siguiente:

Los síntomas no cumplen los criterios para un episodio mixto

Los síntomas provocan malestar clínicamente significativo o deterioro social, laboral o de otras áreas importantes de la activiada del individuo

Los síntomas no se deben a los efectos fisiológicos directos de una sustancia (por ejemplo droga o medicamento) o a una enfermedad médica (por ejemplo, hipotiroidismo).

Los síntomas no se explican mejor por la presencia de un duelo (por ejemplo, después de la pérdida de un ser querido), los síntomas persisten durante más de dos meses o se caracterizan por una acusada incapacidad funcional, preocupaciones mórbidas de inutilidad, ideación suicida, síntomas psicóticos o enlentecimiento psicomotor.

Adaptado de Baldwin and Birtwistle, 2005

TABLA 2: Diagnóstico de depresión según criterios CIE-10.

A. Criterios generales para episodio depresivo

El episodio depresivo debe durar al menos dos semanas.
El episodio no es atribuible a abuso de sustancias psicoactivas o a trastorno mental orgánico

B. Presencia de al menos dos de los siguientes síntomas:
Humor depresivo de un carácter claramente anormal para el sujeto, presente durante la mayor parte del día y casi todos los días, que se modifica muy poco por las circunstancias ambientales y que persiste durante al menos dos semanas.
Marcada pérdida de los intereses o de la capacidad de disfrutar de actividades que anteriormente eran placenteras.
Falta de vitalidad o aumento de la fatigabilidad.

C. Además debe estar presente uno o más síntomas de la siguiente lista, para que la suma total sea al menos de 4:
Pérdida de confianza y estimación de sí mismo y sentimientos de inferioridad.
Reproches hacia sí mismo desproporcionados y sentimientos de culpa excesiva e inadecuada.
Pensamientos recurrentes de muerte o suicidio o cualquier conducta suicida.
Quejas o disminución de la capacidad de concentrarse y de pensar, acompañadas de falta de decisión y vacilaciones.
Cambios de actividad psicomotriz, con agitación o inhibición.
Alteraciones del sueño de cualquier tipo.
Cambios del apetito (disminución o aumento) con la correspondiente modificación del peso.

D. Puede haber o no síndrome Somático.

Episodio depresivo leve: Están presentes dos o tres síntomas del criterio B. La persona con un episodio leve probablemente está apta para continuar la mayoría de sus actividades.

Episodio depresivo moderado: Están presentes al menos dos síntomas del criterio B y síntomas del criterio C hasta sumar un mínimo de 6 síntomas. La persona con un episodio moderado probablemente tendrá dificultades para continuar con sus actividades ordinarias.

Episodio depresivo grave: Deben existir los 3 síntomas del criterio B y síntomas del criterio C con un mínimo de 8 síntomas. Las personas con este tipo de depresión presentan síntomas marcados y angustiantes, principalmente la pérdida de autoestima y los sentimientos de culpa e inutilidad. Son frecuentes las ideas y acciones suicidas y se presentan síntomas somáticos importantes. Pueden aparecer síntomas psicóticos tales como alucinaciones, delirios, retardo psicomotor o estupor grave. En este caso se denomina como episodio depresivo grave con síntomas psicóticos. Los fenómenos psicóticos como las alucinaciones o el delirio pueden ser congruentes o no congruentes con el estado de ánimo.

Adaptado de Baldwin and Birtwistle, 2005

Ambas clasificaciones difieren ligeramente, pero ambas recogen la exigencia de que, para generar un diagnóstico de depresión, deben existir al menos dos síntomas nucleares (humor deprimido y pérdida de la capacidad de obtener placer por las cosas) y al menos tres síntomas acompañantes (que marcan la gravedad del trastorno), presentes la mayoría de los días durante al menos dos semanas. Estos síntomas tienen, además, que interferir considerablemente en las actividades de la vida diaria del individuo induciendo discapacidad.

En la práctica clínica, y sobre todo en el contexto de la investigación, normalmente se usan entrevistas diagnósticas estructuradas que generan un diagnóstico psiquiátrico según criterios DSM-IV o CIE-10. En el caso concreto de la depresión, existen entrevistas diagnósticas diseñadas especialmente para explorar sintomatología depresiva y generar diagnósticos en DSM-IV o CIE-10. Tal es el caso de la CIDI (*Composite International Diagnostic Interview, CIDI, Robins et.al. 1988),* que consiste en una entrevista psiquiátrica estructurada que se puede aplicar de forma global o sólo secciones específicas y que puede ser utilizada por entrevistadores legos con un cierto entrenamiento previo.

4. ETIOLOGÍA DE LA DEPRESIÓN

4.1. Factores Biológicos de Riesgo

La depresión es una enfermedad compleja en la que convergen numerosos factores que pueden predisponer, desencadenar o perpetuar el trastorno. La etiología de los trastornos afectivos en general, y de la depresión mayor en particular, tiene un componente biológico y otro psicosocial.

Distintos estudios sugieren que las bases biológicas de la depresión tienen que ver con una disfunción inespecífica de la neurotransmisión monoaminérgica (principalmente de serotonina, noradrenalina y dopamina) y un mal funcionamiento del eje hipotálamo-hipófiso-adrenal, además de la existencia de una vulnerabilidad genética en el individuo a padecer la enfermedad. En las Figuras 1 y 2 se representan las principales evidencias neuroquímicas y endocrinológicas, respectivamente, asociadas a depresión.

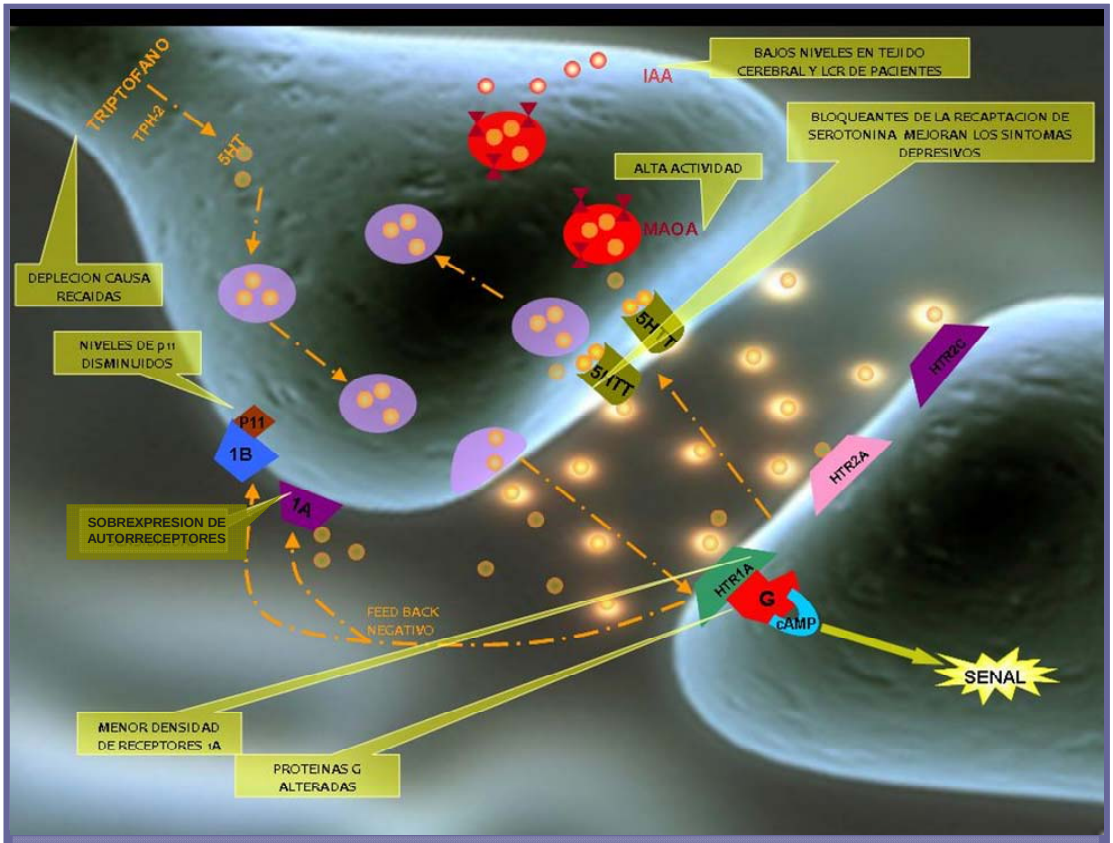


FIGURA 1: Principales hallazgos a favor de la hipótesis de la disfunción serotoninérgica en depresión.

La **hipótesis monoaminérgica** centra la fisiopatología de la depresión en una deficiencia de los neurotransmisores serotonina, noradrenalina y/o dopamina en el sistema nervioso central. Tal idea surge en los años 50 al observarse, de modo casual, una mejora en los síntomas de pacientes depresivos a los que se les había administrado drogas cuyo efecto implicaba la elevación de las concentraciones de tales neurotransmisores en el espacio presináptico *(Axelrod et.al. 1959)*. No tardaron en encontrarse evidencias que apoyaban esta teoría, tales como la existencia de bajos niveles de **serotonina** y de su principal metabolito (5hidroxi-indolacético) en tejido cerebral y líquido cefalorraquídeo y una menor concentración de triptófano (molécula precursora de la síntesis de serotonina) en sangre de pacientes depresivos suicidas *(Arango et.al. 1990)*. Además, la depleción de triptófano induce nuevos episodios en pacientes y ánimo disfórico en sus familiares de primer grado *(Young et.al. 1985)*. Además, se observó un incremento de la densidad de receptores postsinápticos 5HT2 y del transportador de serotonina SERT en la corteza frontal de depresivos suicidas, lo cual podría explicarse por una respuesta adaptativa de estas moléculas ante la reducción sináptica de serotonina (*Arango et.al. 1990*). La densidad de receptores postsinápticos serotoninérgicos tipo 1A está disminuida en individuos deprimidos, mientras que los autorreceptores reguladores 1A y 1B son menos sensibles (*Pitchot et.al. Biol Psychiatry 2005;58:854-8; Svenningsson P, Science 2006;311:77-80)*. Se ha descrito además, una disfunción en la transmisión de señal por parte de la proteínas G.

La implicación de la noradrenalina en la etiología de la depresión se pone de manifiesto al observarse que los fármacos que inhiben la recaptación de noradrenalina y favorecen la neurotransmisión noradrenérgica producen una mejora de los síntomas depresivos *(Langer et.al. 1980)*. Además, se ha observado una disminución de la síntesis de noradrenalina, un descenso de los receptores α y β adrenérgicos y un aumento del metabolito MHPG, marcador de la actividad presináptica de las neuronas noradrenérgicas, en pacientes con depresión *(Schildkrant et.al. 1978)*.

El sistema dopaminérgico muestra también evidencias de estar implicado en la etiología del trastorno depresivo, tales como la existencia de fármacos antidepresivos (bupropión o la nomifensina) y sustancias como las anfetaminas, con efecto bloqueador de la recaptación de dopamina, que producen una elevación del estado de ánimo *(Langer et.al. 1980)*. Así pues, parece claro que las monoaminas, y en especial la serotonina, juegan un papel clave en la etiología de la depresión.

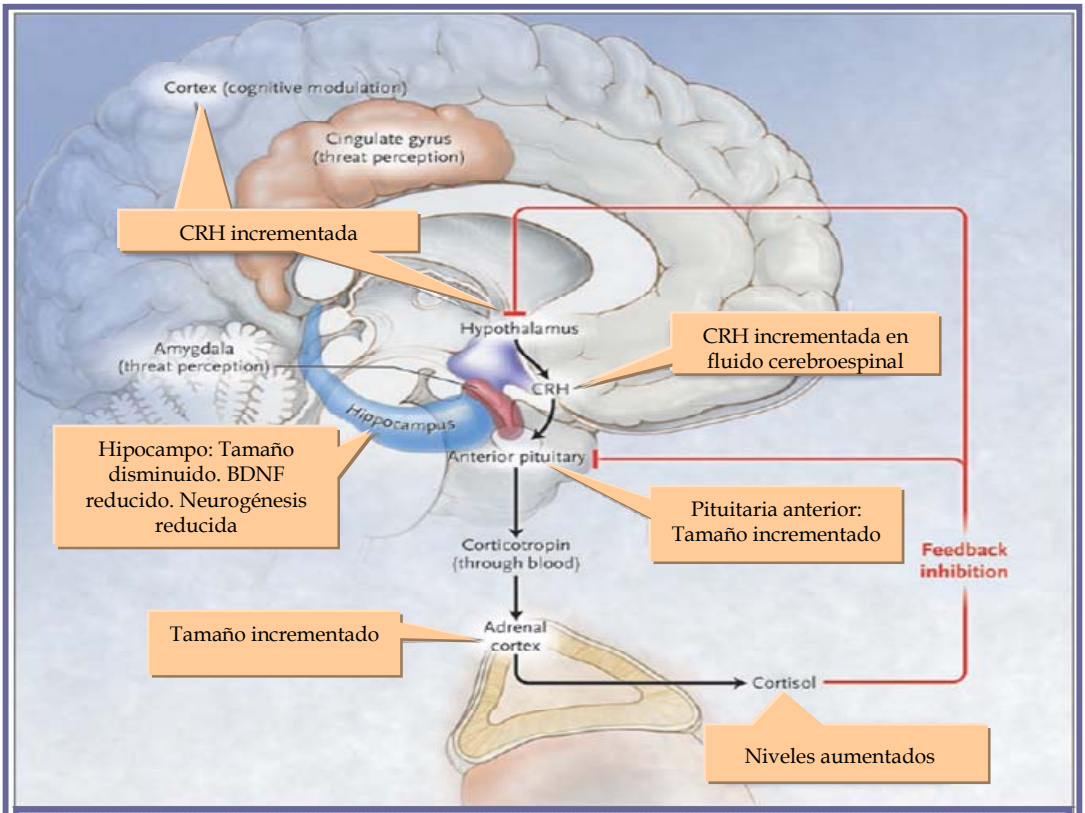


FIGURA 2: Hallazgos principales que involucran al eje hipotálamo-hipófiso-adrenal en la fisiopatología de la depresión. Figura tomada de *Belmaker et.al. 2008*.

Ante situaciones de estrés, el organismo reacciona dando lugar a una serie de respuestas adaptativas a nivel del sistema nervioso central y periférico. *Al*teraciones en el modo en que una persona responde ante las situaciones estresantes que aparecen en su vida, podrían determinar la generación de una patología depresiva *(Duman 1997; Nemeroff 1998; Holsboer 2000)*. Numerosos estudios han puesto de manifiesto una elevación de los niveles de cortisol y de factor liberador de corticotropina (CRH) al menos en un 50% de los pacientes depresivos *(Catalán et.al. 1998)*, no debido al estrés producido por la enfermedad, ya que esta alteración afecta también al ritmo circadiano de secreción de cortisol (alto por la tarde y al principio de la noche), hecho que no ocurre en situaciones de estrés. Estos pacientes presentan una incapacidad de su sistema hipotálamo-hipófiso-adrenal (HHA) para suprimir el cortisol plasmático. Este hecho se ha interpretado como una falta de acoplamiento del sistema simpático-adrenal y el eje HHA debido a una intensa producción de factores liberadores o como una hiperactivación del sistema simpático-adrenal como consecuencia de la hiperactividad del eje HHA *(Dinan 2001)*. Sin embargo, estos hallazgos son controvertidos por su inespecificidad, ya que también se han descrito en manía, esquizofrenia y demencia, por lo que, más bien, parecen ser debidos al padecimiento de enfermedad mental y no específicamente depresión *(Holsboer 1999)*. Sin embargo, quizá el cortisol juegue un papel importante en la etiología de la depresión postraumática. Tal situación estresante induciría una hipersecreción de cortisona, que produciría una elevación sostenida de los niveles de cortisol, lo cual provocaría una alteración del sistema serotoninérgico *(Linkowski 1990)*.

Así pues, respuestas excesivas o bien inadecuadas (en duración o en magnitud) a factores estresantes pueden provocar depresión. De hecho, en el sujeto depresivo aparecen ciertas características comunes a una situación de respuesta mantenida al estrés (anorexia, pérdida de la libido, taquicardias, hipotensión, etc.). Serían, por tanto, los circuitos cerebrales del eje hipotálamo-hipófiso-adrenal encargados de controlar la respuesta al estrés, los involucrados, al menos en parte, en la etiopatogenia de la depresión *(Holsboer et.al. 2000; O´Connor et.al. 2000)*. Algunos autores han demostrado un efecto del estrés mantenido sobre vías de neurotransmisión serotoninérgica, disminuyendo la concentración de serotonina en el espacio intersináptico *(Mossner et.al. 2000)*.

4.1. 1. Bases Genéticas de la Depresión

Durante décadas, estudios de familia, gemelos y adopción han aportado evidencias acerca de la existencia de un componente genético en la etiología de la depresión *(Fañanás 2002).* Sin embargo, a pesar del extraordinario avance de la Biología Molecular, salvo algunas excepciones, no existen a día de hoy resultados concluyentes acerca de qué genes podrían estar implicados en el origen de esta enfermedad. Distintas razones se encuentran detrás de este "fracaso".

La primera y más importante de ellas, es que no existen parámetros biológicos que definan los límites de la enfermedad o los subtipos o categorías que pudieran existir dentro de ésta, por lo que la clasificación se centra fundamentalmente en criterios clínicos que no tienen por qué reflejar una realidad biológica. Además, la complejidad de estas enfermedades, en las que no parece existir un gen concreto responsable, sino probablemente numerosos genes de efecto menor que interaccionan con el ambiente dando lugar al fenotipo final, hace difícil la definición de un modelo de herencia.

En segundo lugar, en algunas ocasiones ocurren cambios en el diagnóstico de la enfermedad del paciente a lo largo de su vida, lo cual da lugar a que, en el momento del estudio, podamos encontrarnos con falsos positivos o falsos negativos que podrían distorsionar el resultado de nuestros análisis.

Por último, las enfermedades mentales, y en especial la depresión, no tienen una edad de inicio concreta, y esto representa un problema a la hora de llevar a cabo un estudio, porque da lugar a falsos negativos, es decir, sujetos que son codificados como sanos pero que más adelante enfermarán.

Con todo, la investigación en genética psiquiátrica se ha tornado fundamental en el entendimiento de la enfermedad mental, teniendo como principales focos de interés la identificación de genes de susceptibilidad para

enfermedades mentales, ayudar a conocer mejor la etiología de determinados trastornos, redefinir fenotipos clínicos y proporcionar las claves para mejorar los tratamientos, identificando de forma individualizada cuál es el fármaco con mayor probabilidad de responder mejor y con menos efectos secundarios (Farmacogenética).

4.1. 1.a Evidencias desde la Genética Cuantitativa

◘ Estudios de Familia

La primera aproximación al estudio de los factores genéticos implicados en la enfermedad mental surge de la observación de la prevalencia de la enfermedad entre los miembros de una familia.

Los primeros estudios de familia en los que se analizaron de manera independiente los trastornos unipolar y bipolar, se realizaron en los años 60 y en ellos se constató una frecuencia incrementada del riesgo tanto para trastorno bipolar como para unipolar en las familias de individuos afectados por cualquiera de los dos trastornos (*Perris et.al. 1966* y *Angst et.al. 1966,* de manera independiente*).* Posteriores estudios han corroborado estas observaciones *(Nurnberger 1992; Maier 1993; Valles et.al. 2000).*

A partir de los estudios de familia, podemos calcular el riesgo mórbido familiar o riesgo relativo (RR), que nos informa de cuánto mayor es el riesgo para padecer la enfermedad en los familiares de un individuo enfermo respecto a la población general. En la Figura 3 se recogen los riesgos relativos para trastorno bipolar y unipolar en población general y en familiares de primer grado de individuos afectados por ambos trastornos.

Como se puede observar, el riesgo para padecer trastorno bipolar entre los familiares de primer grado de individuos con esta enfermedad es casi 10 veces superior al de la población general, pero además estos individuos tienen también un riesgo de casi el doble para padecer depresión. Entre los familiares de individuos con trastorno unipolar, existe aproximadamente tres veces más riesgo de padecer este trastorno y también un ligero incremento del riesgo para trastorno bipolar.

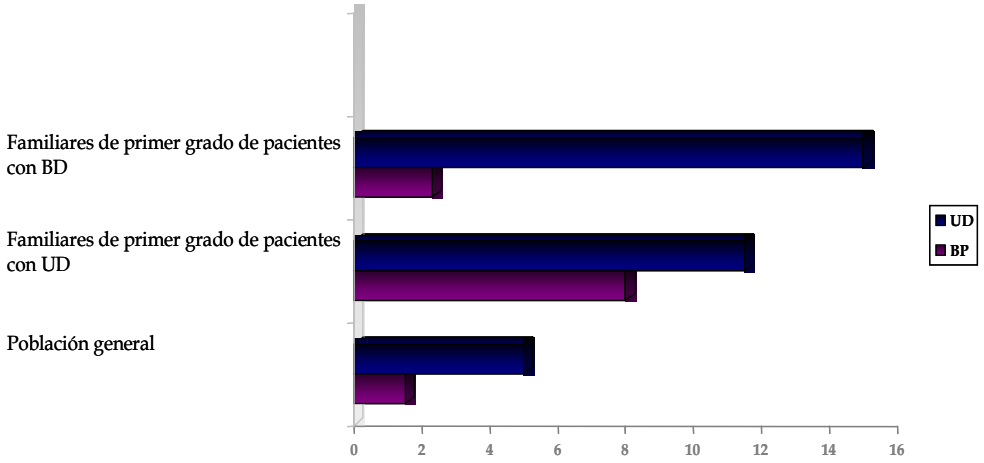


FIGURA 3: Riesgos incrementados entre los familiares de primer grado de individuos afectados de trastorno bipolar (BD) y unipolar (UD) en comparación con la población general.

Podemos decir que existe un riesgo de entre 1.5 y 3 veces superior para padecer depresión entre los familiares de primer grado de pacientes depresivos, según informan la mayoría de los autores *(Jones et al. 2002).*

Numerosos estudios han descrito, además, un efecto de la edad de inicio en la agregación familiar, de modo que edades de inicio tempranas se han relacionado con un riesgo en torno a 7 veces mayor en los familiares. Estos autores han descrito además un riesgo mayor para depresión en familiares de primer grado de pacientes con depresión recurrente en comparación con el riesgo que presentan familiares de pacientes que han sufrido un solo episodio *(Bland et al. 1986; Sullivan et al.,, 2000; Weissman et al. 1984)*. Diversos autores concluyen que el rango de riesgos incrementados para depresión en familiares de sujetos

afectados está entre un 3.4%, cuando el probando ha debutado tardíamente y ha tenido pocos episodios, y un 17.4%, en el caso de familiares de pacientes con una forma recurrente de la enfermedad que comenzó a una edad temprana. Parece que las formas de aparición temprana y recurrentes de la enfermedad tendrían una mayor familiaridad que el resto (*Shih, R.A. et.al. 2004).*

Todos estos hallazgos sugieren la existencia de agregación familiar, pero no demuestran si este hecho es debido a un componente genético de riesgo común o a aprendizajes adquiridos en el entorno familiar que pudieran hacer vulnerables a los miembros de una misma familia.

Con los estudios de gemelos y de adopción es posible controlar el factor ambiental y separarlo del genético para así esclarecer cuál es la aportación real de la genética en la etiología de las enfermedades mentales. Mediante este tipo de estudios se puede descomponer la etiología de la enfermedad en tres variables, a saber, la carga genética de riesgo, la carga ambiental común a los individuos que crecen con una misma familia y los efectos ambientales únicos de cada individuo.

◘ Estudios de Gemelos y Adopción

En los estudios de adopción se estima el riesgo para la enfermedad en hijos de padres biológicos afectados que han sido adoptados por personas sanas e hijos de padres afectados que han sido criados en el seno de su familia natural. El riesgo para la enfermedad se estima en la edad adulta de estos sujetos. De este modo, podremos determinar la causa responsable de la agregación familiar observada en los estudios de familias mediante la evaluación de la influencia del factor ambiental. Así, un riesgo incrementado para la enfermedad en sujetos

adoptados con padres biológicos afectados nos estaría indicando la existencia de una carga genética sobre el fenotipo final de enfermedad.

Son escasos los estudios de adopción realizados para depresión y no reportan una contribución clara de la carga genética a la etiología de la enfermedad, aunque en algunos de ellos se describen riesgos relativos mayores en sujetos adoptados con padres biológicos deprimidos que en hijos adoptados con padres biológicos sanos *(Cadoret et.al. 1985; Wender et.al. 1986)*. Otros estudios, sin embargo, no encontraron diferencias significativas en las incidencias de trastorno afectivo entre individuos adoptados de padres biológicos afectados y de padres sanos *(Von Knorring et.al. 1983)*. Los estudios de adopción no han obtenido, por tanto, resultados concluyentes.

Otra herramienta de aproximación a la genética de las enfermedades mentales son los estudios de gemelos. Si existe una base genética en la etiología de una enfermedad, esperaríamos que las tasas de concordancia para la misma fueran significativamente mayores entre gemelos monozigóticos (que comparten el 100% de sus genes) que entre gemelos dizigóticos o no idénticos (que comparten el 50% de los genes). Las tasas de concordancia para depresión en gemelos monocigóticos se sitúan alrededor del 46% mientras que en gemelos dicigóticos son significativamente menores, situándose aproximadamente en el 20% (*McGuffin and Katz, 1989*). Estos valores nos permiten también calcular la heredabilidad, esto es, la proporción de varianza genética implicada en la varianza fenotípica del trastorno. Los autores dan cifras de heredabilidad para el trastorno depresivo en torno al 40% *(Kendler et.al. 1992; Kendler 1999; Sullivan 2000)*.

Algunos estudios han sugerido la existencia de diferencias en las tasas de heredabilidad entre mujeres y hombres (*Beirut et.al. 1999),* encontrando tasas más altas en mujeres, pero estos hallazgos no han sido replicados *(McGufin et.al. 1996; Kendler et.al. 1999; Sullivan et.al. 2000)* por lo que el efecto de los genes en la vulnerabilidad a depresión parece ser similar en hombres y en mujeres (*McGuffin and Katz, 1988*).

No está claro tampoco si la edad de inicio de la enfermedad podría determinar diferencias en las tasas de heredabilidad. Algunos estudios han sugerido una relación entre la edad de inicio de la depresión y la heredabilidad del trastorno (*Lyons et.al. 1998)* aunque otros no encuentran esta relación (*Kendler et.al. 1999).* Otras características clínicas como la recurrencia del trastorno, la duración del episodio más largo o el número de síntomas han sido evaluadas como predictores de agregación familiar en depresión. De ellas, la depresión recurrente parece ser el subtipo de depresión mayor que más consistentemente se asocia con una heredabilidad elevada (*Kendler et.al. 1999*), sugiriendo que aquellos pacientes en los que los episodios depresivos se suceden de forma recurrente constituirían un subgrupo etiológicamente más homogéneo.

Podemos decir que, aunque los estudios de familias, adopción y gemelos proporcionan resultados controvertidos, apuntan hacia la existencia de un componente genético en la etiología de la enfermedad. Con las nuevas técnicas de Biología Molecular, ha sido posible explorar el genoma en busca de estos genes de susceptibilidad para depresión. Este abordaje genético se lleva a cabo mediante estudios de ligamiento y de asociación.

4.1. 1.b Evidencias desde la Genética Molecular

La búsqueda de cuáles son y dónde se encuentran localizados los genes que parecen jugar un papel importante en la etiología de los trastornos afectivos y, concretamente, de la depresión, se lleva a cabo utilizando, fundamentalmente, dos tipos de estrategias diferentes: los estudios de ligamiento y los de asociación.

◘ Estudios de ligamiento

Los estudios de ligamiento se fundamentan en la existencia de secuencias de ADN variables entre los individuos que se encuentran dispersas por todo el genoma humano. Tales secuencias constituyen el punto de referencia para la localización de la secuencia génica responsable de la enfermedad. Si uno de estos marcadores genéticos cosegrega junto con la enfermedad en proporción mayor a lo esperable por azar en los individuos de una familia, podremos decir que marcador y enfermedad están ligados, es decir, se encuentran muy próximos en el mismo cromosoma, tan cerca que no se produce recombinación meiótica entre ellos, razón por la cual no se heredan independientemente. En este caso podremos sugerir la existencia de un gen para la enfermedad situado cerca del polimorfismo usado como marcador.

Son escasos los estudios de ligamiento realizados en depresión *(Neiswanger et.al. 1998; Holmans et.al. 2004; Camp et.al. 2005)* y los resultados no han sido concluyentes, quizá debido a que el diseño de estos estudios sea más apropiado para enfermedades monogénicas, con un modelo sencillo de herencia (mendeliano) que para enfermedades multigénicas en las que cada gen tiene un efecto menor modificado además por el ambiente.

Aún así, algunos loci han sido propuestos para la depresión desde estudios de ligamiento, loci situados en los cromosomas 1, 3, 4, 6, 7, 9, 11, 12, 13,

15, 16, 18 y 21 (*Wilson et al., 1989; Lim et al., 1993; Kawada et al., 1995b; Neiswanger et al., 1998; Balciuniene et al., 1998; Serretti et al., 2000; Abkevich et al., 2003; Fullerton et al., 2003; Zubenko et al., 2003; Holmans et al., 2004; Nash et al., 2004; Camp et al., 2005; McGuffin et al., 2005*). No obstante, los resultados no son del todo concluyentes, quizá debido a que la depresión es un carácter complejo y en ellos la replicación del ligamiento es difícil.

◘ Estudios de asociación

En ellos se compara la frecuencia de del supuesto genotipo de riesgo de un gen candidato entre los individuos afectados por la enfermedad y los sujetos sanos, teniendo siempre ambos grupos la misma procedencia poblacional. En el supuesto de que existiera una frecuencia significativamente mayor del genotipo de riesgo entre los individuos afectados respecto a los sanos, podríamos afirmar la existencia de una asociación entre ese genotipo y la enfermedad.

El término odds ratio (OR) indica cuántas veces es más frecuente la enfermedad en individuos que poseen el genotipo de riesgo que en los que no lo poseen. Valores de OR significativamente por encima de 1 indican que el marcador confiere susceptibilidad para la enfermedad.

En los estudios de asociación el gen candidato es elegido por su implicación en la neurobiología de la enfermedad o porque se encuentra localizado en una zona que ha sido descrita como ligada a la aparición del trastorno. Así, debido a las evidencias a favor de una hipótesis monoaminérgica en la etiología de la depresión (Ver Figura 1), los investigadores han centrado sus esfuerzos en genes que codifican para proteínas clave de los sistemas de neurotransmisión serotoninérgico, dopaminérgico, noradrenérgico o gabaérgico (receptores de serotonina, especialmente 5HT2A, 5HT2C y 5HT1A, transportador de serotonina y receptores de dopamina, en especial, DRD3 y DRD4) y en los

genes implicados en la metabolización de los neurotransmisores de estos sistemas (como el gen de la tirosina hidroxilasa, TH; el de la monoamino-oxidasa A, MAOA; o la catecol-O-metil transferasa, COMT).

Los estudios de asociación genética suponen una herramienta útil en el estudio de la genética de la depresión, siendo más adecuados que los estudios de ligamiento para detectar genes de efecto menor *(Fañanás et.al., 2002).*

Se resume a continuación cuáles han sido los principales hallazgos sobre las bases genéticas de la depresión, aportados desde estudios de asociación.

4.1. 1.c Principales Hallazgos En Genética Psiquiátrica

En general, los estudios que desde la Genética Psiquiátrica se han realizado, han encontrado una asociación entre depresión y algunos marcadores genéticos polimórficos, fundamentalmente en el gen del transportador de serotonina (SERT o SLC6A4) *(Collier et al., 1996; Gutierrez et al., 1998; Steffens et al., 2002; Hauser et al., 2003; Willeit et al., 2003; Lotrich et.al. 2004; Hoefgen et al., 2005; Cervilla et al., 2006; Grunblatt et al., 2006; Munafo et al., 2006; Ramasubbu et al., 2006; Dick et al., 2007; Jarrett et al., 2007)*, en el gen del enzima monoamino-oxidasa A (MAOA), aunque de forma menos concluyente *(Schulze et.al. 2005; Yu et.al. 2005; Rivera et.al. 2008)* y en los genes de receptores serotoninérgicos tipo 5HT2A *(Johansson et al., 2001; Fañanas, 2002; Hamet and Tremblay, 2005; Levinson, 2006)*, tipo 5HT2C *(Lerer B et.al. 2001; Iwamoto et.al., 2005)* y tipo 5HT1A *(Lemonde et.al. 2003: Parsey et al., 2006; Lenze et al., 2008).*

A continuación, se describen brevemente los hallazgos principales en cuanto al posible papel de algunos de estos genes en al etiología de la depresión.

◘ Gen del Transportador de Serotonina (SERT)

Existen numerosas evidencias que apuntan hacia el gen del transportador de serotonina como uno de los principales candidatos para ser analizado en depresión.

Numerosos estudios sugieren que la serotonina tiene un papel destacado en la regulación del humor, el sueño, o el apetito (*Meltzer, 1989*). Como se ha comentado, la depleción de triptófano induce nuevos episodios en pacientes y ánimo disfórico en sus familiares de primer grado (*Young et.al. 1985*). Algunos estudios en animales han evidenciado la aparición de rasgos de comportamiento asociados a depresión después de la interrupción de la función del transportador de serotonina en el periodo neonatal *(Ansorge et al., 2004).* También se ha visto que fármacos que bloquean la acción del transportador de serotonina mejoran los síntomas depresivos *(Goodnick and Goldstein, 1998).*

El gen del transportador de serotonina (gen SERT o SLC6A4), codifica para una proteína de 630 aminoácidos clave en la neurotransmisión serotoninérgica, ya que se encarga del transporte activo de la serotonina que ha sido liberada al espacio intersináptico de vuelta al interior de la neurona. Esta proteína es la responsable de la eliminación y reciclaje rápidos de la serotonina liberada tras la estimulación neuronal, limitando su acción a un corto periodo de tiempo. Por tanto, tiene un papel crítico en la regulación homeostática de la magnitud, duración y distribución espacial de las señales que alcanzan los receptores serotoninérgicos *(Murphy et al., 2004).*

El transportador de serotonina se localiza tanto en la membrana presináptica de los terminales nerviosos como en las dendritas. Se incluye dentro de una familia de proteínas transportadoras Na^+/Cl^- dependientes que presentan una estructura con 12 dominios hidrofóbicos transmembrana *(Murphy et al., 2004).*

El gen del transportador de serotonina (gen SERT o SLC6A4) está localizado en el cromosoma 17, en la región 17q11.1-q12 *(Ramamoorthy et al., 1993)* y está constituido por 14 exones que se extienden a lo largo de 31 Kb *(Lesh et.al. 1994)* (Ver Figura 4). Se han descrito varios polimorfismos a lo largo de su secuencia *(Lesch et al., 1994; Gelernter and Freimer, 1994; Heis et al., 1995; Di Bella et al., 1996; Nakamura et al., 2000)*. De todos ellos, hay dos de particular interés por su relación con la vulnerabilidad a los trastornos afectivos:

i) un VNTR (variable number of tandem repeat) que contiene 9, 10 ó 12 copias de un fragmento de 17 pares de bases, localizado en el segundo intrón del gen *(Lesch et al., 1994)*.

ii) una inserción/deleción (5-HTTLPR) de 44 pares de bases en la región promotora del mismo *(Heils et al., 1996)* (Ver Figura 4).

La actividad trascripcional del gen del transportador de serotonina está modulada, en parte, por la variación en la longitud del polimorfismo 5-HTTLPR, que presenta dos variantes alélicas, una corta (484 ó S) y otra larga (528 ó L). La variante corta (alelo S) se ha relacionado con una menor eficiencia trascripcional del gen, que se traduce en una menor presencia de proteína transportadora en la neurona *(Lesh et.al. 1996)*. El ser portador de esta variante menos eficiente en la trascripción del gen no es poco probable, ya que este polimorfismo es muy frecuente en todas las poblaciones humanas *(Gelernter et al., 1999)*. Concretamente, en población española, las frecuencias alélicas descritas para cada variante son del 57% para la variante larga (L) y del 43% para la variante corta (S) y las frecuencias genotípicas descritas en esta población son del 32% para el genotipo L/L , 51% para los heterocigotos L/S y 17% para los S/S (*Gutierrez et al., 1998*).

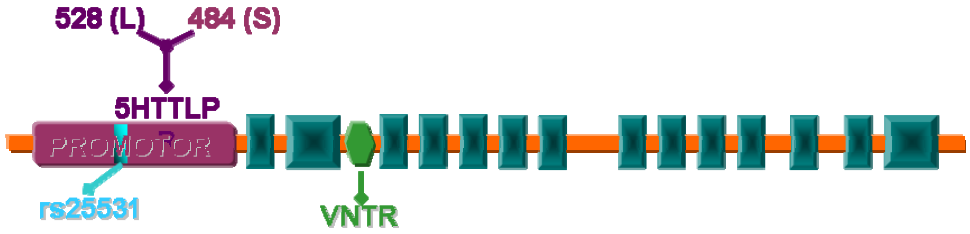


FIGURA 4: Representación gráfica del gen del transportador de serotonina.

Recientemente, se ha descrito un nuevo SNP (*Single Nucleotide Polymorphism*) en este gen, identificado como rs25531, que consiste en una sustitución de un nucleótido de adenina (A) por otro de guanina (G) dentro de la variante alélica larga (L) del polimorfismo 5HTTLPR *(Hu et al., 2006).* Las frecuencias alélicas descritas en población caucasoide son del 9-15% para el alelo L_G y del 49-51% para el alelo L_A *(Hu et al., 2006; Wendland et al., 2006*). Los alelos S y L_G han sido asociados con niveles disminuidos de expresión del gen del transportador de serotonina comparados con el alelo L_A *(Hu et al., 2006),* por lo que los alelos S y L_G parecen comportarse de forma similar en términos de expresión génica, aunque esto no está aún del todo claro (*Martin et.al. 2007*). Además, tampoco está claro si el SNP rs25531 tiene algún impacto en la funcionalidad del alelo corto (S).

Numerosos autores, en muestras independientes, han asociado el genotipo S/S del polimorfismo 5HTTLPR del gen SERT con riesgos incrementados para padecer depresión *(Collier et al., 1996; Gutierrez et al., 1998; Steffens et al., 2002; Hauser et al., 2003; Willeit et al., 2003; Lotrich et.al. 2004; Hoefgen et al., 2005; Cervilla et al., 2006; Grunblatt et al., 2006; Munafo et al., 2006; Ramasubbu et al., 2006; Dick et al., 2007; Jarrett et al., 2007)*, si bien otros trabajos no han conseguido replicar estos hallazgos *(Kunugi et al., 1997; Rees et al., 1997; Bellivier et al., 1998; Hoehe et al., 1998; Ohara et al., 1998; Frisch et al., 1999; Serretti et al., 1999; Furlong et al., 1999b; Oliveira et al., 2000; Kim et al., 2000; Shcherbatykh et al., 2000; Mellerup et al., 2001;*

Minov et al., 2001; Serretti et al., 2002b; Mendlewicz et al., 2004; Willis-Owen et al., 2005; Bozina et al., 2006).

En la Tabla 3 se muestran los principales estudios de asociación entre el polimorfismo 5HTTLPR del gen del transportador de serotonina y depresión y los metanálisis realizados hasta el momento.

Los estudios de asociación que han analizado el posible papel de este polimorfismo en la etiología de la depresión, han producido resultados dispares y no del todo concluyentes. Los metanálisis publicados hasta el momento tampoco han arrojado demasiada luz al respecto, ya que algunos sugieren que el alelo S confiere un riesgo incrementado para depresión (*Furlong et al, 1998; Lotrich & Pollock, 2004*) y otros apuntan a que el alelo S se encuentra más bien asociado a trastorno bipolar, y no tanto a depresión mayor (*Anguelova et al, 2003, Lasky-Su et al, 2005*).

Las discrepancias en cuanto a los resultados podrían tener que ver con aspectos metodológicos.

En muchos de estos trabajos, el tamaño muestral utilizado no es lo suficientemente amplio como para que proporcione poder estadístico suficiente para detectar el efecto del gen, o la muestra es heterogénea *(Angelova et.al. 2003; Hoefgen et al., 2005; Lasky-Su et.al. 2005),* o ha sido seleccionada con un objetivo de estudio diferente.

En otras ocasiones, existen diferencias en la definición del fenotipo, o en los criterios diagnósticos utilizados entre los diferentes estudios de asociación *(Murphy et al., 2004; Munafo et al., 2006),* o hay una coexistencia con otros trastornos psiquiátricos, o bien, no se ha tenido en cuenta en el análisis de los datos el efecto de posibles factores confusores, como pueden ser el sexo o la edad, entre otros.

TABLA 3: Principales estudios de asociación genética y meta-análisis entre el polimorfismo 5HTTLPR de gen del transportador de serotonina y depresión realizados hasta el momento.

ESTUDIO	POBLACIÓN	MUESTRA	ASOCIACIÓN
Collier et al, 1996	Inglaterra, Italia y Alemania	454 casos: depresión y trastorno bipolar. 570 controles	Sí, asociación del genotipo S/S con depresión y trastorno bipolar.
Kunugi et al, 1997	Japón	191 casos: 49 depresión y 142 trastorno bipolar. 212 controles	No, con depresión y trastorno bipolar.
Rees et al, 1997	Reino Unido	251 casos: 80 depresión mayor; 171 trastorno bipolar 121 controles	No, con depresión y trastorno bipolar
Bellivier et al, 1998	Francia	- 253 casos: 45 depresión; 208 trastorno bipolar - 99 controles	No, con depresión. Sí, del genotipo S/S con trastorno bipolar
Gutiérrez et al, 1998	España	74 casos: depresión mayor con melancolía. 84 controles	Sí, del haplotipo S - 10 de los polimorfismos 5HTTLPR - STin2 con depresión mayor con melancolía
Hoehe et al, 1998	Francia y Alemania	115 casos: 36 depresión mayor; 79 trastorno bipolar. 294 controles	No, con depresión y trastorno bipolar
Ohara et al, 1998	Japón	80 casos: 46 depresión; 34 trastorno bipolar. 92 controles	No, con depresión y trastorno bipolar No encuentran asociación con características subclínicas como la recurrencia, el comportamiento suicida o la edad de inicio.

Frisch et al, 1999	Israel	102 casos: depresión mayor. 172 controles	No, con depresión mayor
Furlong et al, 1998	Reino Unido	212 casos: 125 depresión y 87 trastorno bipolar 174 controles	No, con depresión y trastorno bipolar
Serretti et al, 1999	Italia	230 casos: 70 depresión mayor; 160 trastorno bipolar	No, con síntomas depresivos, de excitación, delirios y desorganización
Kim et al, 2000	Corea	120 casos: depresión mayor. 252 controles	No, con depresión mayor
Oliveira et al, 2000	Brasil	192 casos: 66 depresión mayor, 64 trastorno bipolar y 62 distimia. 152 controles	No, con depresión mayor, trastorno bipolar y distimia
Shcherbatykh et al, 2000	Rusia	423 casos: psicosis endógenas. 277 controles	No, con depresión mayor y trastorno bipolar
Mellerup et al, 2001	Dinamarca	158 casos: 92 depresión; 66 trastorno bipolar. 108 controles	No, con depresión y trastorno bipolar
Minov et al, 2001	Alemania	173 casos: depresión mayor. 121 controles	No, con depresión mayor, aunque encuentran un exceso de genotipos S/S entre los deprimidos que no llega a ser significativo.
Serretti et al, 2002	Italia	1820 casos: 667 de depresión mayor; 789 de trastorno bipolar; 261de esquizofrenia; 66 de delirios y 37 de psicosis.	No, con depresión mayor y trastorno bipolar

		457 controles.	
Steffens et al, 2002	Estados Unidos	182 casos de depresión 107 controles. Todos ancianos	Sí, con depresión hombres Sí, con nº episodios depresivos mujeres
Hauser et al, 2003	Polonia	226 casos: 94 de depresión; 132 de trastorno bipolar 213 controles	Sí, con depresión y trastorno bipolar
Willeit et al, 2003	Austria	138 casos de trastorno afectivo estacional. 146 controles.	No, con depresión estacional. Sí, del alelo L con depresión melancólica. Sí, del alelo S con depresión atípica
Mendlewicz et al, 2004	Europa (8 nodos)	1111 casos: 539 depresión; 572 trastorno bipolar. 821 controles	No, con depresión y trastorno bipolar No asociación con características subclínicas como comportamiento suicida, presencia de rasgos psicóticos. No avocación con historia familiar de trastorno afectivo.
Hoefgen et al, 2005	Alemania	466 casos: depresión mayor (severos). 836 controles	Sí, del alelo S con depresión mayor
Willis-Owen et al, 2005	Inglaterra	MIRAR	No, con depresión mayor, depresión mayor recurrente y neuroticismo
Bozina et al, 2006	Croacia	114 casos: depresión mayor. 120 controles	No, con depresión mayor
Cervilla et al, 2006	España	262 casos: depresión 475 controles Procedentes de atención primaria	Sí, del alelo S con depresión

Grünblatt et al, 2006	Austria	544 casos y controles Todos mayores de 75 años sin demencia.	Sí, con depresión en edad adulta
Munafò et al, 2006	Reino Unido	251 participantes	Sí, con neuroticismo y depresión a lo largo de la vida. Sugieren que neuroticismo media la asociación del polimorfismo 5HTTLPR con depresión.
Ramasubbu et al, 2006	Canadá	26 casos: depresión mayor. 25 controles. Todos han sufrido accidente cerebrovascular (ACV).	Sí, con depresión mayor después de haber sufrido un ACV.
Dick et al, 2007	Estados Unidos	1913 participantes: 679 con depresión, 882 con problemas de abuso alcohol y 411 con comorbilidad.	Sí, con depresión
Jarrett et al, 2007	Estados Unidos	138 participantes todos con síndrome de colon irritable	Sí, con historia de depresión No, con otras variables (ansiedad, ideación suicida o angustia psicológica)
METANÁLISIS:			
Furlong et al, 1998		392 casos con trastorno bipolar 275 con depresión 739 controles.	Sí, del alelo S con depresión y trastorno bipolar

Anguelova et al, 2003		45 estudios. 941 casos de depresión mayor 2110 controles 1382 casos de trastorno bipolar 2085 controles	No, del alelo S con depresión mayor Sí, del alelo S con trastorno bipolar
Lotrich & Pollock, 2004		910 casos de depresión mayor 2017 controles 1356 casos de trastorno bipolar 1953 controles	Sí, del genotipo S/S con depresión mayor No, del genotipo S/S con trastorno bipolar aunque detectan una tendencia a la asociación.
Lasky-Su et al, 2005		1961 casos: depresión y trastorno bipolar 3402 controles	No, del alelo S con depresión Sí, del alelo S con trastorno bipolar
López-León et al, 2007		3752 casos: depresión mayor 5707 controles	Sí, del alelo S y el genotipo S/S con depresión mayor

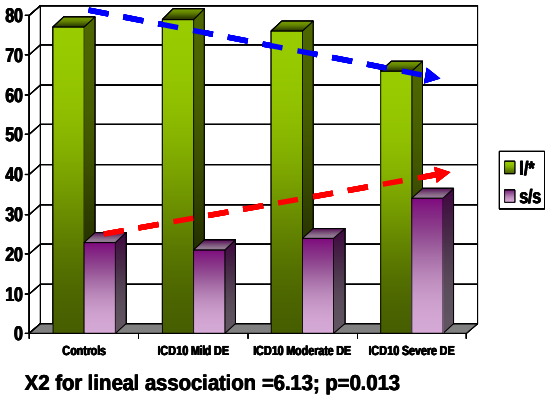
Todos estos factores han provocado que, si bien como se ha mencionado existen numerosos trabajos a favor de un papel de este polimorfismo en el riesgo para padecer depresión, la existencia de trabajos en los que no se ha conseguido replicar tales hallazgos hace que, la asociación entre el polimorfismo 5HTTLPR del gen SERT y depresión no sea todavía hoy del todo concluyente *(Lim et.al. 2006).*

Con el objetivo de clarificar el posible papel del polimorfismo 5HTTLPR del gen SERT en el riesgo para desarrollar depresión, nuestro grupo de investigación publicó, recientemente, los resultados de un estudio de asociación caso-control *(Cervilla et.al. 2006)*.

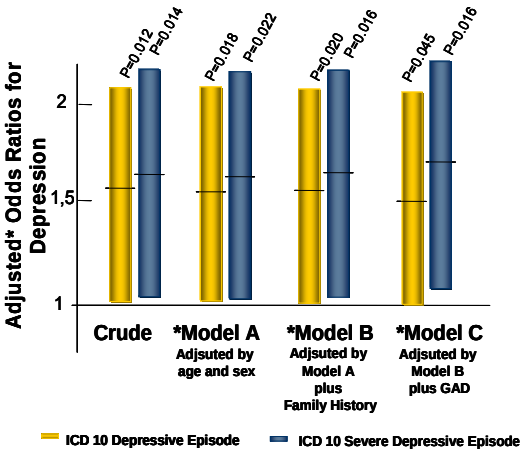
Este estudio se llevó a cabo en el contexto del estudio PREDICT-GENE, que consiste en un seguimiento longitudinal de una cohorte de individuos usuarios de los servicios de atención primaria de distintos centros de salud del país, con el objetivo de establecer perfiles genéticos de vulnerabilidad a depresión y analizar posibles interacciones entre factores genéticos y ambientales determinantes de la aparición de episodios depresivos (*Cervilla et.al. 2006).* El estudio PREDICT-GENE se enmarca, a su vez, en el contexto de otro proyecto más ambicioso, el proyecto PREDICT, desarrollado a nivel europeo, con el objetivo de identificar predictores de episodios de depresión entre los usuarios de los servicios de atención primaria de centros de salud de distintos países europeos (*King et.al. 2006, 2008, Bellón et.al. 2008).*

Cervilla y colaboradores (2006) usaron una muestra de 737 individuos que fueron evaluados para toda una batería de variables sociodemográficas y psicológicas, mediante entrevistas estructuradas y validadas, administradas por entrevistadoras previamente entrenadas (*King, M. et.al. 2006*). En esta muestra amplia, representativa y bien caracterizada, describieron riesgos incrementados para depresión (según criterios CIE-10), en los individuos homocigotos para el alelo S, independientemente de posibles factores confusores tales como el sexo, la edad, la historia familiar de problemas psicológicos o la frecuente

comorbilidad existente entre la depresión y los trastornos de ansiedad generalizada. Además, encontraron una asociación lineal entre la severidad del trastorno depresivo y el genotipo S/S, de modo que la asociación se hacía más fuerte cuanto mayor era la severidad del diagnóstico de depresión utilizado, sugiriendo un efecto dosis dependiente del riesgo para depresión conferido por el genotipo S/S *(Cervilla et.al. 2006)* (Ver Figura 5).



Asociación lineal entre la severidad del episodio depresivo (CIE-10) y el genotipo S/S del polimorfismo 5-HTTLPR del gen del transportador de serotonina. X2 = 6.13; DF= 1; p=0,013 para la asociación lineal.



Valores de odds ratio ajustados (95% CI) para la asociación entre el genotipo S/S y depresión.

FIGURA 5 Asociación entre el genotipo S/S del polimorfismo 5-HTTLPR del gen SERT y depresión (CIE-10). Tomado de *Cervilla et.al. 2006*

Otros trabajos han aportado evidencias neurobiológicas a favor de un papel clave de este polimorfismo en la etiología de la depresión.

Estudios de farmacogenética describen una peor respuesta al tratamiento con antidepresivos ISRS en los individuos portadores de la forma corta del gen SERT (*Zanardi et.al. 2000; Arias et.al. 2003; Serretti et.al. 2004*). Además, el alelo S ha sido asociado con una respuesta alterada del sistema serotoninérgico *(Smith et al., 2004*) y con una síntesis mayor y más prolongada de los niveles de cortisol en respuesta al estrés (*Gotlib et al., 2008)*.

Estudios de neuroimagen han descrito volúmenes del hipocampo reducidos en pacientes con depresión mayor portadores del alelo S *(Frodl et al., 2004; Taylor et al., 2005; Frodl et al., 2008*) y un incremento de la reactividad

amigdalar en estos pacientes *(Hariri et al., 2002; Hariri et al., 2005; Bertolino et al., 2005; Pezawas et al., 2005; Heinz et al., 2005; Dannlowski et al., 2008; Munafo et al., 2008*).

El alelo S ha sido también asociado con un procesamiento alterado de la emoción en pacientes que sufren de depresión *(Whalen et al., 2002)*, y con un elevado recambio de la serotonina en el cerebro de pacientes con depresión *(Barton et al., 2008).*

A pesar de la existencia de estas evidencias neurobiológicas a favor de la existencia de una asociación entre la variabilidad contenida en el polimorfismo 5HTTLPR y depresión, futuras investigaciones en cuanto al papel de este polimorfismo en la etiología de la depresión, son necesarias. Dado que, como se ha comentado, la depresión es una enfermedad con un claro componente ambiental, tales investigaciones deberán dirigirse, además, a explorar el papel de este gen en interacción con factores de riesgo de tipo ambiental.

◘ Gen del Enzima Monoamino Oxidasa A (MAOA)

La monoamino oxidasa (MAO) es una enzima mitocondrial que cataliza la degradación de los neurotransmisores serotonina, noradrenalina y dopamina, entre otras aminas biológicas *(Berry et al., 1994)*. En el cerebro existen dos tipos de esta enzima: la monoamino oxidasa A (MAOA) y la monoamino oxidasa B (MAOB). La MAOA metaboliza preferentemente serotonina y norepinefrina, mientras que la MAOB actúa sobre las feniletilaminas y la benzilamina *(Bach et al., 1988; Berry et al., 1994).*

La implicación de la MAOA en la etiología de la depresión vino determinada por el hecho de que, como ya se ha comentado, la MAOA es el enzima encargado de la degradación de monoaminas y éstas han sido ampliamente implicadas en la etiología de la depresión (*Berry et.al. 1994*).

Además, fármacos inhibidores de la MAOA son, desde hace tiempo, utilizados como antidepresivos *(Nolen, 2003; Frieling and Bleich, 2006; Papakostas and Fava, 2006).* Estas observaciones han hecho que los investigadores dirijan sus esfuerzos a la investigación del posible papel de esta molécula en la etiología de la depresión.

El gen que codifica para la MAOA ha sido también objeto de estudio por parte de los investigadores, ya que, variaciones en las secuencias estructurales o reguladoras de este gen podrían estar asociadas con alteraciones en la funcionalidad de la proteína.

El gen que codifica para la MAOA está localizado en el brazo corto del cromosoma X, entre las bandas Xp11.23 y Xp11.4 y se extiende a lo largo de más de 70 Kb. Está constituido por 15 exones *(Chen et al., 1991; Chen et al., 1992).*

Varios polimorfismos han sido descritos en el gen de la MAOA *(Hinds et al., 1992; Black et al., 1991; Hotamisligil and Breakefield, 1991; Brunner et al., 1993a; Brunner et al., 1993b)*, sin embargo, ninguno de ellos ha mostrado estar relacionado de forma directa con los niveles de expresión del gen ni con susceptibilidad para los trastornos afectivos, salvo el descrito por *Sabol y col*. en 1998.

Este polimorfismo, denominado uMAOA, es un VNTR localizado en la región promotora del gen, que está formado por una secuencia de 30 pb que puede repetirse 3, 3.5, 4 ó 5 veces. Otros autores han descrito alelos de 2 repeticiones *(Kunugi et.al. 1999)* y de 6 *(Huang et.al. 2004*), aunque con frecuencias muy bajas.

Los alelos 3.5, 4 y 5 han sido asociados a una eficiencia trascripcional de 2 a 12 veces mayor que la que muestran los alelos con 3 copias *(Sabol et.al. 1998;*

Deckert et.al. 1999; Denney et.al. 1999), quizá debido a que proporcionan una longitud optima de la región reguladora de la trascripción génica *(Sabol et al., 1998).* Ser portador de las variantes de alta actividad no es raro. Las frecuencias alélicas descritas en población española son: 31% para el alelo 3, 0.8% para el alelo 3.5, 67.8% para el alelo 4 y 0.4% para el alelo 5 *(Gutierrez et al., 2004).*

En la Figura 6 se muestra un esquema de la estructura del gen de la MAOA.

FIGURA 6. Estructura del gen MAOA y posibles alelos para el polimorfismo uMAOA.

Los investigadores se han interesado por este polimorfismo en relación a su posible implicación en la etiología de la depresión, ya que los alelos de alta actividad del gen darían lugar a una mayor tasa de degradación de monoaminas, provocando una reducción de los niveles monoaminérgicos en el espacio intersináptico, lo cual ha sido asociado con depresión *(Yu et.al. 2005).*

Así, algunos autores han descrito un exceso de alelos de alta actividad trascripcional en mujeres deprimidas *(Schulze et.al. 2000; Rivera et.al. 2009)* y han encontrado estos alelos asociados a una peor respuesta al tratamiento con antidepresivos *(Yu et.al. 2005).* Además los alelos de alta actividad han sido asociados a otros correlatos clínicos tales como suicidio *(Ho et.al. 2000),* neuroticismo *(Eley et.al. 2003)* o estacionalidad *(Manuck et.al. 2000; Eley et.al. 2003; Gutierrez et al. 2004).* Sin embargo, otros estudios no han replicado la asociación con depresión *(Muramatsu et al., 1997; Kunugi et al., 1999; Syagailo et al., 2001; Gutierrez et al., 2004; Huang et al., 2004; Christiansen et al., 2007)* e

incluso hay hallazgos en la dirección opuesta, es decir, que describen asociación entre los alelos de baja actividad (3 copias) y riesgos incrementados para depresión *(Brummett et.al. 2007).*

En la Tabla 4 se muestran los estudios de asociación llevados a cabo hasta el momento sobre el polimorfismo uMAOA y depresión. Las discrepancias en los resultados pueden deberse, nuevamente, al uso de muestras heterogéneas o no demasiado amplias.

Nuestro grupo de investigación también ha explorado, recientemente, el posible papel del polimorfismo uMAOA en la etiología de la depresión. De nuevo, usuarios de los servicios de atención primaria fueron ampliamente evaluados para una serie de variables psicológicas y sociodemográficas, en el contexto del estudio PREDICT-Gene (Cervilla et.al. 2006).

Rivera et.al. (2009), sobre una muestra de 1228 usuarios de atención primaria, describen riesgos incrementados para depresión (evaluada para tres fenotipos diferentes y cada vez más estrictos: episodio depresivo según criterios CIE-10, episodio depresivo severo CIE-10 y depresión según criterios DSM-IV) en mujeres portadoras de los alelos de alta actividad del polimorfismo uMAOA. En hombres describen una tendencia a la asociación que no llega a ser significativa, probablemente debido al escaso número de hombres en la muestra (Ver Figura 7).

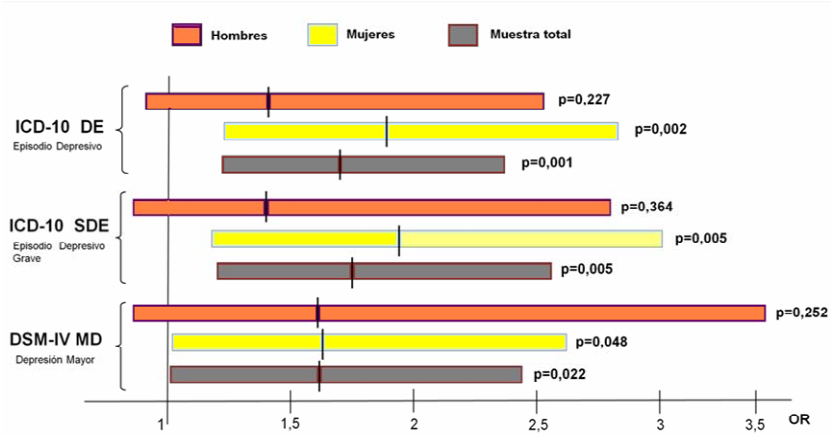


FIGURA 7. Odds ratios ajustados (por edad, al usar por separado la muestra de mujeres y la de hombres, y por edad y sexo, al usar toda la muestra) para la asociación entre depresión y los genotipos de alta actividad del polimorfismo uMAOA. Tomado de *Rivera et.al. 2009*)

TABLA 4: Principales Estudios de asociación genética y meta-análisis entre el polimorfismo uMAOA del gen de la MAOA y depresión.

ESTUDIO	POBLACIÓN	MUESTRA	ASOCIACIÓN
Muramatsu et al, 1997	Japonesa	112 casos: 52 depresión; 60 trastorno bipolar. 100 controles	No, de alelos de alta actividad con trastornos afectivos (depresión o trastorno bipolar).
Furlong et al, 1999	Caucásica y japonesa	231 casos: 125 depresión; 106 trastorno bipolar. 215 controles	No, de alelos de alta actividad con depresión o trastorno bipolar
Kunugi et al, 1999	Japonesa	259 casos: 98 depresión; 161 trastorno bipolar. 258 controles	No, de alelos de alta actividad con depresión, trastorno bipolar o intento de suicidio
Ho et al, 2000	Reino Unido	270 casos: 139 depresión; 131 trastorno bipolar.	Sí, de alelos de alta actividad con historia de suicidio y abuso de sustancias en la muestra de bipolares.
Schulze et al, 2000	Alemania	146 casos con depresión. 101 controles	Sí, de alelos de alta actividad con depresión mayor recurrente en mujeres.
Syagailo et al, 2001	Alemania	432 casos: 174 depresión mayor; 100 trastorno bipolar; 258 esquizofrenia. 229 controles	No, de alelos o genotipos de alta actividad con depresión mayor, trastorno bipolar y esquizofrenia.
Ono et al, 2002	Japón	155 víctimas suicidas 162 controles	No, de alelos y genotipos de alta actividad con suicidio.

Serretti et al, 2002	Italia	134 familias: 58 sujetos con depresión; 103 con trastorno bipolar	No, de alelos de alta actividad con depresión y trastorno bipolar.
Eley et al, 2003	Alemania	57 alto neuroticismo 62 bajo neuroticismo	Sí, de alelos de alta actividad con alto neuroticismo en hombres
Huang et al, 2004	Estados Unidos	663 casos con depresión mayor, trastorno bipolar, historia de suicidio o de abuso 104 controles	No, de alelos de alta actividad con trastornos del humor o historia de suicidio. Sí, del alelo de baja actividad con historia de abuso y su efecto en el desencadenamiento de alta impulsividad en hombres.
Gutiérrez et al, 2004	España	389 casos: 301 depresión mayor; 88 trastorno bipolar 156 controles	No, de alelos de alta actividad con depresión mayor y trastorno bipolar Sí, con estacionalidad y con sintomatología psicótica en mujeres con depresión
Courtet et al, 2005	Francia	738 casos con historia de suicidio 222 controles	No, de alelos de alta actividad con comportamiento suicida
Yu et al, 2005	China	230 casos con depresión mayor 217 controles	Sí, de alelo 4 con depresión mayor especialmente en mujeres. Asociación del alelo 4 con peor respuesta al tratamiento con antidepresivos (fluoxetina) en mujeres y no en hombres.
Brummett et al, 2007	Estados Unidos	42 cuidadores de parientes con demencia	Sí, del alelo de baja actividad con síntomas depresivos y mala calidad del sueño

		32 controles. * Solo hombres	
Christiansen et al, 2007	Dinamarca	684 gemelos *edad > 75 años	No, de alelos de alta actividad con síntomas depresivos.
Jollant et al, 2007	Francia	168 pacientes con historia de intento de suicidio	Sí, de alelos de alta actividad con la decisión de suicidarse en mujeres.
Rivera et.al. 2009	España	1228 usuarios de atención primaria: 243 con Depresión DSM-IV 421 Depresión CIE-10 de los que 243 tenían depresión severa CIE-10.	Si, de alelos de alta actividad con depresión (CIE-10, depresión severa CIE-10 y DSM-IV)

Este trabajo supuso el primer estudio de asociación con el polimorfismo uMAOA llevado a cabo en una amplia muestra (la más grande utilizada hasta ese momento), representativa de la población y en el que se realizaba una validación interna de los resultados mediante el análisis de las asociaciones a través de tres definiciones de depresión cada vez más estrictas.

El trabajo de *Rivera y colaboradores (2009)* arroja luz sobre el papel que este gen parece tener en la etiología de la depresión, aunque futuros estudios son necesarios.

Tales estudios deberían incluir una revisión sistemática, en forma de meta-análisis, de los trabajos publicados hasta este momento en relación al polimorfismo uMAOA y depresión. Futuras investigaciones deberán responder a ciertas cuestiones, tales como: ¿Variaciones en el polimorfismo uMAOA confieren realmente un riesgo incrementado para padecer depresión?; ¿Podría este polimorfismo tener algún efecto en el riesgo conferido por otros genes?; ¿Modifica este polimorfismo el riesgo para depresión conferido por los factores ambientales? Así, los futuros análisis deben testar la existencia de interacciones gen- gen o interacciones genético-ambientales.

◘ Gen del Receptor de Serotonina Tipo 1A (5HT1A)

El gen del recetor 5HT1A (HTR1A) se localiza en el cromosoma 5, concretamente en 5q11.2-q13 *(Kobilka, 1987)* y codifica para una proteína, de 422 aminoácidos, receptora de moléculas de serotonina. Los receptores 1A son receptores acoplados a proteína G, y presentan una estructura parecida a la de los receptores 5HT2A, con siete dominios transmembrana.

A nivel presináptico, los receptores 5HT1A se localizan en los cuerpos neuronales de los núcleos del rafe y actúan como autorreceptores somatodendríticos. Postsinápticamente, se encuentran principalmente en el

sistema límbico y actúan como heterorreceptores. Debido a esta doble localización de los receptores 5HT1A, su estimulación tiene un efecto dual en la transmisión serotoninérgica ya que, por una parte, la estimulación de los autorreceptores inhibe la actividad eléctrica de las neuronas serotoninérgicas de los núcleos del rafe, reduciendo así la liberación distal de serotonina y la neurotransmisión serotoninérgica, y, por otro lado, la estimulación a nivel postsináptico, facilita la neurotransmisión serotoninérgica.

El papel regulador de la neurotransmisión serotoninérgica desempeñado por los autorreceptores 5HT1A, el hecho de que estos receptores constituyen dianas de algunos de los fármacos antidepresivos más utilizados y la localización de los receptores postsinápticos 5HT1A en áreas corticales cerebrales y regiones límbicas implicadas en los estados emocionales, ha despertado un interés por conocer el posible papel que estos receptores podrían jugar en la etiología de la depresión y otros trastornos afectivos.

Así, estudios de neuroimagen *(Drevets et al., 1999; Drevets et al., 2000; Hasler et al., 2007; Hirvonen et al., 2008)* y estudios sobre muestras de cerebro postmortem *(Stockmeier et al., 1998; Boldrini et al., 2008)* han sugerido una implicación de estos receptores en la fisiopatología de la depresión, que podría estar determinada, al menos en parte, por la variabilidad genética contenida en el gen que codifica para los mismos.

Hasta ahora un total de 23 SNPs han sido descritos a lo largo del gen que codifica para los receptores serotoninérgicos tipo 1A. En 1999, *Wu y Comings* describieron un polimorfismo localizado en la región promotora del gen 5HT1A, concretamente en la posición -1019, que consta de una variación de un solo par de bases (C→G) *(Wu and Comings, 1999).*

Años más tarde, *Lemonde y colaboradores (2003)* describieron la existencia de un mecanismo alelo-dependiente de unión de factores de trascripción génica a la zona promotora del gen. Demostraron que ciertos factores de trascripción génica sólo se unen a la zona promotora del gen en presencia del alelo C de este polimorfismo y no lo hacen en presencia del alelo G.

Estos factores de trascripción tienen además una acción inhibidora o activadora de la trascripción génica que depende de su localización pre o postsináptica, respectivamente. Así, presinapticamente, el alelo G (-1019) no permite la unión de factores de trascripción que, en esa localización, tienen un efecto inhibitorio de la trascripción del gen 5HT1A. Por tanto, en presencia del alelo G, no hay represión de la expresión del gen 5HT1A, y se produce una sobre-expresión de autorreceptores 5HT1A y, como consecuencia, una reducción de la liberación de serotonina y de la neurotransmisión serotoninérgica, lo cual ha sido asociado a depresión. Sin embargo, a nivel postsináptico, la presencia del alelo G no permite la unión de factores de trascripción génica que, en esa localización, tienen un efecto potenciador de la expresión del gen 5HT1A.

Esta situación produce una reducción de la densidad de receptores 5HT1A postsinápticos y por tanto una disminución de la excitabilidad serotoninérgica en la neurona postsináptica *(Lemonde et.al. 2003)*. En definitiva, este trabajo describió el mecanismo molecular por el que el polimorfismo G (-1019) C del gen 5HT1A parecía conferir riesgos incrementados para depresión.

Posteriormente, otros autores describieron riesgos incrementados para depresión en individuos portadores del alelo G de este polimorfismo *(Parsey et al., 2006; Lenze et al., 2008)*, aunque otros no habían encontrado resultados en la misma dirección *(Arias et al., 2002; Huang et al., 2004)*. En la Tabla 5 se muestra un resumen de los principales estudios de asociación del este gen 5HT1A y depresión.

La depresión suele cursar muy frecuentemente de forma comórbida con ansiedad, y muy especialmente con trastorno de ansiedad generalizada *(Alonso et.al. 2004).* Ambos trastornos constituyen la mayor parte de la psicopatología más frecuente en atención primaria. Quizá, ambos trastornos se produzcan como resultado de una desregulación en la actividad serotoninérgica *(Millan, 2004),* es decir, que tengan una etiología compartida *(Anderson y Hope, 2008*) ya que, por ejemplo, fármacos que potencian la neurotransmisión serotoninérgica tienen un efecto tanto antidepresivo como ansiolítico *(Sthal, 2008).*

El gen 5HT1A ha sido también explorado en relación a su posible papel en el riesgo para trastornos de ansiedad, aunque nuevamente los resultados son poco concluyentes. Así, algunos autores sugieren que este gen podría conferir cierto riesgo para rasgos de personalidad tales como el neuroticismo *(Strobel et.al. 2003)* o para trastorno de pánico *(Rothe et.al. 2004),* aunque estos resultados no han sido aún replicados *(Hettema et.al. 2008).* Diversos estudios ponen de manifiesto que ratones knockout para el gen 5HT1A exhiben un comportamiento más ansioso que los ratones normales (*Lesh & Mossner 1999: Toth et.al. 2003).* Como consecuencia de la falta del mecanismo de feed-back negativo ejercido por los autorreceptores 1A, se produciría un aumento de la disponibilidad de serotonina a nivel de la neurotransmisión, que explicaría ese fenotipo más ansioso *(Lesch & Mossner 1999).* Otros trastornos de ansiedad relativamente frecuentes, tales como el trastorno de ansiedad generalizada (TAG), no han sido aún explorados en relación a su posible asociación con la variabilidad contenida en el gen 5HT1A *(Hettema et.al. 2008).*

Como ya se ha comentado, genes serotoninérgicos tales como el gen del transportador de serotonina o genes que codifican para receptores postsinápticos serotoninérgicos (5HT2A, 5HT2C, etc.) han sido estudiados en relación a su posible implicación tanto en depresión como en trastornos de ansiedad. Sin embargo, y a pesar de la frecuente comorbilidad de ambos trastornos, hasta ahora ningún estudio ha explorado el posible papel del gen

5HT1A en la etiología de la depresión, descontando el efecto de la comorbilidad con ansiedad generalizada, ni el papel de este gen en el riesgo para depresión comórbida con ansiedad. Dado el importante papel regulador que los receptores serotoninérgicos tipo 1A tienen en la neurotransmisión serotoninérgica, y dado que estos receptores son dianas de algunos de los fármacos antidepresivos actualmente en uso, es importante clarificar cuál es el verdadero papel, si existe, de este gen en el origen de la depresión.

TABLA 5: Principales Estudios de asociación genética entre el polimorfismo C (-1019) G del gen 5-HT1A y depresión y ansiedad.

ESTUDIO	POBLACIÓN	MUESTRA	ASOCIACIÓN
Zill et al. 2001		n=342 C-C	No con depresión
Arias et al. 2002	España	249 pacientes con depresión 170 controles	No con depresión, estacionalidad, melancolía, comp. suicida, sínt. psicóticos
Strobel et al. 2003	Alemania	284 participantes sanos	Si, con Depresión, Neuroticismo, ansiedad
Lemonde et al. 2003	Francia	129 pacientes con depresión 134 controles	Sí, con depresión y suicidio
Huang et al. 2004	Caucásicos, Africanos, hispanos, asiáticos y otros	696 pacientes psiquiátricos 107 controles 241 postmortem: 85 víctimas suicidas 156 controles no suicidas	No, con depresión Si con ataques de pánico y esquizofrenia
Rothe et al. 2004	Alemania	134 pacientes con trastorno de pánico 134 controles	No, con Trastorno de pánico Si, con Trastorno de pánico con agorafobia
Koller et al. 2006	Alemania	185 individuos con dependencia alcohólica	No, con rasgos de personalidad, temperamento, intento de suicidio
Serretti et al. 2007	Italia y Alemania	Grupo Alemania: 167 pacientes con intentos de suicidio (con depresión mayor: 107, espectro	No, con comportamiento suicida No, con intento suicidio, violencia, severidad de las consecuencias

		esquizoide 35, borderline 25), 92 víctimas suicidas y 312 controles. Grupo Italia: 152 pacientes con intentos de suicidio (con depresión mayor 68 y bipolares 84), y 131 controles.	

4.2. Factores Ambientales y Riesgo de Depresión

Como se ha comentado con anterioridad, la depresión es una enfermedad compleja y multifactorial, en la que no sólo los factores de riesgo biológicos (vulnerabilidad genética, disfunción monoaminérgica, disfunción del eje HPA, etc.) son los responsables de la aparición de la enfermedad, sino que, además, existen otros factores de tipo psicológico y social que juegan un importante papel en la aparición del fenotipo depresivo.

4.2.1. Factores Psicológicos

Numerosos estudios han asociado la personalidad premórbida del paciente con una mayor predisposición a padecer un trastorno depresivo. Ciertos rasgos neuróticos de personalidad (cluster C), una baja autoestima, la dependencia social, la introversión o el comportamiento obsesivo, han sido asociados a riesgos incrementados de depresión (*Hirschfeld et al 1989; Krieg et al., 1990; Boyce et.al., 1991; Kendler et.al, 1993).* Se trata de personas inseguras, con tendencia a la ansiedad, dependientes, con una elevada necesidad de aprobación, perfeccionistas y con bajos niveles de tolerancia al estrés, que desarrollan vínculos ansiosos no adaptativos que, quizá, los predisponen a padecer depresión *(Mazure and Maciejewski, 2003).* En estas personas, la personalidad podría jugar un importante papel en la sensibilización del paciente a determinados acontecimientos que otro tipo de personalidad no encontraría estresantes.

Además, el aprendizaje de comportamientos y conductas "negativas" a lo largo de la vida, les haría ver la realidad desde una perspectiva cada vez más pesimista y les predispondría a la enfermedad, ya que poco a poco dejarían de poner en marcha mecanismos de adaptación y aceptación frente a aquellas circunstancias que generan estrés, haciéndose más vulnerables a los efectos perjudiciales provocados por las adversidades.

4.2.2. Factores Sociales

Se ha comentado ya la importancia de la identificación de los factores de riesgo de depresión como herramienta de prevención de una enfermedad tan frecuente en nuestras poblaciones. Tales factores son muy numerosos e influyen sobre el individuo de muy diversas formas. Se ha sugerido un modelo explicativo que postula la existencia de factores sociales de riesgo predisponentes, factores precipitantes y factores perpetuantes que actuarían sobre el individuo aumentando la probabilidad de padecer la enfermedad y de que ésta se cronifique.

◘ Factores de riesgo predisponentes

Los factores de riesgo predisponentes son aquellos acontecimientos vitales que ocurrieron en el periodo prenatal, en la infancia o en la adolescencia y que tendrán su influencia en la edad adulta, como por ejemplo, la pérdida de un progenitor a edad temprana *(Lloyd,C. 1980-a.; Lloyd,C. 1980-b).*

Numerosos trabajos han sugerido un importante papel del abuso físico, sexual o psicológico durante la infancia, como factor de riesgo para el padecimiento de trastornos psiquiátricos en el adulto, incluyendo la depresión mayor *(Famularo et al., 1992; Mulder et al., 1998; Brown et al., 1999; Canetti et al., 2000; Kaufman et al., 2000; Harris, 2001; Harkness and Monroe, 2002; Bifulco et al., 2002; Gibb et al., 2003; Kaplow and Widom, 2007; Widom et al., 2007; Lenze et al., 2008).*

Se ha demostrado que un estrés temprano, generalmente asociado con circunstancias de deprivación maternal o de abuso, produce cambios celulares y neuroquímicos en ciertas partes del cerebro que alteran la excitabilidad neuronal (*Kaufman et.al 1998*) facilitando en el futuro la aparición de disfunciones de unión en esas áreas.

Otros hechos tales como la sobreprotección de los hijos por parte de los padres o, por el contrario, la falta de cuidado de los mismos, se han relacionado también con la aparición de depresión en la edad adulta *(Canetti et al., 2000; Enns et al., 2000; Enns et al., 2002).*

La presencia de estresores fuertes y repetitivos en ciertas circunstancias, da lugar a la formación de conexiones entre ciertas emociones y cogniciones que después, podrán repetirse juntas más frecuentemente (*Mandell 1995*). Experiencias traumáticas durante la niñez, son una de las circunstancias que pueden originar estas conexiones, que pueden ser después evocadas por mínimos estímulos ambientales y pueden ser reactivadas de una forma desproporcionada, provocando finalmente la enfermedad (*Wals et.al., 2005)*.

◘ Factores de riesgo precipitantes

Los factores de riesgo precipitantes tienen que ver con acontecimientos vitales estresantes (AVEs) que ejercen su efecto provocando el desencadenamiento del episodio depresivo. Los AVEs son fenómenos externos bruscos, de tipo psicológico, social, económico o familiar, que producen desadaptación social y distrés psicológico y que son percibidos como una amenaza por el sujeto sobre el que recaen *(Catalán et.al. 2005)*. Tales circunstancias corresponden con la muerte de un familiar, la pérdida de relaciones íntimas, dificultades de pareja, el padecimiento de una enfermedad o la discapacidad, entre otras.

El porcentaje de riesgo atribuible para estas circunstancias se estima entre el 37 y el 58%, es decir, que los AVES representan el 37-58% del total de eventos necesarios para que el paciente sufra un trastorno depresivo. *(Finlay-Jones et.al. 1981).*

Desde que a finales de los años 70, algunos autores asociaron el padecimiento de un episodio depresivo con la presencia previa de acontecimientos vitales estresantes *(Brown and Harris 1978),* diversos estudios

han puesto de manifiesto que estos acontecimientos pueden tener un efecto acumulativo, por lo que varios acontecimientos negativos tienen mayor efecto que uno solo *(Catalán Campos, 2004).* A su vez, el riesgo para depresión en individuos expuestos a AVES es siete veces mayor que en individuos no expuestos a eventos estresantes ambientales *(Paykel 1978).* Además, estudios prospectivos establecen que la acción de un acontecimiento estresante perdura hasta los siguientes seis meses o un año. *(Bebbington et al. 1993; Kendler and Karkowski-Shuman 1997; Kendler, Karkowski, and Prescott 1999).*

Diversos autores han demostrado que tales adversidades ambientales no actúan en solitario, sino en interacción con factores de vulnerabilidad personal (genéticos y de personalidad) que modularían su efecto, tal y como se describe más adelante (Caspi et.al. 2002, 2003; Zamit et.al. 2006; *Cervilla et.al. 2007).*

◘ Factores de riesgo perpetuantes

Los factores de riesgo perpetuantes son aquéllos que aumentan la probabilidad de instauración de una forma recurrente del trastorno depresivo. El déficit de apoyo social es uno de ellos, es decir, la falta de una relación emocional íntima que amortigüe situaciones de estrés. Numerosos trabajos han demostrado que el tener un confidente es un factor de protección contra el inicio y la recurrencia de episodios depresivos *(Paykel et al. 1996).*

El aprendizaje de comportamientos y conductas "negativas" a lo largo de la vida, es otro factor de riesgo perpetuante, que predispone a la cronificación de la enfermedad.

Factores perpetuantes de un trastorno depresivo son, además, aquellos que generan discapacidad (el padecimiento de una larga enfermedad, como la fibromialgia o algunos tipos de cáncer) o el consumo prolongado de tóxicos o fármacos que inducen depresión del sistema nervioso central (como el cannabis o las benzodiacepinas).

Sin embargo, las experiencias negativas ocurridas a lo largo de la vida no siempre desencadenan enfermedad mental. De hecho, lo normal es que el individuo pueda superar el efecto perjudicial de tales circunstancias. No es difícil percatarnos de que existen personas más resistentes que otras al efecto de estos acontecimientos estresantes, debido a la existencia de factores intrínsecos (como la carga genética, la personalidad o el sexo femenino) que interaccionan con los factores ambientales determinando la vulnerabilidad a la enfermedad.

En resumen, podemos decir que la depresión es el resultado de la interacción de factores predisponentes, precipitantes y perpetuantes y que, por tanto, los acontecimientos estresantes raramente son los únicos responsables de la aparición del episodio depresivo, más bien ejercen su acción sobre la predisposición intrínseca de cada individuo, lo que provoca el aumento de los niveles de vulnerabilidad hasta la aparición de la enfermedad.

4.2.3 Modelos Predictivos de Depresión

Como ya se ha comentado, la depresión es una enfermedad compleja y multifactorial, en la que factores de riesgo de distinta naturaleza (genéticos, psicológicos, sociales, etc.) están interrelacionados.

Los investigadores han dedicado sus esfuerzos a intentar comprender cómo esos factores etiológicos se combinan para dar lugar al fenotipo final de enfermedad. Así, han intentado elaborar modelos predictivos de depresión que integren tales factores, que expliquen las relaciones existentes entre ellos y que profundicen en la etiología de esta enfermedad, para desarrollar a partir de ellos medidas de prevención más eficaces.

- **Modelo de Akiskal y McKinney**

Akiskal y McKinney (1973) elaboraron un modelo predictivo de depresión que supuso el primer intento de integración de los datos clínicos,

experimentales, bioquímicos y neurofisiológicos disponibles hasta ese momento.

El modelo sugería que la depresión debe ser entendida como una combinación de factores químicos, de desarrollo, y de relaciones interpersonales, que ocurren de forma simultánea y no independientemente, y que convergen en los centros diencefálicos de refuerzo, provocando un deterioro reversible de estos centros neurofisiológicos, lo cual se traduciría en la aparición de la enfermedad.

Este modelo no incluía los factores genéticos como elementos de riesgo para depresión.

- **Best-Fitting Model (Kendler y colaboradores)**

El siguiente intento vino de la mano de *Kendler y colaboradores (1993; 2000; 2002; 2006),* quienes desarrollaron un modelo de predicción de depresión a un año, en el que analizaban un total de 18 predictores, que se estructuraban en cinco niveles, correspondiendo con cinco periodos del desarrollo del individuo (Ver Tabla 6).

Kendler y colaboradores comparaban, por primera vez, la importancia relativa de cada uno de los factores de riesgo respecto al resto, de modo que el modelo predictivo no sólo permitía conocer cuánto efecto tenía un factor determinado sobre la vulnerabilidad a padecer depresión, sino que permitía establecer si ese efecto era independiente o dependiente del resto de factores de riesgo.

El modelo predecía la aparición de depresión mayor en los siguientes 12 meses con un nivel de predicción del 52.1% en mujeres y de un 48.7% en hombres.

TABLA 6: Factores de riesgo analizados en el modelo de predicción de depresión Best-Fitting Model (*Kendler et al., 2006*).

PERIODO	PREDICTOR
Infancia	Riesgo genético (tasas de concordancia)
	Ambiente familiar perturbado (calor paternal y ambiente familiar)
	Abuso sexual en la infancia (self-report)
	Pérdida de alguno de los padres (antes de los 17 años)
Adolescencia temprana	Neuroticismo
	Baja autoestima
	Inicio temprano de trastornos de ansiedad
	Desórdenes de conducta
Adolescencia tardía	Nivel educativo
	Traumas a lo larga de la vida (muerte inesperada de un ser querido, aborto, asalto físico, etc.)
	Soporte social
	Abuso de sustancias (alcohol, tabaco y otras drogas)
Adulto	Haber estado alguna vez divorciado
	Historia previa de depresión
En el último año	Problemas de pareja
	Dificultades en el último año
	AVEs dependientes
	AVEs independientes

Este modelo sugiere que la depresión mayor es una enfermedad compleja y multifactorial y que la vulnerabilidad a tal enfermedad es el resultado del efecto sobre el individuo de una amplia matriz de factores de riesgo internos (carga genética o factores temperamentales, como el neuroticismo o el inicio temprano de trastornos de ansiedad), factores externos (desórdenes de conducta y abuso de sustancias) y de adversidad (un ambiente familiar adverso, el abuso sexual en la infancia, la pérdida temprana de alguno de los progenitores, un nivel educativo bajo, haber sufrido algún trauma, un bajo

soporte social o haber estado divorciado alguna vez), que actúan en distintos momentos de la vida del individuo.

Estos factores están interrelacionados de modo que, por ejemplo, sufrir adversidades durante la infancia, contribuye posteriormente a la aparición de desordenes de conducta y abuso de sustancias y éstos, a su vez, son predictores de adversidad. Además, las variables de internalización (neuroticismo e inicio temprano de trastornos de ansiedad) también contribuyen a adversidad en el futuro (ver Figura 8).

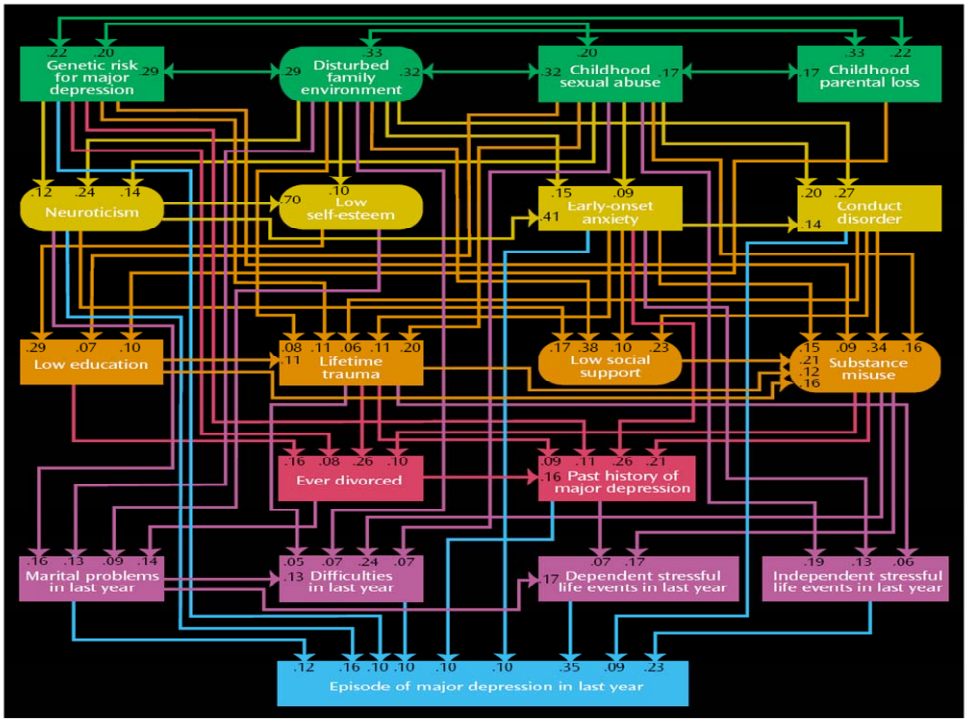


FIGURA 8. Representación del modelo Best-Fitting para la predicción de depresión en el siguiente año. Se representan las intrconexiones entre los predictores evaluados en el modelo y los valores de correlación estimados entre ellos. Tomado de *Kendler et al., 2005*

Aquellos individuos con una elevada carga genética de riesgo para depresión mayor (evaluada aquí como tasas de concordancia entre gemelos monozogóticos y dizigóticos), presentan también riesgos incrementados para estar expuestos a altas tasas de adversidad infantil, tienen mayores niveles de neuroticismo, presentan mayores tasas de inicio temprano de trastornos de

ansiedad y abuso de sustancias y son más propensos a verse afectados por dificultades y acontecimientos estresantes cuando son adultos, incrementando, todos estos factores, el riesgo para padecer depresión.

◘ Modelo PREDICT-D

Recientemente, se ha desarrollado un nuevo modelo predictivo de aparición de depresión, denominado PREDICT-D, que incluye un algoritmo compuesto por diez predictores y que proporciona un valor de probabilidad de aparición de depresión en los siguientes 12 meses.

Este modelo surge en el contexto de un proyecto internacional pionero denominado PREDICT-D, desarrollado a nivel europeo *(King et.al. 2006, 2008)*. Como se ha comentado anteriormente, se trata de un estudio prospectivo cuyo principal objetivo era desarrollar un inventario multifactorial predictor del riesgo de aparición de episodios depresivos a un año en usuarios de los servicios de atención primaria, y evaluar su poder predictivo en una muestra no europea para validar el modelo. El proyecto se desarrolló en seis nodos (o países) europeos y un nodo externo en Latino América, concretamente en Chile.

Tras una sistemática revisión bibliográfica, se seleccionaron treinta y nueve factores potenciales de riesgo para padecer depresión, para ser incluidos en la evaluación de los participantes del estudio PREDICT-D. Los factores seleccionados correspondían tanto a factores intrínsecos al individuo, como a factores que tienen que ver con el contexto social (ver Tabla 7). Además, se tuvo en cuenta el país de procedencia de cada participante.

TABLA 7: Factores de riesgo evaluados inicialmente, en la elaboración del modelo predictivo PREDICT-D para la predicción de depresión a un año.

FACTORES DE RIESGO PARA DEPRESIÓN EVALUADOS EN EL MODELO PREDICT-D	
Sexo	Historia previa de problemas con el alcohol
Edad	Historia previa de uso de drogas
Trabajo	Calidad de las relaciones sexuales y emocionales con la pareja
Nivel de estudios	Presencia de enfermedad física o mental grave, o problemas de abuso de sustancias, o incapacidad en personas con una estrecha relación con el entrevistado
Estatus en el trabajo	Dificultades en conseguir amigos o mantener relaciones cercanas
Etnia	Experiencia de abuso físico y/o emocional y sexual en la infancia
Propietario-ocupante de la vivienda	Creencias religiosas
Vive solo	Historia de problemas psicológicos graves en familiares de primer grado
Nacido en el país de residencia o extranjero	Historia de suicidio en familiares de primer grado
Satisfacción con las condiciones de vida	Síntomas de ansiedad en los 6 meses previos
Padecimiento de una larga enfermedad física	Síntomas de pánico en los 6 meses previos
Historia previa de depresión	Satisfacción con la zona donde vive
Dificultades en el trabajo remunerado o no remunerado	Sensación de seguridad en el hogar
Situación financiera	Acontecimientos vitales estresantes en los 6 mese previos a la entrevista
Autoconcepto de la salud física y mental	Experiencia de discriminación en los 6 meses previos a la entrevista por sexo, edad, etnia, discapacidad u orientación sexual
Uso de alcohol en los seis meses previos a la entrevista	Soporte social de amigos y familia

Un total de 5216 individuos procedentes de los servicios de atención primaria de diferentes centros de salud de Europa fueron evaluados para los treinta y nueve potenciales factores de riesgo de depresión. Todos los participantes fueron, además, evaluados a tiempo 0 para depresión mayor, según criterios DSM-IV, mediante la Entrevista Diagnóstica Compuesta Internacional (CIDI) (*Robins et.al. 1988; WHO, 1997*) repitiéndose la evaluación a los seis y a los doce meses de seguimiento, con el objetivo de identificar los nuevos casos de depresión.

A continuación, se midió cuáles de esas treinta y nueve variables predecían más potentemente la aparición de nuevos episodios. Siete de los treinta y nueve factores de riesgo evaluados fueron incluidos en el modelo final por ser los que más fuertemente se asociaban a depresión y, por tanto, los más potentes predictores de depresión mayor a un año (el criterio de inclusión en el modelo final se fijó en un valor de $p<0.01$ para la asociación de cada factor de riesgo con depresión). Se incluyeron en el modelo, además, las variables edad, sexo y país de procedencia

Tres de los diez predictores identificados en el modelo PREDICT-D correspondían a eventos ocurridos en el pasado (nivel educativo, historia previa de depresión, historia familiar de problemas psicológicos), dos correspondían a características del paciente (sexo y edad), cuatro predictores se enmarcaban en el momento actual (salud física, mental, dificultades en el trabajo remunerado o no remunerado y discriminación) y uno se refería al país de residencia.

El índice C nos informa sobre el poder predictor del modelo final. El valor del índice C global para todos los países europeos participantes fue del 0.79, lo cual significa que el modelo predecía nuevos casos de depresión a un año con una sensibilidad del 79%. En la Tabla 8 se muestran los valores del índice C para cada uno de los países europeos participantes en el proyecto.

TABLA 8: Valores del índice C para cada uno de los países europeos participantes en el estudio PREDICT. Tomado de King et al., 2008.

INDICE C PARA CADA PAIS	
PAIS	**INDICE-C (95% CI)**
Reino Unido	0.765 (0.705-0.808)
España	0.793 (0.746-0.840)
Eslovenia	0.833 (0.775-0.891)
Estonia	0.761 (0.690-0.833)
Países Bajos	0.852 (0.799-0.905)
Portugal	0.747 (0.693-0.800)
Índice C medio para todos los aíses	0.790 (0.767-0.813)

Para evaluar la bondad de ajuste del modelo, se dividió la muestra en deciles de probabilidad de riesgo para depresión dada por el modelo PREDICT-D. Se comparó, dentro de cada decil, la probabilidad de depresión dada por el modelo con la probabilidad observada de depresión, encontrándose que ambas tenían valores muy similares.

Además este modelo fue validado en una muestra externa, compuesta por 1732 usuarios de los servicios de atención primaria de Chile. El índice C para este país fue de 0.71 (95% CI 0.670-0.749).

El precedente en modelos predictivos de enfermedad más importante, es el modelo predictivo de riesgo cardiovascular, desarrollado por *Anderson y y colaboradores* en el año 1991 *(Anderson et.al 1991).* Este modelo fue desarrollado sobre una cohorte procedente de 12 países europeos y en la actualidad, es uno de los más utilizados en la prevención de enfermedad. Produce unos valores de índice C de entre 0.71 y 0.82.

El modelo PREDICT-D predice depresión con una probabilidad de acierto muy comparable a la del modelo predictivo de riesgo cardiovascular, por lo que supone una herramienta predictiva de depresión equiparable a dicho modelo, tan utilizado actualmente.

Cabe destacar que cuatro de los factores de riesgo incluidos en el modelo PREDICT-D son factores modificables y corresponden al momento actual. La identificación de estas variables como predictores de depresión y su naturaleza modificable, abre la puerta a la aplicación de estrategias de intervención y prevención de la enfermedad. Así, los esfuerzos para reducir la incidencia de la depresión podrían dirigirse a hacer frente a esos factores modificables, a través de una combinación de intervenciones médicas y psicológicas.

El modelo PREDICT-D proporciona una herramienta predictiva de episodios de depresión mayor antes de que éstos aparezcan, por lo que supone un valioso instrumento para la prevención de tales episodios.

4.3. Modelos de Interacción Genético-Ambiental en Depresión

Las investigaciones sobre los factores genéticos en los trastornos mentales que se han llevado a cabo desde la genética cuantitativa y molecular, han mostrado la discrepancia existente entre las altas tasas de heredabilidad estimadas y la escasez de resultados genéticos concluyentes (McGuffin et al., 2001; *Uher & McGuffin 2008; Insel & Collins 2003).* Una de las posibles explicaciones es que los genes implicados podrían no actuar directamente en relación a la etiología de la enfermedad, sino más bien modulando el riesgo conferido por determinados factores de tipo ambiental.

Es bien sabido que la enfermedad mental tiene un importante componente ambiental en su etiología. El estrés maternal durante el desarrollo embrionario (*Hernández-Martínez et al., 2008*), las complicaciones obstétricas (*Guth et al., 1993*), la deprivación del cuidado parental en la infancia (*Kaufman et.al 1998*), la exposición a conflictos familiares (*Cina et al., 2009*), o el padecimiento de acontecimientos vitales estresantes (*Brown and Harris 1978)*, son algunos de los reconocidos factores ambientales de riesgo para enfermedad mental. Sin embargo, hasta ahora, las investigaciones realizadas desde la genética en psiquiatría no han tenido demasiado en cuenta este componente ambiental.

El hecho observable de que los individuos muestran una heterogeneidad en sus respuestas a estos factores ambientales adversos, siendo unos individuos más susceptibles que otros al efecto ambiental, ha obligado a los investigadores a incorporar una visión integradora en el estudio de la etiología de la enfermedad mental, teniendo en cuenta conjuntamente el componente genético y el ambiental.

En esta nueva aproximación genético-ambiental, la enfermedad mental se produciría como resultado del efecto de intrincadas redes de cientos o miles de genes que actúan en interacción con múltiples factores ambientales, dando lugar al fenotipo final de enfermedad *(Hamer et.al. 2002).* Así, factores ambientales adversos serían responsables de la enfermedad y la carga genética determinaría una susceptibilidad individual a ese efecto perjudicial del ambiente *(Caspi & Moffitt 2006).*

De este modo, podemos definir la interacción genético-ambiental como una situación en la que el riesgo conferido por la exposición a un factor ambiental varía en función de la carga genética del individuo, o por el contrario, la situación en la que el efecto del genotipo varía de acuerdo con el ambiente *(Zammit and Owen, 2006)*.

Algunos autores han sugerido que, en muchos casos, el gen analizado deja de mostrar una asociación significativa directa con la enfermedad al descontar el efecto ambiental, es decir, que la asociación del componente genético con la enfermedad puede anularse erróneamente si no se usa una aproximación genético-ambiental *(Moffit et.al. 2005)*.

Esta podría ser la razón por la que los estudios de asociación genética, hasta ahora, han producido resultados no del todo concluyentes. Debemos tener en cuenta que el hecho de que un gen no haya sido asociado de forma concluyente a la enfermedad de interés, no implica necesariamente que ese gen no juegue un papel en la etiología de tal carácter, ya que éste puede estar actuando a través de su interacción con factores ambientales que, si no son tomados en cuenta, pudieran enmascarar su efecto *(Moffit et.al. 2005)*.

Las investigaciones realizadas desde la aproximación genético-ambiental constituyen una importante herramienta para un mejor conocimiento de la etiología de las enfermedades complejas, tales como las enfermedades mentales *(Taylor and Kim-Cohen, 2007)*. Además, aportarán una estimación más precisa del riesgo atribuible a la población tanto de factores genéticos como ambientales, siendo de gran ayuda en el diseño de estrategias de tratamiento y prevención de tales enfermedades.

Sin embargo, los estudios de interacción genético-ambiental tienen que ser abordados con prudencia, debido a que necesitan de un mayor número de tests estadísticos, lo cual supone un mayor riesgo de cometer errores de tipo I. Además, en estos estudios el número pequeño de acontecimientos dentro de los grupos de comparación, lleva a obtener un poder estadístico reducido, lo cual sólo será compensado con la presencia de un fuerte efecto de la interacción (*Zammit and Owen, 2006*).

Por tanto, la metodología y la interpretación de resultados en este tipo de estudios son complicadas. *Moffit y colaboradores (2005*) plantean una serie de

pasos estratégicos para llevar a cabo de una forma adecuada los experimentos de interacción genético-ambiental (ver Figura 9).

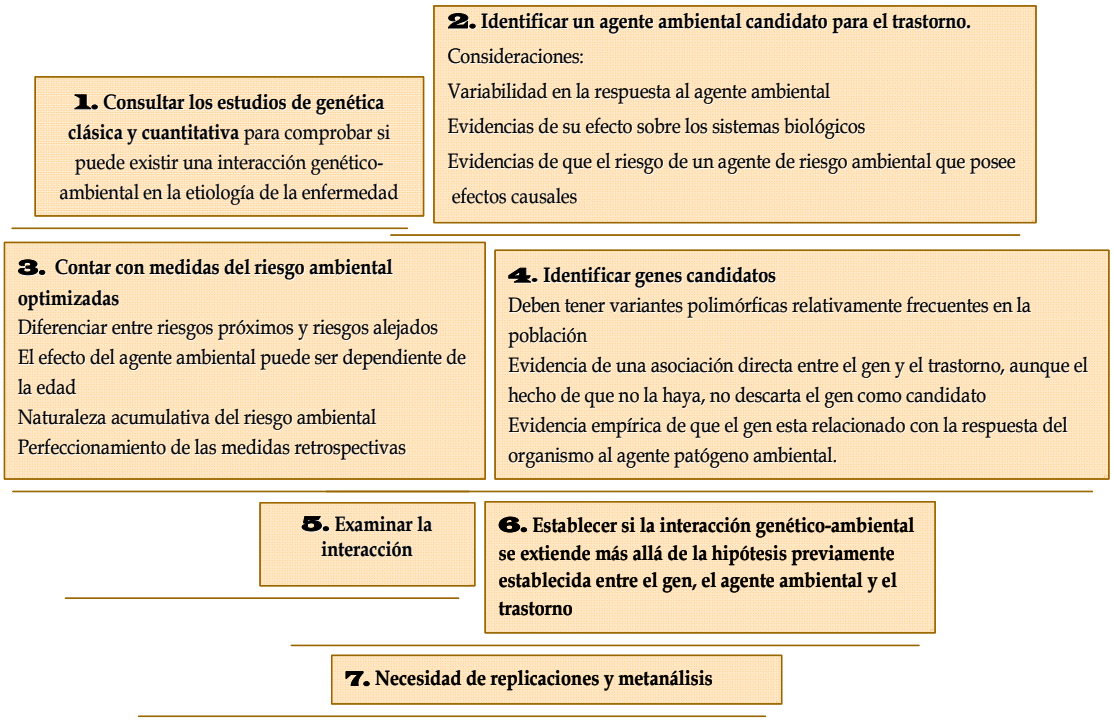


FIGURA 9. Pasos para realizar correctamente un estudio de interacción genético-ambiental. *(Moffit et.al. 2005)*

En el caso concreto de la depresión, la naturaleza de la relación entre los acontecimientos estresantes y la vulnerabilidad genética no está del todo bien establecida.

No se conoce bien si los genes predisponen a un individuo a encontrarse con eventos adversos o si ,una vez que éstos se producen, el efecto de los genes se limita a hacer que el individuo con la carga genética de riesgo sea más susceptible a ellos *(Farmer et al. 2005)*, aunque parece que los genes hacen que los individuos sean más susceptibles a la adversidad, más que influir en la exposición a ésta *(Farmer et.al.2000)*.

4.3.1. Modelos de interacción SERT y ambiente.

La primera evidencia empírica de la existencia de una interacción entre genes y ambiente en depresión, fue descrita por *Caspi* y colaboradores (*Caspi et.al Science 2003*).

Previamente, se había sugerido que la vulnerabilidad de los individuos a los acontecimientos estresantes estaba relacionada con su carga genética *(Costello et.al. 2002)* y algunos investigadores habían documentado un mayor riesgo de depresión, tras sufrir un acontecimiento estresante, en los individuos con una carga genética de riesgo, en comparación con aquéllos sin ese riesgo genético *(Kendler et.al. 1995)*. Sin embargo, no se conocía cuáles podrían ser los genes que modulaban el efecto de los AVEs en la vulnerabilidad a padecer depresión.

Algunas evidencias apuntaban al gen que codifica para el transportador de serotonina (SERT) como uno de los genes candidatos para ejercer este efecto.

Murphy y colaboradores (2001) habían demostrado la existencia de una respuesta al estrés diferenciada en ratones knock-out para este gen. Así, frente a un estímulo estresante, los ratones knock-out para el transportador de serotonina, tanto homocigotos (5HTT -/-) como heterocigotos (5HTT +/-), exhibían un comportamiento más atemorizado y mayores niveles de hormona adrenocorticotropina en comparación con los controles.

Posteriormente, en un estudio realizado sobre primates (*Rhesus machaques*) sometidos a estrés, se había asociado el alelo S de este gen con una función serotoninérgica disminuida (una menor concentración de 5-hidroxindol-acético en líquido cefalorraquídeo), respecto a aquellos animales con el genotipo L/L *(Bennett et.al. 2002)*.

Por último, *Hariri y colaboradores (2002),* en un estudio de neuroimagen, asociaban el polimorfismo 5HTTLPR del gen del transportador de serotonina con la respuesta al estrés, demostrando que los individuos portadores del alelo S de este polimorfismo, exhibían mayor actividad amigdalar en situaciones de miedo que los individuos L/L.

Estos hallazgos sugerían que variaciones en el gen del transportador de serotonina podrían modular las reacciones psicopatológicas a acontecimientos estresantes.

El trabajo de *Caspi y colaboradores (2003)* supuso la primera evidencia empírica de la existencia de una interacción genético-ambiental y un riesgo incrementado para padecer depresión.

Se realizó sobre una cohorte de 1037 individuos (52% hombres) de la misma edad, que fueron seguidos y evaluados para toda una serie de variables socio-ambientales y psicológicas a las edades de 3, 5, 7, 9, 11, 13, 15, 18, 21, 26 años.

Se evaluaron dos factores ambientales concretos: la presencia de acontecimientos vitales estresantes (AVEs) en los últimos 5 años (relativos al empleo, la economía, la salud, las relaciones y el hogar) y el maltrato en la infancia.

La aparición de depresión mayor (según criterios DSM-IV) en el ultimo año se evaluó, al final del seguimiento, mediante la Diagnostic Interview Schedule *(Robins et.al. 1998).*

Los resultados de este estudio mostraban, en primer lugar, y tal y como se esperaba, la existencia de una asociación entre el número creciente de AVEs y un riesgo incrementado para padecer depresión ($p<0.001$). En segundo lugar, no encontraron un efecto del polimorfismo 5HTTLPR del gen que codifica para

el transportador de serotonina en la aparición de depresión (p=0.29). Sin embargo, cuando se valoró la posible interacción entre las diferentes variantes polimórficas de ese gen y los acontecimientos vitales estresantes, se encontró un efecto global estadísticamente significativo (P=0.056). Por genotipos, la significación aumentaba, de modo que los individuos doblemente portadores del alelo corto (S) del gen y que además acumulaban más acontecimientos vitales estresantes, eran los que presentaban un mayor riesgo para padecer depresión (p=0.002) (Figura 10-a).

Respecto al efecto del maltrato en la infancia en el riesgo para padecer depresión, encontraron que el maltrato sufrido durante la infancia incrementaba significativamente la probabilidad de desarrollar depresión en la vida adulta en individuos portadores del alelo corto (S) (p=0.05 para la interacción) en comparación con los no portadores. (Figura 10-b).

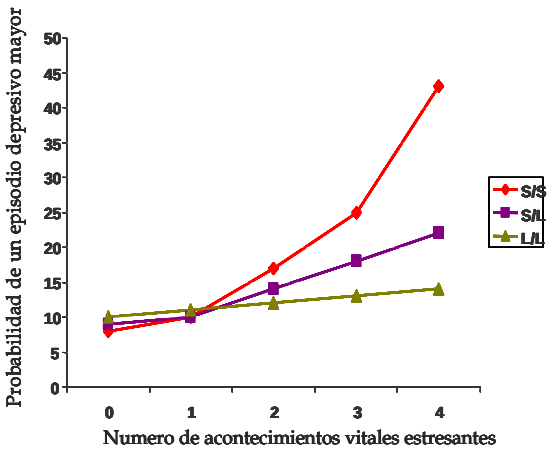


FIGURA 10-a. Probabilidad de un Episodio Depresivo Mayor en función del número de acontecimientos vitales estresantes y de la variabilidad del gen SERT (Caspi et al., 2003).

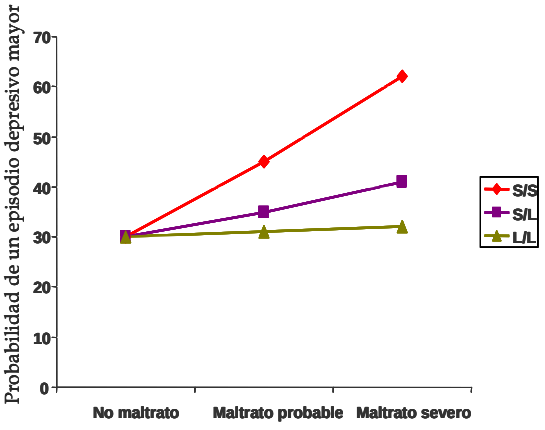


FIGURA 10-b. Probabilidad de un Episodio Depresivo Mayor en función del tipo de maltrato y de la variabilidad del gen SERT (Caspi et al., 2003).

En resumen, los individuos portadores del alelo corto (S), transcripcionalmente menos activo *(Lesh et.al. 1996),* del gen del transportador de serotonina, tienen una mayor probabilidad de desarrollar depresión, después de sufrir acontecimientos vitales estresantes o abuso durante la

infancia, que aquellos individuos homocigotos para el alelo largo (L). Estos hallazgos demuestran que el polimorfismo funcional 5-HTTLPR de la región promotora del gen SERT modula el efecto de la adversidad ambiental en depresión (*Caspi et.al. 2003*).

Desde los hallazgos de *Caspi y colaboradores (2003)*, la interacción entre el polimorfismo 5HTTLPR y la exposición a AVEs en la aparición de depresión ha estado siendo profundamente examinada. Numerosos estudios han replicado estos hallazgos (*Grabe et.al. 2005; Eley et al 2004, Kaufman et.al. 2004; Kendler et.al. 2005; Nakatani et.al. 2005; Sjoberg et.al. 2006; Jacobs et.al. 2006; Kaufman et.al. 2006; Wilhelm et.al. 2006; Cevilla et.al 2007; Kim et.al. 2007; Mandelli et.al 2007; Lazary et.al. 2008),* mientras que otros no lo han conseguido *(Gillespie et.al. 2005; Surtees et.al. 2006; Chipman et.al. 2007; Covault et.al. 2007; Power et.al.2008*).

En la Tabla 9 se resumen los principales estudios de interacción genético-ambiental entre el polimorfismo 5HTTLPR del gen SERT, los AVEs y depresión.

Las discrepancias en los resultados probablemente tienen que ver con el uso de diferentes metodologías.

Algunos trabajos han sido desarrollados sobre una muestra de sólo mujeres (Eley et.al. 2004; Sjöberg et.al. 2006; Chorvov et.al. 2007; Wichers et.al. 2008), o de gemelos *(Gillespie et al., 2005; Kendler et al., 2005; Jacobs et al., 2006; Chorbov et al., 2007;* Wichers et al, 2008*),* o sólo de niños *(Kaufman et al., 2004; Kaufman et al., 2006)*, sólo adolescentes *(Eley et al., 2004; Chipman et al., 2007; Cicchetti et.al. 2007),* sólo estudiantes o profesores *(Covault et al., 2007; Sjöberg et al., 2006; Taylor et al., 2006; Wilhelm et al., 2006)*, sólo ancianos *(Kim et al, 2007; Power et.al. in press)*, sólo mujeres embarazadas *(Scheid et al, 2007),* o sólo individuos afectados por la enfermedad *(*deprimidos o bipolares en *Mandelli et.al 2007)*.

Tabla 9. Estudios de interacción genético-ambiental entre el polimorfismo 5-HTTLPR, los AVEs y la depresión.

ESTUDIO	POBLACIÓN	MUESTRA	METODOLOGÍA	INTERACCIÓN
Caspi et al, 2003	Nueva Zelanda	847 participantes de 26 años	Estudio longitudinal. Seguimiento a 23 años. Evaluados en 10 tiempos. AVEs en los últimos 5 años Diagnostic Interview Schedule para evaluar depresión (DSM-IV).	Sí, interacción genotipos(S/S y L/S) con AVEs, maltrato infantil y riesgo para depresión. No asociación S/S y depresión
Kaufman et al, 2004	Estados Unidos: Origen Euro-americano Hispano y Afro-americano	57 casos: niños con historia de maltrato 44 niños controles 5-15 años de edad	Estudio transversal. Mood and Feelings Questionary (MFQ) para depression en niños. Schedule for Affective Disorders and Schizophrenia for School Aged Children (K-SADS-PL) y Child Behavior Checklist (CBCL) para evaluar la presencia de enfermedad psiquiátrica. Maltrato: se midió abuso físico, sexual, maltrato emocional y negligencia usando múltiples informantes. Arizona Social Support Interview	Sí, triple interacción genotipo S/S, historia de maltrato, déficit de apoyo social y riesgo para depresión. Si asociación S/S y depresión

			Schedule (ASSIS) para evaluar el soporte social.	
Eley et al, 2004	Reino Unido	377 adolescentes 12-19 años: 93 con altos niveles de depresión y de adversidad, 117 con altos niveles de depresión y bajos de adversidad, 57 con bajos niveles de depresión y altos de adversidad y 110 con bajos niveles de depresión y de adversidad.	Estudio transversal caso-control. Se seleccionaron los que puntuaron entre 3 y 12 en la Mood and Feelings Questionary (MFQ) para depresión. Social Problems Questionary (SPQ) para adversidad familiar y social. Nivel educativo List of Threatening Events (LTE) en los 6 meses previos	Sí, interacción genotipo S/S y AVEs y riesgo para depresión solo en mujeres. Si asociación S/S y depresión sólo en mujeres
Gillespie et al, 2005	Australia	1091 gemelos entre 19-78 años.	Estudio longitudinal. Pare evaluar depresión (DSM-IV) se usó SCL-90 y DSSI/sAD. LTE en los 12 meses previos.	No, interacción genotipo S/S y AVEs y riesgo para depresión. No asociación S/S y depresión.
Grabe et al, 2005	Alemania	976 participantes (población	Estudio transversal.	Sí, interacción alelo S con desempleo y

		general) entre 20-79 años: un grupo con altas tasas de estrés físico y mental vs. grupo control, con sexo, estado civil y nivel educativo similares.	Estrés físico y mental (incluido depresión) medido con BL-38, "Tangible Suport Subscale" fue usada para evaluar soporte social	enfermedades crónicas y riesgo de ansiedad física y mental en mujeres. No asociación S/S con salud física ni mental.
Kendler et al, 2005	Estados Unidos	549 gemelos de 31-71 años	Estudio longitudinal. Depresión DSM-III-R. 14 AVEs ocurridos en los 2 últimos meses.	Sí, interacción S/S, AVEs y riesgo para depresión. El genotipo S/S modula el efecto ejercido por los AVEs recientes de intensidad baja sobre el riesgo para depresión, pero no tanto el efecto que ejercen los AVEs de intensidad moderada o alta.
Nakatani et al, 2005	Japón	2509 pacientes con infarto de miocardio	Estudio transversal. Zung Self-rating Depresión Scale (SDS) para depresión	Sí, interacción alelo S e infarto de miocardio y riesgo para depresión

Jacobs et al, 2006	Bélgica	374 gemelos (mujeres)	Estudio longitudinal a 6 meses en 4 tiempos 90-Items Symptom Check List para evaluar síntomas depresivos. Interview for Life Events para evaluar AVEs en los últimos 6 meses.	Sí, interacción genotipo S/S y AVEs y riesgo para depresión
Kaufman et al, 2006	Estados Unidos	109 niños maltratados 87 niños no maltratados 5-15 años	Estudio transversal Mood and Feelings Questionary (MFQ) para depression en niños. Schedule for Affective Disorders and Schizophrenia for School Aged Children (K-SADS-PL) y Child Behavior Checklist (CBCL) para evaluar la presencia de enfermedad psiquiátrica. Maltrato: se midió abuso físico, sexual, maltrato emocional y negligencia usando múltiples informantes. Arizona Social Support Interview Schedula (ASSIS) para evaluar el	Sí, interacción genotipo S/S, alelo Met del gen BDNF, historia de maltrato y riesgo para depresión. Además, describen una cuádruple interacción: S/S x Met/* x historia de maltrato x soporte social y riesgo para depresión.

			soporte social.	
Sjöberg et al, 2006	Suecia	180 estudiantes (16-19 años)	Estudio longitudinal No se usaron entrevistas estructuradas para medir AVEs (tipo de residencia, familia separada, presencia de conflictos traumáticos dentro de la familia), sino una serie de preguntas. Depresión Self-Rating Scale para medir depresión (DSM-IV).	Sí, interacción genotipo S/S, AVEs, sexo y riesgo para depresión. Los AVES que incrementaban el riesgo para depresión en hombres tienen que ver con el estatus social y en mujeres con las relaciones personales.
Surtees et al, 2006	Reino Unido	4175 participantes (41-80 años). Extremos de neuroticismo	Estudio transversal. Depresión en el último año (DSM-IV). Health and Life Experiences Questionary (HLEQ) para evaluar las circunstancias sociales y psicológicas. List of Threatening Experiences Questionary (LTE-Q) para evaluar los AVEs en el último año y en la infancia.	No, interacción genotipo S/S y adversidad social y riesgo para depresión mayor. No asociación S/S y depresión.

Taylor et al, 2006	Estados Unidos	116 participantes	Estudio transversal. Risk Families Questionary para evaluar el ambiente familiar en la infancia. Se uso una lista de 10 LEs para evaluar la adversidad reciente.	Sí, interacción genotipo S/S, adversidad (tanto reciente como en la infancia) y una mayor sintomatología depresiva.
Wilhelm et al, 2006	Australia	127 participantes	Estudio longitudinal a 5 años. Diagnostic Interval Schedule (DIS) y Composite Internacional Diagnostic Interview (CIDI) para depresión. Entrevista semiestructurada para medir AVEs, maltrato en la infancia y deficiencias en el cuidado parental.	Sí, interacción genotipo S/S y adversidad (reciente y en la infancia) y riesgo para depresión mayor.
Zalsman et al, 2006	Caucásico	191 casos con depresión 125 controles	Estudio transversal (caso-control). Structured Clinical Interview para depression. Hamilton Depression Ratting Scale y	Sí, interacción de los genotipos de baja expresión génica (S/S y L_G/L_G), AVEs y severidad de depresión. No, interacción de estos genotipos con

			Beck Depression Inventory para severidad de depression. St. Paul-Ramsey Scale para medir LEs en los 6 meses previos.	abuso en la infancia y severidad de depresión.
Cervilla et al, 2007	España	737 usuarios de atención primaria	Estudio transversal. List of Threatening Events para evaluar LEs en los últimos 6 meses. Sección de depression del Composite International Diagnostic Interview (CIDI) para evaluar prevalencia de depresión y depresión severa (CIE-10) en los 6 últimos meses.	Sí, interacción del genotipo S/S, AVEs y riesgo para depresión (tanto severa como moderada), incluso ajustando por edad, sexo e historia familiar de problemas psicológicos entre los familiares de primer grado. Si, asociación.
Chipman et al, 2007	Australia	2095 y 674 adolescentes y adultos jóvenes	Estudio longitudinal. Goldberg Depresión and Anxiety Scalespara evaluar síntomas de depresión y ansiedad. AVEs se evaluaron mediante una lista	No, interacción genotipo S/S con AVEs o adversidad en la infancia y síntomas de depresión y ansiedad

			de 12 AVEs referida a los 6 meses anteriores. Adversidad en la infancia se evaluó mediante una lista de 17 experiencias traumáticas.	
Chorbov et al, 2007	Estados Unidos	247 gemelos (mujeres, adolescentes y adultas jóvenes)	Estudio longitudinal. Para evaluar hitoria de problemas médicos o psiquiátricos usaron la Child Semi-Structured Assesment for the Genetics of Alcoholism (CSSAGA). Usaron una lista de 10 items autoadministrada para evaluar AVEs traumáticos.	Si, interacción, pero con alelo L_A (alta expresión génica), trauma y riesgo para depresión mayor.
Covault et al, 2007	Estados Unidos	295 estudiantes de universidad	Estudio longitudinal. Life Events Scale for Students (LESS) adaptada de la Social Readjustement Rating Scale, para evaluar AVEs.	No, interacción genotipo S/S, AVEs con síntomas depresivos Sí, interacción genotipo S/S y AVEs y consumo habitual de drogas y alcohol

			Beck Depression Inventory para evaluar depresión. NEO Personality Inventory para evaluar neuroticismo.	
Kim et al, 2007	Corea	732 participantes de edad avanzada (65+)	Estudio longitudinal. Seguimiento a 2.5 años. Geriatric Mental State (GMS) para depresión. List of Threateing Events para AVEs.	Sí, interacción genotipos (S/S y S/L), AVEs y riesgo para depresión, incluso ajustando por edad, sexo y discapacidad. Además encuentran una triple interacción del genotipo para 5HTTLPR, BDNF (Val66Met), AVEs y riesgo para depresión. Replican los hallazgos de Kaufman et.al. 2006 pero con AVEs y en una muestra de ancianos.
Scheid et al, 2007	Estados Unidos	568 mujeres embarazadas	Estudio transversal. Center of Epidemiologic Studies Depresión Scale (CES-D) evaluó síntomas depresivos en la última semana. AVEs desde la infancia hasta la adultez	Sí, interacción genotipo S/S, violencia física o abuso sexual y riesgo para depresión

			se evaluaron con la Turner, Wheaton and Lloyd Checklist.	
Mandelli et al, 2007	Italia	686 casos: 323 depresión mayor; 363 trastorno bipolar	Estudio de casos, transversal. Structured Clinical Interview (SCID) se usó para evaluar trastorno afectivo según criterios DSM-IV. AVEs ocurridos en el último año se evaluaron mediante la Life-Events and Difficulty Schedule (LEDS).	Sí, interacción alelo S, AVEs y riesgo para trastornos afectivos, principalmente depresión mayor y en mujeres. Si, interacción alelo Met en el gen COMT, AVEs y trasntornos afectivos. Triple interacción alelo S, alelo Met, AVEs y trastornos afectivos.
Cicchetti et al 2007	Origen mayoritario afro-americano. Algunos de origen Hispano y Caucásico	339 adolescentes con bajo nivel socioeconómico: 207 con historia de maltrato, 132 sin maltrato.	Diagnostic Interview Schedule for Children (DISC) para síntomas de depression (DSM-IV). La version Young Self Report (YSR) de Child Behavior Checklist (CBCL) para evaluar síntomas ansioso-depresivos o somáticos. Adolescent Doping Orientation for Problem Experiences (A-COPE) para	Si, interacción genotipo S/S con abuso sexual y depresión. No, interacción 5HTTLPR con otros tipos de maltrato y depresión. Si, interacción alelo de baja actividad de uMAOA con maltrato y depresión. Si, triple interacción, genotipo S/S, uMAOA baja actividad y abuso sexual con depresión.

			evaluar experiencias para hacer frente a acontecimientos estresantes. Maltreatment Classification System para codificar el tipo de maltrato sufrido.	
Wichers et al, 2008	Bélgica	394 mujeres gemelas	Estudio longitudinal a 15 meses con 5 tiempos. Structured Clinical Interview (SCID) se usó para evaluar trastorno afectivo según criterios DSM-IV. Para evaluar adversidad infantil se usó la versión corta del cuestionario CTQ.	Si, triple interacción del genotipo Met/Met del BDNF, el genotipo S/S del 5HTTLPR, adversidad infantil y síntomas de depresión.
Lazary et al, 2008	Hungría (origen caucásico)	567 usuarios de atención primaria	Cuestionario estructurado autoadministrado para historia médica y psiquiátrica, personal y familiar y datos socioeconómicos. Con la Zung Self-Rating Depresión	Si, interacción del genotipo S/S del 5HTTLPR con AVEs y depresión. Interacción del genotipo G/G del rs140700 del SLC6A4 con AVEs y depresión. No, interacción 5HTTLPR, rs140700 y

			Scale (ZSDS) se evaluaron síntomas depresivos (DSM-IV). List of Threatening Life Events para evaluar AVEs en los dos años anteriores.	depresión. Triple interacción 5HTTLPR x rs140700 x AVEs y depresión. Interacción haplotipos 5HTTLPR-rs140700 con altas dosis de adversidad y depresión.
Brummett et al, 2008	USA. Caucásicos y Afro-americanos	430 voluntarios sanos	Center Epidemiologic Studies Depresión Scale (CES-D) y Obvious depresión Scale (OBS) para medir síntomas depresivos.	Si, interacción 5HTTLPR x sexo x estrés x depresión: En mujeres, interacción alelo S, elevados niveles de estrés y depresión. En hombres, es el genotipo L el que modifica el riesgo para depresión conferido por elevados niveles de estrés. No, interacción 5HTTLPR con bajos niveles de estrés y depresión. No, interacción genotipo S/S, etnia y nivel de estrés con depresión.
Power et al, in press	Francia	1421 individuos mayores de 65 años procedentes de la	Estudio longitudinal. Seguimiento a 2 años.	No, interacción polimorfismo 5HTTLPR con AVEs y depresión

		Comunidad	MINI y CES-D para evaluar depresión.	
Zhang et al, 2009	China	401 casos con depresión 391 controles	Estudio transversal. Hamilton Rating Scale for Depresión (HAM-D) para severidad de depresión Life Events Scale by Yang & Zhang para medir AVEs en el último año. Chinese Social Support Rating Scale (SSRS) para evaluar el soporte social.	Si, interacción genotipo L/L, altas dosis de adversidad y depresión. Sin embargo, es el genotipo S/S el que modifica el efecto de los AVEs en el riesgo para depresión cuando éstos no son severos. Además describen una cuádruple interacción del genotipo L/L de 5HTTLPR, el genotipo G/G del rs6295 del gen 5HT1A, AVEs y deficiente soporte social, con síntomas de depresión (solo en el grupo de 20-29 años).
METANÁLISIS				
Munafo et al, 2009		15 artículos de interacción 5HTTLPR y AVEs con depresión.	Metanálisis Criterios de inclusión de artículos: Evaluación del genotipo para 5HTTLPR Evaluación de los AVEs de forma	La interacción genotipo S/S con AVEs y síntomas de depresión es insignificante. Los hallazgos positivos de interacción 5HTTLPR x AVEs son, por ahora, comparables a los que pudieran darse

			comparable a Caspi et al, 2003. Evaluación de depresión mayor o de ánimo deprimido.	por azar. Pocos estudios son comparables metodológicamente con el de Caspi et al, 2003. Los estudios hasta ahora publicados tienen poco poder estadístico para evaluar la interacción gen-ambiente.

Las diferencias metodológicas radican también en el tamaño muestral. Así, trabajos como los de *Gillespie et al., 2005; Grabe et.al. 2005; Nakatani et.al. 2005; Surtees et al., 2006; Chipman et al., 2007; Cervilla et.al. 2007; Kim et.al. 2007; Zhang et.al. 2009; Power et.al. in pres,* usan un elevado tamaño de muestra, mientras que otras investigaciones cuentan con tamaños muestrales más reducidos *(Kaufman et.al. 2004; Eley et.al. 2004; Kendler et.al. 2005; Jacobs et.al. 2006; Kaufman et.al. 2006; Sjöberg et.al. 2006; Taylor et.al. 2006; Wilhelm et.al. 2006; Zalsman et.al. 2006; Chorvov et.al. 2007; Covault et.al. 2007; Scheid et.al. 2007;Cicchetti et.al. 200; Wichers et.al. 2008; Lazary et.al. 2008; Brummett et.al. 2008)*.

El tamaño muestral es el principal factor limitante para detectar una interacción genético-ambiental *(Luan,J.A. et.al. 2001)*, pero no es el único factor a tener en cuenta. Así, por ejemplo, las investigaciones de *Gillespie et al., 2005, Surtees et al., 2006* y *Chipman et al., 2007,* aunque realizadas sobre muestras amplias, obtienen resultados negativos. Esto podría ser debido a que el reclutamiento y evaluación de la muestra se haya realizado con otros propósitos diferentes al de testar la existencia de una interacción genético-ambiental en depresión *(*en el caso de *Gillespie et.al. 2005; Surtees et.al. 2006)*, lo cual disminuye el poder estadístico de forma sustancial, ya que las evaluaciones psicopatológicas no están diseñadas con ese propósito. Por tanto, son preferibles muestras más pequeñas pero evaluadas de una forma precisa *(Wong et al., 2003).*

Otro problema es que la variable dependiente (diagnóstico de depresión) y los AVEs no se evalúan de una forma estandarizada en todas las investigaciones. Así, por ejemplo, *Surtees y cols. (2006),* evalúan los AVEs y los síntomas de depresión de forma auto-administrada, como parte del mismo cuestionario; sin embargo, *Gillespie et al., (2005)* obtienen el diagnóstico de depresión de forma retrospectiva.

Las asociaciones débiles o no significativas que se encuentran entre los AVEs y la depresión en estos estudios sugieren la existencia de problemas con

la validez de las medidas auto-administradas de los AVEs y con la posible falta de precisión de la evaluación retrospectiva de la depresión *(Uher and McGuffin, 2008)*. En el estudio realizado por *Chipman y cols. (2007),* las limitaciones incluyen la falta de equilibrio Hardy-Weinberg en las frecuencias genotípicas y la ausencia de datos longitudinales en los AVEs.

Todos estos trabajos han utilizado diferentes medidas de depresión y estrés ambiental, y han utilizado diferentes diseños experimentales. Aún así, la evidencia más fuerte está a favor de una interacción entre el polimorfismo 5-HTTLPR y distintos factores estresantes y un riesgo incrementado para depresión *(Zammit and Owen, 2006).*

Recientemente, nuestro grupo de investigación ha llevado a cabo un estudio en el que se intentaba replicar los hallazgos de *Caspi et.al. 2003* en una muestra amplia, representativa de la población general y en la que se han usado herramientas estandarizadas y validadas para efectuar el diagnóstico de depresión y la presencia de AVEs *(Cervilla et.al. 2007).*

Los resultados de este trabajo fueron publicados en una de las revistas con mayor impacto en el área de la Psiquiatría, la revista Molecular Psychiatry (ver Anexo 1).

El trabajo de *Cervilla y colaboradores (2007)* surge en el contexto del estudio PREDICT-Gen *(Cervilla et al., 2006)* que, como ya se ha comentado, es un estudio de asociación caso-control desarrollado sobre la cohorte española de participantes en otro estudio más amplio, el estudio PREDICT-D *(King et al., 2006, 2008)*, cuyo objetivo es la identificación de predictores de depresión en los usuarios de los servicios de atención primaria.

Cervilla y colaboradores (2007) evaluaron una muestra de 737 participantes, para variables sociodemográficas y psicológicas, mediante entrevistas estructuradas y validadas (*King, M. et al., 2006*). En el caso en que no estuviera

disponible una herramienta validada para explorar alguna variable en concreto (como es el caso de la historia familiar de problemas psicológicos), se llevaba a cabo una prueba de fiabilidad.

La presencia de AVEs en los seis meses anteriores a la entrevista, fue evaluada mediante la List of Threaning Events (*Brugha et al., 1990*). Se trata de una lista de eventos estresantes, que incluye acontecimientos estresantes serios, como la muerte uno de los padres, esposo/a o hijo/a u otro pariente, ruptura con la pareja o problemas económicos. Se dividió a los participantes en tres niveles de exposición a AVEs (ninguno, un AVE, dos o más AVEs).

La sección de depresión de la Entrevista Diagnóstica Compuesta Internacional (CIDI) *(Robins et al., 1989)* fue usada para generar el diagnóstico de depresión y de gravedad del episodio depresivo, según criterios CIE-10, referido a un periodo de seis meses previos a la entrevista. Esta herramienta fue administrada por entrevistadoras entrenadas previamente.

Como se ha comentado con anterioridad, *Cervilla y colaboradores (2006)* habían descrito previamente, en esta misma muestra, riesgos incrementados para padecer depresión entre los individuos con el genotipo S/S del polimorfismo 5HTTLPR del gen del transportador de serotonina. Tal asociación fue independiente de la edad, el sexo, la historia familiar de problemas psicológicos en los familiares de primer grado de los participantes y de la comorbilidad con trastorno de ansiedad generalizada. Además, la asociación se hacía más robusta a medida que aumentaba la severidad del trastorno depresivo.

Cervilla et.al. (2007), describieron una asociación independiente entre el diagnóstico de depresión (y de depresión severa) con la exposición previa a AVEs. Esta asociación también era independiente de la edad, el sexo, y la historia familiar de problemas psicológicos.

Por último, replicando los hallazgos de *Caspi et al., 2003,* en este trabajo se encontró que el genotipo para el polimorfismo 5HTTLPR modificaba el riesgo para depresión conferido por los AVEs. Así, los individuos homocigotos para el alelo S, sólo necesitaban estar expuestos a un AVE para elevar su riesgo de padecer depresión (o depresión severa) al mismo nivel que los individuos con otras combinaciones alélicas, expuestos a más de dos AVEs. Esta interacción era independiente de la edad, el sexo y la historia familiar de problemas psicológicos en los familiares de primer grado de los participantes (Ver Figura 8).

Estos resultados supusieron la primera replicación de los resultados de *Caspi et.al. 2003*, ya que se trataba del primer estudio, después del original, realizado sobre una muestra representativa de la población general. Hasta ese momento, como se ha comentado con anterioridad, las replicaciones que se habían publicado habían sido desarrolladas sobre muestras sólo de mujeres *(Eley et al., 2004; Grabe et al., 2005; Sjoberg et al., 2006),* o sólo de gemelos *(Kendler et al., 2005),* etc.

Además, fue el primer estudio que examinaba la existencia de una interacción genético-ambiental en una muestra española, con frecuencias genotípicas y prevalencias de exposición a AVEs similares a otras poblaciones europeas.

Por último, se trataba de la primera vez que un estudio de interacción tenía en cuenta el efecto de posibles confusores, tales como el sexo, la edad y la historia familiar de problemas psicológicos entre los familiares de primer grado de los participantes.

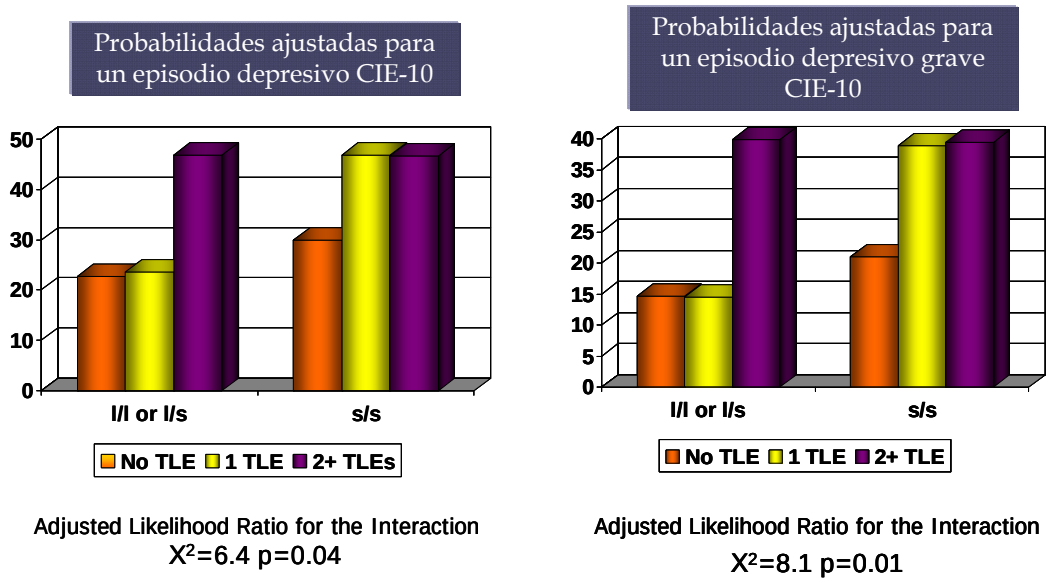


FIGURA 11. Interacción entre el genotipo S/S, los acontecimientos vitales estresantes (TLEs) y riesgo para depresión. Tomado de *Cervilla et,al 2007.*

Con todo, *Munafò et al. (2009),* en un reciente metanálisis, concluyen que pocos de los estudios de interacción entre el polimorfismo 5HTTLPR del gen SERT, AVEs y depresión son comparables metodológicamente con el de *Caspi et.al. 2003* y que los estudios hasta ahora realizados tienen poco poder estadístico para evaluar una interacción genético-ambiental, por lo que los resultados a favor de la existencia de tal interacción pueden haber sido fruto del azar *(Munafò et.al. 2009).*

Existen hasta ahora pocos estudios con un diseño longitudinal que aborden la interacción 5HTTLPR y AVEs en depresión. Quizá estos estudios sean la clave. Los estudios longitudinales proporcionan un mayor poder que los transversales y posibilitan determinar la dirección de la causalidad puesto que permiten relacionar la presencia del hipotético factor de riesgo con casos incidentes de la variable resultado (outcome) que se esté considerando.

4.3.2 Modelos de interacción MAOA y ambiente.

Los trabajos publicados que exploran la posible existencia de una interacción genético-ambiental en depresión no son muy numerosos para el caso del gen de la monoamino-oxidasa A (MAOA) (Ver Tabla 10).

Caspi et.al. (2002) publicaron el primer estudio de interacción con el gen MAOA. Los resultados mostraron que los niños que habían sido maltratados y que portaban los genotipos que confieren baja actividad del enzima MAOA, desarrollaban en la edad adulta trastornos de la conducta, personalidad antisocial y delitos violentos, de una forma significativamente más frecuente que aquéllos niños que también sufrieron malos tratos pero que portaban otra forma del gen *(Caspi et al., 2002)*.

Estudios posteriores han conseguido replicar estos hallazgos *(Foley et al., 2004; Kim-Cohen et al., 2006; Nilsson et al., 2006; Widom and Brzustowicz, 2006; Frazzetto et al., 2007; Ducci et al., 2008)*.

Dos recientes metanálisis analizan la interacción entre el gen de la MAOA y el maltrato en la infancia en la etiología del comportamiento antisocial en la edad adulta *(Kim-Cohen et al., 2006; Taylor and Kim-Cohen, 2007)*. Ambos describen la existencia de una interacción fuerte entre la MAOA y el maltrato en la niñez como factor de riesgo para el desarrollo de un comportamiento antisocial.

Sin embargo, existe poca evidencia acerca de la existencia de una posible interacción entre el gen MAOA, el ambiente y depresión.

Hasta ahora, existen dos trabajos publicados que han explorado, aunque de forma muy sutil, esta potencial interacción. *Eley et.al. 2004*, en un estudio realizado sobre una muestra de 377 adolescentes, no encontró asociación entre el genotipo para el polimorfismo uMAOA, AVEs y depresión. Sin embargo, años más tarde, *Cicchetti et.al. 2007,* en una muestra de adolescentes, describe la existencia de una interacción entre los genotipos de baja actividad del enzima

MAOA, maltrato y depresión. Además, describe una triple interacción entre genotipo S/S del gen del transportador de serotonina, el genotipo de baja actividad del enzima MAOA, abuso sexual y depresión.

Una vez más, y como ocurriera en el caso de los estudios de interacción para el gen del transportador de serotonina (SERT), el tamaño muestral en estos estudios no es demasiado amplio. Además, las muestras utilizadas no son representativas de la población general (sólo se consideraron adolescentes).

Se hace necesario, por tanto, el desarrollo de nuevos estudios de interacción genético-ambiental en depresión para clarificar el posible papel que el gen de la MAOA pudiera tener en el origen de la enfermedad.

Tabla 10. Estudios de interacción genético-ambiental entre el polimorfismo uMAOA, los AVEs y la depresión.

ESTUDIO	POBLACIÓN	MUESTRA	METODOLOGÍA	INTERACCIÓN
Eley et al, 2004	Reino Unido	377 adolescentes 12-19 años: 93 con altos niveles de depresión y de adversidad, 117 con altos niveles de depresión y bajos de adversidad, 57 con bajos niveles de depresión y altos de adversidad y 110 con bajos niveles de depresión y de adversidad.	Estudio transversal caso-control. Se seleccionaron los que puntuaron entre 3 y 12 en la Mood and Feelings Questionary (MFQ) para depresión. Social Problems Questionary (SPQ) para adversidad familiar y social. Nivel educativo List of Threatening Events (LTE) en los 6 meses previos	No, interacción uMAOA, AVEs y depresión.
Cicchetti et al 2007	Origen afro-americano. Algunos de origen Hispano y Caucásico	339 adolescentes con bajo nivel socioeconómico: 207 con historia de maltrato, 132 sin maltrato.	Diagnostic Interview Schedule for Children (DISC) para síntomas de depression (DSM-IV). La version Young Self Report (YSR) de Child Behavior Checklist (CBCL) para evaluar síntomas ansioso-depresivos o somáticos.	Si, interacción alelo de baja actividad de uMAOA con maltrato y depresión. Si, triple interacción, genotipo S/S, uMAOA baja actividad y abuso sexual con depresión.

			Adolescent Doping Orientation for Problem Experiences (A-COPE) para evaluar experiencias para hacer frente a acontecimientos estresantes. Maltreatment Classification System para codificar el tipo de maltrato sufrido.	

II. Hipótesis y Objetivos

HIPÓTESIS

Los factores genéticos individuales (por ejemplo, el polimorfismo 5-HTTLPR del gen SERT o el polimorfismo uMAOA del gen MAOA), en interacción con factores de adversidad psicosocial (en concreto, aquellos integrados en el modelo de predictivo de depresión PREDICT-D, o aquellos que suponen la exposición previa a acontecimientos vitales estresantes), pueden aumentar el riesgo para sufrir episodios depresivos.

OBJETIVOS GENERALES

1. Estudiar si los factores de riesgo genéticos (genes clave de la neurotransmisión serotoninérgica, tales como el gen SERT y el gen MAOA) y ambientales (factores de adversidad social como los que componen el modelo predictivo de depresión PREDICT-D, o los acontecimientos vitales estresantes) interaccionan entre sí para determinar la aparición prospectiva de episodios depresivos.

2. Establecer perfiles genéticos de vulnerabilidad a depresión.

OBJETIVOS ESPECÍFICOS

1. Estudiar la asociación entre la depresión y la variabilidad en genes clave de la neurotransmisión setoroninérgica, tales como el gen 5-HT1A.

2. Realizar un estudio de interacción gen-gen entre la variabilidad contenida en el gen SERT y la del gen MAOA, como determinantes de la aparición prospectiva de episodios de depresión.

3. Explorar, mediante la realización de un estudio de interacción genético-ambiental, si la variabilidad contenida en los genes SERT y MAOA interacciona con la exposición a acontecimientos vitales estresantes en la aparición prospectiva de episodios depresivos.

4. Realizar un estudio de interacción genético-ambiental entre la variabilidad contenida en los genes SERT y MAOA y los factores adversidad social incluidos en el modelo predictivo de depresión PREDICT-D, como determinantes de la aparición prospectiva de episodios depresivos.

III. Muestra y Métodos

Esta Tesis Doctoral se presenta por el innovador formato de artículos, modalidad contemplada en las Normas Reguladoras de los Estudios del Tercer Ciclo y del Título de Doctor de la Universidad de Granada (http://www.ugr.es/~docto/guia.html), según la cual: "podrá constituir la tesis doctoral, el reagrupamiento en una memoria de los trabajos de investigación publicados por el doctorando".

Así, todos los resultados derivados del trabajo desarrollado por la doctoranda, e incluidos en la presente Tesis, ya han sido publicados o están en vías de publicación en revistas indexadas y de referencia en el área de la Psiquiatría y las Neurociencias. Por este motivo tal y como se estipula que debe hacerse en estos casos, el capítulo de Muestra y Métodos no es un capítulo redactado al estilo convencional, sino un breve resumen del contexto y diseño del estudio, de la muestra sobre la que se ha basado el trabajo y de los métodos utilizados. Cada uno de los artículos científicos que conforman los distintos capítulos de la sección de Resultados detalla en más profundidad todos esos aspectos.

1. CONTEXTO DEL ESTUDIO

La presente tesis doctoral se ha desarrollado en el contexto del estudio PREDICT-Gene *(Cervilla et.al. 2006).* Se trata un estudio de seguimiento longitudinal a tres años de una cohorte de individuos usuarios de los servicios de atención primaria de distintos centros de salud del país con el objetivo de establecer perfiles genéticos de vulnerabilidad a depresión e identificar posibles interacciones entre factores genéticos y ambientales determinantes de la aparición prospectiva de episodios depresivos (*Cervilla et.al. 2006*).

El estudio PREDICT-Gene se origina y se enmarca, a su vez, en el contexto de otro estudio, el proyecto PREDICT-D.

El PREDICT-D es un estudio prospectivo de cohorte en el que distintos usuarios de los servicios de atención primaria de seis países europeos participantes fueron reclutados y evaluados en tres ocasiones a lo largo de un año.

El objetivo principal era desarrollar un inventario de riesgo multifactorial predictor de la aparición de episodios de depresión (*King et al., 2006*). Como objetivo secundario del estudio estaba el que ese inventario pudiera ser utilizado en el futuro como una herramienta de trabajo que permitiera, por un lado, detectar individuos de alto riesgo para depresión y, por otro, que facilitara la elaboración de estrategias de intervención y prevención (*King et.al. 2006).*

El estudio PREDICT-D, cuyos resultados principales han sido publicados recientemente (*King et al., 2008*), no contemplaba la inclusión de factores biológicos, algo que sí contempla el estudio PREDICT-Gene.

El estudio PREDICT-Gene se ha desarrollado sobre la muestra de Málaga que formaba parte del estudio europeo originario y, siguiendo exactamente el mismo diseño, sobre una muestra más amplia procedente de Granada y otras

cinco provincias españolas (Madrid, Zaragoza, Logroño, Las Palmas de Gran Canaria y Mallorca) que forman parte de una extensión española del estudio PREDICT-D (*Bellón et al., 2008*).

En la presente tesis doctoral se incluyen los individuos procedentes de los centros de salud de Málaga y Granada, dado que la muestra restante no ha sido todavía analizada genéticamente. Así, de los 2761 individuos que a día de hoy han donado muestra biológica para el estudio PREDICT-Gene, en este trabajo se presentan datos sólo para 1319 sujetos, que son los procedentes de los nodos andaluces del proyecto.

2. MUESTRA

Componen nuestra muestra aquellos individuos, usuarios de los servicios de atención primaria de quince centros de Atención Primaria de las provincias de Granada y Málaga, que participaron en el estudio PREDICT-Gene en periodos discretos desde Septiembre de 2003 a Diciembre de 2007.

En la Figura 12 se muestra los centros de Atención Primaria que participaron en el estudio PREDICT.

Todos los individuos fueron invitados a participar voluntariamente en el estudio, siendo previamente informados acerca de los objetivos de éste y de su naturaleza. Aquellas personas que accedieron a participar, dieron su consentimiento informado por escrito.

Fueron criterios de exclusión: ser mayor de 75 años (debido a la alta prevalencia de trastornos cognitivos a partir de esta edad) o menor de 18 años,

sufrir una enfermedad terminal, un trastorno mental de naturaleza orgánica, no entender español.

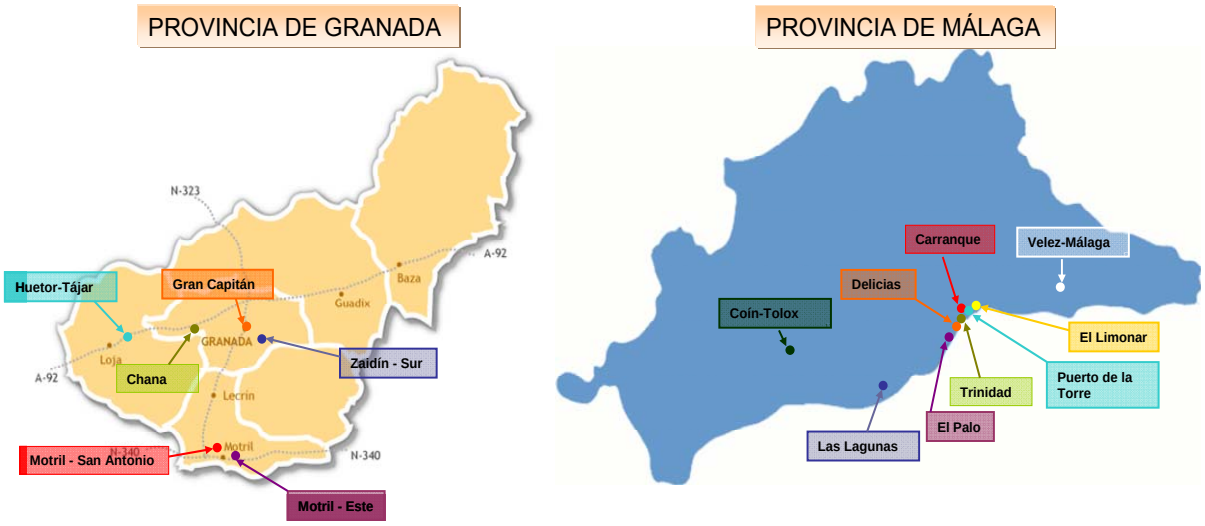


FIGURA 12. Centros de Atención Primaria de las provincias de Granada y Málaga que participaron en el estudio PREDICT

El nodo español del proyecto PREDICT-D, con sede en la Universidad de Granada, recavó una muestra inicial diana de 1278 individuos procedentes de nueve centros de salud de la provincia de Málaga. (Ver Figura 13) Todos ellos fueron seguidos a lo largo de un año y evaluados a tres tiempos (T1: inicio, T2: 6 meses, T3: 12 meses) para toda una batería de variables sociodemográficas y psicológicas, mediante entrevistas estructuradas y validadas administradas por entrevistadoras previamente entrenadas (*King, M. et.al. 2006*). De los 1278 individuos que iniciaron el estudio, 960 completaron el seguimiento a un año.

Posteriormente, 783 usuarios de seis centros de Atención Primaria de la provincia de Granada accedieron a participar en el estudio PREDICT y fueron, igualmente, seguidos a lo largo de un año y evaluados a tres tiempos para las mismas variables. De estos 783 sujetos que iniciaron el estudio en Granada, 564 completaron el seguimiento a 6 meses, momento en el que se les solicitó donar una muestra biológica para el análisis genético.

Aunque la muestra diana final estaba, por tanto, formada por 1524 individuos (960 de Málaga y 564 de Granada, ver figura 12), los trabajos aquí presentados pueden presentar oscilaciones en el número final de individuos incluidos en cada análisis, puesto que dichos trabajos se fueron desarrollando en distintos momentos temporales. De esta forma, aquellos análisis desarrollados antes incluirán menos individuos que los que se desarrollaron más tarde, cuando la muestra se había incrementado.

En el apartado de resultados se detallan las características metodológicas y muestrales concretas de cada uno de los trabajos que componen esta tesis.

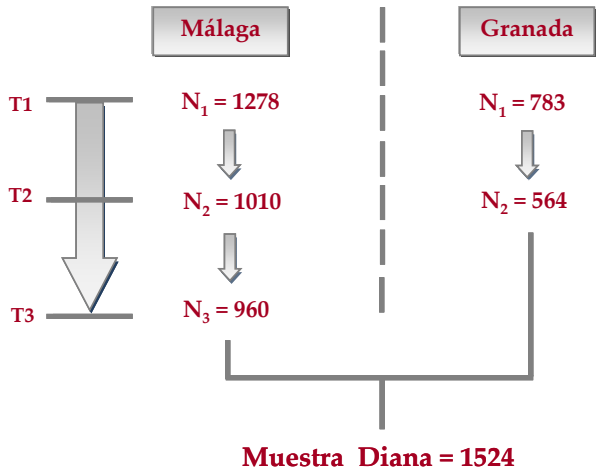


FIGURA13. Participación y seguimiento del estudio

3. VARIABLE DEPENDIENTE: DIAGNÓSTICO DE DEPRESIÓN

Las variables dependientes o variables resultado utilizadas en los trabajos de investigación que se incluyen en esta tesis doctoral fueron:

1) Depresión mayor según criterios diagnósticos DSM-IV a T0 (en el primero de los trabajos)

2) Depresión incidente en cualquier momento a lo largo del primer año de seguimiento (en el resto de trabajos incluidos).

El diagnóstico de depresión se hizo usando la sección de depresión de la Entrevista Compuesta Diagnóstica Internacional *(Composite International Diagnostic Interview, CIDI, Robins et.al. 1988).* Esta herramienta consiste en una entrevista psiquiátrica estructurada dirigida a evaluar trastornos psiquiátricos según criterios CIE-10 y DSM-IV. Contiene una serie de preguntas clave que establecen el camino a seguir en la exploración. Se puede aplicar la herramienta global o sólo secciones específicas y puede ser utilizada por entrevistadores sin conocimientos psicopatológicos y con un entrenamiento no superior a una semana.

La sección de depresión de la CIDI nos permitió generar tanto diagnósticos de episodio depresivo según criterios ICD-10 (episodio depresivo leve, moderado o grave), como diagnósticos de depresión según criterios DSM-IV. Para ello, las puntuaciones obtenidas en la subescala de depresión de la CIDI se analizaron a través de un algoritmo de decisión validado por *King y colaboradores (2008).* Este algoritmo permitió transformar la información recogida mediante la CIDI en un diagnóstico, es decir, habilitar esa información para establecer un diagnóstico de depresión (bien de depresión mayor según criterios DSM-IV, o bien de episodio depresivo, siguiendo criterios ICD-10). Este algoritmo pasa por alto algunos datos perdidos, incluso si se responden positivamente, siempre que esas respuestas no den lugar al diagnóstico, es decir, no deja de tomar en cuenta a un individuo por que tenga un ítem perdido, siempre que ese ítem no dé lugar al diagnóstico (*King et.al. 2008)*.

En el primero de los trabajos incluidos en esta tesis, sólo los diagnósticos a tiempo 0 (T0) fueron tenidos en cuenta, ya que el estudio se diseñó según un modelo caso-control, transversal, anidado en un estudio de cohorte.

Para el resto de trabajos, se tuvieron en cuenta los diagnósticos generados en los tres tiempos dentro del primer año de seguimiento.

4. VARIABLES INDEPENDIENTES

Como se ha comentado con anterioridad, todos los participantes en el estudio PREDICT-Gene fueron evaluados prospectivamente (T0, T1 y T2) para una batería de variables sociodemográficas (como el sexo, edad, estado civil, nivel educativo, nivel ingresos o profesión) y psicológicas (relaciones familiares y sociales, historia psiquiátrica familiar, acontecimientos vitales estresantes y problemas de discriminación, entre otros) que constituyen potenciales factores de riesgo individuales o ambientales para depresión, según la literatura previa.

Para evaluar estos factores, se utilizó un inventario derivado de medidas autoaplicadas de validez y fiabilidad reconocidas. En los casos en los que esto no fue posible, los instrumentos utilizados fueron adaptados de cuestionarios estandarizados, o bien fueron desarrollados específicamente para este estudio. Los instrumentos utilizados fueron sometidos a pruebas de fiabilidad, de acuerdo a lo estipulado por *King y cols. (2006).*

De todos los factores de riesgo evaluados en el contexto del estudio PREDICT-D (ver Tabla 7 del capítulo de introducción), a continuación se describen sólo aquéllos que se usaron en los análisis incluidos en la presente Tesis Doctoral.

Factores Sociodemográficos

Edad

Sexo

Nivel académico: doctorado, licenciado, diplomado, secundaria (Bachiller/COU/FP2), primaria (EGB/ESO/FP1), sabe leer y escribir pero no tiene estudios, analfabeto, otros.

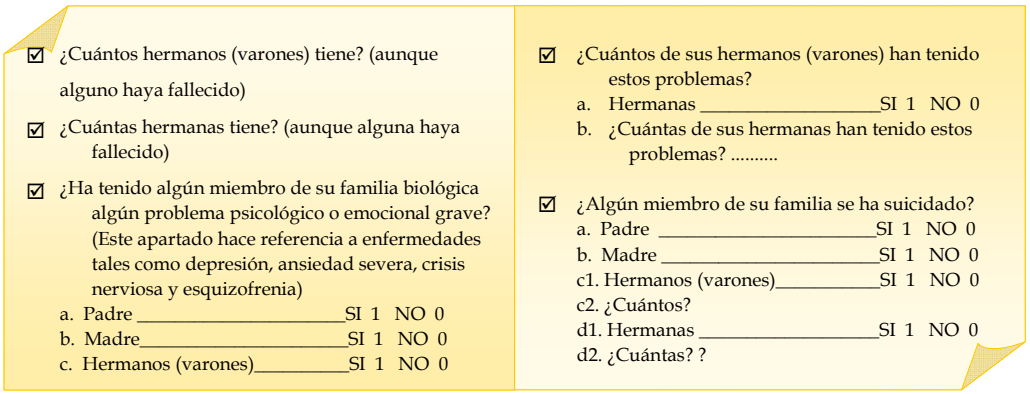
Estado civil: casado, viviendo en pareja, separado, divorciado, viudo, soltero.

Situación profesional: trabajando, en paro, jubilado, incapacitado para trabajar, cuidando el hogar, estudiando, otros.

Historia Familiar de Problemas Psicológicos

Se evaluó la presencia de problemas psicológicos que requirieran tratamiento psicológico o farmacológico en los familiares de primer grado del individuo entrevistado. Para ello se administró un cuestionario tomado de *Qureshi et.al. (2005)* que incluía las preguntas que se indican en la Figura 14.

Se llevaron a cabo pruebas de fiabilidad test-retest que nos informaron de la idoneidad de este cuestionario para la exploración de historia familiar de problemas psicológicos.



☑ ¿Cuántos hermanos (varones) tiene? (aunque alguno haya fallecido)

☑ ¿Cuántas hermanas tiene? (aunque alguna haya fallecido)

☑ ¿Ha tenido algún miembro de su familia biológica algún problema psicológico o emocional grave? (Este apartado hace referencia a enfermedades tales como depresión, ansiedad severa, crisis nerviosa y esquizofrenia)

a. Padre _____SI 1 NO 0
b. Madre_____SI 1 NO 0
c. Hermanos (varones)_____SI 1 NO 0

☑ ¿Cuántos de sus hermanos (varones) han tenido estos problemas?

a. Hermanas _____SI 1 NO 0
b. ¿Cuántas de sus hermanas han tenido estos problemas? .....

☑ ¿Algún miembro de su familia se ha suicidado?

a. Padre _____SI 1 NO 0
b. Madre _____SI 1 NO 0
c1. Hermanos (varones)_____SI 1 NO 0
c2. ¿Cuántos?
d1. Hermanas _____SI 1 NO 0
d2. ¿Cuántas? ?

FIGURA 14 Cuestionario para evaluar la historia familiar de problemas psicológicos

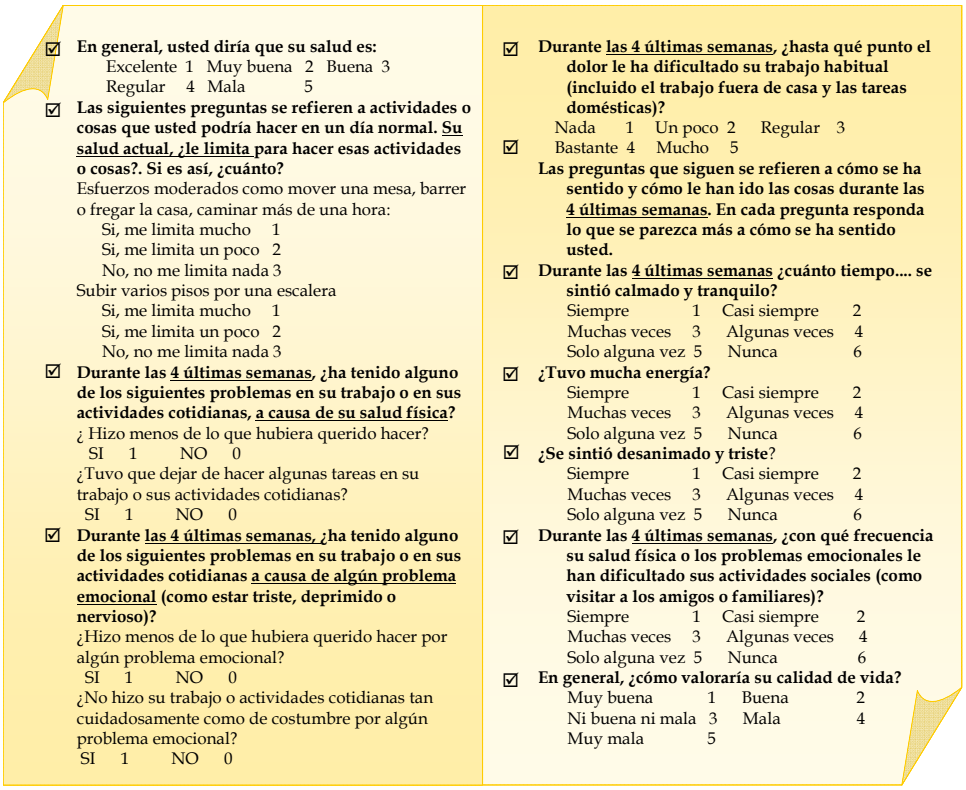
Dificultades en el Trabajo Remunerado o no Remunerado

La presencia de dificultades en el trabajo remunerado o no remunerado, en los seis meses anteriores a la entrevista, fue evaluada mediante una serie de preguntas tomadas del instrumento diseñado por *Karasek y cols (1990)*.

Los pacientes se clasificaron como: i) aquellos con sensación de control en el trabajo remunerado o no remunerado; ii) aquellos con dificultades y sin apoyo en el trabajo remunerado o no y iii) aquellos con sensación de angustia y sin sensación de respeto en el trabajo.

Salud Física y Mental Subjetivas

Los participantes fueron, además, evaluados para la sensación de salud física y mental, mediante la escala auto-informada de discapacidad Short Form 12 (*Jenkinson C et.al.1997*).



☑ **En general, usted diría que su salud es:**
Excelente 1 Muy buena 2 Buena 3
Regular 4 Mala 5

☑ **Las siguientes preguntas se refieren a actividades o cosas que usted podría hacer en un día normal. Su salud actual, ¿le limita para hacer esas actividades o cosas?. Si es así, ¿cuánto?**
Esfuerzos moderados como mover una mesa, barrer o fregar la casa, caminar más de una hora:
Si, me limita mucho 1
Si, me limita un poco 2
No, no me limita nada 3
Subir varios pisos por una escalera
Si, me limita mucho 1
Si, me limita un poco 2
No, no me limita nada 3

☑ **Durante las 4 últimas semanas, ¿ha tenido alguno de los siguientes problemas en su trabajo o en sus actividades cotidianas, a causa de su salud física?**
¿ Hizo menos de lo que hubiera querido hacer?
SI 1 NO 0
¿Tuvo que dejar de hacer algunas tareas en su trabajo o sus actividades cotidianas?
SI 1 NO 0

☑ **Durante las 4 últimas semanas, ¿ha tenido alguno de los siguientes problemas en su trabajo o en sus actividades cotidianas a causa de algún problema emocional (como estar triste, deprimido o nervioso)?**
¿Hizo menos de lo que hubiera querido hacer por algún problema emocional?
SI 1 NO 0
¿No hizo su trabajo o actividades cotidianas tan cuidadosamente como de costumbre por algún problema emocional?
SI 1 NO 0

☑ **Durante las 4 últimas semanas, ¿hasta qué punto el dolor le ha dificultado su trabajo habitual (incluido el trabajo fuera de casa y las tareas domésticas)?**
Nada 1 Un poco 2 Regular 3
Bastante 4 Mucho 5

☑ **Las preguntas que siguen se refieren a cómo se ha sentido y cómo le han ido las cosas durante las 4 últimas semanas. En cada pregunta responda lo que se parezca más a cómo se ha sentido usted.**

☑ **Durante las 4 últimas semanas ¿cuánto tiempo.... se sintió calmado y tranquilo?**
Siempre 1 Casi siempre 2
Muchas veces 3 Algunas veces 4
Solo alguna vez 5 Nunca 6

☑ **¿Tuvo mucha energía?**
Siempre 1 Casi siempre 2
Muchas veces 3 Algunas veces 4
Solo alguna vez 5 Nunca 6

☑ **¿Se sintió desanimado y triste?**
Siempre 1 Casi siempre 2
Muchas veces 3 Algunas veces 4
Solo alguna vez 5 Nunca 6

☑ **Durante las 4 últimas semanas, ¿con qué frecuencia su salud física o los problemas emocionales le han dificultado sus actividades sociales (como visitar a los amigos o familiares)?**
Siempre 1 Casi siempre 2
Muchas veces 3 Algunas veces 4
Solo alguna vez 5 Nunca 6

☑ **En general, ¿cómo valoraría su calidad de vida?**
Muy buena 1 Buena 2
Ni buena ni mala 3 Mala 4
Muy mala 5

FIGURA 15 Cuestionario para evaluar la salud física y mental

Historia Personal de Depresión

Al comienzo del estudio, en T0, todos los participantes fueron preguntados sobre depresión a lo largo de la vida, mediante las dos primeras preguntas de la sección de depresión del CIDI. (*Robins et.al. 1988*)

Experiencias de Discriminación

Se evaluó la presencia de experiencias de discriminación, en términos de sexo, edad, etnia, apariencia, discapacidad u orientación sexual, en los seis meses previos a la entrevista, mediante las mismas cuestiones que *Janssen, et.al. (2003*).

Acontecimientos Vitales Estresantes (AVEs).

Para evaluar la presencia de AVEs en los seis meses previos a la entrevista se usó la List of Threatening Experiences (TLEs) *(Brugha et.al. 1985),* una herramienta estructurada y validada que incluye acontecimientos altamente amenazantes para el individuo (Ver Figura 16).

Debido a que había muy pocos individuos que acumularan 3 ó más AVEs (dada la particular severidad de los eventos medidos en esta escala), se establecieron tres grupos de exposición a acontecimientos vitales estresantes, según si no habían sufrido ningún AVE en los seis meses anteriores a la entrevista, si habían sufrido sólo uno, o si habían sufrido dos o más AVEs.

- ☑ ¿Ha sufrido usted mismo una enfermedad, lesión o agresión grave?
- ☑ ¿Algún familiar cercano ha sufrido una enfermedad, lesión o agresión grave?
- ☑ ¿Ha muerto uno de sus padres, hijos o su pareja/cónyuge?
- ☑ ¿Ha muerto un amigo cercano a la familia o algún otro familiar (tíos, primos, abuelos)?
- ☑ ¿Se ha separado a causa de problemas en su matrimonio?
- ☑ ¿Ha roto una relación estable?
- ☑ ¿Ha tenido algún problema grave con algún amigo cercano, vecino o familiar?
- ☑ ¿Se ha quedado sin empleo o ha buscado empleo durante más de un mes sin éxito?
- ☑ ¿Le han despedido de su trabajo?
- ☑ ¿Ha tenido una crisis económica grave?
- ☑ ¿Ha tenido problemas con la policía o ha comparecido ante un tribunal?
- ☑ ¿Le han robado o ha perdido algún objeto de valor?

FIGURA 16 Listado de acontecimientos vitales estresantes incluidos en la List of Threatening Expierences (TLEs) (Brugha et.al. 1985

Abuso en la Infancia

Los participantes furon preguntados acerca de la existencia de experiencias de abuso físico, psicológico o sexual en al infancia. Para ello, se utilizó una entrevista estructurada y validada (*Fink et.al. 1995*).

Variables Genéticas Independientes: Análisis de Genes de Riesgo

Se exploró la posible asociación entre la variabilidad de tres genes clave de la depresión. En concreto, analizamos el polimorfismo 5-HTTLPR del gen del transportador de serotonina (SERT), el polimorfismo uMAOA del gen MAOA y el polimorfismo rs6295 del gen HTR1A.

En todos los casos, se trataba de polimorfismos funcionales situados en las zonas promotoras de sus respectivos genes (se comentan en detalle más adelante).

5. ANÁLISIS MOLECULARES

Tal y como se ha señalado anteriormente, los análisis moleculares llevados a cabo para la elaboración de los trabajos de investigación contenidos en la presente tesis doctoral consistieron en la exploración de la variabilidad contenida en los genes SERT, MAOA y HTR1A. Dichos análisis se llevaron a cabo a partir del ADN extraído de las muestras biológicas (saliva y/o sangre) donadas por los individuos participantes en el estudio.

Los análisis fueron desarrollados en distintas etapas, que se describen a continuación.

Toma de la Muestra Biológica

La toma de muestras de sangre de los participantes en el proyecto PREDICT-Gene, corrió a cargo del personal de enfermería de los centros de Atención Primaria donde se realizaban las entrevistas.

En los casos en los que el participante donaba una muestra de saliva, el frotis bucal era recogido por el propio entrevistador, previo entrenamiento en la recogida de este tipo de muestras, y mediante un kit de hisopos estériles diseñado especialmente para este estudio.

Transporte y Recepción de la Muestra en el Laboratorio

Las muestras eran custodiadas por el entrevistador hasta su traslado al laboratorio para su procesamiento y análisis.

A su llegada al laboratorio, cada muestra biológica era registrada de manera informatizada en una base de datos, con el objetivo de monitorizar la fase de recogida de muestras biológicas.

Extracción de ADN Genómico.

Para la extracción de ADN genómico a partir de las muestras de saliva, se usaron técnicas convencionales de extracción con fenol-cloroformo.

El ADN procedente de las muestras de sangre se extrajo utilizando un kit comercial de extracción (SSS, Durviz).

Genotipado

En el presente estudio se han analizado tres polimorfismos funcionales situados en tres genes clave de la neurotransmisión de serotonina:

i) El polimorfismo **5-HTTLPR**, situado en el gen del transportador de serotonina (SERT)

ii) El polimorfismo **uMAOA** del gen de la monoamino oxidasa A (MAOA)

iii) El polimorfismo **C (-1019) G (rs 6295)** localizado en el gen que codifica para el receptor serotoninérgico tipo 1A.

Todos ellos son polimorfismos de la región promotora del gen en el que se encuentran y se han asociado a una alteración en la expresión génica *(Lesch et al. 1996; Sabol et.al. 1998; Lemonde et.al. 2003).*

i) Genotipado del polimorfismo 5-HTTLPR

Para el análisis de este polimorfismo, se llevó a cabo una amplificación del fragmento de ADN mediante la técnica de la PCR (Polymerase Chain Reaction) bajo las siguientes condiciones: volumen final de 25µl; 50 ng de ADN, 0,25 µM de cada *primer*, 250 µM de cada uno de los dNTPs, 1 µl de Cl_2Mg (1,5 mM), 50mM de ClK, 10mM de Tris ClH y 0,3 unidades de Taq-polimerasa.

La secuencia de cada *primer* utilizado fue: 5´- GGC GTT GCC GCT CTG AAT GCC- 3´ y 5´- CAG GGG AGA TCC TGG GAG AGG T- 3´. Las condiciones de temperatura utilizadas fueron las siguientes:

95ºC	15 segundos	1 ciclo
94ºC	30 segundos	35 ciclos
66º C	30 segundos	
72ºC	40 segundos	
72ºC	15 minutos	1 ciclo
4ºC	indefinido	

La técnica de electroforesis en gel de agarosa (al 2% peso/volumen) permitió la separación de los productos de PCR según su peso molecular y su carga eléctrica. Así, los fragmentos de ADN se desplazarán desde el polo negativo hacia el positivo debido a su naturaleza aniónica. Aquellos fragmentos con menor peso molecular (correspondientes, en este caso, a la forma corta, S, del polimorfismo 5HTTLPR), migrarán más lejos en el gel de

agarosa en comparación con los de mayor peso molecular (que corresponden con la forma larga o L del polimorfismo).

La visualización de los fragmentos de ADN amplificados y separados en el gel de agarosa se realizó con la ayuda del compuesto bromuro de etidio y utilizando un transiluminador de luz ultravioleta.

Los individuos fueron clasificados, según el patrón de bandas visualizado, en homocigotos para el alelo corto (S/S), heterocigotos (L/S), y homocigotos para el alelo largo (L/L) (Figura 17).

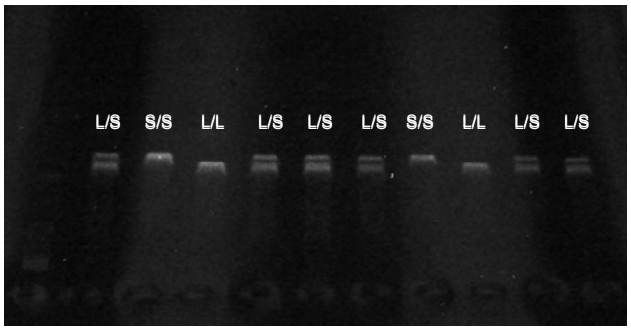


FIGURA 17. Gel de agarosa al 2% en el que se distinguen los genotipos posibles (s/s, s/l y l/l) para el polimorfismo 5HTTLPR del gen SERT.

ii) Genotipado del polimorfismo u MAOA

El genotipado de cada individuo para el polimorfismo uMAOA se realizó de forma análoga al caso anterior, es decir, mediante la tecnología de amplificación por PCR y electroforesis en gel de agarosa (en este caso al 3% p/v) y utilizando bromuro de etidio para la visualización de los fragmentos de ADN, mediante un transiluminador.

La PCR se llevó a cabo en un volumen final de 15µl. Se usaron 50 ng de ADN, 0,25 µM de cada *primer*, 250 µM de cada uno de los dNTPs, 1 µl de Cl_2Mg (1,5 mM), 50mM de ClK, 10mM de Tris ClH y 0,2 unidades de Taq-polimerasa. La secuencia de cada *primer* utilizado fue: 5´- ACA GCC TGA CCG TGG AGA AG- 3´ y 5´- GAA CGG ACG CTC CAT TCG GA- 3´. Las condiciones de temperatura utilizadas fueron las siguientes:

94ºC	2 minutos	1 ciclo
94ºC	60 segundos	
66º C	60 segundos	30 ciclos
72ºC	60 segundos	
72ºC	15 minutos	1 ciclo
4ºC	indefinido	

Los individuos fueron clasificados, según el patrón de bandas visualizado en el gel de agarosa, en individuos homocigotos para el alelo de baja actividad (3/3), homocigotos para alelos de alta actividad (3.5/3.5; 4/4; 5/5) e individuos heterocigotos (Ver Figura 18).

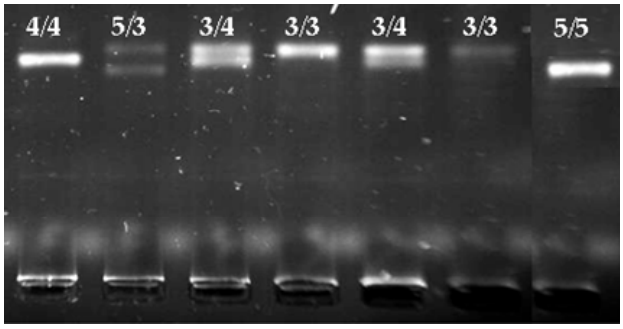


FIGURA 18. Gel de agarosa al 3% en el que se distinguen los genotipos posibles para el polimorfismo uMAOA del gen MAOA.

Posteriormente, para llevar a cabo los análisis estadísticos, los individuos fueron clasificados, al igual que habían hecho otros autores (*Deckert et.al, 1999; Schulze et.al. 2000; Gutiérrez et.al, 2004; Rivera et.al. 2009*) en:

a) Portadores de alelos de baja actividad (3 copias de la secuencia repetida de 30 pb)
b) Portadores de alelos de alta actividad (3.5, 4 ó 5 repeticiones).

En cuanto a su genotipo, los individuos se clasificaron en:

a) Individuos homocigotos para el alelo de baja actividad (3/3)

b) Individuos portadores de alelos de alta actividad, es decir, homocigotos (3.5/3.5; 4/4; 5/5) o heterocigotos para alelos de alta actividad.

iii) Genotipado del polimorfismo C (-1019) G

Este polimorfismo fue genotipado, junto con otros polimorfismos de interés en el contexto del estudio PREDICT-Gene, mediante la tecnología de MassARRAY (Sequenom). Esta técnica de genotipado masivo permite genotipar hasta 36 polimorfismos (SNPs) en una misma reacción.

En primer lugar, se amplifican los fragmentos de ADN que contienen los polimorfismos de interés, mediante una PCR multiplex. A continuación se lleva a cabo la reacción de discriminación alélica, mediante la tecnología MALDI-TOF Iplex Gold, que consiste en una reacción de minisecuenciación que termina tras la extensión de una base (Single Base Extensión, SBE). El uso de cebadores de minisecuenciación con distintas longitudes permite identificar los picos de los productos de cada SNP.

Esta tecnología se encuentra disponible en la plataforma de genotipado masivo del Centro Nacional de Genotipado (CEGEN) (www.cegen.org).

6. CONSIDERACIONES ESTADÍSTICAS

Se utilizó el paquete estadístico SPSS 15.0 (2006) para llevar a cabo los análisis estadísticos. En primer lugar, se analizó la distribución de las distintas variables utilizadas en los diferentes estudios. En segundo lugar, se utilizó el test de Chi cuadrado para comprobar que las frecuencias genotípicas para cada uno de los polimorfismos analizados se encontraban en equilibrio Hardy-Weinberg, tanto en los pacientes como en los controles. El mismo test fue usado para comparar la distribución de frecuencias alélicas y genotípicas de cada uno de los polimorfismos analizados en los casos y los controles.

Se aplicaron modelos de regresión logística para testar perfiles genéticos concretos de vulnerabilidad a la depresión, definidos a priori. Dichos

modelos se usaron para ajustar las asociaciones por posibles factores confusores tales como el sexo, la edad, la historia familiar de problemas psicológicos entre familiares de primer grado y/o el trastorno de ansiedad generalizada (GAD). Se estimaron también los riesgos que conferían para los fenotipos clínicos considerados las variantes genéticas analizadas.

Para el análisis longitudinal de los datos se llevó a cabo un análisis multivariante empleando un modelo de *Generalized Estimating Equations* (GEE) en el que la variable dependiente pasó a ser la depresión incidente en los tres instantes del año (T1, T2 y T3). Se empleó este método para poder analizar con rigor las tres medidas de que disponíamos que, dentro de cada individuo, obviamente estaban relacionadas, no permitiendo, por tanto, un análisis clásico de datos independientes. Este modelo GEE es un modelo de efectos aleatorios que permite ajustar por la variabilidad intraindividual los análisis de comparación que se hagan posteriormente. Un modelo GEE logístico binario se empleó para los diferentes análisis estimándose con él las Odds Ratio para la asociación entre genotipos y la depresión en cualquiera de los tres instantes y sus intervalos de confianza al 95%. Con objeto de medir el efecto ambiental se empleó el índice definido por el estudio PREDICT-D (King et al., 2008) aplicado a nuestra muestra. El análisis final se centró primero en el análisis de la posible interacción Gen x Ambiente (SERT x índice PREDICT o MAOA x índice PREDICT) y posteriormente en la posible interacción Gen x Gen x Ambiente (SERT x MAOA x índice PREDICT). Para valorar la significación de las interacciones se usó el test de razón de verosimilitudes. En el caso en el que la interacción resultó ser significativa, se llevó a cabo un estudio detallado de la misma a partir de las comparaciones del efecto de la variable ambiental (índice PREDICT) en los distintos genotipos.

Gráficamente la interacción se representó usando como variable dependiente la probabilidad de deprimirse en alguno de los tres instantes (eje de abcisas) frente al índice PREDICT (eje de ordenadas) en función de los genotipos para los genes SERT o/y MAOA.

IV. Resultados

1. RESUMEN DE LOS RESULTADOS

En este capítulo se resumen los principales resultados de los artículos de investigación que conforman esta Tesis Doctoral.

1.1. Descriptivos de la Muestra y Participación

De los 1524 individuos que fueron invitados a participar en el estudio PREDICT-Gene, 1319 (86.55%) aceptaron, dieron su consentimiento informado y donaron una muestra biológica (Ver Figura 19).

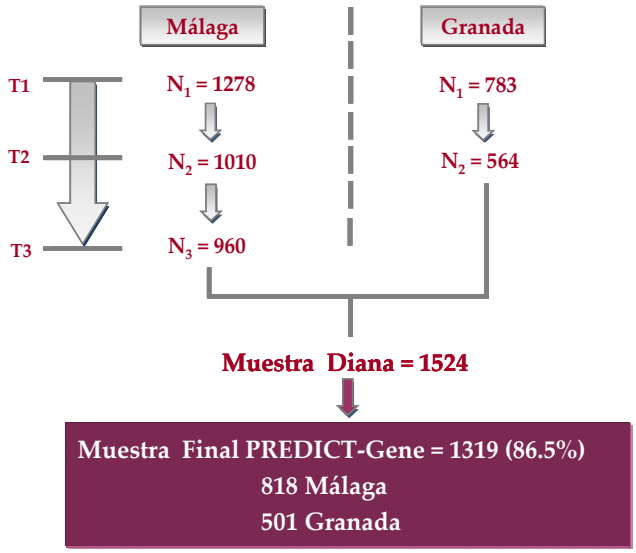


FIGURA 19. Éxito de participación y seguimiento del estudio

En la Tabla 11 se muestra el éxito de participación en el estudio PREDICT-Gene por cada uno de los Centros de Atención Primaria.

TABLA 11. Éxito de participación por centros de Atención Primaria.

CENTRO DE SALUD	PROVINCIA	Nº DE PARTICIPANTES
El Palo	Málaga	141
Trinidad	Málaga	74
Carranque	Málaga	103
Delicias	Málaga	91
Limonar	Málaga	73
Puerto de la Torre	Málaga	59
Coín-Tolox	Málaga	104
Vélez-Málaga	Málaga	98
Las Lagunas	Málaga	75
Doctor Caballero	Granada	119
Chana	Granada	42
Huétor-Tájar	Granada	107
Motril-San Antonio	Granada	127
Motril-Este	Granada	64
Gran Capitán	Granada	10
Zaidin Sur	Granada	32

Todos los individuos que formaron la muestra eran de origen caucasoide español. En la Tabla 12 se muestra el perfil sociodemográfico y clínico de la muestra.

No se encontraron diferencias en la frecuencia de Depresión Mayor, entre aquellos individuos que participaron en el estudio, los que rehusaron participar y aquellos que no completaron el seguimiento. Tampoco hubo diferencias en el perfil sociodemográfico de la muestra.

Como ya se ha mencionado con anterioridad, los trabajos presentados en el presente capítulo de resultados pueden presentar oscilaciones en el número final de individuos incluidos en cada análisis, puesto que dichos trabajos se fueron desarrollando en distintos momentos temporales. Así, los

análisis realizados antes incluirán un menor número de individuos que aquellos que fueron realizados posteriormente, cuando la muestra se había incrementado.

TABLA 12. Perfil sociodemográfico y clínico de la muestra

VARIABLES	FRECUENCIAS / MEDIAS
Sexo (mujeres / hombres)	954 (72.3%) / 365 (27.7%)
Edad Media	50.33 (SD 15.12)
Estado Civil	
Casado/pareja	922 (69.9%)
Separado/divorciado	75 (5.7%)
Viudo	117 (8.9%)
Soltero	205 (15.5%)
Nivel Académico	
Secundaria o superior	442 (33.5%)
Primaria	530 (40.2%)
Sin estudios/analfabeto	347 (26.3%)
Profesión	
Trabajando	407 (30.8%)
Minusválido / jubilado	418 (31.7%)
Labores del hogar	373 (28.3%)
Estudiando o en formación	46 (3.5%)
Otros	75 (5.7%)
Vive solo	
Si	103 (7.8%)
No	1216 (92.2%)
Depresión mayor DSM-IV To	
Sin depresión	1039 (78.8%)
Con depresión	257 (19.5%)
Datos no disponibles	23 (1.7 %)
Depresión mayor DSM-IV T_6	
Sin depresión	1021 (77.4%)
Con depresión	196 (14.9%)
Datos no disponibles	102 (7.7%)
Depresión mayor DSM-IV T_{12}	
Sin depresión	1026 (77.8%)
Con depresión	156 (11.8%)
Datos no disponibles	137 (10.4%)

A continuación se describen, mediante un breve resumen, los principales resultados de cada uno de los trabajos de investigación incluidos en la presente Tesis Doctoral.

1.2. Estudio de Asociación Genética entre el Polimorfismo C (-1019) G del gen 5-HT1A y la Depresión y Ansiedad comórbidas.

The Association of C (-1019) G Serotonin 1A Promoter Polymorphism with Comorbid Anxiety and Depression. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. (En consideración editorial).

1.2.1. Contexto y Diseño del Estudio

El presente trabajo se trata de un estudio de asociación genética con un diseño transversal, en el que se analizan los datos correspondientes al comienzo ($T_{0)}$ del seguimiento a un año de la cohorte de individuos participantes en el estudio PREDIC-Gene.

El objetivo fue identificar factores de riesgo genético para depresión y, concretamente, explorar si la variabilidad contenida en el gen que codifica para el receptor de serotonina tipo 1A, podía constituir un factor de riesgo genético para depresión.

1.2.2. Características Sociodemográficas y Clínicas

Este estudio contó con una muestra de 1059 individuos, usuarios de los servicios de Atención Primaria de las provincias de Málaga y Granada que participaron en el estudio PREDICT-Gen.

En la Tabla 13 se muestra el perfil sociodemográfico y clínico de la muestra en cuanto a las variables analizadas en este estudio.

TABLA 13. Perfil sociodemográfico y clínico de la muestra

VARIABLES	FREQUENCIAS/MEDIAS
Sexo (masculino/femenino)	301 (28.4%) / 758 (71.6%)
Edad media	49.88 años (SD15.30)
Historia familiar de problemas psicológicos entre familiares de primer grado (FH)*	
FH +	387 (48.25%)
FH -	502 (62.59%)
Depresión Mayor DSM-IV ¤	
Deprimido	206 (19.95%)
No deprimido	827 (80.05%)
Trastorno de Ansiedad Generalizada (GAD)	
GAD	85 (8.03%)
No GAD	974 (91.37%)
Comorbilidad MD/GAD ◊	
Depresión/GAD	58 (5.5%)
No Depresión/GAD	804 (90.23%)
Depresión o GAD	170(16%)
C (-1019) G	
G/*	762 (73.67%)
C/C	271 (26.33%)

***** FH: Historia Familiar de Problemas Psicológicos entre familiares de primer grado: Información no disponible para 170 participantes.

¤ Diagnóstico de Depresión Mayor DSM-IV: Información no disponible para 26 participantes.

◊ Comorbilidad MD/GAD: Comorbilidad entre Depresión Mayor DSM-IV y Trastorno de Ansiedad Generalizada: información no disponible para 27 participantes.

No se encontraron diferencias para variables sociodemográficas ni para la prevalencia de Depresión Mayor, Trastorno de Ansiedad Generalizada (GAD) o para la comorbilidad entre Depresión Mayor y GAD, entre los

individuos que participaron en el estudio, los que rehusaron participar y aquellos que no completaron el seguimiento.

1.2.3 Asociación del Polimorfismo C (-1019) G con Depresión Mayor

La distribución de frecuencias genotípicas entre el grupo de pacientes y el de controles se encontraba en equilibrio Hardy-Weinberg.

En un primer análisis, ser portador del alelo G del polimorfismo C (-1019) G fue encontrado asociado, de forma estadísticamente significativa, con Depresión Mayor (OR = 1.67, 95%CI = 1.14 - 2.44, p = 0.008), aunque al ajustar por la presencia de Trastorno de Ansiedad Generalizada, tal asociación perdió su significación (OR = 1.43, 95%CI = 0.96-2.14, p = 0.080).

Posteriormente, al incluir además en el análisis de asociación otros posibles factores confusores, tales como el sexo, la edad y la presencia de historia familiar de problemas psicológicos entre los familiares de primer grado, la asociación se hizo aún más débil (*OR* = 1.37; 95% CI = 0.87-2.16; p = 0.173). (Ver Figura 20).

1.2.4. Asociación del Polimorfismo C (-1019) G con Trastorno de Ansiedad Generalizada (GAD)

La distribución de frecuencias genotípicas entre el grupo de pacientes y el de controles se encontró, nuevamente, en equilibrio Hardy-Weinberg.

Aquellos individuos con, al menos, un alelo G del polimorfismo C (-1019) G, presentaban un riesgo significativamente mayor para Trastorno de Ansiedad Generalizada (OR = 2.54; 95% CI = 1.28-4.86; p = 0.003) que los individuos homocigotos para el alelo C. Sin embargo, al incluir en el análisis el diagnóstico de Depresión Mayor, tal asociación se hizo más débil (OR =1.97; 95% CI = 0.99-3.91; p =0.050).

Al ajustar, además, por sexo, edad y presencia de historia familiar de problemas psicológicos entre los familiares de primer grado, la asociación quedó fuera de los niveles de significación (OR = 1.62; 95% CI = 0.79-3.30; p =0.184). (Ver Figura 20).

1.2.5. Asociación del Polimorfismo C (-1019) G con la Comorbilidad Depresión/GAD

De nuevo, en este caso, la distribución de frecuencias genotípicas entre el grupo de pacientes y el de controles se encontraba en equilibrio Hardy-Weinberg.

Se detectó la existencia en nuestra muestra de una asociación estadísticamente significativa entre ser portador del alelo G del polimorfismo C (-1019) G y la comorbilidad de Depresión Mayor y Trastorno de Ansiedad Generalizada (OR =3.41; 95% CI = 1.44-8.05; p = 0.005).

Tal asociación mantuvo su robustez incluso al ajustar por los posibles confusores: sexo, edad e historia familiar de problemas psicológicos entre los familiares de primer grado de los participantes (OR =2.82; 95% CI = 1.18-6.77; p = 0.020). (Ver Figura 20).

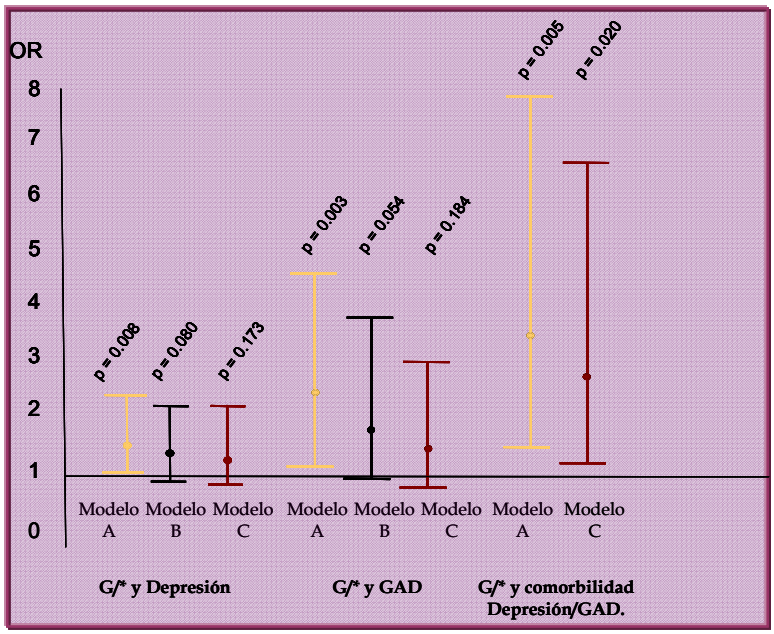


Figure 20: Odds ratios (OR) para la asociación entre los portadores del alelo G del polimorfismo C (-1019) G y Depresión Mayor, Trastorno de Ansiedad Generalizada (GAD) y la comorbilidad entre ambas (Depresión/GAD).
Modelo A:Odds ratios crudas.
Modelo B: Odds ratios para Depresión Mayor y Trastorno de Ansiedad Generalizada (GAD) ajustando una por la otra.
Modelo C: Odds ratios para el modelo B y ajustando además por sexo, edad e historia familiar de problemas psicológicos.

1.3. Interacción del Gen SERT y el Gen MAOA con el Estrés Psicosocial Previo en la Aparición Prospectiva de Depresión.

Gene by Gene by Environment Interactions as Determinants of Major). Depression: A Prospective Analysis of the PREDICT-Gene Cohort

1.3.1. Contexto y Diseño del Estudio

En este estudio se analizan los datos derivados del seguimiento a un año (con tres tiempos de corte: al comienzo en T_0, a los seis meses en T_1 y a los 12 meses en T_2) de la cohorte formada por los individuos de la provincias de Málaga y Granada que participaron en el estudio PREDIC-Gene (*Cervilla et al., 2007*).

Nuestro objetivo fue analizar si los polimorfismos 5-HTTLPR del gen SERT y el uMAOA del gen MAOA interactuaban conjuntamente (Interacción Gen-Gen) confiriendo un riesgo incrementado para padecer depresión y si tales

polimorfismos modificaban el riesgo para padecer depresión conferido por el estrés psicosocial y las experiencias de abuso en la infancia (Interacción Gen–Gen–Ambiente).

1.3.2. Características Sociodemográficas y Clínicas

La muestra utilizada en el presente trabajo consistió en 1319 usuarios de los servicios de Atención Primaria de las Provincias de Málaga y Granada que habían participado en el estudio PREDICT-Gene.

El perfil sociodemográfico y clínico de la muestra se detalla en la Tabla 14.

No hubo diferencias estadísticamente significativas para las variables sociodemográficas, la prevalencia de Depresión Mayor, el número de AVEs sufridos, o para la presencia de experiencias de abuso entre los individuos que aceptaron participar en el estudio PREDICT-Gene y los que rehusaron. Tampoco las hubo entre los individuos que comenzaron el seguimiento y los que llegaron al T3.

Tabla 14. Perfil sociodemográfico y clínico de la muestra

Variables	Frecuencias / Medias
Sex (hombre/mujer)	365 (27.7%) / 954(72.3%)
Edad media	50.33 years (SD 15.12)
Nivel Académico	
Secundaria o superior	442 (33.5%)
Primaria	530 (40.2%)
Sin estudios/analfabeto	347 (26.3%)
Estado Civil	
Casado/pareja	922 (69.9%)
Separado/divorciado	75 (5.7%)
Viudo	117 (8.9%)
Soltero	205 (15.5%)
Profesión	
Trabajando	407 (30.8%)
Minusválido / jubilado	418 (31.7%)
Labores del hogar	373 (28.3%)
Estudiando o en formación	46 (3.5%)

Otros	75 (5.7%)
Vive solo	
Si	103 (7.8%)
No	1216 (92.2%)
Exposición a Acontecimientos Vitales Estresantes (AVES)	
No AVEs	378 (28.7%)
1 AVE	453 (34.3%)
≥ 2 AVEs	483 (36.6%)
Datos no disponibles	5 (0.4%)
Abuso Sexual en la Infancia	
No	1271 (96.3%)
Si	43 (3.3%)
Datos no disponibles	5 (0.4%)
Abuso Físico en la Infancia	
No	1164 (88.2%)
Si	150 (11.4%)
Datos no disponibles	5 (0.4%)
Abuso Psicológico en al Infancia	
No	1075 (81.5%)
Si	239 (18.1%)
Datos no disponibles	5 (0.4%)
Cualquier tipo de abuso	
No	1027 (77.9%)
Si	287 (21.7%)
Datos no disponibles	5 (0.4%)
Depresión mayor DSM-IV To	
Sin depresión	1039 (78.8%)
Con depresión	257 (19.5%)
Datos no disponibles	23 (1.7 %)
Depresión mayor DSM-IV T_6	
Sin depresión	1021 (77.4%)
Con depresión	196 (14.9%)
Datos no disponibles	102 (7.7%)
Depresión mayor DSM-IV T_{12}	
Sin depresión	1026 (77.8%)
Con depresión	156 (11.8%)
Datos no disponibles	137 (10.4%)
5-HTTLPR	
S/S	329 (24.9%)

L/*		990 (75.1%)
Homocigotos HA/HA uMAOA		
Hombres	HA/HA	245 (70%)
	LA/*	121 33.2%)
Mujeres	HA/HA	431 (45.2%)
	LA/LA	522(54%)

1.3.3 Asociaciones de factores genéticos con Depresión Mayor.

Encontramos que el genotipo s/s del polimorfismo 5-HTTLPR confería un riesgo significativamente incrementado para padecer depresión, al ser comparado con otros genotipos (OR = 1.3, 95%CI 1.05-1.55, p = 0.008). De amanera análoga encontramos que el alelo de alta actividad del polimorfismo uMAOA se encontraba asociado a depresión, sobre todo en las mujeres (Mujeres: OR = 1.5, 95% CI 1.12-2.05, p = 0.003; Hombres: OR = 1.4, 95% CI 0.89-2.29, p = 0.079). Ser homocigoto para el alelo de alta actividad se encontró asociado a depresión, tras ajustar por sexo (OR = 1.25 95% CI 1.03-1.51, p = 0.024). Ver Figuras 2.a – 2.c. del artículo 2.

1.3.4 Asociaciones de factores ambientales con Depresión Mayor.

Nuestros resultados mostraron evidencia de una asociación lineal entre el número de acontecimientos vitales estresantes (AVEs) y el riesgo para padecer depresión (OR = 1.5 95%CI 1.35-1.64, p = 0.000). Además la prevalencia de depresión resultó estar asociada a las experiencias de abusos en la infancia, tanto abuso sexual (OR = 2 95%CI 1.41-2.91, p = 0.000), como abuso físico (OR = 2.5 95%CI 2.06-3.12, p = 0.000 y psicológico (OR = 2.6 95%CI 2.17-3.07, p = 0.000). Ver Figuras 3.a. – 3.e. del Artículo 2.

1.3.5. Interacción Gen-Gen

Encontramos una interacción estadísticamente significativa entre el genotipo para el polimorfismo 5-HTTLPR del gen SERT y el polimorfismo uMAOA del gen MAOA. De este modo, aquellos individuos con el genotipo s/s

y que además eran homocigotos para el alelo de alta actividad del polimorfismo uMAOA (HA/HA), tenían mayor riesgo de padecer depresión que los individuos con otras combinaciones genotípicas (Interacción χ_2 = 6.65, p = 0.0098) (Ver Figura 4 del Artículo 2).

1.3.6. Interacción Gen-Gen-AVEs en Depresión.

Nuestros resultados muestran, además, que los polimorfismos 5-HTTLPR y uMAOA modifican el riesgo para depresión conferido por los acontecimientos vitales estresantes. Así, dentro de cada nivel de estrés psicosocial (ningún AVE, 1 AVE o dos o más AVEs), aquellos individuos con el genotipo s/s y HA/HA tienen riesgos significativamente mayores para padecer depresión que los individuos con otras combinaciones genéticas (Interacción χ_2 = 13.1, p = 0.069) (Ver Figura 5 del Artículo 2).

1.3.7. Interacción Gen-Gen-abuso en Depresión.

Por último, encontramos que estas regiones genéticas polimórficas también modificaban el riesgo para padecer depresión conferido por las experiencias de abuso en la infancia.

De este modo, entre los individuos que habían sufrido abuso sexual en la infancia, aquellos con el genotipo de riesgo s/s del polimorfismo 5-HTTLPR y que, además, portaban en homocigosis el alelo HA del polimorfismo uMAOA, presentaban riesgos significativamente mayores para padecer depresión que los individuos que habían sufrido abuso sexual pero que no portaban esas variantes genéticas de riesgo (Interacción χ_2 = 9.15, p = 0.057). Ver Figura 6 del Artículo 2.

Idénticos resultados obtuvimos al incluir otros tipos de abuso durante la infancia (sexual, físico o psicológico) (Interacción χ_2 = 9.06, p = 0.059). Ver Figura 7 del Artículo 2.

1.4. Estudio de Interacción de los Genes SERT y MAOA con el Algoritmo PREDICT-D en la Predicción de la Aparición de Episodios Depresivos.

Multifactor Prediction of Incident Major Depression is Significantly Modified by Genetic Variation at both SERT and uMAOA Loci.

1.4.1. Contexto y Diseño del Estudio

En el presente trabajo se analizan los datos derivados del seguimiento a un año (con tres tiempos de corte: al comienzo en T_0, a los seis meses en T_1 y a los 12 meses en T_2) de la cohorte de individuos de la provincia de Málaga que participaron en el estudio PREDICT-Gene (*Cervilla et al., 2007).*

Nuestro objetivo fue identificar posibles interacciones entre los factores de riesgo genéticos y los ambientales, como predictoras de la aparición de depresión, y en concreto, analizar si la variabilidad contenida en los genes SERT y MAOA modifica la predicción de depresión a un año hecha por el algoritmo predictor PREDICT-D (Ver artículo 3 del Anexo II).

1.4.2. Características Sociodemográficas y Clínicas

En este estudio, la muestra utilizada consistió en un total de 775 individuos usuarios de los servicios de Atención Primaria de la Provincia de Málaga y que habían aceptado participar en el estudio PREDICT-Gen. Todos ellos fueron evaluados tres veces durante un año de seguimiento y aceptaron donar una muestra biológica de sangre y/o saliva para la realización de los análisis genéticos.

El perfil sociodemográfico y clínico de la muestra se detalla en la Tabla 15. No se encontraron diferencias para las variables sexo, edad, estado civil, prevalencia de depresión mayor o valores medios del índice-C entre los individuos que comenzaron el seguimiento y los que llegaron al T_3.

Tabla 15. Perfil Sociodemográfico y clínico de la muestra

Variables	**Frecuencias/medias**
Sexo (masculino / femenino)	217 (28%) / 558 (72%)
Edad media	50.53 años (SD 15.21)
Nivel Educativo	
Estudios superiores	100 (12.9%)
Secundaria	183 (23.6%)
Primaria, sin estudios	491 (63.4%)
Otros	1 (0.1%)
Estado Civil	
Soltero / no vive con pareja	230 (29.7%)
Casado / vive con pareja	544 (70.2%)
Perdidos	1 (0.1%)
Profession	
Trabajando o estudiando	267 (34.5%)
Retirado	131 (16.9%)
Otros	377 (48.6%)
Vive solo	
Si	46 (5.9%)
No	729 (94.1%)
Dificultades en el trabajo remunerado y no remunerado	640 (82.6%)
Sin dificultades	135 (17.4%)
Con dificultades	
Discriminación	
En ningún área	687 (88.6%)
En un área	64 (8.3%)
En más de un área	24 (3.1%)
Puntuación Física Media en la escala de Salud Física Subjetiva (SF-12)	41.90 (SD 11.67)
Puntuación Física Media en la escala de Salud Mental Subjetiva (SF-12)	44.42 (SD 12.81)
Historia Familiar de Problemas Psicológicos en familiares de primer grado	
Sin problemas	442 (57%)

Con problemas		333 (43%)
Depresión a lo largo de la vida		215 (27.7%)
No		560 (72.3%)
Si		
Depresión Mayor (DSM-IV) a tiempo cero		
Depresión Mayor		135 (17.4%)
No Depresión Mayor		624 (80.5%)
Perdidos		16 (2.1%)
Depresión Mayor (DSM-IV) a los seis meses		
Depresión Mayor		110 (14.2%)
No Depresión Mayor		619 (79.9%)
Perdidos		46 (5.9%)
Depresión Mayor (DSM-IV) a los doce meses		
Depresión Mayor		95 (12.2%)
No Depresión Mayor		626 (80.8%)
Perdidos		54 (7.0%)
Genotipos 5-HTTLPR		
S/*		564 (72.8%)
L/L		221 (27.2%)
Genotipos uMAOA		
Hombres	Alta/*	145 (18.7%)
	Baja/Baja	72 (9.3%)
Mujeres	Alta/*	470 (60.6%)
	Baja/Baja	88 (11.4%)

1.4.3 Asociaciones con Genótipos de Riesgo

El genotipo s/s del polimorfismo 5-HTTLPR del gen SERT se encontró asociado a Depresión Mayor (DSM-IV), en cualquiera de los tres tiempos (OR= 1.51; 95% CI 1.17-1.93; $\chi 2$=10.6; p=0.001).

Análogamente, los individuos portadores del alelo de alta actividad (alelo high) del polimorfismo uMAOA del gen MAOA, tuvieron riesgos incrementados para Depresión Mayor en comparación con aquellos individuos homocigotos (OR = 1.74; 95% CI 1.26-2.39; $\chi 2$=11.62; p=0.001).

Por otro lado, el genotipo s/s del polimorfismo 5-HTTLPR se encontró asociado a valores del índice C de predicción de Depresión significativamente más altos (media del índice C =0.22, SD=0.16; t=-4.1; p=0.0001) que los valores de índice C del resto de combinaciones alélicas (mean c-Index=0.18, SD=0.16).

De forma similar, los individuos portadores del alelo de alta actividad del polimorfismo uMAOA tenían valores medios de índice C significativamente mayores (t=0.54; p=0.0001; media del índice C=0.20; SD=0.17) que los individuos homocigotos para el alelo de baja actividad (media del índice C =0.16; SD=0.14).

1.4.4 Interacción del genotipo de riesgo con la predicción de depresión por el índice C.

Encontramos una interacción estadísticamente significativa entre el genotipo para el polimorfismo 5-HTTLPR del gen SERT y el riesgo para depresión predicho por el índice C (LR para la interacción = 4.63; p = 0.031), de modo que el valor del índice C fue significativamente mayor en aquellos individuos portadores de, al menos, una copia del alelo de riesgo (alelo s) (OR = 5.25; 95%CI = 4.00-6.88) en comparación con los que no tenían ninguna copia de esta variante alélica (los l/l) (OR= 3.34 95% CI= 2.36-4.72) (Ver Figura 21).

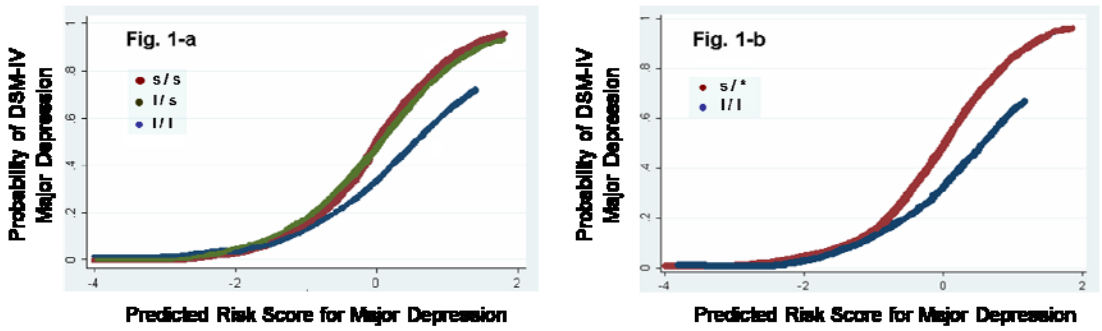


Figura 21. Interacción para depresión mayor entre el genotipo para el polimorfismo 5-HTTLPR del gen SERT y los factores de riesgo ambientales incluidos en el modelo predictivo de depresión PREDICT-D.

Encontramos además, una tendencia no significativa a la interacción entre el polimorfismo uMAOA y el riesgo para depresión predicho por el índice C (LR para la interacción = 2.12; p = 0.14), al comparar el efecto de tal índice en los portadores del alelo de alta actividad (high) de este polimorfismo (OR = 4.87; 95%CI = 3.79-6.26) *versus* los no portadores de esa variante alélica (low) (OR = 3.37; 95%CI = 2.15-5.28). (Ver Figura 22).

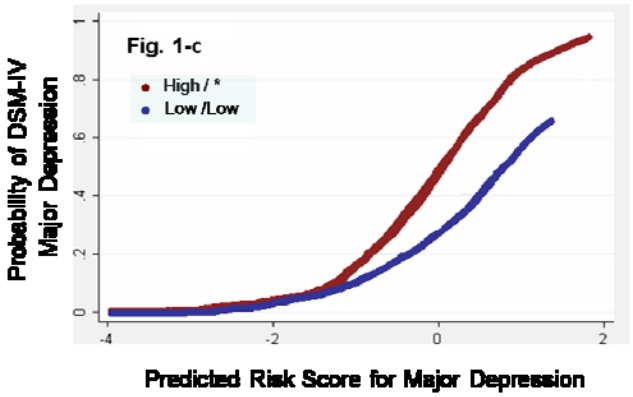


Figura 22. Interacción para depresión mayor entre el genotipo para el polimorfismo uMAOA del gen MAOA y los factores de riesgo ambientales incluidos en el modelo predictivo de depresión PREDICT-D.

Por último, encontramos que el genotipo para los dos polimorfismos analizados anteriormente, modificaba el efecto del índice C en el riesgo para padecer depresión (LR para la interacción = 17.12; p = 0.038). De este modo, mientras el valor del índice C fue moderado en aquellos individuos sin genotipos de riesgo (ie, l/l y low/low) (OR=3.02; 95%CI=1.40-6.51), éste era significativamente mayor en aquellos individuos que portaban, al menos, una de las variantes alélicas de riesgo de cualquiera de los dos polimorfismos (el alelo s del polimorfismo 5-HTTLPR o el alelo high del polimorfismo uMAOA) (OR=3.06; 95% CI=1.16-8.05). El índice C alcanzaba los valores más altos en aquellos individuos portadores de los dos alelos de riesgo de ambos polimorfismos (alelo s del 5-HTTLPR y el alelo high del polimorfismo uMAOA) (OR= 6.70; 95%CI= 2.68-16.77). (Ver Figura 23).

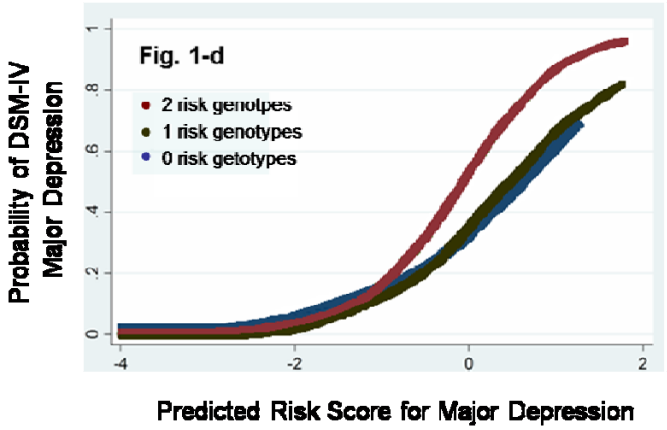


Figura 23. Interacción para depresión mayor entre los genotipos para los polimorfismos 5-HTTLPR y uMAOA y los factores de riesgo ambientales incluidos en el modelo predictivo de depresión PREDICT-D.

Por último, dividimos las puntuaciones de riesgo de depresión predichas por el índice C en cinco grupos, para explorar si esa interacción entre el riesgo de depresión predicho por el índice C y la presencia de uno o dos alelos de riesgo, era particularmente fuerte en alguno de los grupos. Así, se observó que la interacción era mayor en el grupo de individuos con mayor número de alelos de riesgo y para los que el valor del índice C era más alto ($\chi 2$= 16.16; p=0.040) (Ver Tabla 16).

Tabla 16. Grado de Interacción entre el estatus de riesgo de depresión calculado mediante el índice C y el status de riesgo genético conferido por los polimorfismos 5-HTTLPR y uMAOA.

c-Index Risk Prediction Effect	0 Risk Allele	1 Risk Allele	2 Risk Alleles
1	1	OR = 0.07; 95% CI 0.005-1.214; p = 0.069	OR = 0.13 ; 95% CI 0.122-1.379 ; p = 0.090
2	1	OR = 0.40; 95% CI 0.046-3.497; p = 0.411	OR = 0.42 ; 95% CI 0.054-3.324 ; p = 0.414
3	1	OR = 2.64; 95% CI 0.371-18.756; p = 0.332	OR = 1.92; 95% CI 0.277-13.382; p = 0.508
4	1	OR = 9.06; 95% CI 1.287- 63.83; p = 0.027	OR = 4.86; 95% CI 0.725- 32.564; p = 0.103
5	1	OR = 12.66; 95% CI 1.802-89.001; p = 0.011	OR = 34.89; 95% CI 5.162-235.784; p = 0.000

RESULTADOS

ARTÍCULOS CIENTÍFICOS

A continuación se presentan tres artículos científicos, en los que la doctoranda es coautora, que describen los últimos hallazgos derivados del estudio PREDICT-Gene y que componen el capítulo de resultados de la presente Tesis Doctoral.

Artículo 1

The Association of C (-1019) G Serotonin 1A Polymorphism with Comorbid Anxiety and Depression.

***Esther Molina**, Jorge Cervilla, Margarita Rivera, Francisco Torres, Juan Ángel Bellón, Berta Moreno, Michael King, Irwin Nazareth, Blanca Gutiérrez.*

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RESUMEN

Los receptores de serotonina tipo 1A constituyen moléculas clave reguladoras de la actividad serotoninérgica y su disfunción podría estar involucrada en la etiología de la Depresión Mayor (MD) y del Trastorno de Ansiedad Generalizada (GAD). Los resultados desde los estudios de asociación genética realizados hasta ahora no han sido del todo concluyentes en cuanto al posible papel del gen del receptor de serotonina tipo 1A en la etiología de la depresión. Además, ningún estudio hasta el momento ha estudiado este polimorfismo en depresión mayor o en ansiedad teniendo en cuenta la frecuente comorbilidad existente entre esos dos trastornos.

Nuestro objetivo fue clarificar el posible papel del polimorfismo C (-1019) G del gen del receptor de serotonina tipo 1A (gen 5-HT1A) en la etiología tanto de MD, como de GAD, además de su papel en el origen de las formas comórbidas de MD con GAD.

Un total de 1059 individuos que estaban participando en el estudio PREDICT-Gene (*Cervilla et.al. 2006*) fueron evaluados mediante la Entrevista Diagnóstica Compuesta Internacional y la Primary Care Evaluation of Mental Disorders Patient Health Questionnaire para generar diagnósticos de Depresión Mayor (MD) y Trastorno de Ansiedad Generalizada (GAD), según criterios DSM-IV.

Estos sujetos donaron una muestra biológica a partir de la cual se realizaron los análisis genéticos llevados a cabo en la plataforma Sequenom.

En un primer análisis crudo, encontramos que ser portador del alelo G del polimorfismo C (-1019) G se asociaba con un riesgo incrementado de Depresión Mayor (OR = 1.67, 95%CI = 1.14 - 2.44, p = 0.008), aunque esta asociación dejó de ser significativa tras ajustar por la presencia de Trastorno de

Ansiedad Generalizada (OR = 1.43, 95%CI = 0.96-2.14, p = 0.080) y también cuando además se controlaba el posible efecto de factores confusores tales como el sexo, la edad o la presencia de historia familiar de problemas psicológicos entre los familiares de primer grado (OR = 1.37; 95% CI = 0.87-2.16; p = 0.173).

De manera análoga, este polimorfismo resultó estar significativamente asociado con GAD en un primer análisis crudo (Crude: OR = 2.54, 95%CI = 1.28-4.86, p = 0.003), aunque al tener en cuenta en el análisis el diagnóstico de MD, esa asociación se hizo más débil (OR =1.97, 95%CI = 0.99-3.91, p=0.050), y se alejó de los niveles de significación estadística al ajustar por otros posibles confusores (OR = 1.62; 95% CI = 0.79-3.30; p =0.184).

Sin embargo, al analizar la posible asociación entre este polimorfismo y la comorbilidad entre MD y GAD, encontramos que el ser portador del alelo G estaba significativamente asociado a la comordilidad MD/GAD (OR =3.41, 95% CI = 1.44-8.05, p = 0.005), que incluso se mantuvo significativa al ajustar por factores confusores tales como el sexo, la edad o la presencia de historia familiar de problemas psicológicos entre los familiares de primer grado OR =2.82, 95%CI = 1.18-6.77, p = 0.020).

Nuestros resultados sugieren que el polimorfismo C (-1019) G del gen 5HT1A podría determinar un riesgo diferencial para la presentación comórbida de la MD y la GAD, pero no para cada uno de estos trastornos por separado. En concreto, los individuos portadores del alelo G tendrían 2.82 veces más riesgo de desarrollar un cuadro ansioso-depresivo que los sujetos con otros genotipos.

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THE ASSOCIATION OF C (-1019) G SEROTONIN 1A PROMOTER POLYMORPHISM WITH COMORBID ANXIETY AND DEPRESSION

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Keywords:	5-HT1A, rs6295, major depression, generalized anxiety disorder, comorbidity

THE ASSOCIATION OF C (-1019) G SEROTONIN 1A PROMOTER POLYMORPHISM WITH COMORBID ANXIETY AND DEPRESSION

Esther Molina[1], Jorge Cervilla[1], Margarita Rivera[1], Francisco Torres[1], Juan Ángel Bellón[2], Berta Moreno[3], Michael King[4], Irwin Nazareth[5], Blanca Gutiérrez[1,*].

[1] CIBERSAM University of Granada. Sección de Psiquiatría e Instituto de Neurociencias. Universidad de Granada. Granada. Spain.

[2] Centro de Atención Primaria "El Palo", Unidad de Investigación de Atención Primaria del Distrito Málaga, grupo SAMSERAP. Departamento de Medicina Preventiva. Universidad de Málaga. Málaga, Spain.

[3] Departamento de Personalidad, Evaluación y Tratamiento Psicológico. Universidad de Málaga. Málaga, Spain.

[4] Department of Mental Health Sciences. University College London, Royal Free Campus, London, UK.

[5] Research Department of Primary Care & Population Health, University College London & MRC General Practice Research Framework, London, UK.

Keywords: 5-HT1A, C (-1019) G polymorphism, rs6295, major depression, generalized anxiety disorder, comorbidity

Corresponding author
Blanca Gutiérrez, PhD. Section of Psychiatry and Institute of Neurosciences, School of Medicine, University of Granada. Avda. Madrid 11, 18012 Granada, Spain. E-mail: blancag@ugr.es

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ABSTRACT

Serotonin 1A receptors are key regulators of serotonin activity and their dys-regulation might be involved in the pathology of major depression (MD) and generalized anxiety disorder (GAD). Previous studies have yielded inconclusive results as to whether the 5-HT1A receptor gene has a role in the aetiology of MD and no study up to date had studied this polymorphism on either pure MD or MD comorbid with GAD. We aim to clarify the putative role of the C (-1019) G polymorphism at the serotonin 1A receptor gene in the aetiology of both MD and GAD and comorbid MD/GAD states. DSM-IV MD and GAD diagnoses were ascertained using the Composite International Diagnostic Interview and the Primary Care Evaluation of Mental Disorders Patient Health Questionnaire, respectively. 1059 sujects who took part in the PREDICT-GENE study (Cervilla et al., 2006) were included for molecular analyses using Sequenom platform. Carrying the G allele of the C (-1019) G polymorphism was associated with MD (OR = 1.67, 95%CI = 1.14 - 2.44, p = 0.008) but this association became non-significant after adjusting by presence of GAD (OR = 1.43, 95%CI = 0.96-2.14, p = 0.080). Similarly, the C (-1019) G polymorphism was univariately associated with GAD (OR = 2.54, 95%CI = 1.28-4.86, p = 0.003), although adjusting by MD made results no longer significant (OR =1.97, 95%CI = 0.99-3.91, p=0.050). However, we found a solid significant association between carrying the G allele and comorbid MD and GAD (OR =3.41, 95% CI = 1.44-8.05, p = 0.005) which remained robust and statistically significant after adjusting by sex, age and family history of psychological problems (OR =2.82, 95%CI = 1.18-6.77, p = 0.020). Our results suggest that the C (-1019) G serotonin 1A polymorphism confers a risk for the frequent clinical presentation of comorbid MD and GAD but not for either of these disorders alone after adjusting by presence of one another.

INTRODUCTION

Major depression (MD) and generalized anxiety disorder (GAD) are the most common psychiatry disorders found among primary care attendees (King et al., 2008-a*)*. Both conditions frequently co-occur possibly as a consequence of a shared psychopathology (Anderson and Hope, 2008) and common dys-regulation in serotonergic activity (Copen et al., 1973; Millan, 2004). Involvement of the serotonergic system is also strongly supported by psychopharmacological studies which show that drugs enhancing serotonergic transmission are as effective as antidepressants and anti-anxiety agents (Sthal, 2008). Many previous studies have focused on genetic variability at loci encoding for serotonin receptors and enzymes implicated in serotonin metabolism (Naughton et al., 2000). Among these, the serotonin transporter gene and certain post-synaptic serotonin receptors (i.e., 5-HT2A, 5-HT2C) have been implicated in the origin of both depression and anxiety. Although these results do not conclusively suggest an association with post-synaptic receptor genes (Angelova et al., 2003)*,* the evidence for the serotonin transporter gene (Gutierrez et al., 1998; Munafo et al., 2005; Lasky-Su et al., 2005; Cervilla et al., 2006) is stronger. Little is known about other serotonin receptors, such as 5HT1A, despite their potential role as critical regulators of serotonergic transmission, in the emergence of such disorders.

The role of 5HT1A in depression has been demonstrated by neuroimaging, post-mortem and expression studies. Neuroimaging studies have shown decreased pre-synaptic 5-HT1A binding in cortical and raphe regions of depressed patients compared to controls (Drevets et al,. 1999; Drevets et al., 2000; Hasler et al., 2007; Hirvonen et al., 2008). However*,* post-mortem analyses of brain samples of people who have committed suicide while depressed have shown significant increases in presynaptic levels of the 5-HT1A autoreceptors compared to healthy controls (Stockmeier et al., 1998; Boldrini et al., 2008). On the other hand, post-mortem and neuroimaging studies of RNA expression have shown decreased levels of postsynaptic 5-HT1A receptors *(*Drevets et al., 2000; Hsiung et al., 2003; Lopez-Figuera et al., 2004; Drevets et al., 2007). Moreover, people with high rates of neuroticism (Tauscher et al.,

2001) and anxious depression (Sullivan et al., 2005) have altered 5-HT1A receptor binding. Additionally, 5-HT1A receptor knock-out mice exhibit increased anxiety-related behaviour (Toth, 2003) and manipulation of the 5-HT1A receptor agonists/antagonists alters anxiety behaviours in experimental animals (Vicente et al., 2008).

This body of research suggests that 5-HT1A receptors could play a key role in the aetiology of MD and GAD and this could, in part, be determined by variability at the 5HT1A receptor gene. Currently, 23 single nucleotide polymorphisms (SNPs) have been described and validated on the 5-HT1A receptor gene (The International HapMap Consortium, 2003). Some of them, such as Ile28Val, Arg219Leu, and Gly22Ser have been found to have low heterozygosity in Caucasian populations (Rotondo et al., 1997; Bruss et al., 2005). However, a common polymorphism in the promoter region of the 5-HT1A receptor gene was described by Wu and Commings (1999). This polymorphism, the so-called C (-1019) G polymorphism (also rs6295), is located in a 26-bp palindrome region which is recognized by transcription factors such as NUDR (nuclear deformed epidermal autoregulatory factor (DEAF-1)) (Lemonde et al., 2003). Lemonde et al., (2003) found that NUDR can only bind with the C allele of the C (-1019) G polymorphism and cannot bind with the G allele (Lemonde et al., 2003). In addition, NUDR has either an enhancer or a repressive activity depending on its postsynaptic or presynaptic localization, respectively (Czesak et al., 2006). The presence of the G (-1019) variant would result in both an increase in presynaptic 5HT1A autoreceptor expression and a reduction in post-synaptic receptor expression. This, in turn, could contribute to low levels of serotonin neurotransmission which is associated with depression (Lemonde et al., 2003).

Some researchers (Parsey et al., 2006; Lenze et al., 2008) have demonstrated the existence of an association between the G allele of the C (-1019) G polymorphism and high risks for major depression. However, others have failed to find such an association (Arias et al., 2002; Huang et al., 2004). Similarly, the role of the C (-1019) G polymorphism in the aetiology of anxiety disorders has remained inconclusive. Whilst some authors reported a significant association

between the G allele at this locus and anxiety related traits of neuroticism (Strobel et al., 2003), more recent studies have not replicated such association (Hettema et.al. 2008-a). To our knowledge, no study to date has examined this polymorphism on either pure or comorbid GAD. Yet, as suggested by Middeldorp et al., (2005), a shared genetic substrate may play a role in comorbid depressive and anxiety states. Furthermore, despite the frequent co-occurrence of MD and GAD (Alonso et al., 2004), no previous researchers have explored the potential association between the C (-1019) G polymorphism and comorbid depression with GAD, or indeed with depression or GAD adjusting by one another.

This study aimed to clarify the putative role of the C (-1019) G polymorphism in the aetiology of MD and/or GAD using the enlarged PREDICT-Gene cohort *(*Cervilla et al., 2006). Our objective was to explore whether the previous controversial findings on the association between the G allele and depression or anxiety could be explained by the lack of analyses considering the frequent comorbidity associated with both mental states.

METHODS

Study design

The PREDICT-GENE study *(*Cervilla et al., 2006; Cervilla et al., 2007; Rivera et al., 2008) is a case-control association study nested in the Spanish subsample of a larger European study (Predict-D) on predictors of depression onset in primary-care attendees (King et al., 2006; King et al., 2008-b; Bellón et al., 2008). We used only baseline assessments for the present analysis. Both the PREDICT-D and PREDICT-GENE studies were approved by the relevant research ethics committees.

Sample

Consecutive attendees to thirteen primary care centres in the provinces of Málaga (9) and Granada (4), in Andalusia (Spain), were asked to participate in this genetic sub-study by their family doctors between April 2004 and December 2007. After informed consent was obtained, interviews were undertaken by trained researchers. Only those participants aged 18 to 75 were included in our study. Attendees unable to understand Spanish, as well as those with an organic mental disorder and/or any terminal illness, were not included in the study. Participants agreeable to participate in the genetic sub-study (1059) gave further specific informed consent to provide the biological sample for genetic analyses, consisting of 10 cm^3 of blood and/or up to 4-mouth swabs for saliva collection.

Sociodemographic data

The full PREDICT risk factor assessment was shown to have adequate test-retest reliability. Sociodemographic data (such sex, age, education, marital status, living arrangement and profession) were gathered using previously validated measures *(*King et al., 2006*)*.

HTR1A genotyping

Genomic DNA from both blood and saliva samples was extracted following standard procedures. The (C-1019G) polymorphism in the promoter region of the serotonin receptor type 1A was genotyped by MassArray (Sequenom)

technology which is based on the detection of the products of an allelic discrimination reaction using MALDI-TOF at the Spanish National Genotyping Centre (CEGEN) (www.cegen.org).

Outcome measures: Major Depression, Generalized Anxiety Disorder and Co-morbid MD and GAD.

DSM-IV Major Depression (MD) was ascertained using the depression section of the Composite International Diagnostic Interview (CIDI) (Robins et al., 1988). Participants were also assessed to establish DSM-IV criteria for Generalized Anxiety Disorder (GAD) using the Anxiety Section of the Spanish Version of the Primary Care Evaluation of Mental Disorders Patient Health Questionnaire (PHQ PRIME-MD) (Spitzer et al., 1999; Diez-Quevedo et al., 2001). Patients were considered to suffer from comorbid MD and GAD only if they fulfilled the criteria for both MD and GAD using the above diagnostic tools.

Statistical Analyses

The statistical analyses were performed using the SPSS 15.0 package (2006). Firstly, we performed an exploratory analysis to study the distribution of all the variables. Secondly, we used the Chi squared test to compare genotype and allele frequencies between patients and controls. Finally, using binary logistic regression analyses, we estimated the odds ratios (OR), with 95% confidence intervals (95% CI), comparing allele G (-1019) carriers versus non-carriers. These analyses were conducted first in people who only had MD (with no GAD), repeated in those only with GAD (with no MD) and finally conducted in those with comorbid MD and GAD. Logistic regression models were built up with a progressive number of potential confounders. When comorbid MD and GAD was used as an outcome, we excluded non-comorbid MD or GAD subjects from the control group.

RESULTS

Demographic and clinical characteristics

Out of a total number of 1531 participants approached to take part in the genetic sub-study, 1110 agreed to provide a biological sample and full data was obtained on 1059 participants. Frequencies of all the clinical and socio-demographic variables assessed in our study are summarized in Table I. No statistical differences were found for socio-demographic factors (sex, living arrangements, educational level and main occupation) or for the prevalence of depression, GAD or comorbid depression and GAD between the participants (1059), those who refused (421) or those that did not complete assessment (51).

758 participants were women (71.6%) and 301 were men (28.4%) and overall mean age was 49 years (SD=15.3). All participants were of Spanish ethnic origin. 387 participants (48.3%) had a family history of psychological problems amongst at least one first degree relative. The 6-month prevalence of DSM-IV Major Depression (MD) was 20% (206) that of DSM-IV Generalized Anxiety Disorder Diagnosis was 8% (85), whilst 58 participants (5.5%) fulfilled criteria for comorbid MD and GAD.

Association C (-1019) G polymorphism and DSM-IV Major Depression

Genotype frequencies were in Hardy-Weinberg equilibrium. Allele and genotype frequencies across study groups are shown on Table II. We found a significant excess of G allele carriers in the group with MD when compared to the control group (Cases: G/* 167 (81%), C/C 39 (19%); Controls: G/* 595 (72%), C/C 232 (28%); OR = 1.67; 95% CI = 1.14 - 2.44; p = 0.008) (see Figure 1). This association was no longer significant after adjusting for GAD (OR = 1.43; 95% CI = 0.96-2.14; p = 0.080) and appeared even weaker after we further adjusted for other potential confounders such as sex, age and family history of psychological problems (OR = 1.37; 95% CI = 0.87-2.16; p = 0.173).

Association C (-1019) G polymorphism and GAD

Genotype frequencies for GAD were also in Hardy-Weinberg equilibrium. G carriers were significantly more frequent among GAD patients than in the control group *(*Cases*: G/** 74 (87%), C/C 11 (13%); Controls: G/* 707 (73%), C/C 267 (27%)*; OR* = 2.54; 95% CI = 1.28-4.86; p = 0.003) (see Figure 1). When we adjusted for the presence of DSM-IV Major Depression Diagnosis, the above association became less apparent (OR =1.97; 95% CI = 0.99-3.91; p =0.050) and this grew even weaker after adjusting for sex, age and family history of psychological problems (OR = 1.62; 95% CI = 0.79-3.30; p =0.184).

Association C (-1019) G polymorphism and Comorbidity MD/GAD

We found a statistically significant association between carrying the G allele and comorbid MD and GAD (Cases: G/*:52 (90%); C/C: 6 (10%); Controls: G/*: 577 (72%), C/C: 227 (28%); *OR* =3.41; 95% CI = 1.44-8.05; p = 0.005) (see Figure 1). This association remained significant after adjusting for the potentially confounding effects of sex, age and family history of psychological problems (OR =2.82; 95% CI = 1.18-6.77; p = 0.020).

DISCUSSION

We aimed to clarify the potential role of the C (-1019) G polymorphism in the 5-HT1A gene in the aetiology of MD and GAD or their comorbid states. We found that G allele carriers have a higher risk for comorbid MD and GAD, but not for either MD or GAD alone. This association was independent of possible confounders such as age, sex, and family history of psychological problems among first degree relatives (FH). This is the first study to explore the nature of the association between the 5-HT1A gene and comorbid MD and GAD. It is also the first to test the association with MD or GAD taking each other into account and further adjusting by other confounders, including FH.

One of the main advantages of the study is that we used the largest sample (N=1059) to date to test these associations. This provided sufficient statistical power to allow us to detect a small effect conferred by polymorphic variability at just one genetic locus. The sample was likely to be representative of the general population as it was derived from consecutive primary care attendees. Additionally, the sample was from a genetically homogenous population avoiding potential population stratification that could have presented difficulties in previous studies (Huang et al., 2004). Finally, we assessed the prevalence of MD and GAD using validated instruments which enabled us to generate both MD and GAD diagnoses according to DSM-IV criteria.

We could not rule out selection bias, as the final sample represents both 69.2% of those who agreed to participate at baseline and 95.4% of those who consented to provide a biological sample twelve months later. Nevertheless, we did not detect statistically significant differences in terms of sex, mean age, living arrangements, educational level, main occupation, prevalence of MD, GAD or comorbid MD and GAD either between the cohort at baseline, the study participants who gave a biological sample and those who refused to do so.

C (-1019) G and Major Depression

Most previous studies looking into the association between G carrier status and MD have reported positive findings (Lemonde et al., 2003; Parsey et al., 2006;

Lenze et al., 2008) although some studies failed to find an effect (Arias et al., 2002; Huang et al., 2004). To our knowledge, no previous published study has explored these associations with adjustments for potential confounders, particularly coexisting GAD. Indeed, we also found a crude association between being a G carrier and MD, but this did not hold true after taking GAD into account. Hence, we found no major specific effect for G allele status on MD and hypothesize that previous associations might have been observed on account of failure to control for GAD. This is an important issue as GAD is frequently seen together with MD in daily clinical practice (Alonso et al., 2004).

C (-1019) G and Generalized Anxiety Disorder

Lesch and Mössner (1999) found that 5-HT1A knock-out mice demonstrated enhanced anxiety-related behaviour. This could be as a consequence of increased terminal 5-HT availability resulting from the lack of reduction in presynaptic somatodendritic 5-HT1A autorreceptor negative feed-back function *(*Lesh and Mossner, 1999*)*. In addition, previous studies have reported associations between this polymorphism and panic disorder (Rothe et al., 2004) or personality traits such as neuroticism (Strobel et al., 2003). Such results suggest a potential role for this gene in the aetiology of these conditions, although this may not hold true for GAD. To our knowledge, few previous studies exist on the association between the C (-1019) G polymorphism and anxiety disorders (Toth, 2003; Hettema et.al, 2008-a) and no other studies have focused specifically on GAD alone. We found a statistically significant crude association between being a G carrier and GAD, however this association weakened to non-significant levels after adjusting for the frequently comorbid MD condition. These crude associations between G carrier status and MD or GAD suggest that failure to adjust for relevant confounders could lead to spurious positive findings. However, this is not the case for comorbid MD and GAD states where our findings held after adjustment.

C (-1019) G and comorbid Major Depression and Generalized Anxiety Disorder

Despite the frequent clinical presentation of comorbid MD and GAD, no previous study had explored the putative role of the 5-HT1A gene in comorbid MD and GAD, particularly after exploring such role on both disorders alone. We

relatives but that this does not hold true for either MD or GAD when considered alone.

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report here a robust association between G carrier status and comorbid MD and GAD which is independent of age, sex and a positive family history of psychological problems. Our results suggest that changes in 5-HT1A gene transcription, as those induced by G carrier status (Lemonde et al., 2003; Czesak et al., 2006), may increase the risk for comorbid MD and GAD or for a latent psychopathological state that we diagnose as two conditions. Evidence for the latter comes from variety of fields including at least psychopathology (Strobel et al., 2003), genetics (Gorwood, 2004; Kronenberg et al., 2007; Verhagen et al., 2009), neurochemistry (Sthal, 2008), neuroimaging (Mathew et al., 2004; Campbell and McQueen, 2006) and neuroendocrinology (Boyer et al., 2000).

In brief, variation at the C (-1019) G polymorphism in the 5-HT1A gene has been linked to neuroticism (Strobel et al., 2003). Interestingly, neuroticism is considered a potential underlying psychopathological core shared by both MD and GAD (Clark and Watson, 1991; Brandes and Bienvenu, 2006), supporting our results from a psychopathological viewpoint. Our results are further supported by other genetic association studies (involving genes different from 5-HT1A) that report increased risk for comorbid MD and GAD rather than each condition alone (Kronenberg et al., 2007; Verhagen et al., 2009). Moreover, the monoaminergic deficit hypothesis putatively involved in the aetiology of both MD and GAD (Sthal, 2008) suggests a neurochemical basis for such comorbidity. Indeed, selective serotonin reuptake inhibitors (SSRI) can be as efficacious in the treatment of GAD as MD. Neuroimaging studies are starting to identify some overlap of specific areas of the brain that are jointly implicated in both MD and GAD (Mathew et.al, 2004; Campbell and McQueen, 2006; Hettema, 2008-b). Finally, our results are further supported by neuroendocrine findings such as corticotrophin releasing hormone (CRH) hypersecretion and disruption of neurosteroid functions, as a consequence of hypothalamic pituitary axis (HPA) dysfunction, in both MD and GAD (Boyer, 2000).

In conclusion, our results suggest that the variability at the 5-HT1A gene plausibly plays a role in the aetiology of comorbid MD and GAD, independent of sex, age and family history of psychological problems among first degree

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TABLE I. Demographic and clinical characteristics

Variables	Frequencies/means
Sex (male/female)	301 (28.4%) / 758 (71.6%)
Mean age	49.88 years (SD15.30)
Family History of Psychological Problems (FH)*	
FH +	387 (48.25%)
FH -	502 (62.59%)
DSM-IV Major Depression ¤	
Depressed	206 (19.95%)
Not depressed	827 (80.05%)
Generalized Anxiety Disorder (GAD) ▪	
GAD	85 (8.03%)
Not GAD	974 (91.37%)
Co morbidity MD/GAD ◊	
DEP/GAD	58 (5.5%)
Not DEP/GAD	804 (90.23%)
DEP or GAD	170(16%)

* FH: Family history of psychological problems amongst first degree relatives. Data missing -170 participants

¤ DSM-IV Major Depression Diagnosis was not available for 26 participants.

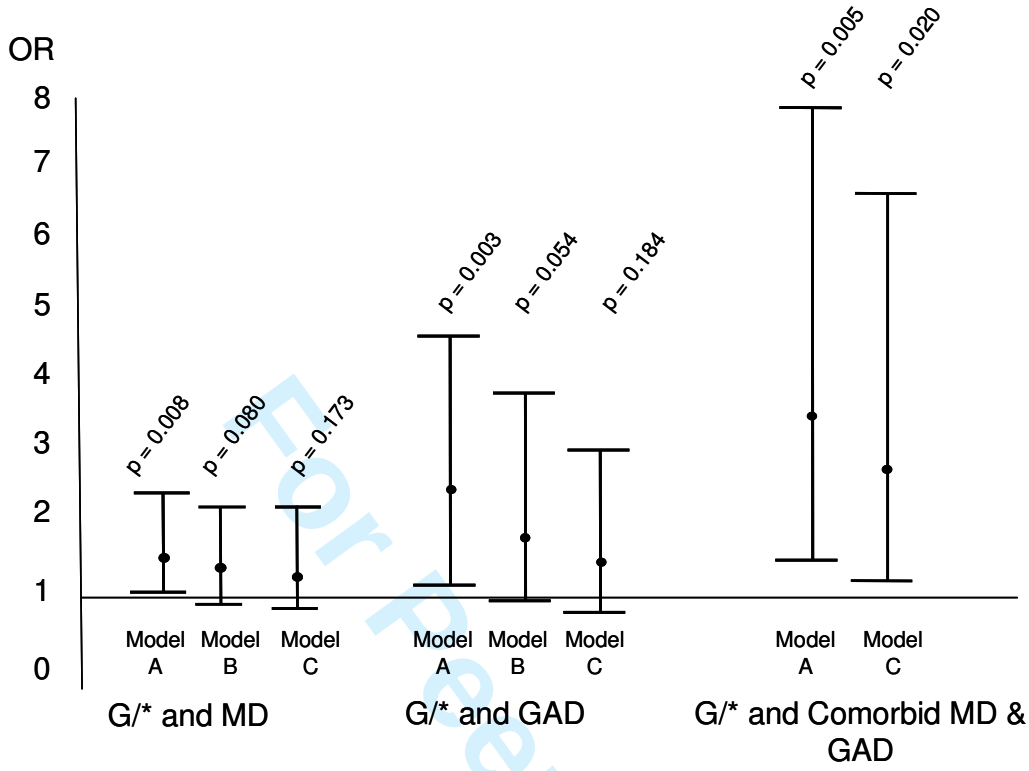
• GAD: Generalized Anxiety Disorder.

◊ Comorbidity MD/GAD: Comorbidity DSM-IV Major Depression and Generalized Anxiety Disorder. Diagnosis was not available for 27 participants.

TABLE II. Allele and genotype frequencies distribution in participants with DSM-IV Major Depression, those with Generalized Anxiety Disorder and those with comorbid MD and GAD *versus* controls.

	Alleles						Genotypes			
	G	C	X^2	OR	95% CI	*p*	G/G	G/C	C/C	*p*
DSM-IV Major Depression (MD) n=206	217 (53%)	195 (47%)	2.05	1.17	0.94 - 1.46	0.152	50 (24%)	117 (57%)	39 (19%)	0.011
Controls n=827	806 (49%)	848 (51%)					211 (26%)	384 (46%)	232 (28%)	
GAD n = 85	96 (57%)	74 (43%)	3.71	1.36	0.98-1.89	0.054	22 (26%)	52 (61%)	11 (13%)	0.010
Controls n = 974	950 (49%)	998 (51%)					243 (25%)	464 (48%)	267 (27%)	
Comorbid MD/GAD n = 58	65 (56%)	51 (44%)	2.41	1.35	0.91-2.01	0.120	13 (22%)	39 (67%)	6 (10%)	0.003
Controls n = 804	781 49%)	827 (51%)					204 (25%)	373 (46%)	227 (28%)	

FiG.1. Odds ratios (OR) for the association between G allele carriers and MD, GAD and comorbid MD and GAD.



Model A: Crude odds ratios.

Model B: Odds ratios for both MD and GAD adjusting one by another.

Model C: Odds ratios for model B + adjusting by sex, age and FH

Artículo 2

Gene by Gene by Environment Interactions as Determinants of Major Depression: A Prospective Analysis of the PREDICT-Gene Cohort

Blanca Gutiérrez, Jorge Cervilla, Margarita Rivera, ***Esther Molina****, Juan de Dios Luna, Francisco Torres, Juan Ángel Bellón, Berta Moreno, Michael King, Irwin Nazareth.*

RESUMEN

La Depresión es una enfermedad compleja con una etiología multifactorial, en la que diversos factores ambientales, tales como el estrés psicosocial o las experiencias traumáticas en la infancia, confieren un riesgo incrementado para padecer la enfermedad. Sin embargo, recientemente se ha sugerido que este riesgo conferido por el ambiente parece ser modificado por la carga genética individual (Caspi et.al. 2003, Cervilla et.al. 2007), pero no está del todo claro cómo se produce tal interacción y cuáles son los factores genéticos y ambientales implicados.

Nuestro objetivo es analizar si los polimorfismos 5-HTTLPR del gen SERT y el uMAOA del gen MAOA, ampliamente estudiados en relación a la etiología de la depresión, interactúan conjuntamente (Interacción Gen-Gen) confiriendo un riesgo incrementado para padecer depresión. Además, pretendemos analizar si tales polimorfismos modifican el riesgo para padecer depresión conferido por el estrés psicosocial y las experiencias de abuso en la infancia (Interacción Gen–Gen–Ambiente).

1319 usuarios de los servicios de Atención Primaria de las Provincias de Málaga y Granada fueron seguidos durante un año y evaluados a tres tiempos para toda una batería de variables sociodemográficas y psicológicas en el contexto del estudio PREDICT-Gene. Todos ellos aceptaron, además, donar una muestra biológica para llevar a cabo los análisis genéticos.

Nuestros resultados mostraron que, tanto el genotipo s/s del polimorfismo 5-HTTLPR como el alelo de alta actividad en homocigosis del polimorfismo uMAOA, conferían riesgos incrementados para padecer Depresión ((OR=1.3; 95%CI:1.05-1.55; p=0.008 para el polimorfismo 5-HTTLPR; OR= 1.25; 95%CI: 1.03-1.51; p=0.024 para el polimorfismo uMAOA).

De manera análoga, el número de acontecimientos vitales estresantes (AVEs) y las experiencias de abuso durante la infancia, también se encontraron asociados a depresión (AVEs: OR = 1.5 95%CI 1.35-1.64, p = 0.000. Abuso sexual :OR = 2 95%CI 1.41-2.91, p = 0.000. Abuso físico: OR = 2.5 95%CI 2.06-3.12, p = 0.000. Abuso psicológico: OR = 2.6 95%CI 2.17-3.07, p = 0.000).

Los análisis de interacción mostraron, en primer lugar, la existencia de una interacción Gen-Gen, de modo que, aquellos individuos con el genotipo s/s del polimorfismo 5-HTTLPR y que portaban en homocigosis el alelo de alta actividad del polimorfismo uMAOA (alelo HA), eran los que tenían mayor riesgo de padecer depresión (Interacción $\chi_2 = 6.65$, p = 0.0098).

En segundo lugar, al analizar si la variabilidad contenida en los genes SERT y MAOA modificaba el riesgo conferido por la presencia de AVEs en la aparición de depresión, encontramos que, dentro de cada nivel de estrés psicosocial (ningún AVE, 1 AVE o dos o más AVEs), aquellos individuos con el genotipo s/s y portadores en homocigosis del alelo HA, presentaron riesgos significativamente mayores para padecer depresión que los individuos con otras combinaciones alélicas (Interacción $\chi_2 = 13.1$, p = 0.069) (Interacción Gen-Gen-ambiente).

Por último, encontramos que estas regiones genéticas polimórficas también modificaban el riesgo para padecer depresión conferido por las experiencias de abuso en la infancia, tanto abuso sexual (Interacción $\chi_2 = 9.15$, p = 0.057), como cualquier tipo de abuso: sexual, físico o psicológico (Interacción $\chi_2 = 9.06$, p = 0.059).

Gene by Gene by Environment Interactions as Determinants of Major Depression: A Prospective Analysis of the PREDICT-Gene Cohort

Blanca Gutiérrez[1,*], Jorge A. Cervilla[1,*], Margarita Rivera[1], Esther Molina[1], Juan de Dios Luna[2], Francisco Torres[1], Juan A. Bellón-Saameño[3], Berta Moreno[4], Michael King[5], Irwin Nazareth[6]

1 CIBERSAM University of Granada. Sección de Psiquiatría e Instituto de Neurociencias. Universidad de Granada. Granada. Spain

2 Departamento de Bioestadística. Universidad de Granada. Granada. Spain

3 Centro de Atención Primaria "El Palo", Unidad de Investigación de Atención Primaria del Distrito Málaga, grupo SAMSERAP. Departamento de Medicina Preventiva. Universidad de Málaga. Málaga. Spain

4 Departamento de Personalidad, Evaluación y Tratamiento Psicológico. Universidad de Málaga. Málaga. Spain

5 Department of Mental Health Sciences. University College London. Royal Free Campus. London. UK

6 Research Department of Primary Care & Population Health. University College London & MRC General Practice Research Framework. London. UK

*Both authors have contributed equally to this paper and act as corresponding authors. Please, address correspondence to Dr. Blanca Gutiérrez or Dr. Jorge Cervilla, CIBERSAM University of Granada. Sección de Psiquiatría y Psicología Médica, Facultad de Medicina, Universidad de Granada. Avda. Madrid 11, 18012 Granada, Spain.

Keywords: Major depression, 5-HTTLPR, uMAOA, gene-by-gene interaction, gene-by-gene-by-environment interaction, longitudinal analysis

Introduction

Depression is a common complex disease in which genetic and environmental risk factors seem to be involved. It is considered a multifactorial and polygenic trait, with each gene contributing only with a small portion of variance (Kendler et al., 2005; Levinson, 2006). The high prevalence of depression in general population (ranging from 5 to 12%, Kessler 2007) suggests that the risk variants of genes involved should be common in human populations instead of unfrequent mutations. The search for such genes has been one of the main goals of psychiatric genetics for decades, and it´s still nowadays one of its major challenges, although a limitation exists in the number of studies exploring complex interplay between different genes or between a variety of genetic markers and environmental risk factors. Both the serotonin transporter gene (also called SLC6A4, 5-HTT gene or SERT) and the monoamine oxidase (MAOA) gene are among the most widely studied genetic risk factors for depression (Collier et al., 1996; Gutierrez et al., 1998; Steffens et al., 2002; Hauser et al., 2003; Willeit et al., 2003; Lotrich et.al. 2004; Hoefgen et al., 2005; Cervilla et al., 2006; Grunblatt et al., 2006; Munafo et al., 2006; Ramasubbu et al., 2006; Dick et al., 2007; Jarrett et al., 2007; Schulze et.al. 2000; Rivera et.al. 2009).

Evidence involving SERT and MAOA Genes in Depression

The SERT gene (Cr. 17) encodes a key molecule of the serotonin neurotransmission pathway that, interestingly, is the site of action of most antidepressive drugs. The short variant (S allele) of the 5-HTTLPR polymorphism, located in the promoter region of such gene, has consistently been associated with an increased risk for depression (Cervilla et al., 2006; Collier et al., 1996; Dick et al., 2007; Grunblatt et al., 2006; Gutierrez et al., 1998; Hauser et al., 2003; Hoefgen et al., 2005; Jarrett et al., 2007; Munafo et al., 2006; Ramasubbu et al., 2006; Steffens et al., 2002; Willeit et al., 2003), although there are some studies yielding negative results (Bellivier et al., 1998; Bozina et al.,

2006; Frisch et al., 1999; Furlong et al., 1999; Hoehe et al., 1998; Kim et al., 2000; Kunugi et al., 1997; Mellerup et al., 2001; Mendlewicz et al., 2004; Minov et al., 2001; Ohara et al., 1998; Oliveira et al., 2000; Rees et al., 1997; Seretti et al., 1999; Serretti et al., 1999; Serretti et al., 2002b; Shcherbatykh et al., 2000; Willis-Owen et al., 2005). This controversy is also found among the metaanalyses about the topic published up till now (Furlong et al, 1998; Lotrich & Pollock, 2004; Anguelova et al, 2003, Lasky-Su et al, 2005). The S allele encodes a particular promoter segment associated to a reduced transcripcional activity of SERT (Lesch et al., 1995; Lesch & Hiels, 2000). Such genetic variant has been related to depression, as stated above, and also to worse antidepressant treatment response (Zanardi et.al. 2001; Arias et.al. 2003; Serretti et.al. 2004) and certain clinical phenotypes, including anxiety disorders (Lesch et al., 1996).

The monoamine oxidase (MAO) is a key enzyme in the metabolism of several different biological amines including monoaminergic neurotransmitters such as serotonin, dopamine and norepinephrine. MAO is expressed in the outer mitochondrial membrane of specific cells in main brain and peripheral tissues (Konradi et al. 1988) and acts oxidising and degrading both endogenous and xenobiotic amines. The implication of MAO, and in particular MAOA (that preferentially deaminates serotonin and norepinephrine), in both altered human behavior and physiology has extensively been reported in the scientific literature (de la Chapelle et al. 1985; Brunner et al. 1993a, 1993b; Cases et al. 1995). In fact, MAOA inhibitors (IMAOs) are extensively used to treat depression (Murphy et al. 1994). The gene encoding MAOA is located on the chromosome Xp11.23-p11.4 (Ozelius et al. 1988). Several polymorphisms have been described in that gene (Black et al. 1991, Hotamisligil and Breakefield, 1991, Hinds et al. 1992), including a 30 bp repeat polymorphism (uMAOA) in the promoter region (Sabol et al. 1998) which has been demonstrated to affect MAOA transcriptional activity (Deckert et al. 1999, Denney et al. 1999). Some authors have described an excess of high activity uMAOA alleles in depressed women (Schulze et.al. 2000; Rivera et.al. 2009). These alleles have also been reported to be associated to both, a worse response to antidepressant treatment

(Yu et.al. 2005) and to certain clinical phenotypes or correlates, such as suicide (Ho et.al. 2000; Hollant et.al. 2007), neuroticism (Eley et al., 2003) and seasonality (Manuck et.al. 2000; Eley et.al. 2003; Gutierrez et al. 2004). Overall, results are not conclusive enough as there are also studies that have failed to identify the putative association between variability at MAOA gene and depression (Christiansen et al., 2007; Gutierrez et al., 2004; Huang et al., 2004; Kunugi et al., 1999; Muramatsu et al., 1997; Serretti et al., 2002a; Syagailo et al., 2001) and there are even some contradictory reports in which low activity alleles, instead of the high activity ones, are those found to increase the risk for the illness (Brummett et.al. 2007).

Although most evidence favours associations between depression and these or other genetic risk factors, inconsistencies in findings from genetic association studies may be related with methodological aspects, such as, the use of an inadequate phenotype characterization, small sample sizes with insufficient statistical power, heterogeneity of the sample, lack of adjustment for potential non-genetic confounders or the fact that many of these studies use standard single-locus tests.

Gene by Gene Interactions in Depression

Exploring the possible interactions between different loci may increase the power to detect specific effects that might be missed if each locus is analyzed separately (Cordell 2009). Hence, such approach may allow us to give some light to the biological and biochemical pathways that underpin disease. There are few studies analyzing gene-by-gene interaction in depression, none focused in a large and well characterized sample followed prospectively along time.

MAOA appears to have a particular affinity for degrading serotonin overall of the other neurotransmitters. The preference of MAOA for the degradation of serotonin suggests that uMAOA and 5-HTTLPR polymorphisms may be mutually influential and may work collectively to affect a variety of behaviors

and disorders, including depression. In previous studies, our own group has demonstrated a robust and significant association between 5-HTTLPR and depression (Cervilla et al., 2006) and uMAOA and depression (Rivera et al., 2009). In both studies, analyses were performed in a large and homogeneous sample of primary care attendees, assessed for major depression using the depression section of the CIDI and ICD-10 and/or DSM-IV diagnostic criteria.

Three-Way Gene by Gene by Environment Interactions in Depression

Finally, some studies have reported a three-way interaction (G x G x E). Thus, the interaction between 5HTTLPR genotype and social distress or abuse in predicting depression, could be modified by the variability at other candidate genes for depression, such as MAOA gene (Cicchetti et al., 2007), brain-derived neurotrophic factor gene (BDNF) (Kaufman et al., 2006; Kim et al., 2007; Wichers et al., 2008) or serotonin receptor type 1A gene (5HT1A) (Zhang et al., 2009).

Kaufman et.al. (2006) found that maltreated children carrying the Met allele of the Val66Met (196 G/A) polymorphism in the BDNF gene and the s/s genotype of the 5-HTTLPR polymorphism in the SERT gene had the highest levels of depressive symptomatology, when comparing to no maltreatment children**.** In addition, they found that the presence of positive social supports buffered the above mentioned genetic and environmental risk effect (four-way interaction). Later, Wichers et.al. (2008) replicated this three-way interaction in a sample of female twins. Furthermore, Kim et.al. (2007) had found the above mentioned polymorphisms to play a G x G x Threat Life Events (TLEs) interaction with depression in an elderly sample. In addition, recently, Zhang et.al. (2009) have suggested that the G (-1019) C polymorphism in the 5-HT1A gene seems to modify the effect of the interaction between the s/s genotype of the 5-HTTLPR and physical abuse in the prediction of depression. Finally, a G x G x E interaction evolving the s/s genotype of the 5-HTTLPR polymorphism, the low

activity allele of the uMAOA polymorphism in the MAOA gene and experiences of sexual abuse was found to be interplaying in the predicting risk for major depression and anxiety (Cicchetti et al., 2007).

All the above evidences support that a G x G x E interaction seem to be involved in the aetiology of depression. However, more studies are necessary to replicate these findings.

We hypothesize that both polymorphisms examined in conjunction in relation to major depression will reveal a GxG effect, demonstrating a moderating effect of one polymorphism on another. In particular, we hypothesize that homozygous for the short allele (s/s) of 5-HTTLPR polymorphism, carrying also two high activity alleles of the uMAOA polymorphism at the MAOA gene, will present the highest risk for major depression.

Sample and Methods

Study context and design

The PREDICT-Gene study (Cervilla et al., 2006) is a genetic sub-study nested in a larger cohort study on prediction of incident depressive episodes among primary care attendees, The PREDICT-D study (King et al., 2006; Bellón et al., 2008). A full descritption of the latter study has been reported elsewhere (King et al., 2006; Bellón et al. 2008). In summary, the PREDICT-D is a 1-year prospective study assessing consecutive general practice attendees at three different times: at the beginning of the follow-up (time 1), six months later (time 2) and 12 months after the first interview (time 3).

Sample

Consecutive attendees to sixteen primary care centres in the provinces of Málaga (9) and Granada (7), in Andalusia (Spain), were asked to participate in the PREDICT-Gene study between April 2003 and September 2004 (as for Málaga centres) and September 2006- December 2007 (in Granada centres). After informed consent was obtained, interviews were undertaken by trained researchers. Only those participants aged 18 to 75 were included in the study. Attendees unable to understand Spanish, as well as those with an organic mental disorder and/or any terminal illness, were not included in study. Participants agreeable to participate in the genetic sub-study (1319) gave further specific informed consent to provide a biological sample, consisting of 10 cm^3 of blood and/or up to 4-mouth swabs for saliva collection. Biological samples were obtained at time 3 in the case of Málaga centres and at time 2 for Granada centres.

Assessing Depression

DSM-IV Major Depression (MD) was ascertained using the depression section of the Composite International Diagnostic Interview (CIDI) which was designed to provide six month and lifetime psychiatric diagnoses (Robins et al., 1988). Prevalence of depression was assessed at baseline and at each follow-up point. We used the variable "depression at any time" (along the one-year of follow-up) as outcome variable. Then, although the sample consisted of 1314 individuals, the final number of observations generated by the statistical analyses (detailed bellow) involves 3681 observations.

Molecular analyses

DNA from blood and saliva was obtained by standard procedures. The 5-HTTLPR polymorphism at the serotonin transporter gene was genotyped

through amplification of genomic DNA and electrophoresis in 2% agarose gels stained with ethidium bromide. PCR reaction was performed using 50 ng of DNA, 0.25 uM of each primer (forward: 5´- GGC GTT GCC GCT CTG AAT GCC-3´; reverse: 5´- CAG GGG AGA TCC TGG GAG AGG T-3´), 250 uM each of dATP, CTP, dGTP and dTTP, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl and 0.3 units of DNA polymerase in a total volume of 25 ul. Samples were amplified for an initial cycle of 8 min at 95ºC followed by 35 cycles each consisting of 30 seconds at 95ºC, 30 seconds at 62ºC and 1 minute at 72ºC.

Genotypes for the uMAOA polymorphism were assessed in both patients and controls using a modified version of the protocol previously described by Deckert et al. (1999). Briefly, PCR fragments were amplified from genomic DNA using as primers oligonucleotides MAOAFor (5´-CCCAGGCTGCTCCAGAAAC) and MAOARev (5´-GGACCTGGGCAGTTGTGC). PCR (45 sec at 94ºC, 45 sec at 57ºC, 45 sec at 72 ºC for 35 cycles) was performed in a final volume of 25 ul containing 50 ng of genomic DNA, 10 pmol of each primer, 200 uM of each dNTP, 0.5 mM $MgCl_2$, 75 mM Tris-HCl (pH 9.0 at 25ºC), 20 mM $(NH_4)_2SO_4$, 0.01 % Tween 20 and 0.5 u of Taq DNA polymerase. The PCR products were separated by electrophoresis on a 3% agarose gel and visualized by ethidium bromide staining. The DNA molecular weight marker VIII from Roche was employed as size standard in each gel run.

Randomised individuals (about 10% of the total sample) were re-tested for their genotype, at both SLC6A4 and MAOA loci, in order to confirm the validity and accuracy of the methods. In all cases patterns were reproducible.

Statistical analyses

The SPSS 15.0 (2006) and STATA 10 (Stata Corp., 2007) statistical packages were both used. Initially, variable distributions were analysed and, then, χ^2 tests were used to check, firstly, whether genotype frequencies for both polymorphisms were in Hardy-

Weinberg equilibrium among both cases and controls; secondly, allelic and genotype distribution of both polymorphisms, and thirdly, univariate associations between DSM-IV major depression and environmental risk factors. Logistic regression was then used to test whether a-priory-defined particular genetic profiles associated with MD after adjusting by potential confounders such as sex. Odds ratios and 95% confidence intervals were then calculated by univariate and multivariate associations.

Longitudinal data analyses entailed a multivariate Generalized Estimating Equations (GEE) modeling using incident depression at any time over the one year follow-up period. This method was employed to enable a thorough analysis of three consecutive measures of depression that were interrelated not allowing a classical independent data analysis. This GEE model is a random effect model that allows adjustments by intraindividual variability. A logistic binary GEE model was, thus, used to calculate odds ratios and 95% confidence intervals for the association between genotypes and MD as measured along the three assessment times, and to test the presence of significant two-way (gene by gene) and three way (gene by gene by environment) interactions. The significance of interactions were then tested using the likelihood ratio test, considering significant any significance p value under the 0.10 level. In the case of a significant interaction, a detailed assessment was made to compare the different risk effect for MD conferred by the environmental factors across different genotype.

Results

The Sample

Table 1 shows the sample's sociodemographic, clinical and risk characteristics. Out of 1524 GP practice attendees, who were approached to provide a biological sample, 1319 (n=1319) consented to provide such sample and were included as participants in our genetic sub-study. No statisticaly significant differences were found among those who were included (86.5%) and those who were not (13.5%) with regards of sex, educational level or prevalence of DSMIV MD over the one year period. Those who did participate tended to be, on

average, three years older than those who refused (50 vs. 47 years old, respectively). 72.3% of participants were female, mean age was 50.33 years (SD 15.12 years), most were married or living with a partner (70%), did not live alone (92.2%) and worked either outside (34.3%) or at home (28.3%); 36.6% had experienced more than two threatening life events (TLEs) during the six-month period prior to the interview and 3.3% had suffered sexual abuse during childhood. Finally, as for genotype frequencies, 24.9% were short/short SERT homozygous, whilst 66.9 % of men and 45.2% of women were high activity MAOA homozygous.

Genetic and Environmental Risk Factors Associations with Major Depression

Genotype frequencies were found to be Hardy-Weinberg equilibrium. Table 2 shows univariate associations with MD. Figures 2a through to 2c show the associations between MD and both SERT and MAOA genotypes. The risk for MD was significantly increased among s/s SERT homozygous (OR=1.3; 95%CI:1.05-1.55; p=0.008) and HA uMAOA allele carriers (OR=1.7; 95% CI: 1.35-2.21; p=0.0001) (Figure 2b shows different strenght for the latter association in men and women). Additionally, a significant association was found between MD and being HA MAOA homozygous when sex was taken into account (OR= 1.25; 95%CI: 1.03-1.51; p=0.024) (Figure 2c, show the different pattern of this association for men, women and the whole sample). Regarding environmental risk factors, MD was found to statistically associate with increasing exposure to TLEs (OR=1.5; 95%CI:1.35-1.64; p=0.0001) or previous experience of abuse, including sexual abuse (OR=2.0; 95% CI: 1.41-2.91; p=0.0001), physical abuse (OR=2.5; 95% CI: 2.06-3.12; p=0.0001), psychological abuse (OR=2.0; 95% CI: 1.41-2.91; p=0.0001) or any abuse (OR=2.6; 95%CI: 2.21-3.08; p=0.0001). Please see Figure 3a through to 3e for a graphical summary of the above associations between MD and environmental risk factors under study.

Gene by Gene Interaction and Risk for MD

We found a significant effect modification by HA MAOA homozygous status on the risk for MD conferred by s/s SERT genotype. Figure 4 shows that the risk for MD among s/s SERT homozygous is significantly higher for HA MAOA homozygous (OR= 2.66; 95% CI 1.35-5.25; p= 0.004) whilst such difference does not exist among l/* participants (OR= 0.96; 95%CI 0.64-1.43; p=0.83; Likelihood ratio for the interaction = 6.65, p = 0.0098).

Three-Way Gene by Gene by Environment Interactions in Major Depression

Three-way significant Gene by Gene by Environment (GxGxE) interactions were found in that the genetically vulnerable individuals on both genotypes (i.e., those who were both s/s SERT and HA MAOA homozygous) were more susceptible to the deletereous risk effect conferred by a variety of environmental factors. Thus, significant interactions in the hypothesised direction were for both genotypes and increasing number of TLEs (Figure 5 (LR = 13.1, p = 0.069), sexual abuse (Figure 6 (LR = 9.15, p = 0.057) and any abuse (Figure 7 (LR = 9.06, p = 0.059).

DISCUSSION

Summary and Innovation of Results

We demonstrate that the combined presence of two distinct genetic risk factors for depression, interact significantly conferring a higher risk for MD than that provided by any of those genetic risk factors taken separatedly. In addition, such combined genetic load also significantly modifies the deleterious effect of well-knonw psychosocial environmental risk factors for depression as proven consistently by our three-way interactions analyses. This is the first study to demonstrate such two-way (gene by gene) and three-way (gene by gene by environment) in a representative and large adult sample followed-up for a year.

Sample and Desing

Our sample is a large consequtive one of primary care attendees and we obtained positive response in most of the PREDICT-D cohort approached to proivide a biological sample (86.5%). Apart from this high response rate, the sample is representative of the cohort as no differences were found for most socio-demographic variables tested (except age) as well as for prevalences of MD. Additionally, the prospective design of the study warrants the adequate means to establish direction of causality, plus it helps to minimise spurious characterisation of MD which would be more likely in a cross-sectional or case-control design. Adequate characterisation of MD is also a strenght of this study as it uses repeated measures of a validated diagnostic interview which were later computed into a diagnostic algorithm to establish prospective MD status using DSMIV diagnostic criteria (Robins et al., 1988); APA, 2002; King et al., 2006). Among limitations in the study, though, we can self-critise the use of self-report retrospective, yet valid, measures for environmental factor when measured at baseline, as this can convey a potential degree of recall bias.

Univariate Associations with Major Depression

We found prospective associations between two different genetic risk factors and MD. This results validate previous cross-sectional findings obtained using shorter samples in the same cohort (Cervilla et al., 2006; Rivera et al., 2009) and further support the idea that these particular loci do constitute modest but significant genetic markers for depression (Collier et a., 1996; Gutiérrez et al., 1998; Hoefgen et al., 2005; Schulze t al., 2000; Yu et al., 2005). Similarly, our findings that exposure to environmental factors, such as TLEs or different kinds of childhood abuse, increase the risk for MD are consistent with most of the previous literature on this topic (Famularo et al., 1992;Cervilla et al., 1997; Farmer eta l., 2000).

Gene by Gene interaction in Major Depression

It has been recently posed that studying gene by gene (GxG) interactions can be an appropriate, yet underused, method to explore genetic risk factors in complex disorders (Cordell et al., 2009). Thus, these authors suggest that GxG analyses can be a means to increase statistical power in detecting specific genetic effects that could be overlooked when a single polymorphism is studied. Our clear and positive results that having both the s/s SERT genotype and being uMAOA HA homozigous, as considered toghether, boost the risk for MD are a rare and highly significant example of GxG interaction in MD. We strongly believe these results are of particular value provided the prospectiveness of the assessments, the representativeness of the sample and the thorough characterization of the depressive phenotype used here. Indeed, to the best of our knowledge no previous study has ever reported a GxG interaction using MD as main outcome, although some positive and negative reports have been published for neuroticism or antidepressant response (Ritchei et al., 2004; Huennerkopf et al., 2007; Motsinger et al., 2006).

Most previous studies had concentrated on GxE interactions but have not tended to demonstrate three-way GxGxE interactions, or indeed repeat analyses using different environmental exposures as we do in the present study (Caspi et al., 2003; Kaufman et al., 2004; Kendler et al., 2005; Schied et al., 2007). Nonetheless, a few reports had previous shown similar three-way interactions when using samples of more limited number of participants and representativeness, such as youngsters (Kaufmann et al., 2006; Cicchetti et al., 2007), older adults (Kim et al., 2007), female twins (Wichers et al., 2008) or Chinese adults in their twenties (Zhang et al., 2009). Our study is the first one to demonstrate in a large prospective adult sample a variety of GxGxE interactions that were re-tested using exposure to several environmental risk factors, including TLEs, sexual abuse or any abuse (physical, sexual or psychological). Whilst our findings are entirely in line with previuos positive reports of GxE as causes of MD Caspi et al., 2003; Kaufman et al., 2004; Kendler et al., 2005; Schied et al., 2007), they constitute one step forward in demonstrating increasing complex (i.e., GxGxE) interactions in MD. Thus, we show the synegic action of two well-known genetic risk factors, taken at a time, as effect modifiers of three different measures of environmental risk factors.

Table 1. Demographic and clinical characteristics

Variables	Frequencies/means
Sex (male/female)	365 (27.7%) / 954(72.3%)
Mean age	50.33 years (SD 15.12)
Educational level	
Above school	169 (12.8%)
Secondary	273 (20.7%)
Primary, no education	530 (40.2%)
Trade, other	347 (26.3%)
Marital Status	
Not married/living with a partner	205 (15.5%)
Married/living with a partner	922 (69.9%)
Other	192 (14.6%)
Profession	
Employed or student	453 (34.3%)
Disabled/Retired	418 (31.7%)
Housekeeping	373 (28.3%)
Other	75 (5.7%)
Living alone	
Not Alone	1216 (92.2%)
Alone	103 (7.8%)

Exposure to Threatening Experiences	
No TLE	378 (28.7%)
1 TLE	453 (34.3%)
≥ 2 TLE	483 (36.6%)
Data no available	5 (0.4%)
Sexual Abuse during Childhood	
No	1271 (96.3%)
Yes	43 (3.3%)
Data no available	5 (0.4%)
Physical Abuse during Childhood	
No	1164 (88.2%)
Yes	150 (11.4%)
Data no available	5 (0.4%)
Psychological Abuse during Childhood	
No	1075 (81.5%)
Yes	239 (18.1%)
Data no available	5 (0.4%)
Any abuse	
No	1027 (77.9%)
Yes	287 (21.7%)
Data no available	5 (0.4%)

Baseline DSM-IV Major Depression		
Major depression		257 (19.5%)
Not depressed		1039 (78.8%)
Data no available		23 (1.7%)
6 month DSM-IV Major Depression		
Major depression		196 (14.9%)
Not depressed		1021 (77.4%)
Data no available		102 (7.7%)
12 month DSM-IV Major Depression		
Major depression		156 (11.8%)
Not depressed		1026 (77.8%)
Data no available		137 (10.4%)
5-HTTLPR genotypes		
S/S		329 (24.9%)
L/*		990 (75.1%)
uMAOA HA homozygous		
Male	HA /HA	245(70%)
	LA/ *	121 (33.2%)
Female	HA /HA	431 45.2%)
	LA/ *	522 (54.8%)

uMAOA HA carriers		
Male		
	HA/*	245 (70%)
	LA/LA	121 (33.2%)
Female		
	HA / *	811 (85.1%)
	LA/LA	142 (14.9%)

Table 2. Univariate associations with DSM-IV Major Depression

	OR	95 % CI	p
Genetic Risk Factors			
5-HTTLPR			
S/S *vs.* L/*	1.3	1.05-1.55	0.008
uMAOA HA/* *vs.* LA/LA			
Women	1.5	1.28-2.05	0.003
Men	1.4	0.89-2.29	0.079
uMAOA HA/HA *vs.* LA/ *			
Women	1.12	0.92-1.37	0.126
Men	1.4	0.89-2.29	0.079
Entire sample	1.25	1.03-1.51	0.024
Environmental Risk Factors			
TLEs	1.5	1.35-1.64	0.000
Sexual Abuse during Childhood	2	1.41-2.91	0.000
Physical Abuse during Childhood	2.5	2.06-3.12	0.000
Psychological Abuse during Childhood	2.6	2.17-3.07	0.000
Any Abuse	2.61	2.21-3.08	0.000

Figure 1. Sample and Participation

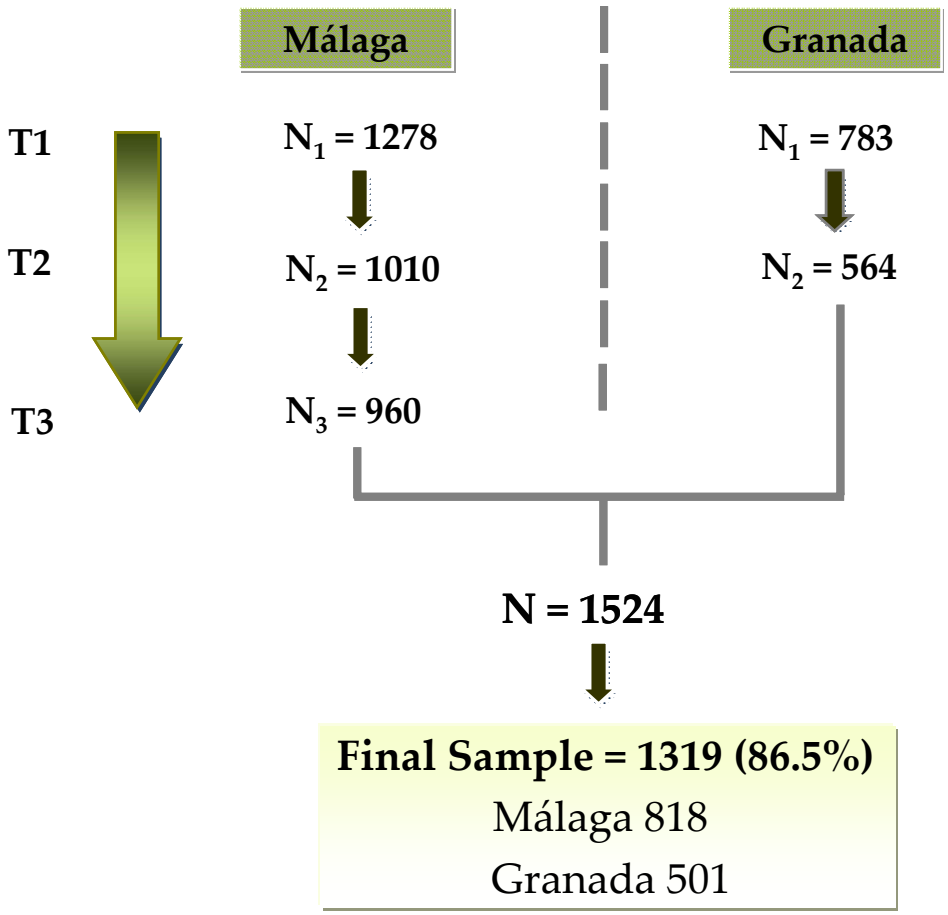


Figure 2. Univariate Associations between genetic risk factors and DSM-IV Major Depression

Figure 2.a. Association with s/s 5-HTTLPR genotype

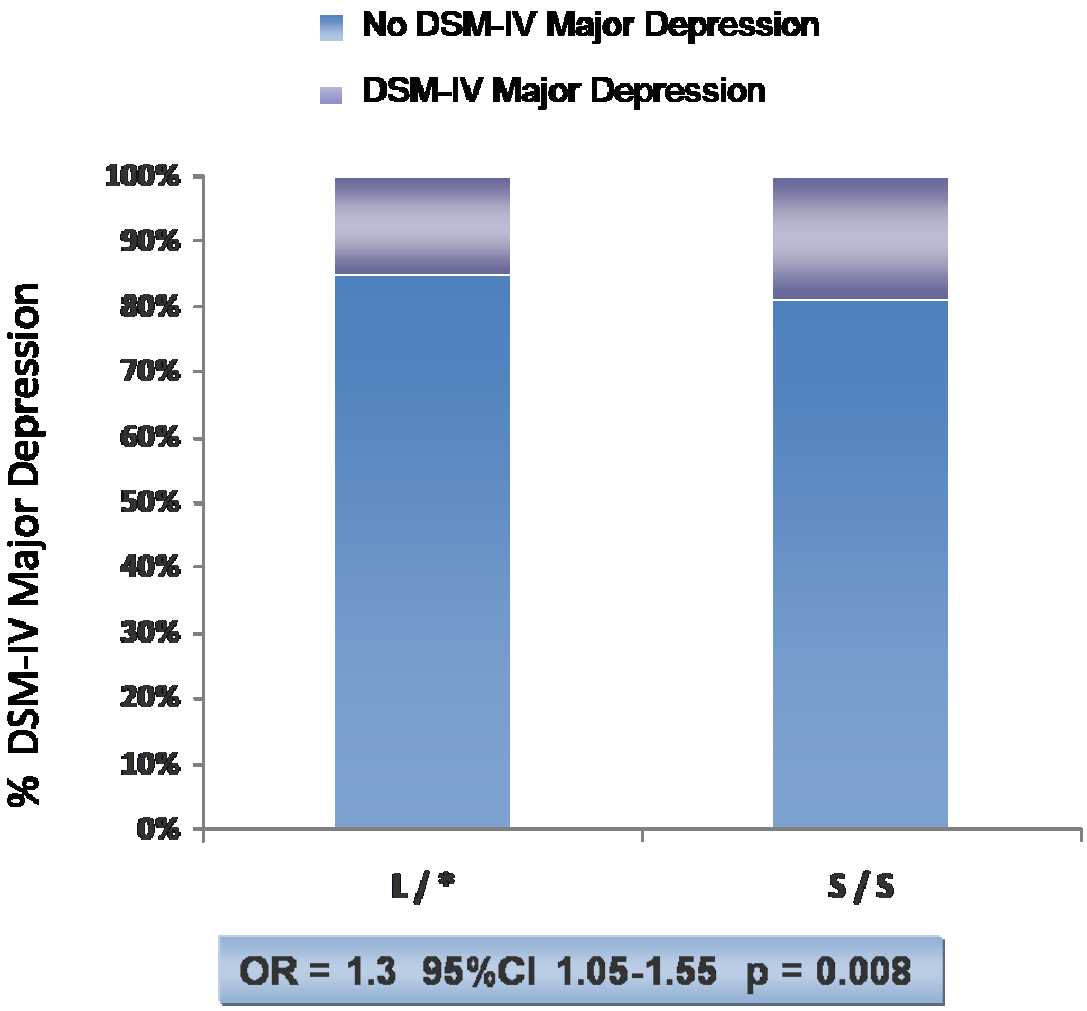


Figure 2.b. Association with HA/ * uMAOA genotype

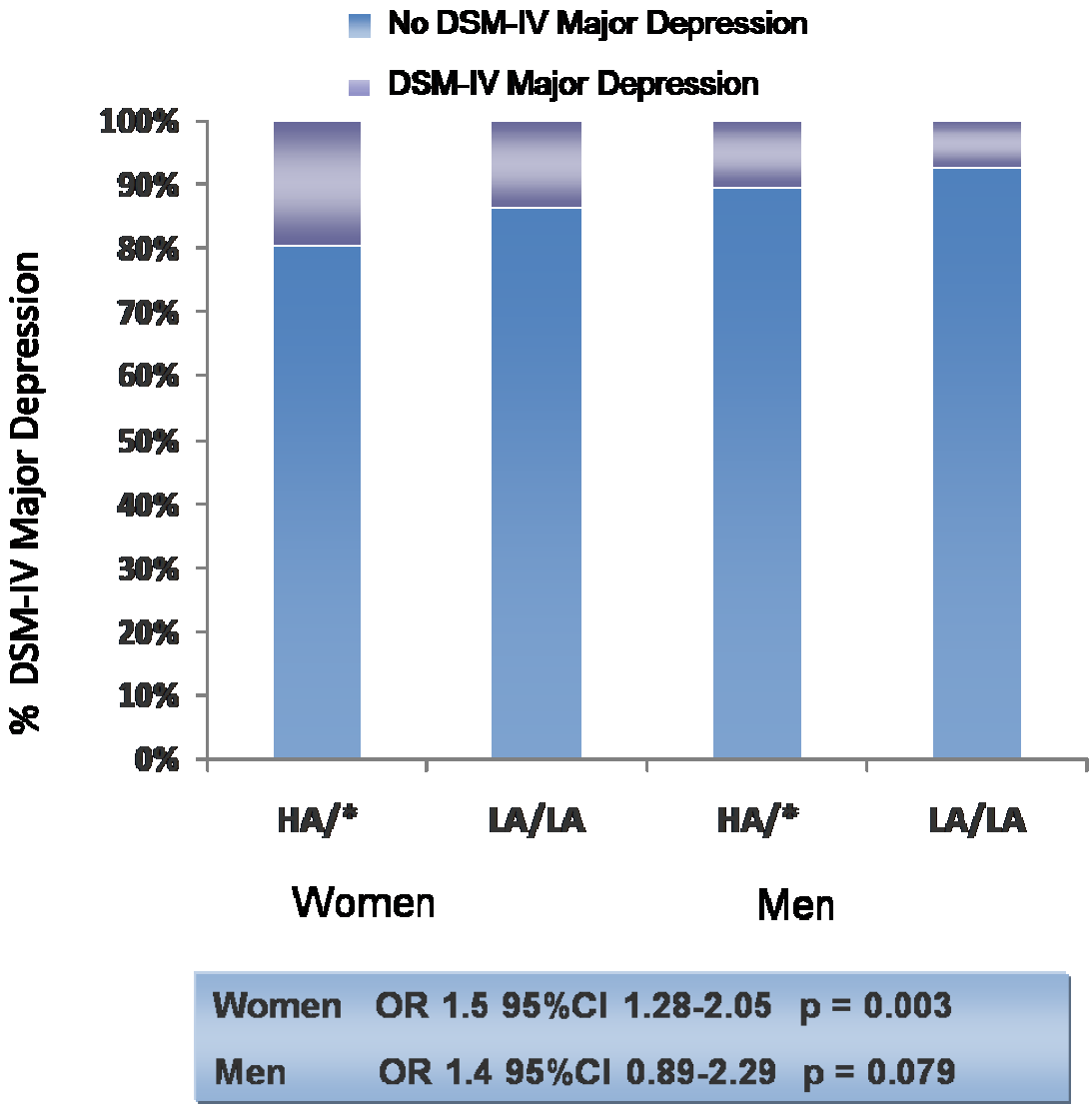


Figure 2.c. Association with HA/HA uMAOA genotype

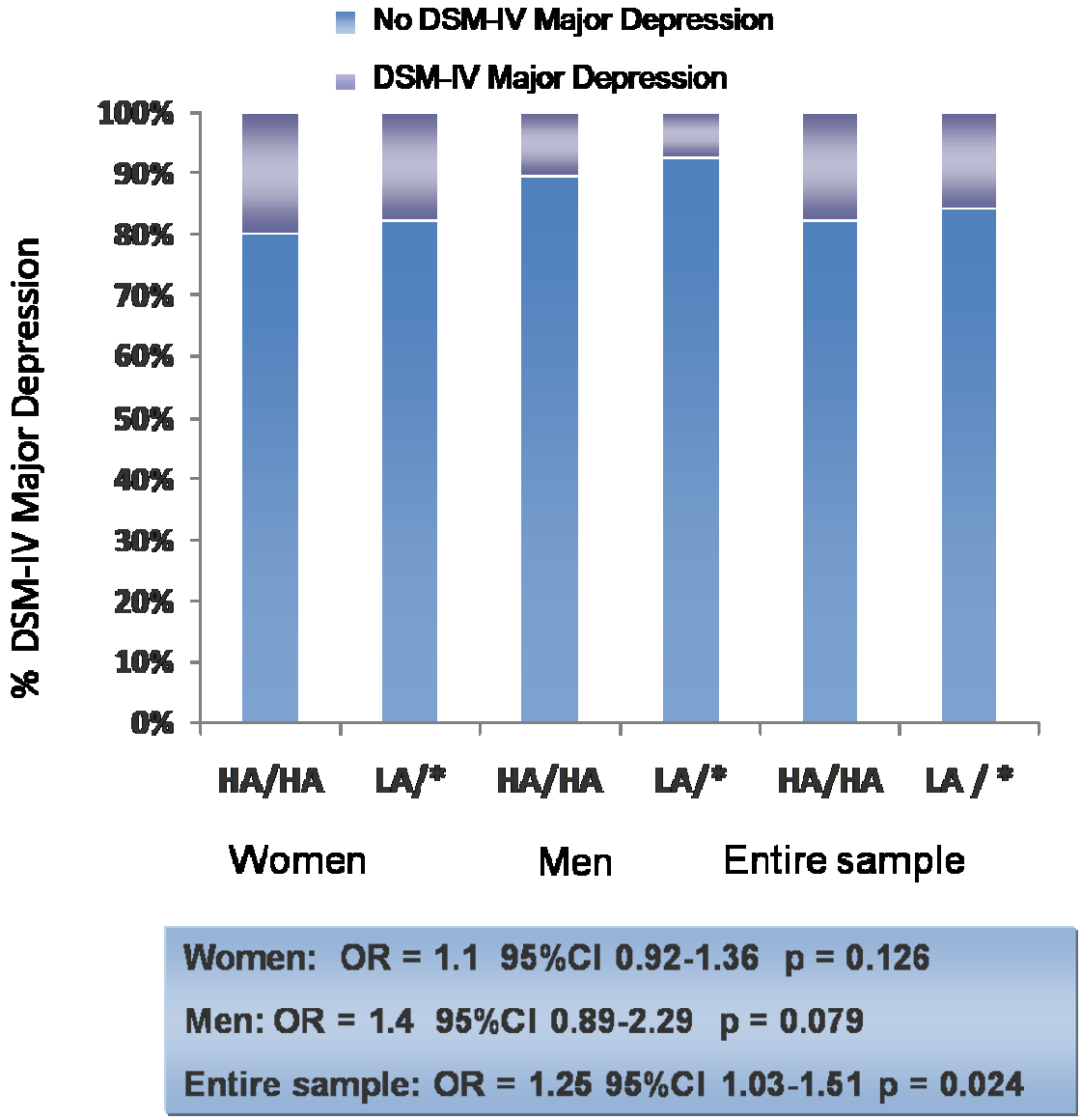


Figure 3. Univariant Associations between Environmental risk factors and DSM-IV Major Depression

Figure 3.a. Association with TLE

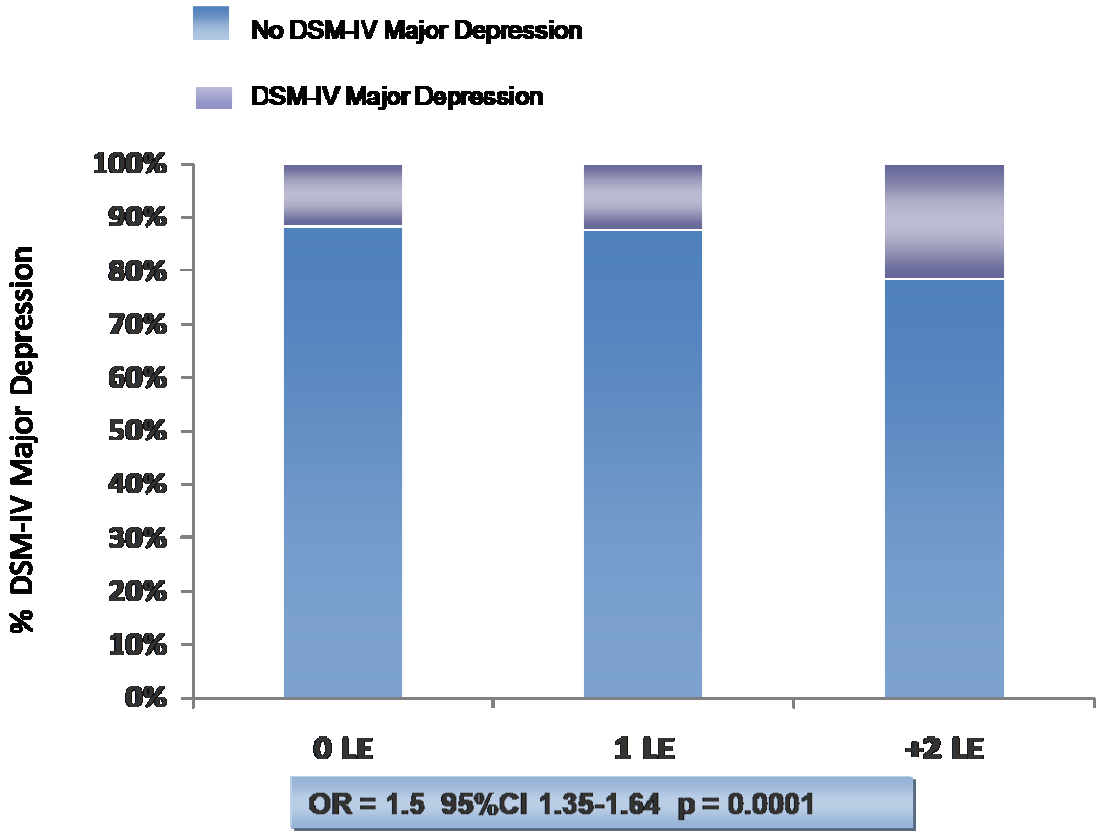


Figure 3.b. Association with sexual abuse

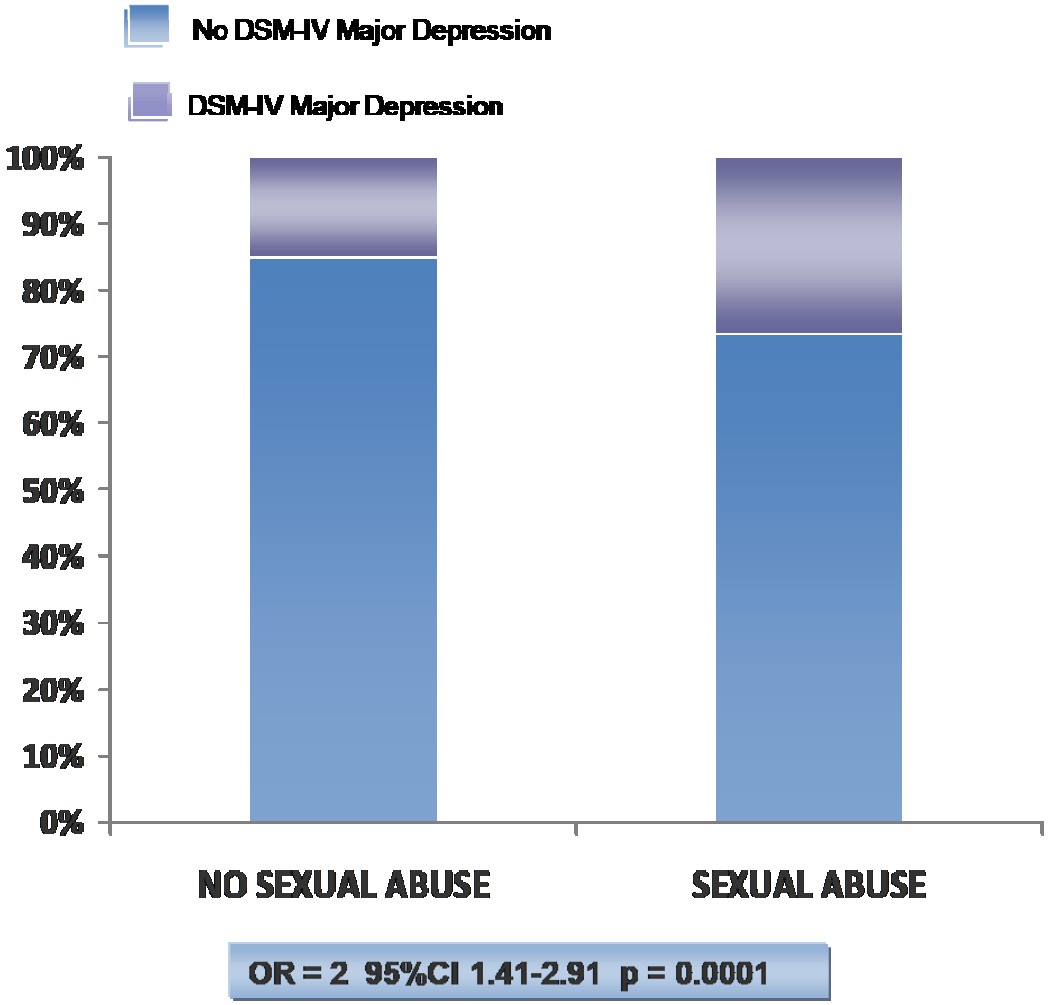


Figure 3.c. Association with physical abuse

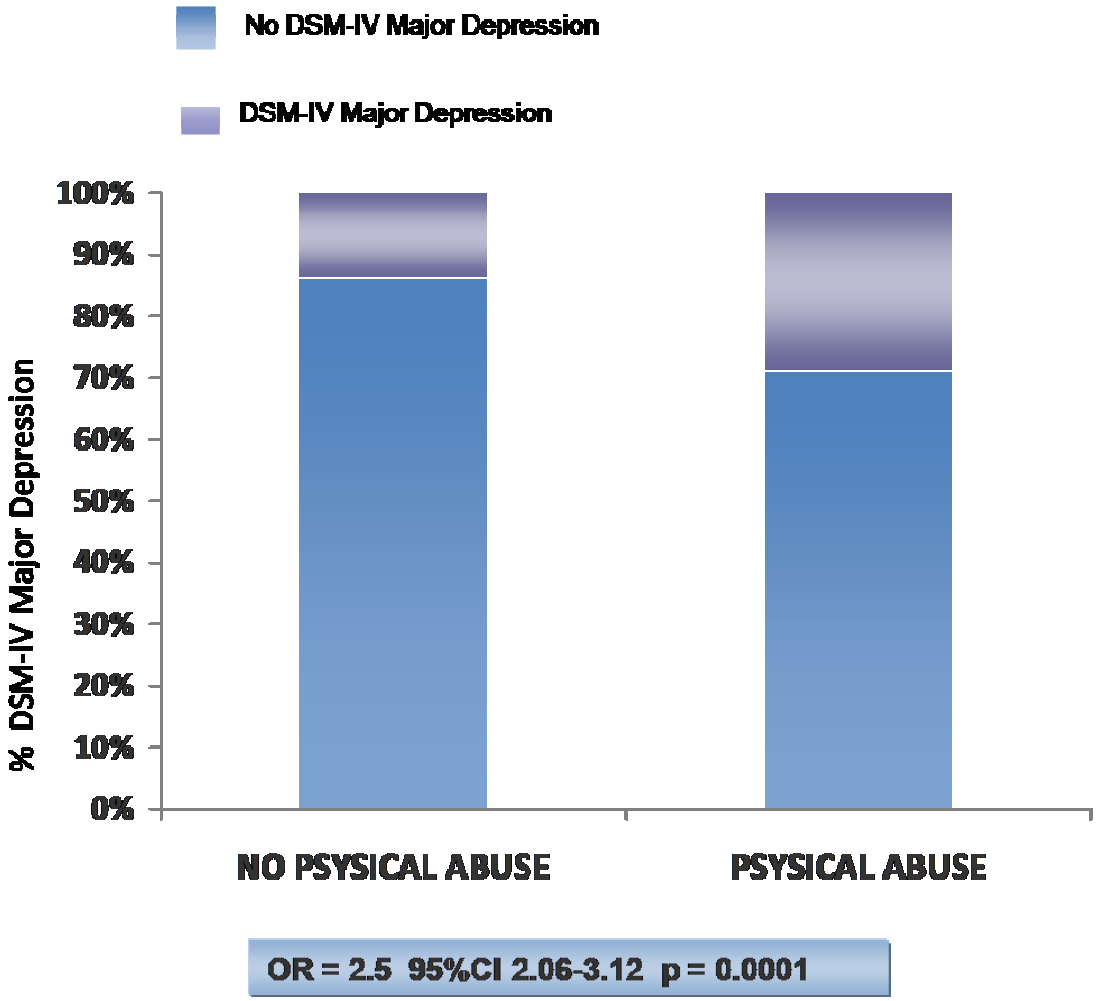


Figure 3.d. Association with physiological abuse

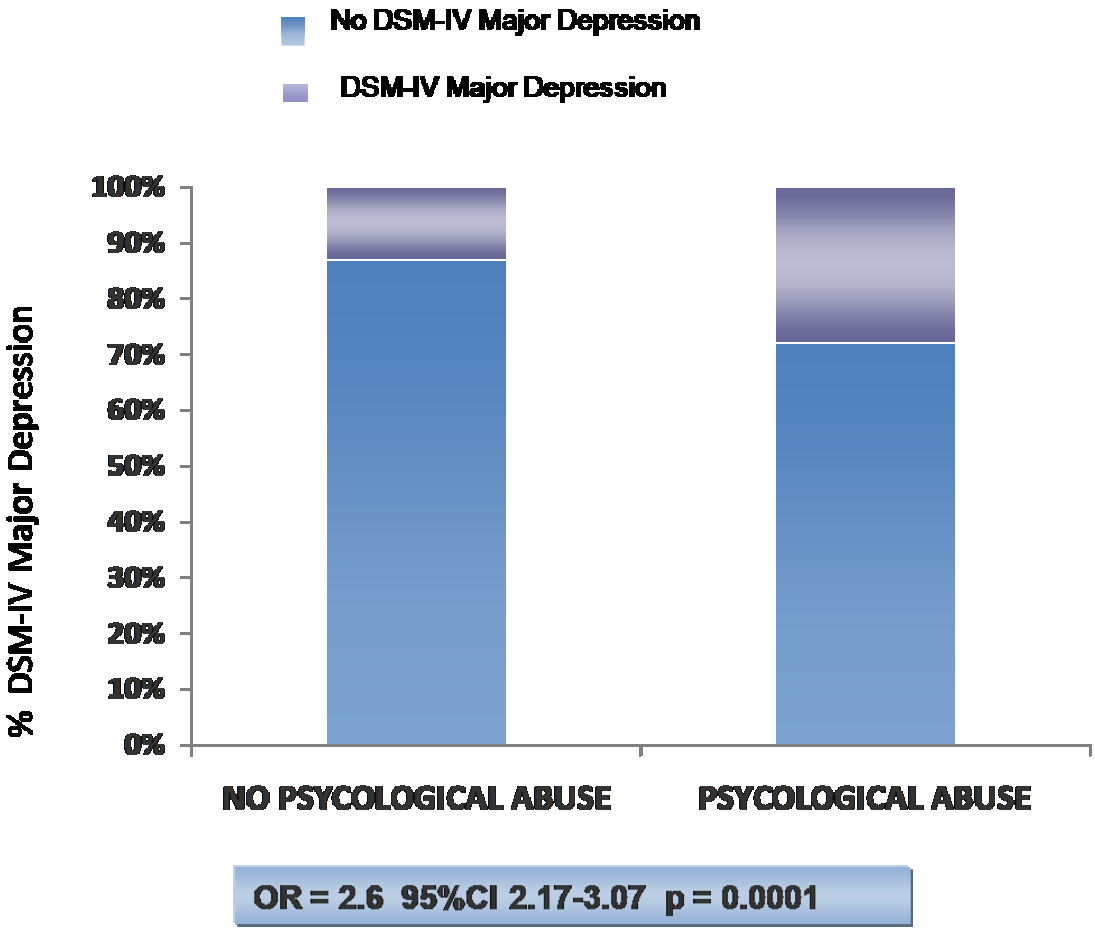


Figure 3.e. Association with any abuse

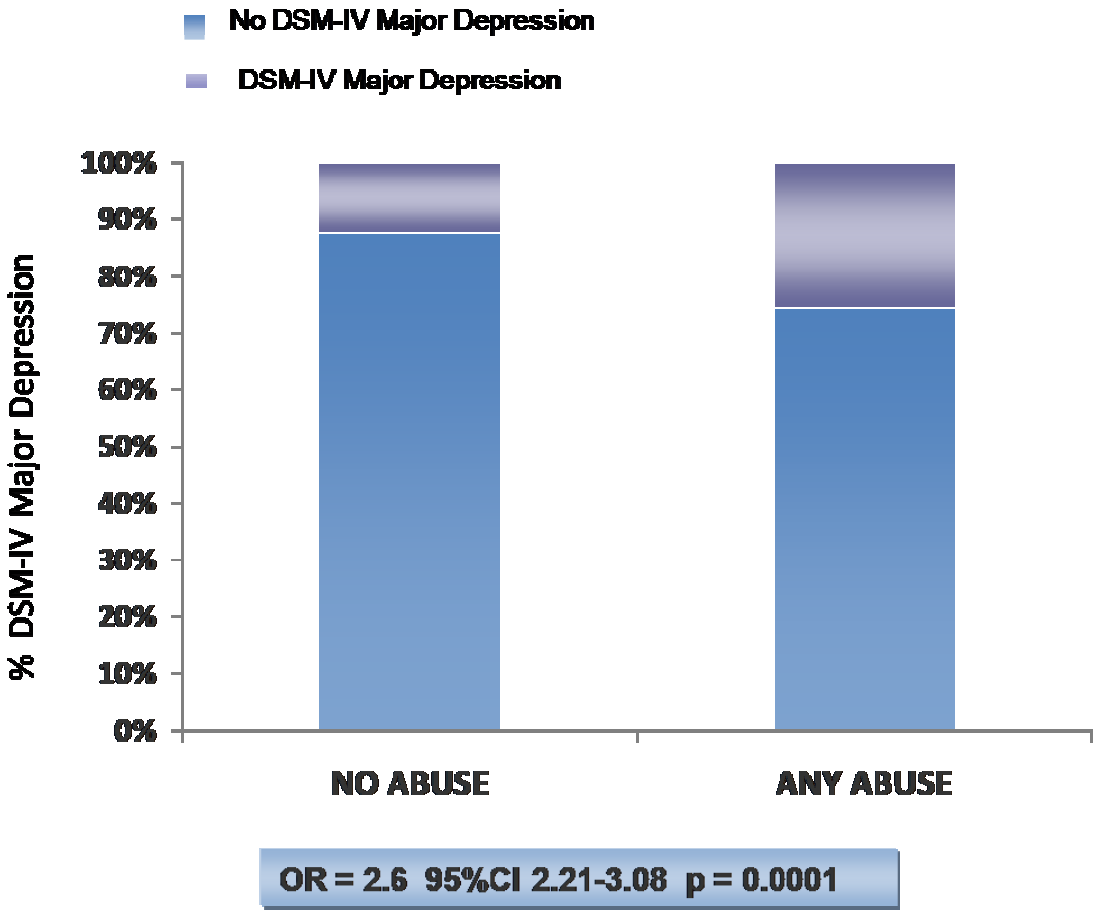
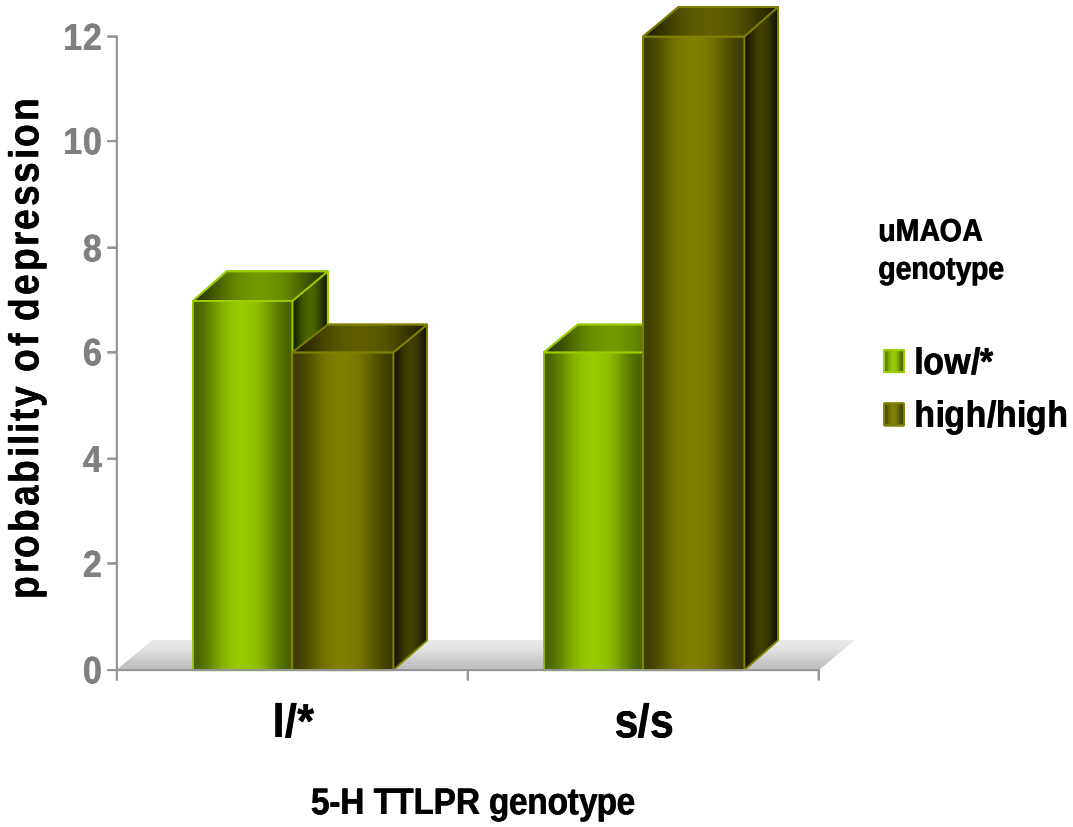


Figure 4. uMAOA (HA) polymorphism by 5HTTLPR polymorphism Significant Interaction in DSM-IV Major Depression



Likelihood Ratio for the Interaction X2=6.65 p= 0.0098

Figure 5. Three Way G x G x TLEs interaction

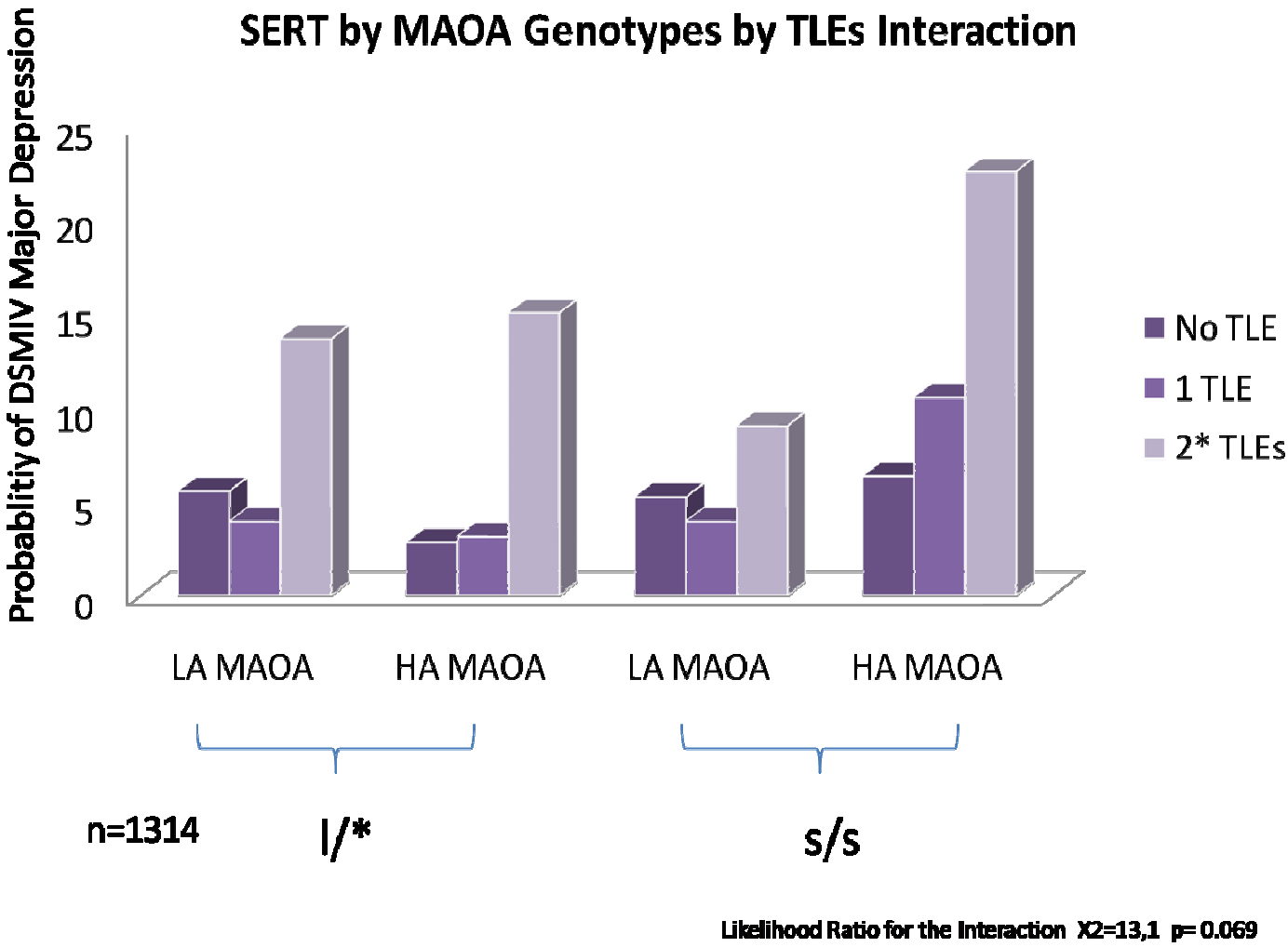


Figure 6 Three Way G x G x Sexual Abuse Interaction

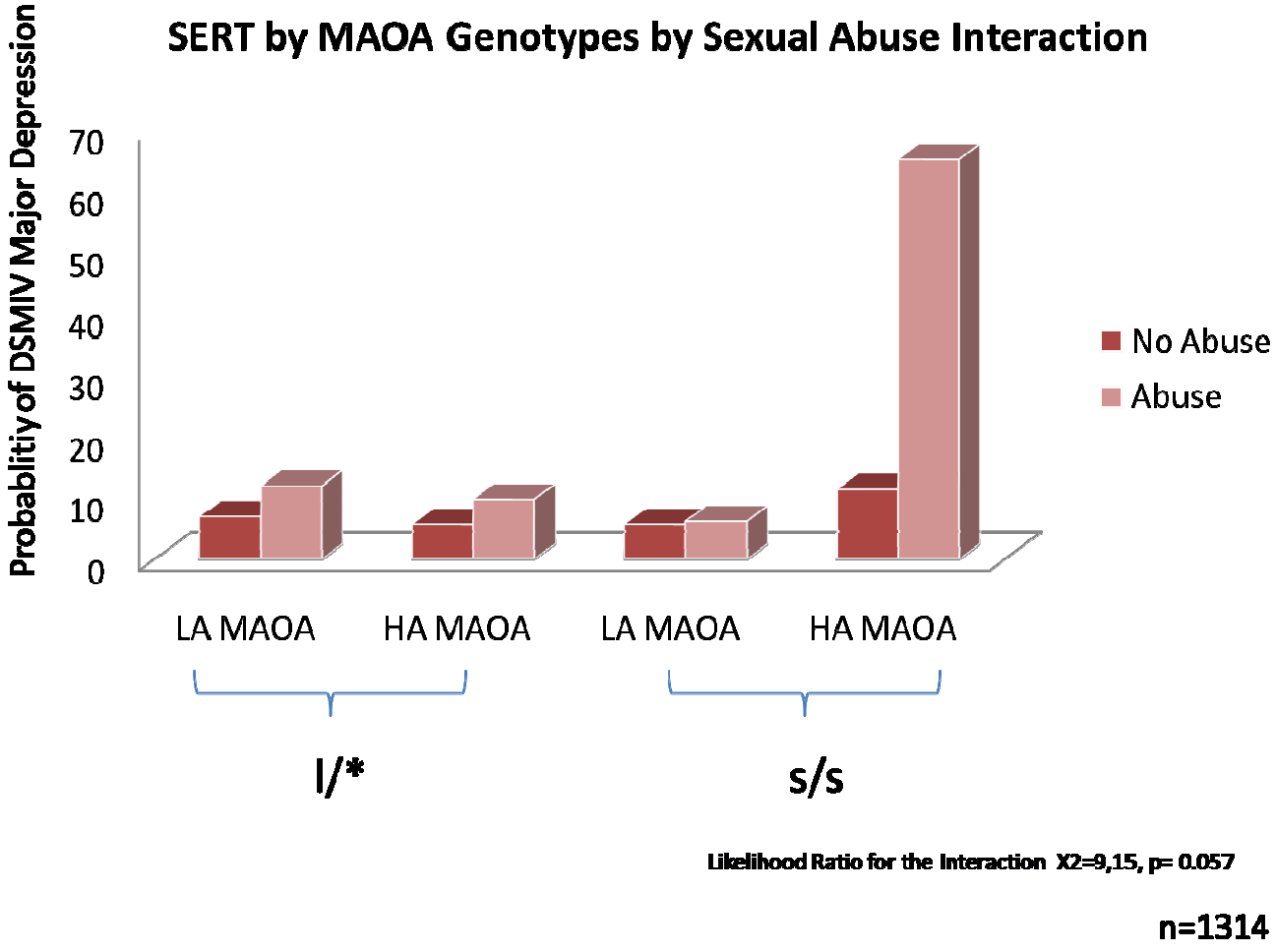
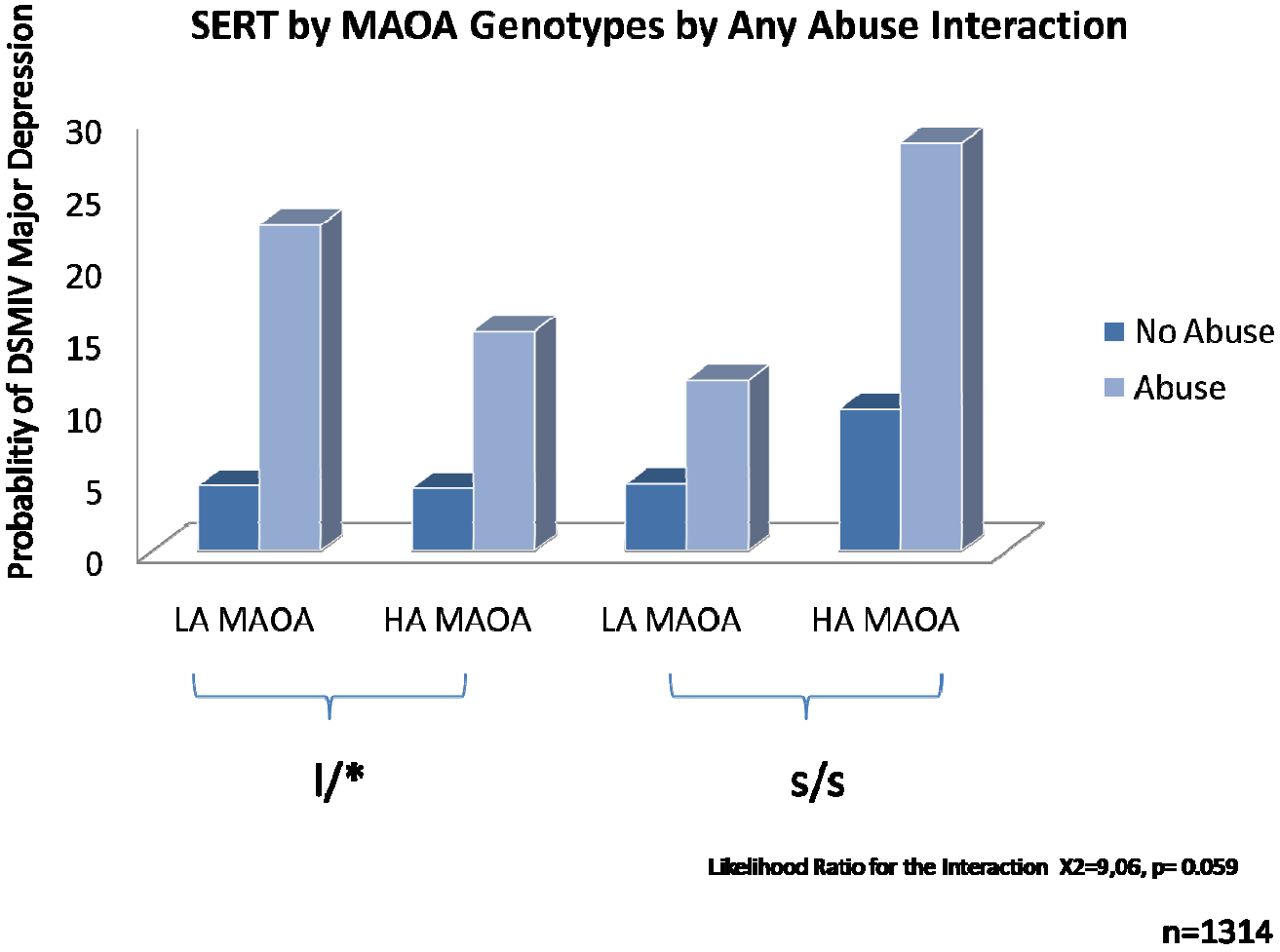


Figure 7. Three Way G x G x Any Abuse Interaction



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Artículo 3

Multifactor Prediction of Incident Major Depression is Significantly Modified by Genetic Variation at both SERT and MAOA loci

*Jorge Cervilla, Blanca Gutiérrez, **Esther Molina**, Margarita Rivera, Juan de Dios Luna, Francisco Torres-González, Irwin Nazareth, Juan Ángel Bellón, Berta Moreno, Michael King.*

RESUMEN

Recientemente se ha publicado un algoritmo predictor del riesgo para padecer depresión en un periodo de un año y con un poder predictivo del 0.79. Este modelo, llamado modelo PREDICT-D, está compuesto por diez factores de riesgo para depresión, en su mayoría factores ambientales.

Además de la influencia ambiental, numerosos estudios han puesto de manifiesto la existencia de un componente genético en la etiología de la depresión. Sin embargo, no existen a día de hoy demasiados trabajos en los que se haya tenido en cuenta la posible interacción entre los factores de riesgo ambientales y los genéticos en la aparición del fenotipo depresivo.

Nuestro objetivo fue identificar la existencia de posibles interacciones entre los factores de riesgo genéticos (regiones de variabilidad en dos genes clave en la neurotransmisión serotoninérgica: genes SERT y MAOA) y los factores ambientales integrados en el modelo predictivo de depresión PREDICT-D.

Para ello, un total de 775 participantes del estudio PREDICT-Gene (*Cervilla et.al. 2006*) fueron seguidos durante un año de y fueron evaluados en tres tiempos (al comienzo en T_0, a los seis meses en T_1 y a los 12 meses en T_2), mediante la sección de depresión de la Entrevista Diagnóstica Compuesta Internacional, para generar diagnósticos de Depresión Mayor (MD) según criterios DSM-IV.

Todos ellos aceptaron donar una muestra biológica para la realización de los análisis genéticos.

El genotipo s/s del polimorfismo 5-HTTLPR se encontró asociado tanto a depresión ($\chi 2=10.6$; $p=0.001$) como a valores significativamente incrementados

del índice C de predicción de Depresión por el modelo PREDIT-D (t=-4.1; p=0.0001).

El alelo de alta actividad del polimorfismo uMAOA del gen MAOA se encontró significativamente asociado a Depresión ($\chi 2$=11.62; p=0.001) y a valores incrementados del índice C significativamente (t=0.54; p=0.0001).

En el análisis de interacción genético-ambiental, encontramos que el valor del índice C fue significativamente mayor en aquellos individuos portadores de, al menos, una copia del alelo de riesgo (alelo s) (OR = 5.25; 95%CI = 4.00-6.88) en comparación con los que no tenían ninguna copia de esta variante alélica (los l/l) (OR= 3.34 95% CI= 2.36-4.72) (LR para la interacción = 4.63; p = 0.031).

En el caso del polimorfismo uMAOA, los portadores del alelo de alta actividad tuvieron valores del índice C mayores (OR = 4.87; 95%CI = 3.79-6.26) que los homozigotos para el alelo de baja actividad (OR = 3.37; 95%CI = 2.15-5.28), aunque esa diferencia no llegó a los niveles de significación estadística (LR para la interacción = 2.12; p = 0.14).

Por último encontramos que el valor del índice C se iba haciendo mayor a medida que aumentaba el número de variantes alélicas de riesgo. De este modo, aquellos individuos que no portaban ningún alelo de riesgo (aquellos con los genotipos l/l del polimorfismo 5-HTTLPR y low/low del polimorfismo uMAOA) tenían valores de índice C significativamente más bajos (OR=3.02; 95%CI=1.40-6.51) que los que portaban una de las variantes de riesgo (el alelo s del polimorfismo 5-HTTLPR ó el alelo high del polimorfismo uMAOA: OR=3.06; 95% CI=1.16-8.05), y éstos, a su vez, tenían valores de índice C significativamente menores que los individuos portadores de las dos variantes de riesgo genético (alelo s del 5-HTTLPR y el alelo high del polimorfismo uMAOA: OR= 6.70; 95%CI= 2.68-16.77).

Nuestros resultados demuestran la existencia de una interacción genético-ambiental en la etiología de la Depresión entre dos genes clave en la neurotransmisión serotoninégica y un paquete predictor de factores de riesgo medioambientales

Multifactor Prediction of Incident Major Depression is Significantly Modified by Genetic Variation at both SERT and uMAOA Loci

Jorge A. Cervilla[*1], *Blanca Gutiérrez*[*1], ***Esther Molina***[1], *Margarita Rivera*[1], *Juan D. Luna del Castillo*[2], *Francisco Torres-González*[1], *Irwin Nazareth*[3], *Juan A. Bellón*[4], *Berta Moreno-Küstner*[5] *and Michael King*[6]

[1] CIBERSAM University of Granada. Sección de Psiquiatría e Instituto de Neurociencias. Universidad de Granada. Granada. Spain.

[2] Departamento de Bioestadística, Universidad de Granada, Granada, Spain.

[3] Research Department of Primary Care & Population Health, University College London & MRC General Practice Research Framework, London, UK.

[4] Centro de Atención Primaria "El Palo", Unidad de Investigación de Atención Primaria del Distrito Málaga, grupo SAMSERAP. Departamento de Medicina Preventiva. Universidad de Málaga. Málaga, Spain.

[5] Departamento de Personalidad, Evaluación y Tratamiento Psicológico. Universidad de Málaga. Málaga, Spain.

[6] Department of Metal Health Sciences. University College London, Royal Free Campus, London, UK.

*Both authors have contributed equally to this paper and act as corresponding authors.

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ABSTRACT

Recent studies searching for causes of depression have focused on Gene by Environment (GxE) interactions considering one individual risk factor at a time. This study sets out to investigate whether genetic vulnerability for depression interacts with a group of environmental factors that has recently showed to predict prospective depression highly efficiently. 775 consecutively attended general practice patients from the same Spanish origin were followed-up for a year when three assessments (months 0, 6 and 12) were completed. Using validated measures, diagnoses of DSM-IV major depression (MD) over the one-year period were established. Multifactor (environmental and non-genetic) predicted risk for depression was calculated using a novel validated risk prediction algorithm (PREDICT-D). Genetic variation at two well-known candidate genes for MD (5HTTLPR and uMAO polymorphisms) was established using standard genotyping procedures after DNA was extracted from either blood or saliva samples from consented participants. We found that among those having at least one short (s) allele at the 5HTTLPR polymorphism the predicted risk score for MD using the PREDICT-D algorithm was significantly higher than among subjects with no s alleles (LR for the interaction χ^2=4.63; p = 0.031). Similarly, being a carrier of high activity (HA) MAOA alleles at the uMAOA polymorphism also increased the predicted risk score for MD as compared to low activity MAOA homozygous (LR for the interaction by uMAOA genotype χ^2= 2.12; p = 0.14). Finally, when both genotypes were combined, we found subjects who were s allele and HA allele carriers to also a significantly even higher predicted risk for MD than participants with just one or none of these allelic combinations (LR for the interaction χ^2 = 17.12; p = 0.038). This is the first study to empirically demonstrate statistically significant genetic effect modifications on the risk for incident MD predicted by an unprecedently accurate and comprehensive risk-predicting group of environmental and non-genetic risk factors.

INTRODUCTION

Identification of main risk factors for depression is fundamental to prevent the onset of this common and disabling disorder. A recent report has demonstrated that it is now possible to predict the onset of depression, over a one year period, using a combination of well known risk factors what may open up the scope for designing prevention strategies (*1*). The PREDICT-D that was developed in Europe as a risk prediction algorithm for major depression over 12 months is an integrative model including ten, mostly environmental, risk factors: five concerned with past events or patients characteristics (age, sex, educational level, lifetime depression and family history of psychological difficulties), four regarding current status (physical and mental health, unsupported work difficulties and discrimination) and one relating to country of origin. This first risk algorithm for onset of major depression is comparable to those used to predict cardiovascular events (*2*). The model was generated to be used as a predictive tool for the detection of depression prior to onset and it has an excellent predictive power of 0.79 (*1*).

In addition to environmental risk factors, many studies have shown evidence of existing genetic risk factors for the onset of major depression. The short allele (S allele) at the 5HTTLPR polymorphism in the serotonin transporter gene (5HTT or SLC6A4) is one of the most widely associated with depression (*3-6*) although not all studies concur (*7-9*). Other studies have focused on the genetic variability in loci encoding for other proteins also implicated in the serotonin metabolism, such as the uMAOA polymorphism in the promoter region of the gene encoding for the enzyme monoamine oxidase A (MAOA). However, they have also yielded inconsistent results (*10-14*).

It has been suggested that inconsistent results from genetic association studies may arise from a variety of sources, including difficulties with inadequate characterisation of depression (*15*) or lack of adjustment for potentially confounding non-genetic risk factors (*3*). Recently, a growing number of

researchers have focused on gene by environment interactions (G x E) as a more parsimonious approach to explore risk factors for depression in a more holistic manner and avoid reaching erroneous conclusions that a genetic effect is not present (*16*). Particular attention has been paid to genetic mediated modification on the depressiogenic effect of stressful life events (SLEs). This may reflect the individual's vulnerability to adverse experiences, which seems to be driven by their genetic makeup (*17*). The first empirical evidence for such G x E interaction came from Caspi et al. (2003) who demonstrated an interaction between SLEs and the 5HTTLPR genotype on the risk for DSMIV Major Depression (MD) demonstrating that their risk effect was significantly higher the more short (s) alleles the subject had (*18*). Many other independent studies have now replicated these findings accross a wide variety of samples such as twins (*19*), adolescents (*20*), older adults (*21*), general practice attendees (*22*) and women (*23*). Although, other studies have failed to replicate this particular GxE interaction (*24-27*), overall, the stronger evidence seems to be in favour of the existence of an interaction between 5HTTLPR and social stress (*28*).

Furthermore, interactions by 5HTTLPR genotype have now been demonstrated on the risk for depression conferred by other environmental factors such as a history of childhood maltreatment (*18, 29*), unemployment (*30*) and sexual abuse (*25, 31*). Similarly, other genetic risk factors for depression such as variability at the uMAOA polymorphism have now been shown to modify the risk for depression conferred by maltreatment (*25, 32*) but not that due to SLEs (*20*). Finally, a number of studies have reported three-way interactions (G x G x E) such as the interaction between 5HTTLPR genotype and social distress or abuse as risk factor for depression being further modified by the variability at other genetic risk factors, such as the MAOA gene (*25*), the brain-derived neurotrophic factor gene (BDNF) (*21, 33, 34*) or the serotonin type 1A receptor gene (5HT1A) (*35*).

We hypothesized that genetic variability at two main candidate genes for depression (SERT and MAOA genes) act as a risk modifier on prediction of depression using the PREDICT-D risk algorithm, which can be also considered as a grouped measure of environmental determinants of depression.

METHODS

Study setting and design

The PREDICT-Gene study (*3*) is a longitudinal study which aims to identify genetic risk factors for depression and to assess putative gene by environment interactions as predictors for the onset of depression. This study is nested in the Spanish subsample of a larger prospective European study developed in 6 European centres, the PREDICT-D study (*36, 37*) which has recently reported and developed a risk predicting algorithm for the onset and maintenance of major depression in primary care attendees (*1*). Both of the PREDICT-D study and its genetic sub-study (PREDICT-Gene) were approved by the relevant research ethics committees.

Sample

Consecutive attendees to 9 primary care centres in Andalusia (Spain) were asked to participate in the PREDICT-D study by their family doctors between April 2004 and December 2006. After informed consent was obtained, interviews were undertaken by trained researchers. Participants included were all those aged 18 to 75, able to understand Spanish and without any organic mental disorder and/or any terminal illness.

Consenting participants were recruited and assessed on three occasions: Baseline assessment (time 1), six months follow-up (time 2), and twelve months follow-up (time 3). Participants were asked to participate in our genetic sub-study (PREDICT-GENE study) during and after time 3. Those agreeable to it

gave further specific informed consent to provide the biological sample for genetic analyses (n=775), consisting of 10 cm^3 of blood and/or up to 4-mouth spredict-wabs for saliva collection. Further details on the methodology of both PREDICT-D and PREDICT-Gene studies have been reported earlier (*3, 37*).

Assessment of Depression (outcome variable)

DSM-IV Major Depression (MD) was ascertained using the depression section of the Composite International Diagnostic Interview (CIDI) which was designed to provide six month and lifetime psychiatric diagnoses (*38*). Prevalence of depression was assessed at baseline and at each follow-up point.

Measuring Environmental and Other Non-Genetic Risk Factors

A total number of 39 known risk factors for depression were included in the initial PREDICT-D risk factor assessment protocol. This was developed, where possible, by using standardised self-report measures. Sociodemographic data, such as sex, age or educational level, were also gathered using previously validated measures. All questions on risk factors were either adapted from available standardised instruments or developed for the PREDICT study and shown to have adequate test-retest reliability (*1*).

Analysing the above risk factor data, King et al. (2008) developed and validated the PREDICT-D risk prediction algorithm (PREDICT-D RPA) for MD. This is a is a quantitative multifactor risk prediction tool for the onset of depression over 12 months, developed in general practice attendees (*1*). Using stepwise logistic regression and using a conservative threshold for inclusion ($p<0.01$), they included in the model those risk factors that resulted to be the most predictive for the onset of depression over 12 months, which were: sex, age, educational level, difficulties in paid and unpaid work, physical and mental health subscale

scores on the Short From 12, lifetime depression, first-degree relative with emotional problem, experiences of discrimination, and country of residence (*1*). The resulting risk score provides a predicted probability of depression over 12 months in all European countries of 0.79 (95% CI 0.767-0.813). Effect size for difference in predicted log odds of depression between European attendees who became depressed and those who did not was 1.28 (95% CI, 1.17-1.40). The predictive model was validated in an external population and resulted to have a good external validity (*1*). For the purpose of the present interaction study we used the PREDICT-D RPA as our environmental and non-genetic risk factor package for depression and tested whether the genetic risk factors below modified its effect on predicted risk for MD.

Genetics analyses

Genomic DNA from both blood and saliva samples was extracted following standard procedures. The 5-HTTLPR polymorphism at the SLC6A4 gene was genotyped in all samples. The Polymerase chain reaction (PCR) was performed under the follow conditions: 50 ng of DNA, 0.25 μM of each primer (forward: 5´-GGCGTT GCCGCT CTG AAT GCC-3´ and reverse: 5´-CAGGGG AGATCC TGG GAG AGG T-3´), 250 mM each of dATP, dCTP, dGTP and dTTP, 1.5mM MgCl2, 50mM KCl, 10mM Tris-HCl and 0.3 units of DNA polymerase in a total volume of 25 ml. Samples were amplified for an initial cycle of 8 min at 95ºC followed by 35 cycles each consisting of 30 s at 95ºC, 30 s at 62ºC and 1 min at 72ºC. After amplification, genotypes were resolved by a 2% agarose gel electrophoresis and ethidium bromide staining.

The uMAOA polymorphism at the MAOA gene was also genotyped in all samples. We used 50 ng of DNA, 0.25 μl of each primer (forward: 5´-ACA GCC TGA CCG TGG AGA AG-3´ and reverse: 5´-GAA CGG ACG CTC CAT TCG GA-3´), 250 μM of each dNTP, 1.5 mM MgCl, 50 mM KCL, 10 mM Tris-HCl and 2 U of Taq DNA polymerase. The samples were preheated at 94ºC for 2 min, followed by 30 cycles each one consisting of 1 min at 94ºC, 1 min at 66ºC and 1

min at 72ºC. After amplification, genotypes were resolved by a 3% agarose gel electrophoresis and ethidium bromide staining. For the purpose of this study, alleles were classified into two groups: low-activity alleles (three copies of the 30-bp repeat sequence) and high activity alleles (3.5, 4 or 5 repeats), as was done by previous authors (*10, 12, 13*).

Statistical Analyses

The SPSS 15.0 (2006) and STATA 10 (Stata Corp., 2007) statistical packages were both used. Initially, variable distributions were analysed and, then, χ^2 tests were used to check, firstly, whether genotype frequencies for both polymorphisms were in Hardy-Weinberg equilibrium among both cases and controls; secondly, allelic and genotype distribution of both polymorphisms, and thirdly, univariate associations between DSM-IV major depression and environmental risk factors. Logistic regression was then used to test whether a-priory-defined particular genetic profiles associated with MD after adjusting by potential confounders such as sex. Odds ratios and 95% confidence intervals were then calculated by univariate and multivariate associations.

Longitudinal data analyses entailed a multivariate Generalized Estimating Equations (GEE) modeling using incident depression at any time over the one year follow-up period. This method was employed to enable a thorough analysis of three consecutive measures of depression that were interrelated, not allowing a classical independent data analysis. This GEE model is a random effect model that allows adjustments by intraindividual variability. A logistic binary GEE model was, thus, used to calculate odds ratios and 95% confidence intervals for the association between genotypes and MD as measured along the three assessment times, and to test the presence of significant two-way (gene by gene) and three way (gene by gene by environment) interactions. The significance of interactions was then tested using the likelihood ratio test, considering significant any significance p value under the 0.10 level. In the case of a significant interaction, a detailed assessment was made to compare the

different risk effect for MD conferred by the environmental factors across different genotype.

Fort the purpose of this analysis we considered the environmental risk factors as a grouped continuous measure obtained by using the risk score calculated using the PREDICT-D predicted risk algorithm as recently described by King et al., 2008. Hence, we tested gene by risk score interactions first using both genotypes separatedly and then comparing the environmental risk effect on MD along three genotype combinations (having no risk genotype vs. having just one (any) risk genotype vs. having both risk genotypes). Graphically, the interactions were represented using the probability for MD at any time over the one year period (y axis) by PREDICT-D calculated environmental risk score (x axis), as a function of different SERT and/or MAOA genotypes.

RESULTS

Sample

All 922 participants who took part in the Spanish branch of the PREDICT-D study were approached to participate in the present PREDICT-Gene study. Out of them, 775 (84%) participants provided both informed consent and a valid biological sample for inclusion in this study. Table 1 summarizes the sample's socio-demographic and clinical features, genetic variation for both loci, frequencies of exposure to all components of the PREDICT-D RPA and the mean predicted risk score for MD using the PREDICT-D RPA. When comparing the sample with either T3 participants or all participants included at baseline, no statistically significant differences were found on sex, age, marital status, living arrangement, prevalence of depression (at times 1, 2 or 3) or mean PREDICT-D RPA predicted risk score for MD (data not shown).

Associations with Risk Genotypes

Participants who had the short/short (s/s) genotype at the 5HTTLPR locus had a significantly increased risk for DSM-IV major depression (MD) at any time over the one year period when compared to all other subjects with either

long/long (l/l) or long/short (l/s) genotypes (Odds Ratio with 95% Confidence Intervals (OR: 1.51; 95 CI: 1.17-1.93; $\chi 2$=10.6; p=0.001). Similarly, those subjects who were High Activity (HA) uMAOA allele carriers also showed an increased risk for MD (OR: 1.74; 95% CI: 1.26-2.39; χ^2=11.62; p=0.001) compared to low activity (LA) uMAOA homozygous. Mean predicted risk score was significantly higher (t=-4.1; p=0.0001) among s/s homozigous (mean c-Index=0.22, SD=0.16) versus subjects with other 5HTTLPR genotypes (mean c-Index=0.18, SD=0.16). On the other hand, mean predicted risk score for MD was also significantly higher (t=0.54; p=0.0001) for HA MAOA allele carriers (mean c-Index=0.20; SD=0.17) than among LA MAOA homozigous (mean c-Index=0.16; SD=0.14). Adding as factors either 5HTTLPR or uMAOA genotypes to the PREDICT-D RPA did not alter significantly its predictive ability (data on file).

Risk Genotypes Interaction with Environmental Risk Scores for Depression

A statistically significant interaction by 5HTTLPR genotype (Likelihood Ratio (LR) for the interaction χ^2= 4.63; p= 0.031) was found, on the risk for MD predicted by the score calculated with PREDICT-D RPA, after comparing such risk score effect among those participants who did not have any s allele (OR= 3.34 95% CI= 2.36-4.72) and those with one or two s alleles (OR= 5.25; 95% CI=4.00-6.88). Similarly, we also found a non-significant trend for an interaction by uMAOA genotype (LR for the interaction χ^2=2.12; p=0.14) on the PREDICT-D RPA predicted risk score when LA MAOA homozygous (OR= 3.37; 95%CI=2.15-5.28) were compared versus HA MAOA allele carriers (OR= 4.87; 95%CI= 3.79-6.26).

Finally, when we tested whether combinations of both genes' variations modified the effect of the predicted risk score for depression we found this to be the case. Thus, while predicted risk score for MD was moderate among those having no risk alleles (i.e. neither HA MAOA alleles nor "s" 5HTTLPR allele; OR=3.02; 95% CI=1.40-6.51); the predicted risk score was slightly higher for subjects having at least one risk genotype (i.e., either s/* or HA/*; OR=3.06;

95% CI=1.16-8.05); and it was markedly and significantly higher among individuals with a combination of both a risk genotypes, i.e. both s/* or HA/* (OR= 6.70; 95%CI= 2.68-16.77; LR for the interaction χ^2=17.12; p=0.038). Figure 1 shows the probabilty of DSMIV MD as a function of increasing predicted risk scores for MD by both genotypes alone (Figures 1a, 1b and 1c) and combined (Figure 1d).

DISCUSSION

This study demonstrates that risk variants of two candidate genes for MD significantly modify the risk effect conferred by a multi-factorial risk package that has recently demonstrated unprecedented validity in predicting depression over a one year period. This is, to our knowledge, the first time a study demonstrates G x E interactions to influence the risk for MD considering, at a time, a fully-comprehensive and well-known package of risk factors for MD, rather than just individual risk factors. Such a holistic approach is likely to open a breakthrough channel in understanding the etiology of a complex and common disorder such as MD.

We used a relatively large (775) sample of consecutive primary care attendees from the same Southern Spanish origin who were followed-up for a year. Response rate to participating in the genetic study was high (84%). However, the sample included more women than men, although interactions were adjusted by sex and we showed that those who took part did not differ significantly on socio-demographic characteristics or prevalence of MD from either all who were asked to participate (all time 3 participants) or, indeed, those who started the study at baseline.

Our findings of a significantly increased risk for MD among participants having any or both genetic risk variants are congruent with previous literature (*3, 12, 39*) and are in line with neurobiological studies suggesting a monoaminergic

dysfunction in depression (*40*). Interestingly, we also found these genetic risk factors significantly associated with the predicted risk for MD score calculated using the PREDICT-D RPA. This result further validates externally, from a genetic view-point, the validity of such risk score as a potent tool for prospective prediction of depression. Although we did not find an improvement in the prediction ability after including either of the two genes in the PREDICT-D RPA this was expectable as the latter includes variables that are likely strongly associated with genetic variation such as family history and life-time previous depressive episodes. Furthermore, the predicted risk score can be also conceptualized as a highly explanatory package of risk factors for MD that demonstrated to explain nearly 80% of outcome variance along the one year observation period (*1*). This makes it difficult for significant yet modest genetic risk factors for depression to actually add much predictive power, particularly if they are likely to be in colineality with other factors (e.g., family or personal history of MD).

We demonstrated our a-priory hypothesis that carrying risk alleles of candidate genes for depression should modify the risk for MD conferred by a demonstrated package of risk factors for MD. Although G x E interactions have been reported in depression over the past 6 years (*18, 22, 25, 29, 41*), previous findings have tended to include individual environmental factors in the analyses even when, admittedly, MD seems to be determined by a rather large of identified environmental and non-genetic risk factors (*1, 42*) clearly in interaction with genetic vulnerability (*16, 28, 43*). Our study showed G x E interactions more comprehensively than ever before, using a package of environmental and non-genetic risk factors rather than a single risk factor alone. Moreover, such risk factor package is strongly associated with MD and is likely to represent a more realistic and comprehensive scenario towards a full understanding of the etiology of depression. Nonetheless, the environmental risk package we used includes some elements that can be by-proxy indicators of previously reported environmental factors that have been shown to interact

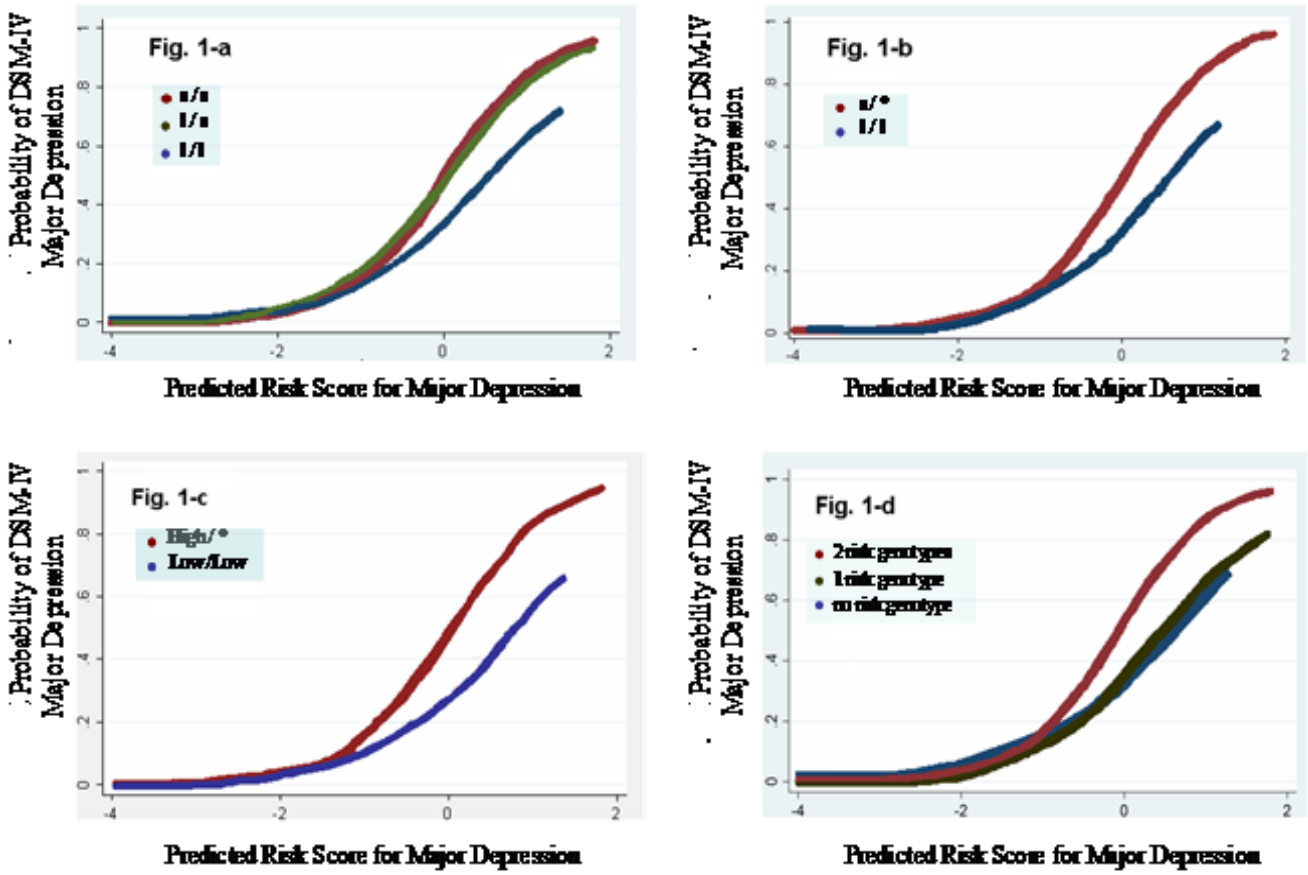
with variability at the 5HTTLPR polymorphism. Thus, discrimination and difficulties with work can constitute serious stressful life events, whilst neuroticism can be part of our measure of psychological health (*44*). Although the ultimate elicitation of the entire spectrum of causes of depression remains to be unveiled, we suggest that our findings constitute one robust step ahead towards a more complete and empirical understanding of such causes. Finally, our results also suggest that identification of individuals who may be genetically more vulnerable to environmentally triggered MD could be a means to sharpen preventive strategies against the disorder among those who seem to be more at ease to suffer from it.

Table I. Demographic and clinical characteristics

Variables	Frequencies/means
Sex (male/female)	217 (28%) / 558 (72%)
Mean age	50.53 years (SD 15.21)
Educational level	
Above school	100 (12.9%)
Secondary	183 (23.6%)
Primary, no education	491 (63.4%)
Trade, other	1 (0.1%)
Marital Status	
Not married/living with a partner	230 (29.7%)
Married/living with a partner	544 (70.2%)
Data no available	1 (0.1%)
Profession	
Employed or student	267 (34.5%)
Retired	131 (16.9%)
Other	377 (48.6%)
Living alone	
Alone	46 (5.9%)
Not Alone	729 (94.1%)
Support at paid and unpaid work	
Support	640 (82.6%)
No support	135 (17.4%)
Number of forms of discrimination	
None	687 (88.6%)
In 1 area	64 (8.3%)
>1 area	24 (3.1%)
Physical Health Mean Score(SF-12)	41.90 (SD 11.67)
Mental Health Mean Score(SF-12)	44.42 (SD 12.81)

Emotional problems among first degree relatives		
No problem		442 (57%)
Emotional problem		333 (43%)
Life-time depression		
No		215 (27.7%)
Yes		560 (72.3%)
Baseline DSM-IV Major Depression		
Major depression		135 (17.4%)
Not depressed		624 (80.5%)
Data no available		16 (2.1%)
6 month DSM-IV Major Depression		
Major depression		110 (14.2%)
Not depressed		619 (79.9%)
Data no available		46 (5.9%)
12 month DSM-IV Major Depression		
Major depression		95 (12.2%)
Not depressed		626 (80.8%)
Data no available		54 (7.0%)
5-HTTLPR genotypes		
S/*		564 (72.8%)
L/L		211 (27.2%)
uMAOA genotypes		
Male	High/*	145 (18.7%)
	Low/Low	72 (9.3%)
Female	High/*	470 (60.6%)
	Low/Low	88 (11.4%)

Figure 1. Genotype Modification of Multifactor* Predicted Risk Score for DSMIV Major Depression.



*Including a group of environmental and other non-genetic risk factors (see text)

Figure 1-a: Likelihood Ratio (LR) for the Interaction by 5HTTLPR χ^2= 3,22; p = 0.07; Figure 1-b: LR for the interaction by s/s genotype χ^2=4.63; p = 0.031.
Figure 1-c: LR for the interaction by uMAOA genotype χ^2= 2.12; p = 0.14.
Figure 1-d: LR for the interaction by both 5HTTLPR and uMAOA genotypes χ^2 = 17.12; p = 0.038

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V. Discusión

La depresión es una enfermedad compleja con una etiología probablemente multifactorial, en la que entrarían en juego tanto factores biológicos (disfunción monoaminérgica, alteraciones endocrinológicas, entre otros) como factores de tipo psicosocial (rasgos de la personalidad, abuso en la infancia, acontecimientos vitales estresantes, etc.).

El hecho de que existan familias con un número incrementado de miembros afectados de depresión (agregación familiar) no ha pasado nunca desapercibido. Los estudios de familia demuestran que entre los familiares de primer grado de individuos con depresión, el riesgo para esta enfermedad es significativamente mayor que el descrito a nivel poblacional (*Perris et.al. 1966* y *Angst et.al. 1966).* Asimismo, los estudios de gemelos y adopción también apoyan la existencia de un componente hereditario en el origen de la depresión, cuyas tasas de heredabilidad se sitúan entre el 35 y el 45 %, según los estudios (*McGuffin and Katz, 1989*).

Numerosos grupos de investigación han centrado sus esfuerzos en la búsqueda de genes de susceptibilidad para depresión. Así, genes que codifican proteínas clave en la neurotransmisión serotoninérgica, tales como el gen del transportador de serotonina (SERT) o el de la monoamino oxidasa A (MAOA), entre otros, han sido intensamente estudiados, debido al papel que la serotonina parece jugar en la modulación del estado de ánimo o el humor.

Sin embargo, y a pesar del gran avance que ha experimentado la Biología Molecular en las últimas décadas, lo cierto es que los resultados de la psiquiatría genética en general han sido contradictorios. Los estudios de ligamiento han servido para identificar algunos puntos del genoma donde parece que podría haber "algo" ligado a la depresión (*Camp et.al., 2005*) y los estudios de asociación genética han permitido detectar algunos genes concretos asociados a la misma (*Gutiérrez et.al. 1998; Cervilla et.al. 2006; Naughton et.al. 2000; Rivera et.al. 2009*). Sin embargo, la ausencia de replicaciones en los

hallazgos (*Lasky-Su et.al. 2005; Huang et.al. 2004; Anguelova et.al. 2003*) y los riesgos descritos, muy pequeños en la mayoría de los casos, determinan que a día de hoy no existan resultados que puedan considerarse concluyentes acerca de cuáles son los genes que confieren susceptibilidad para padecer depresión.

Diversas razones estarían detrás de tales discrepancias en los resultados. El uso de diferentes metodologías en los distintos estudios o de metodologías inadecuadas en algunos de ellos, las posibles deficiencias en la definición de fenotipo, el limitado tamaño muestral o la escasez de estudios que examinen de forma conjunta e integrada los factores de riesgo ambiental y los genético como determinantes de la aparición de la enfermedad, podrían ser los responsables del relativo desconocimiento actual de las bases genéticas y los mecanismos fisiopatológicos últimos de la depresión.

En los artículos científicos que conforman el apartado de resultados de la presente Tesis Doctoral, se hace un esfuerzo por superar esas limitaciones y se intenta explorar las bases etiológicas de la depresión desde un punto de vista integrador. Se ha analizado el posible papel que tres genes clave en la neurotransmisión serotoninérgica (genes 5HT1A, SERT y MAOA) pudieran tener en el origen de dicha enfermedad, en una muestra amplia, representativa de la población general y bien caracterizada procedente del estudio PREDICT-Gene (*Cervilla et.al. 2006*).

A continuación se hace una discusión de cada uno de los trabajos presentados:

1. Asociación del polimorfismo C (-1019) G del gen 5-HT1A con estados comórbidos de ansiedad y depresión.

Nuestro objetivo fue clarificar si el polimorfismo C (-1019) G del gen que codifica para el receptor serotoninérgico tipo 1A (5HT1A), con un reconocido papel regulador de la neurotransmisión serotoninégica, confiere un riesgo incrementado para padecer depresión.

En un intento por definir mejor el fenotipo depresivo, tuvimos en cuenta la alta frecuencia con que la depresión suele ir acompañada por cuadros de ansiedad, concretamente por Trastorno de Ansiedad Generalizada (GAD) (*Anderson y Hope, 2008*), de modo que en los análisis de asociación se ajustó por la presencia de ambos trastornos.

En ninguno de los estudios previos que exploraron el papel de este gen en la etiología de la depresión se tuvo en cuenta la frecuente comorbilidad de ambos trastornos. Por ese motivo, los hallazgos reportados con anterioridad por otros autores bien podrían estar influidos por el efecto de variables confusoras que no se han tenido en cuenta a la hora de analizar los datos y resultados.

Nuestros resultados sugieren que el polimorfismo C (-1019) G del gen 5-HT1A no confiere un riesgo incrementado para padecer depresión o GAD en sus estados puros, al contrario de lo que se desprendía de algunos estudios previos en los que sólo se habían realizado análisis de asociación cruda (*Parsey et.al. 2006; Lenze et.al. 2008*). Sin embargo, encontramos una asociación estadísticamente significativa entre el polimorfismo C (-1019) G y los estados comórbidos de depresión y ansiedad generalizada (GAD). De este modo, encontramos que aquellos individuos portadores del alelo G de este polimorfismo mostraban riesgos incrementados para padecer depresión en comorbilidad con GAD. Esta asociación fue además independiente de posibles

factores confusores tales como sexo, la edad y la presencia de historia familiar de problemas psicológicos en los familiares de primer grado.

Este es el primer estudio que analiza la posible asociación entre el polimorfismo C (-1019) G del gen 5HT1A y los estados comórbidos de depresión y GAD. Además, es también el primero que explora el posible papel de este polimorfismo en la aparición de depresión o de GAD, ajustando por la presencia de una u otra y teniendo en cuenta, además, otros posibles factores confusores, tales como la historia familiar de problemas psicológicos.

Otra posible ventaja del diseño de este estudio es que la muestra utilizada es, hasta el momento, la más amplia en la cual se ha analizado el polimorfismo C (-1019) G del gen 5HT1A en relación a la depresión y la ansiedad generalizada (N=1059), lo cual asegura un poder estadístico suficiente para detectar el efecto conferido por la variabilidad en un solo gen de efecto pequeño.

Además, la muestra utilizada es representativa de la población general, ya que está compuesta por usuarios de servicios de Atención Primaria, tiene un origen genéticamente homogéneo y está bien caracterizada, ya que se utilizaron instrumentos validados para generar los diagnósticos de Depresión y de Ansiedad Generalizada, según criterios DSM-IV.

Nuestros resultados ponen de manifiesto que el polimorfismo C (-1019) G del gen 5-HT1A parece estar asociado con estados comórbidos de depresión y ansiedad generalizada, o bien, con un posible estado psicopatológico latente común a ambos trastornos. Esta asociación es independiente de factores confusores. La existencia de un sustrato biológico de vulnerabilidad común, es decir, compartido por ambos trastornos, es algo que apoyarían estudios provenientes de otros campos de investigación, como la psicopatología (*Strobel*

et.al. 2003), la neuroquímica (*Sthal 2008*), la neuroimagen (*Mathew et.al. 2004; Campbell y Moqueen 2006*) o la neuroendocrinología (*Boyer et.al. 2000*).

El polimorfismo C (-1019) G del gen 5-HT1A ha sido previamente asociado a neuroticismo (Strobel et al., 2003). Se considera que el neuroticismo podría estar en la base tanto de la Depresión como de la Ansiedad Generalizada (*Clark y Watson 1991; Brandes y Vienvenu 2006*), hecho éste que avalaría nuestros resultados desde un punto de vista psicopatológico. Además, nuestros resultados también se verían apoyados por estudios de asociación genética (llevados a cabo con genes diferentes del 5-HT1A) en los que se describen riesgos mayores para depresión comórbida con ansiedad que en cada uno de esos estados por separado (Kronenberg et al., 2007; Verhagen et al., 2009). Desde el punto de vista de las evidencias neuroquímicas, es interesante destacar que la hipótesis de la hipofuncionalidad monoaminérgica se ha postulado tanto en relación a la depresión como en relación a la ansiedad (Sthal, 2008). De hecho, los ISRSs pueden ser fármacos efectivos en el tratamiento de ambos tipos de trastornos. Desde los estudios de neuroimagen, se está empezando también ahora a identificar áreas concretas del cerebro que parecen estar implicadas tanto en depresión como en ansiedad (Mathew et al., 2004; Campbell & McQuee, 2006; Hettama 2008). Por último, nuestros resultados también irían a favor de las evidencias neuroendocrinas que describen una hipersecreción de CRH y una disfunción neuroesteroidea como consecuencia de una alteración en el funcionamiento del eje HPA tanto en depresión como en ansiedad (Boyer 2000).

En conclusión, nuestros resultados sugieren que la variabilidad del gen 5-HT1A juega un papel en la etiología de la depresión mayor comórbida con GAD, independientemente del sexo, la edad y la historia psiquiátrica familiar, algo que no se observa cuando ambos trastornos son analizados por separado.

2. Interacción entre el gen SERT y el gen MAOA con el estrés psicosocial previo en la aparición prospectiva de depresión.

En el segundo artículo de investigación que se presenta en esta Tesis Doctoral, profundizamos en el estudio de la etiología de la depresión sin dejar de lado la perspectiva integradora entre el componente genético y el ambiental.

Haber sufrido algún acontecimiento estresante (AVE) ha sido ampliamente asociado a riesgos incrementados para padecer depresión con porcentajes de riesgo atribuible entre el 37 y el 58 %, es decir, que los AVEs representan el 37-58% del total de eventos necesarios para que un individuo sufra un episodio depresivo (*Finaly-Jones et.al. 1981; Cervilla et.al., 1997*).

Por otro lado, las experiencias adversas sufridas durante la infancia contribuyen, posteriormente, a la aparición de episodios depresivos. Tal es el caso del abuso sexual, físico o psicológico (*Kaufman et.al. 1991*).

Sin embargo, no todas las personas son igualmente vulnerables a las presiones estresantes del ambiente. Se ha sugerido que la carga genética individual puede estar detrás de esta respuesta diferencial al estrés ambiental y, por tanto, puede conferir cierta vulnerabilidad a padecer depresión.

El objetivo de este trabajo fue explorar si los polimorfismos 5-HTTLPR del gen SERT y el uMAOA del gen MAOA, ampliamente estudiados en relación a la etiología de la depresión, interactúan conjuntamente (Interacción Gen-Gen) confiriendo un riesgo incrementado para padecer depresión. Además, se pretendía analizar si tales polimorfismos modifican el riesgo para padecer depresión conferido por el estrés psicosocial y las experiencias de abuso en la infancia (Interacción Gen–Gen–Ambiente).

Para ello, utilizamos una muestra amplia, de 1139 individuos, que fue evaluada hasta tres veces, en un periodo de un año de seguimiento. De esta forma pudimos analizar los nuevos casos de depresión que iban apareciendo y explorarlos desde una perspectiva longitudinal.

Nuestros resultados muestran, en primer lugar, la existencia de una interacción entre los polimorfismos 5-HTTLPR y uMAOA en la aparición prospectiva de episodios depresivos. El análisis de interacción entre diferentes loci puede incrementar el poder estadístico para detectar efectos genéticos específicos que podrían no detectarse al analizar el efecto de un solo polimorfismo (*Cordell et.al. 2009*). A pesar de ello, muy escasos son los estudios que analizan la existencia de interacciones gen-gen, y ninguno de ellos lo ha hecho sobre una muestra amplia, seguida de forma longitudinal, bien caracterizada y representativa de la población general, como es el caso de la muestra utilizada en el presente trabajo.

En segundo lugar, en este trabajo se muestra la existencia de una interacción genético-ambiental entre variantes alélicas de riesgo de dos polimorfismos diferentes y la adversidad social (presencia de AVEs en los seis meses anteriores) en la aparición prospectiva de episodios depresivos.

Por último, nuestros resultados apuntan a que el genotipo para los polimorfismos 5-HTTLPR y uMAOA modifica el riesgo para la aparición prospectiva de depresión conferido por las experiencias de abuso en la infancia.

De esta manera, el riesgo para padecer depresión conferido por la presencia de AVEs o por las experiencias de maltrato en la infancia, fue mayor en aquellos individuos que portaban el genotipo s/s del polimorfismo 5-HTTLPR y el genotipo HA/HA del polimorfismo uMAOA, que en los individuos que portaban otras combinaciones genotípicas.

Las evidencias que a día de hoy existen en cuanto a la interacción entre los factores de vulnerabilidad genética y la presencia de AVEs (*Caspi et.al. 2003, Sjöberget.al. 2006, Zalsman et.al. 2006, Cervilla et.al. 2007*) o de abuso en la infancia (*Kaufman et.al. 2004, Taylor et.la. 2006, Wilhem et.al. 2006*) en depresión son escasas y la mayoría se han centrado en el análisis de un solo gen.

Las escasas evidencias que implican la existencia de una interacción genético-ambiental incluyendo en sus análisis más de un loci en depresión vienen de la mano de autores como Kaufman et.al. (2006), quienes, en una muestra de niños, encontraron riesgos incrementados para padecer depresión en aquellos individuos que habían sufrido experiencias de maltrato en la infancia y que además portaban el genotipo s/s y el alelo Met de los polimorfismos 5-HTTLPR del gen SERT y Val66Met del gen BDNF, respectivamente, en comparación con los individuos que habían sido maltratados pero que no eran portadores de esas variantes genéticas de riesgo. Posteriormente, Wichers et.al. (2008) replicó esos hallazgos en una muestra compuesta por mujeres gemelas. Anteriormente, Kim et.al. (2007) había descrito una interacción entre estos loci y la presencia de AVEs en la aparición de depresión en un estudio longitudinal realizado sobre una amplia muestra de ancianos. Otros estudios han implicado también al polimorfismo G (-1019) G del gen 5-HT1A y al genotipo s/s del polimorfismo 5-HTTLPR en el riesgo para padecer depresión conferido por el abuso en la infancia (Zhang et.al. 2009). Hasta ahora, el único hallazgo de interacción genético-ambiental que implicaba a los polimorfismos uMAOA y 5-HTTLPR era el de *Cicchetti et.al. (2007*), quienes describieron riesgos para padecer depresión significativamente mayores entre los individuos que habían sufrido abuso sexual y portaban el alelo de baja actividad del polimorfismo uMAOA y el genotipo s/s, en comparación con los individuos que habían sufrido abusos sexuales pero que portaban otras combinaciones alélicas.

3. Interacción de los genes SERT Y MAOA con el algoritmo PREDICT-D predictor de la aparición de episodios depresivos.

En este último trabajo presentado en esta Tesis Doctoral, pretendíamos identificar factores de riesgo para depresión, utilizando una perspectiva integradora del componente genético y el ambiental.

Nuestro objetivo fue analizar si la variabilidad genética contenida en dos de los principales genes candidatos para depresión (genes SERT y MAOA) modificaba el riesgo para padecer depresión calculado mediante el algoritmo predictor PREDICT-D (*King et. al. 2008*).

El algoritmo PREDICT-D, recientemente validado por *King et.al. (2008),* es un paquete predictor de depresión a un año compuesto por diez factores de riesgo, en su mayoría medioambientales, que explica aproximadamente el 80% de la variabilidad en la aparición de depresión a lo largo de un periodo de un año.

Este trabajo fue desarrollado sobre una muestra compuesta por 775 usuarios de los Servicios de Atención Primaria, que fueron seguidos durante un año y evaluados hasta tres veces para toda una batería de variables sociodemográficas y psicológicas, en el contexto del estudio PREDIT-Gene (*Cervilla et.al. 2006*).

En primer lugar, encontramos una asociación entre la variabilidad de los genes SERT y MAOA y la depresión. Así, aquellos individuos con una o dos variantes genéticas de riesgo para los polimorfismos 5HTTLPR y uMAOA de los genes SERT y MAOA respectivamente tenían mayor riesgo para padecer depresión que los que portaban cualquier otra combinación genotípica (genotipos l/l y/o Low/*). Estos resultados eran congruentes con los hallazgos previos (*Lotrich y Pollock, 2004; Cervilla et.al. 2006; Rivera et.al. 2009*).

Además, encontramos una asociación entre ambos polimorfismos y las puntuaciones de riesgo para depresión medidas por el algoritmo predictor.

En los análisis de interacción genético-ambiental, encontramos que estas variantes alélicas de riesgo modifican significativamente el riesgo de depresión estimado con el paquete predictor de riesgo multifactorial. Así, aquellos individuos portadores de los dos genotipos de riesgo (s/* y h/h), son los que presentan los mayores valores de riesgo para depresión estimados por el paquete predictor PREDICT.

En los últimos años, algunos trabajos han demostrado la existencia de interacciones genético-ambientales en depresión (*Caspi et.al. 2003; Kaufman et.al. 2004; Cervill et.al. 2007*). Sin embargo, todos ellos han analizado la posible interacción de la carga genética con un solo factor de riesgo ambiental, a pesar de que la depresión parece estar determinada por un amplio conjunto de factores de riesgo ambientales en interacción con los factores genéticos (*Zammit y Owen, 2006*).

Esta es la primera vez que un estudio demuestra la existencia de una interacción genético-ambiental que modifica el riesgo para padecer depresión considerando, no un solo factor de riesgo medioambiental, sino un paquete predictivo de riesgo para depresión. Este paquete de factores de riesgo está fuertemente asociado a depresión y probablemente representa un escenario más realista de la enfermedad.

El paquete de factores de riesgo ambiental que incluye el modelo predictivo de *King et.al. (2008)* incluye algunos elementos que están íntimamente ligados a factores de riesgo cuyo efecto se ha demostrado que es modificado por la variabilidad en el polimorfismo 5-HTTLPR del gen SERT. Así, por ejemplo, la discriminación o las dificultades en el trabajo son elementos incluidos en el algoritmo predictor que pueden constituir acontecimientos

vitales estresantes (AVEs) serios. Algunos autores han sugerido que el riesgo para padecer depresión conferido por los acontecimientos vitales estresantes parece verse modificado por la variabilidad contenida en el polimorfismo 5HTTLPR (Caspi et.al. 2003; *Cervilla et.al. 2007*).

Nuestros hallazgos constituyen un paso importante hacia un mejor entendimiento de cómo se relacionan los distintos factores que causan la aparición de depresión. Asimismo, pueden abrir una puerta a la elaboración de estrategias de intervención y prevención de la depresión en atención primaria. La identificación precoz de individuos con alto riesgo de deprimirse puede facilitar la implantación de este tipo de medidas y puede resultar en una disminución de las tasas de prevalencia de esta enfermedad, que son llamativamente elevadas entre los usuarios de nuestros centros de salud.

Los resultados aquí presentados parten del análisis de las muestras de Málaga y Granada pero, en el contexto del PREDICT-España, otros muchos centros de atención primaria de otras regiones españolas se han sumado al proyecto. En la actualidad contamos con una muestra de aproximadamente 2700 sujetos. Todos ellos han seguido el mismo protocolo PREDICT descrito en detalle en el apartado de material y métodos de la presente tesis doctoral. Sobre esa muestra total se está empezando a trabajar ya para replicar nuestros hallazgos previos y también para elaborar un índice predictor de depresión en el que se incluyan desde el principio las variables genéticas, además de las ambientales, dentro del modelo. El objetivo final es construir un inventario de riesgo multifactorial predictor de la aparición de episodios depresivos en atención primaria basado en variables de tipo medioambiental y genéticas. Hasta el momento, no existen precedentes de estudios semejantes en psiquiatría.

VI. Conclusiones

CONCLUSIONS

A. On Genetic and Environmental Risk Factors for Major Depression

A.1) G allele carriers at the C (-1019) G polymorphism in the 5HT1A gene have an increase the risk for major depression and generalised anxiety disorder when ocurring comorbidly but not when studied separatedly.

A.2) The risk for prospectively assessed major depression is higher among individuals with the short/short allelic combination at the 5HTTLPR polymorphism in the serotonin transporter gene.

A.3) Being a high activity allele carrier at the uMAOA polymorphism, in the MAOA gene, significantly increase the risk for prospectively measured major depression, at least among females. Similarly, after adjusting by sex, being a high activity homozygous, at the same locus, also increase such risk.

A.4) The risk for major depression is significantly increased among individuals who have previously been exposed to threatening life events, sexual abuse, physical abuse and psychological abuse.

B. On Gene by Gene Interaction in Major Depression

B.1) The combined presence of two distinct genetic risk factors for depression (i.e., short/short SERT and high/high activity uMAOA genotypes) significantly interact conferring a higher risk for MD than that provided by any of those genetic risk factors taken separatedly. No previous report had, to the best of our knowledge, ever demonstrated this before.

C. On Three-Way Gene by Gene by Environment Interactions in Major Depression

C.1) The above combined genetic loading (i.e., carrying the short/short SERT genotype and being high activity uMAOA homozygous) also significantly modifies the deleterious effect of psychosocial environmental factors that increase risk for major depression, such as exposure to threatening life events, sexual abuse and any abuse (sexual, physical or psychological).

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VIII. Anexos

ANEXO I

ARTÍCULOS CIENTÍFICOS PREVIOS

A continuación se presentan una serie de artículos científicos, publicados en revistas indexadas de impacto, en los que se describen los primeros hallazgos derivados del estudio PREDICT-Gene en relación a la existencia de factores de riesgo genéticos para depresión y su interacción con factores de riesgo ambientales.

La doctoranda es coautora de tales trabajos.

Artículo 1

The 5-HTTLPR s/s Genotype at the Serotonin Transporter Gene (SLC6A4) Increases the Risk for Depression in a Large Cohort of Primary Care Attendees: The PREDICT-Gene Study

Jorge A. Cervilla, Margarita Rivera, ***Esther Molina****, Francisco Torres-González, Juan A. Bellón, Berta Moreno, Juan de Dios Luna, José A. Lorente, Yolanda de Diego-Otero, Michael King, Irwin Nazareth, Blanca Gutiérrez, and PREDICT Study Core Group*

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The 5-HTTLPR s/s Genotype at the Serotonin Transporter Gene (SLC6A4) Increases the Risk for Depression in a Large Cohort of Primary Care Attendees: The PREDICT-Gene Study

Jorge A. Cervilla,[1,2] Margarita Rivera,[1] Esther Molina,[1] Francisco Torres-González,[1] Juan A. Bellón,[3] Berta Moreno,[1,5] Juan de Dios Luna,[4] José A. Lorente,[1] Yolanda de Diego-Otero,[5] Michael King,[6] Irwin Nazareth,[7] Blanca Gutiérrez,[1,2] and PREDICT Study Core Group†

[1]*Departamento de Medicina Legal, Toxicología y Psiquiatría, Facultad de Medicina, Universidad de Granada, Granada, Spain*
[2]*Instituto de Neurociencias, Universidad de Granada, Granada, Spain*
[3]*Centro de Atención Primaria "El Palo", Departamento de Medicina Preventiva, Universidad de Malága, Málaga, Spain*
[4]*Departamento de Bioestadística, Universidad de Granada, Granada, Spain*
[5]*Fundación IMABIS, Málaga, Spain*
[6]*Academic Department of Psychiatry, University College and Royal Free School of Medicine, London, UK*
[7]*Department of Primary Care & Population Sciences, UCL & MRC General Practice Research Framework, London, UK*

Previous reports and meta-analyses have yielded inconclusive results as to whether the s/s genotype at the 5-HTTLPR serotonin transporter polymorphism confers increased risk for depression. We tested the association between s/s genotype and depression in a large cohort (n = 737) of Spanish primary care consecutive attendees participating in a European study on predictors for depression in primary care (PREDICT study). Participants were genotyped to establish 5HTTLPR genotype. Both univariable and multivariable associations between the s/s genotype and depression were tested twice using two different depressive outcomes (ICD-10 depressive episode and ICD-10 severe depressive episode). We found an association between the s/s genotype and both depressive outcomes that was independent of age, sex, family history of psychological problems among first degree relatives and presence of comorbid generalized anxiety disorder. When comparing s/s homozygous versus the rest, the adjusted odds ratio for any ICD-10 depressive episode and for severe ICD-10 depressive episode were 1.50 (95% CI: 1.0–2.2; $P = 0.045$) and 1.79 (95% CI: 1.1–2.8; $P = 0.016$), respectively. The association was significantly stronger with increasing severity of depression (χ^2 for linear association=6.1; $P = 0.013$) suggesting a dose-dependent relationship. Our results are consistent with previous reports suggesting a small but independent effect by the s/s 5-HTTLPR genotype increasing the risk for depression.

KEY WORDS: depression; 5-HTTLPR; serotonin transporter; association; generalized anxiety

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INTRODUCTION

Family, twin, and adoption studies have shown evidence for a genetic vulnerability to depression although specific genes involved in its aetiology are still to be identified [Jones, 2004]. The serotonin transporter gene (SLC6A4) is a candidate gene for depression as it plays a key role in serotonergic neurotransmission and its protein product is the central target for most antidepressant drugs.

One of the polymorphisms described in that gene, the 5-HTTLPR, consists of an insertion/deletion polymorphism in the promoter region. Its short variant (s allele) reduces the transcriptional efficiency of the gene resulting in decreased serotonin transporter expression in the neuron [Lesch, 1996]. Independent studies have shown that individuals with s alleles tend to have a poorer response to selective serotonin re-uptake inhibitor (SSRI) antidepressant treatment [Zanardi, 2001; Arias et al., 2003; Serretti, 2004]. However, the assumption that expression of variants is associated with mental disorders or response to antidepressants has recently been questioned [Lim et al., 2006; Parsey, 2006]. Consequently, the role of 5HTTLPR genotype in the origin of depression remains

†Miguel Xavier, Igor Slav, Heidi-Ingrid Maaros, Jan Neelman, Francisco Torres-Gonzalez, Irwin Nazareth and Michael King.

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*Correspondence to: Prof. Jorge A. Cervilla, & Dr. Blanca Gutiérrez, Department of Psychiatry and Institute of Neurosciences, Faculty of Medicine, University of Granada, Avenida de Madrid 11, 18012 Granada, Spain. E-mail: jacb@ugr.es

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unclear and controversial. Thus, while some reports describe an association between depression and the s/s genotype at the 5-HTTLPR polymorphism [Kunugi, 1997; Bellivier, 1998; Gutierrez, 1998; Ramasubbu et al., 2006], most studies have failed to report such association [Collier, 1996; Stober et al., 1996; Furlong, 1998]. Meta-analyses have also yielded inconclusive results in that some suggest that s/s individuals have a higher risk for unipolar depression but just a modest trend for bipolar depression [Furlong, 1998; Lotrich and Pollock, 2004] while others indicate that the s/s genotype increases the risk for bipolar depression but not for unipolar depression [Anguelova et al., 2003; Lasky-Su et al., 2005]. An association has also been reported between the 5-HTTLPR polymorphism and anxiety traits and generalized anxiety disorder (GAD) [Ohara et al., 1998; Lesch, 1999; You et al., 2005]. Also recently, the 5HTTLPR genotype has been shown to modulate the risk effect for depression conferred by social adversity [Caspi, 2003; Zammit and Owen, 2006].

Further evidence on the potential association between 5-HTTLPR polymorphism and depression is urgently needed as conflicting results may reflect lack of statistical power and/or heterogeneous sampling [Anguelova et al., 2003; Hoefgen, 2005; Lasky-Su et al., 2005]. The current study utilizes the Spanish cohort of a large European survey of risk factors for depressive episodes among primary care attendees, the PREDICT study [King, 2006]. Our objective was to explore the association between the 5-HTTLPR polymorphism and depression in a large, carefully evaluated cohort. In particular, we hypothesized that the s/s genotype at the 5-HTTLPR locus significantly increases the risk for depression.

METHODS

Study Design

The PREDICT-Gene is a genetic association study using a sample of all Spanish participants in a large study of onset of depression in European primary care attendees (PREDICT study). Both PREDICT and the PREDICT-Gene studies were approved by the relevant research ethics committees. A description of the study design and its method has been reported elsewhere [King, 2006]. In brief, the PREDICT study is a 1-year prospective study assessing consecutive general practice attendees at 0 (Time-1), 6 (Time-2), and 12 months (Time-3). Only cross-sectional (Time-1) data are used for the purpose of this analysis.

Setting and Sample

Consecutive attendees to nine primary care centres in the area of Malaga (Spain) who were aged 18–75 were asked to participate between April 2003 and September 2004. Attendees over 75 were excluded because of higher prevalence of cognitive impairment after that age. Participants unable to understand Spanish, as well as those with an organic mental disorder and/or any terminal illness, were also excluded. The participant's family doctor asked his/her patient to take part and the time-1 interviews, undertaken by three trained researchers, started within 2 weeks of informed consent being provided. No genetic study was initially linked to the PREDICT study protocol, as its aim was to construct a predictive model of depression for use by general practitioners. Consequently, at time-3 further informed consent was requested to obtain a biological sample for genetic analysis consisting of 10 cc of blood and/or up to 4 mouth swabs for saliva collection.

Measuring Non-Genetic Independent Variables

The PREDICT risk factor assessment was shown to have adequate test-retest reliability [King, 2006]. In brief, the risk factors for depression were either based on previously validated measures, concerned exposures (such as socio-demographic data) that are likely to be reported with a high degree of reliability, or (where new questions were developed, e.g., family history of psychological problems and living arrangements) were subjected to reliability testing at the outset of the study [King, 2006]. The anxiety section the Spanish version of the Patient Health Questionnaire (PHQ) was used to develop a DSM-IV diagnosis of GAD [Spitzer et al., 1999; Diez-Quevedo et al., 2001].

SLC6A4 Genotyping

DNA from both blood and saliva was obtained by standard procedures. The 5-HTTLPR polymorphism at SLC6A4 was genotyped in all samples. Amplification of genomic DNA was performed using 50 ng of DNA, 0.25 μM of each primer (forward: 5′-GGC GTT GCC GCT CTG AAT GCC-3′ and reverse: 5′-CAG GGG AGA TCC TGG GAG AGG T-3′), 250 μM each of dATP, dCTP, dGTP, and dTTP, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl and 0.3 units of DNA polymerase in a total volume of 25 μl. Samples were amplified for an initial cycle of 8 min at 95°C followed by 35 cycles each consisting of 30 sec at 95°C, 30 sec at 62°C and 1 min at 72°C. After amplification genotypes were resolved by a 2% agarose gel electrophoresis and ethidium bromide staining.

Outcome Measures of Depression

Six months prevalence of ICD-10 depressive episode (mild, moderate or severe) was ascertained using the depression section of the Composite International Diagnostic Interview (CIDI). The CIDI was administered by trained lay-interviewers. In order to validate our own findings we tested our hypothesis twice performing parallel analyses using first ICD-10 depressive episode of any severity as our depression outcome and then repeating the analyses considering as cases of depression only those that were diagnosed with ICD-10 depressive episode of severe intensity. No measures of bipolarity were used in this study.

Data Quality Control

Data quality was monitored to ensure that the project yielded data of the highest validity and reliability. The Spanish version of the PREDICT risk assessment questionnaires were translated from English and then back translated by professional translators before the coordinating center in London finally verified no major discrepancy in any back-translation. At a local level, each interview was checked for completion by each interviewer, all of whom had previously undergone a standardized training on: administering the CIDI and the risk factor questionnaire; recruitment and interviewing of patients and data management. A Spanish research coordinator made two assessments of each interviewer during the time-1 interviews to monitor the adequacy of the interview and tackle any problems as they arose. Before transferring data to the coordinating center, data quality control sheets were used and progress reports were submitted every 6 months to allow critical assessment by the PREDICT study steering group during regular project management meetings. 10% of data were double-entered and only 0.13% of input errors were detected and corrected.

Statistical Analysis

The data were analyzed using the SPSS 13.0 statistical package. An initial exploratory analysis was performed to study the distribution of both independent and dependent variables. Univariable associations were explored, using

parametric or non-parametric significance tests as appropriate. Bivariable risks were estimated initially using classical stratified analysis. Using a multivariable logistic regression analysis, Odds Ratios (OR) with 95% confidence intervals (95% CI) for depression were calculated in carriers and non-carriers of SLC6A4 allelic risk combinations. Three models containing increasing numbers of potential confounders were used to test the main hypothesis, that is, that depression is associated with s/s/genotype. Model A adjusted the association between depression and s/s genotype by age and gender only; Model B made adjustment for variables included in model A and family history of psychological problems and Model C in addition to the variables explored in Model B included the presence of GAD.

RESULTS

Sample and Frequencies

Out of 922 participants at Time 3, 737 (80%) provided informed consent to be included in the genetic sub-study (PREDICT-Gene) and agreed to provide a biological sample for genetic testing (n = 737). Table I summarizes the frequencies of all the study variables in the analysis. 71.8% of the sample were women (529) whilst 28.2% were men (208). Participants' mean age was 49 years (SD = 15.2). All 737 participants were of Spanish ethnic origin. Most were: married or living with a partner (71%); had primary (60%) or secondary schooling as their highest educational level (33.6%); were working either in (30%) or away from home (30.3%); 15.9% were retired and 5.2% unemployed. A family history of any psychological disorder amongst at least one first degree relative was present in 36.6% of patients. The allelic frequencies found were 50.95% for the l allele and 49.05% for the s allele. The frequencies for the three genotypes at the 5-HTTLPR polymorphism were as follows: long/long (l/l): 192 (26.1%); long/short (l/s): 367(49.88%); and short/short (s/s): 178 (24.1%). One hundred twenty-six participants (17.3%) were detected by the PHQ PRIME-MD interview as having GAD. Participants who agreed to take part in the genetic analysis did not vary systematically in terms of sex (female gender: 74% vs.71%; $\chi^2 = 0.47$; $P = 0.49$), mean age (49 vs. 50 years; Student's $t = 0.87$; $P = 0.38$), marital status (unmarried 33% vs. 29%; $\chi^2 = 4.37$ $P = 0.49$) or prevalence of ICD-10 depressive episode (35.4% vs. 34.7%; $\chi^2 = 0.021$;

TABLE I. predict-Gene Study: Variable Frequencies

Genetic frequencies				
Allelic frequencies	"l" controls	"s" controls	"l" cases	"s" cases
ICD-10 depressive episode	52.8%	47.2%	47.5%	52.5%
ICD-10 severe depressive episode	52.8%	47.2%	46.2%	53.8%
Genotype	l/l	l/s	s/s	
ICD-10 depressive episode				
Cases	64 (24.5%)	120 (46%)	77 (29.5%)	
Controls	128 (27%)	247 (52%)	101 (21%)	
ICD-10 severe depressive episode				
Cases	42 (23%)	85 (46.4%)	56 (30.6%)	
Controls	124 (27%)	236 (51.5%)	98 (21.5%)	
Non-genetic independent variables				
Gender				
Female 529 (71.8%)				
Male 208 (28.2%)				
Mean age				
49.05 years (SD 15.21)				
Education				
Illiterate 24 (3.3%)				
Primary 443 (60.1%)				
Secondary or higher 270 (36.6%)				
Marital status				
Married/couple 522 (70.8%)				
Single 126 (17.1%)				
Other 87 (12.1%)				
Profession				
Housekeeping 221 (29.9%)				
Working 223 (30.2%)				
Disabled/retired 220 (29.8%)				
Other 73 (10.1%)				
Living arrangements				
Alone 38 (5.2%)				
Other 699 (94.6%)				
Generalized anxiety disorder (GAD)				
GAD+ 127 (17%)				
GAD− 610 (83%)				
Family history of psychological problems amongst first degree relatives (FH)				
FH+ 270 (36.6%)				
FH− 467 (63.4%)				
Prevalences of depression				
ICD-10 depressive episode				
Depressed 262 (35.4%)		Not depressed 475 (64.6%)		
ICD-10 Severe Depressive Episode				
Depressed 183 (24.8%)		Not depressed 475 (64.61%)		

$P = 0.88$) from those who refused to give a genetic sample. Neither were there differences on the same variables between participants to the genetic study and those who participated in the initial baseline assessments.

Depressive Outcomes

Depression was common amongst this sample of primary care attendees. The 6-month prevalence of an ICD-10 Depressive Episode was 35.4% (262) and that of ICD-10 depressive episode of severe intensity was 25.4% (183) (see Table I).

Crude Associations With Depression

Both depressive outcomes were significantly associated with female gender, younger age, reporting a family history of psychological difficulties in first degree relatives, having at least one s allele at the 5-HTTLPR locus and being s/s homozygous (see Table I for allele and genotype distributions). The s allele was found to be significantly more frequent in both cases of ICD-10 depressive episode (OR = 1.24 (1–1.54) $\chi^2 = 3.83$; $P = 0.050$) and ICD-10 severe depressive episode (OR = 1.31 (1.02–1.68) $\chi^2 = 4.65$; $P = 0.031$) than in controls. Genotype frequencies were in Hardy–Weinberg equilibrium, both in cases and controls. In addition, there was a statistically significant linear association between an increasing number of s alleles and increasing severity of depression ($\chi^2 = 4.37$, DF=1, $P = 0.037$). This statistically significant linear trend was even more pronounced when the s/s genotype was compared with the aggregate of the other two possible genotypes across depressive episodes of increasing severity (χ^2 for linear trend = 6.13, DF = 1, $P = 0.013$) (Fig. 1). Depression was also associated with having a previous family history of psychological problems among first degree relatives. Hence, whilst 31% of controls had positive family history, 46% of depressed (OR = 1.92, 95% CI: 1.4–2.3; $P = 0.0001$) and 49% of severely depressed (OR = 2.09, 95% CI: 1.4–2.9; $P = 0.0001$) had such positive family history. Depression was not associated with marital status, professional situation, living arrangement or educational level in this sample. Conversely, depression was strongly associated with GAD ($\chi^2 = 104$, DF = 1, $P = 0.0001$ for ICD-10 depressive episode and $\chi^2 = 174$, $P = 0.0001$ for ICD 10 severe depressive episode) which, in turn was not associated with 5HTTLPR s/s genotype ($\chi^2 = 1.6$, DF = 1, $P = 0.19$).

Independent Associations Between s/s Genotype and Depression

Table II shows associations between the s/s genotype and both depressive outcomes adjusted for three progressively different multivariable models. In model A we adjusted the associations by sex and age, in model B we also adjusted for a family history of psychological problems and, finally, in model C we further adjusted for presence of comorbid GAD. Across all three multivariable models the association between the s/s genotype and depression remained robust and statistically significant (Table II). All three multivariable models showed appropriate "goodness of fit" after using Hosmer–Lemershow tests. The associations between the s/s genotype and both depressive outcomes used were also independent of other sociodemographic factors such as marital status, educational level, living arrangement or working situation (data not shown). As none of the latter variables added value to multivariable models (they were not even associated with depression in our univariable analyses), they were excluded from all three final models. The independent associations reported were more robust the more severe the definition of depression used (Fig. 2).

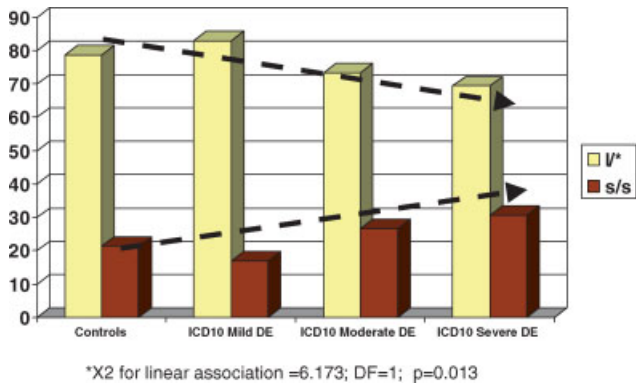

Fig. 1. Lineal association between increasing severity of ICD depressive episode and s/s genotype*. χ^2 for lineal association = 6.13; DF = 1; $P = 0.013$.

DISCUSSION

We have demonstrated an increased risk for depression in participants with the s/s variant at the 5-HTTLPR polymorphism in the serotonin transporter gene, that is, independent of age, gender, family history of psychological problems and comorbid GAD. Our study was named the PREDICT-GENE Study after it was nested in the Spanish sub-cohort of the European PREDICT study [King, 2006]. The large sample size provides sufficient statistical power to detect a small effect conferred by polymorphic variability at just one genetic locus. Lack of power due to small sample size and/or heterogeneous sampling might have contributed to some previous negative reports on the same research question [Furlong, 1998; Anguelova et al., 2003; Hoefgen, 2005]. The majority of people with depression will consult their general practitioners, whether or not they complain of depressive symptoms [Goldberg and Huxley, 1992]. Thus, this sample of consecutive attendees in primary care centers is likely to be fairly representative of the community. However, selection bias cannot entirely be ruled out as the sample studied represents 80% of those who were approached to consent to genetic testing at time-3 within the PREDICT study and loss to follow up during the 1-year study period was almost 25% by the time participants were asked to provide a biological sample for the genetic analysis. Nevertheless, the final sample did not differ significantly in terms of sex, mean age, marital status, working conditions, and prevalence of depression, from either the entire cohort studied at baseline nor the remaining 922 who were still participating at time-3 when biological samples were obtained.

Depression was ascertained following administration of a modified version of the CIDI depression subscale that enabled us to ascertain an ICD-10 depressive episode diagnosis. Nonetheless, the s/s genotype was not associated to mild depression. The latter is consistent with the notion that the CIDI might at times over-diagnose some cases of depression also explaining the high prevalence rates found in this study [Brugha et al., 2001]. We decided a priori to test the hypothesis of a dose-dependent relationship in terms of severity of the diagnosis of depression. The results reveal independent similar associations between the s/s genotype and both depressive outcomes. Furthermore, the association is stronger the greater the severity of the diagnosis of depression used. Similarly, a dose-dependent risk effect for depression was conferred by an increasing number of s alleles.

Our findings are entirely consistent with those suggesting that the s/s genotype exerts a small but independent increased risk effect for depression [Kunugi, 1997; Furlong, 1998; Gutierrez, 1998; Lotrich and Pollock, 2004]. Some authors have reported an effect by sex in the association between the s/s

TABLE II. Adjusted Odds Ratios (95% CI) for Depression and s/s Genotype

Model	ICD-10 depressive episode	ICD-10 severe depressive episode
Crude s/s genotype	1.55 (1.1–2.2) $P = 0.012$	1.62 (1.1–2.4) $P = 0.014$
Model A s/s genotype	1.53 (1.07–2.2) $P = 0.018$	1.58 (1.07–2.3) $P = 0.022$
Female gender	2.49 (1.7–3.6) $P = 0.000$	2.47 (1.6–3.8) $P = 0.000$
Increasing age	0.99 (0.98–1.0) $P = 0.31$	0.99 (0.98–1.00) $P = 0.33$
Model B s/s genotype	1.53 (1.06–2.2) $P = 0.020$	1.65 (1.1–2.5) $P = 0.016$
Female gender	2.37 (1.6–3.5) $P = 0.0001$	2.42 (1.5–3.8) $P = 0.000$
Increasing age	1.0 (0.9–1.01) $P = 0.98$	1.00 (0.99–1.01) $P = 0.95$
FH+	1.88 (1.3–2.6) $P = 0.0001$	2.15 (1.5–3.1) $P = 0.0001$
Model C s/s genotype	1.50 (1.009–2.24) $P = 0.045$	1.79 (1.1–2.8) $P = 0.016$
Female gender	1.91 (1.25–292) $P = 0.003$	1.70 (1.01–2.85) $P = 0.043$
Increasing age	0.99 (0.98–1.0) $P = 0.43$	0.99 (0.97–1.0) $P = 0.16$
FH+[a]	1.29 (1.07–1.54) $P = 0.006$	1.32 (1.07–1.64) $P = 0.01$
DSMIV GAD	9.5 (5.8–15.6) $P = 0.0001$	17.9 (10.3–31.4) $P = 0.0001$

GAD, generalized anxiety disorder.
[a]Family history of psychological problems amongst first degree relatives.

genotype and depression [Sjoberg, 2005]. We adjusted for both sex and age in our first multivariable model but the association remained robust with both depressive outcomes. Furthermore, when we included an additional variable such as family history of psychological problems in our model B, the associations between the s/s genotype and both depressive outcomes also remained significant. This suggests a specific familial effect mediated by the s/s genotype. The specificity of the association reported is also suggested by its independence from GAD which is strongly associated with depression and had been reported linked to the s/s genotype [Ohara et al., 1998; Lesch, 1999; You et al., 2005]. Previous negative results and meta-analyses might be partially due to heterogeneous sampling or not accounting for potential confounders such as those adjusted for by our study. An alternative explanation for previous negative findings has also been recently suggested as a so-called LG functional variant of the long allele has been reported to have comparable lower levels of expression to those linked to the short allele [Hu et al., 2004; Wendland et al., 2006].

We believe our findings add relevant data for future meta-analyses helping to balance a conclusion towards a true association between the s/s genotype and unipolar depression particularly discounting the effect of comorbid GAD. Neurobiological evidence validating such true association between depression and the s/s genotype includes that the serotonin transporter is an important molecule targeted by most antidepressant drugs and potential functional or density changes in such a protein can influence both serotonergic pathways function and response to antidepressant therapy [Zanardi, 2001; Arias et al., 2003; Serretti, 2004]. Moreover, it has been suggested that variability at the 5-HTTLPR locus reduces the transcriptional efficiency of the gene, resulting in decreased SLC6A4 expression in the neuron [Lesch, 1996]. In addition, a genetic modulation by 5-HTTLPR of reactivity in the amygdala to fear-provoking stimuli has been suggested as a potential rationale for increased genetic susceptibility to depression in s/s individuals [Hariri, 2005; Pezawas, 2005].

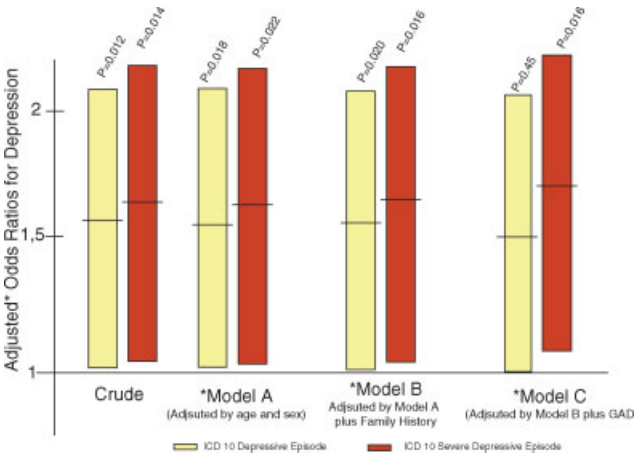

Fig. 2. Association between s/s genotype and depression (adjusted odds ratios with 95% CI).

CONCLUSIONS

We report a consistent and independent association between the 5-HTTLPR genotype and depression in a large community-based primary care sample. Our findings support the notion that functional variability in the serotonin transporter molecule influences the risk for depression.

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We would like to thank all members of the PREDICT Study Core Group (Miguel Xavier, Igor Slav, Heidi-Ingrid Maaros, Jan Neelman, Francisco Torres-Gonzalez, Irwin Nazareth, and Michael King) for allowing us to add a genetic component to their study. We would also like to thank participants for agreeing to provide a biological sample. We thank the three interviewers (Ana Alvarez, Francisca Vidal, and Nuria Lopez) in the Spanish study and all the nurses and GPs at the 9 primary care centres from Malaga (Spain) for their hard work and collaboration. This study was co-financed by the Vth Framework Program of the European Commission, a local grant from the University of Granada (30.PP.00.5000) and by grant number SAF-2004-01310 from the Ministry of Education and Science (Spain). The PREDICT-Gene study is an initiative of the Biological Psychiatry Focus Group included in both the Andalusian Group for Research in Mental Health (GAISAM) and the "Federico Oloriz" Institute of Neurosciences, University of Granada, Spain.

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Artículo 2

The risk for Depression conferred by Stressful life Events is Modified by Variation at the Serotonin Transporte 5-HTTLPR Genotype: evidence from the Spanish PREDICT-Gene Cohort.

Jorge A. Cervilla, ***Esther Molina,*** *Margarita Rivera, Francisco Torres-González, Juan A. Bellón, BertaMoreno-Küstner, Juan D. Luna, José A. Lorente, Fermín Mayoral, Michael King, IrwinNazareth, the PREDICT Study Core Group and Blanca Gutiérrez.*

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ORIGINAL ARTICLE

The risk for depression conferred by stressful life events is modified by variation at the serotonin transporter 5HTTLPR genotype: evidence from the Spanish PREDICT-Gene cohort

JA Cervilla[1,2], E Molina[1], M Rivera[1], F Torres-González[1], JA Bellón[3], B Moreno[1,4], JD Luna[5], JA Lorente[1], F Mayoral[6], M King[7], I Nazareth[8], the PREDICT Study Core Group and B Gutiérrez[1,2]

[1]*Departamento de Medicina Legal, Toxicología y Psiquiatría, Facultad de Medicina, Universidad de Granada, Granada, Spain;* [2]*Instituto de Neurociencias, Universidad de Granada, Granada, Spain;* [3]*Centro de Atención Primaria 'El Palo', Departamento de Medicina Preventiva, Universidad de Málaga, Red de Investigación de Atención Primaria, redIAPP, Málaga, Spain;* [4]*Fundación IMABIS, Málaga, Spain;* [5]*Departamento de Bioestadística, Universidad de Granada, Granada, Spain;* [6]*Hosptial Carlos Haya, Málaga, Spain;* [7]*Department of Mental Health Sciences, Hampstead Campus, Royal Free and University College Medical School, UCL, London, UK and* [8]*Department of Primary Care and Population Sciences, UCL and MRC General Practice Research Framework, London, UK*

We report results from the PREDICT-Gene case-control study nested in a prospective cohort designed to identify predictors of the onset of depression among adult primary-care attendees. We tested the potential gene-by-environment interaction between 5HTTLPR genotype at the serotonin transporter gene and previous exposure to threatening life events (TLEs) in depression. A total of 737 consecutively recruited participants were genotyped. Additional information was gathered on exposure to TLEs over a 6-month period, socio-demographic data and family history of psychological problems among first-degree relatives. Diagnoses of depression were ascertained using the Composite International Diagnostic Interview (CIDI) by trained interviewers. Two different depressive outcomes were used (ICD-10 depressive episode and ICD-10 severe depressive episode). Both the s/s genotype and exposure to increasing number of TLEs were significantly associated with depression. Moreover, the 5HTTLPR s/s genotype significantly modified the risk conferred by TLEs for both depressive outcomes. Thus, s/s homozygous participants required minimal exposure to TLE (1 TLE) to acquire a level of risk for depression that was only found among l/s or l/l individuals after significantly higher exposure to TLEs (two or more TLEs). The interaction was more apparent when applied to the diagnosis of ICD-10 severe depressive episode and after adjusting for gender, age and family history of psychological problems. Likelihood ratios tests for the interaction were statistically significant for both depressive outcomes (ICD-10 depressive episode: LR $X^2 = 4.7$, $P = 0.09$ (crude), LR-$X^2 = 6.4$, $P = 0.04$ (adjusted); ICD-10 severe depressive episode: LR $X^2 = 6.9$, $P = 0.032$ (crude), LR-$X^2 = 8.1$, $P = 0.017$ (adjusted)).
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Keywords: serotonin transporter gene polymorphism; SLC6A4; gene–environment interaction; affective disorders; social stress; primary care

Introduction

The causal processes underlying depression are yet to be identified but, undoubtedly, comprise both genetic and environmental components. One of the environmental risk factors consistently linked to depression is the exposure to stressful life events.[1–4] From the genetic viewpoint, the serotonin transporter gene (SLC6A4) that plays a key role in serotonergic neurotransmission is a candidate gene for depression. Moreover, its protein product is the central target for most antidepressant drugs. One of the polymorphisms described in the gene, the 5-HTTLPR, consists of an insertion/deletion polymorphism in the promoter region. Its short variant (s allele) reduces the transcriptional efficiency of the gene, resulting in decreased serotonin transporter expression in the neuron.[5] Some association studies have reported an increased risk for depression among s/s genotype carriers,[6–10] although others have reported negative results.[11–13]

The interplay between genetic and environmental factors in the aetiology of common and complex

Correspondence: Professor JA Cervilla, Department of Psychiatry, Institute of Neurosciences, Faculty of Medicine, University of Granada, Avenida de Madrid 11, 18012 Granada, Spain.
E-mail: jacb@ugr.es
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diseases has been well recognized,[14] but the technology required to explore this relationship has only become available recently. Research on gene-by-environment interactions can improve our understanding of the aetiology of complex diseases, such as mental disorders by providing a more accurate estimation of population-attributable risks for genetic and environmental risk factors. Such research can also contribute to the design of preventative and therapeutic interventions for depression.[15] Over the past 3 years, the interaction between the 5HTTLPR polymorphism and exposure to stressful life events has been under scrutiny. Animal research has shown a greater likelihood of depressive outcomes in macaques subjected to adverse rearing experiences who carry the risk allele of the rh5HTTLPR polymorphism.[16] Moreover, imaging studies on humans have demonstrated amygdala hyperreactivity in response to fearful stimuli among s allele carriers compared to l/l individuals.[17]

Longitudinal data on people with one or two s alleles at the 5HTTLPR locus indicate that they are more vulnerable to depression than non-s allele carriers for the same level of exposure to stressful life situations.[12] Moreover, variations at the same locus modify the risk effect of developing depression in those maltreated in childhood.[12] These findings have been more recently replicated[18–23] to include: a population-based adult twin study in which people with the s/s genotype were more vulnerable to the depressogenic effects of exposure to stressful life events;[21] research on 101 children in whom the s/s genotype at the 5HTTLPR locus made them more susceptible to depression when they had experienced maltreatment and/or lack of social support[20] and a longitudinal follow-up of 127 people over 25 years in whom the s/s genotype was found to modify the effect of previous exposure to adverse life events as a risk factor for first onset of depression.[23]

Some research has been conducted exclusively in women. Eley *et al*.[18] compared 377 adolescents girls categorized by scores on the self-reported short form of the Mood and Feelings Questionnaire, and found that the risk of social environmental factors was higher among carriers of short (s) alleles. Sjöberg *et al*.[22] reported similar findings in female adolescents, but showed the opposite effects in male adolescents (i.e., male s allele carriers were less likely to develop depression after being exposed to risky environmental factors). There have also been two larger studies that have failed to replicate the gene × environment interaction. The first study was conducted on an adult cohort of 1206 twins,[24] and the other was a 1-year follow-up of 4175 people.[25]

Overall, the strongest evidence is in favour of the effect of an interaction between 5HTTLPR and social distress.[26] However, to date research has been limited by selective sample studies (i.e., children, adolescents or twins) and the use of non-standard depressive outcomes. There is, hence, an urgent need to replicate these findings in large representative adult populations on whom validated measures of depression have been used. The PREDICT-Gene study tests the hypothesis that polymorphic variation at the 5HTTLPR locus interacts with social adversity (exposure to stressful life events), modifying the risk for depression in a Spanish population of primary-care attendees.

Materials and methods

Design

The PREDICT-Gene study[9] is a case-control association study nested in a cohort of Spanish participants who were part of a larger study on prediction of onset of depression in European primary-care attendees (PREDICT study). A detailed description of the PREDICT study design and its method has been reported elsewhere.[27] In brief, the PREDICT study is a 1-year prospective study assessing consecutive general practice attendees at 0 (time-1), 6 (time-2) and 12 months (time-3). Only cross-sectional (time-1) data are used in this analysis. Both PREDICT and the PREDICT-Gene studies were approved by the relevant research ethics committees.

Sample

Consecutive attendees to nine (two rural and seven urban) primary care centres in the area of Málaga (Spain), aged 18–75, were asked to participate between April 2003 and September 2004. The participant's family doctor asked his/her patient to take part, and time-1 interviews were undertaken by three trained researchers within 2 weeks of informed consent being provided. Attendees over 75 were excluded because of higher prevalence of cognitive impairment after that age. Participants unable to understand Spanish, as well as those with an organic mental disorder and/or any terminal illness, were also excluded. This genetic study was not a part of the original PREDICT study protocol, that aimed to construct a predictive model of depression for use by general practitioners. Consequently, at time-3, further informed consent was requested to obtain a biological sample for genetic analysis consisting of 10 cm^3 of blood and/or up to 4-mouth swabs for saliva collection.

Independent measures

The PREDICT risk factor assessment was shown to have adequate test–retest reliability.[27] In brief, the risk factors for depression were either based on previously validated measures, concerned exposures (such as socio-demographic data) that are likely to be reported with a high degree of reliability, or (where new questions were developed, e.g., family history of psychological problems and living arrangements) were subjected to reliability testing at the outset of the study.

Social distress was measured using the List of Threatening Events.[28] This is a list including serious events shown to carry high degrees of contextual

threat. The list includes serious life–events, such as the death of a parent, spouse or child, the death of another relative, the onset of a serious illness or accident affecting a relative, a marital separation, the ending of a friendship or relationship, a serious problem with a close friend, neighbour or relative, a financial crisis, the theft or loss of an item of personal value, having troubles with the police or courts, loss of work through redundancy and loss of work through dismissal. Subjects were asked whether any of these events had occurred within the 6 months before the interview. For the purposes of the analysis, we divided participants into three levels of exposure to threatening life events (TLEs): Having had no TLE, having had just one TLE or having had two or more TLEs, over the 6-month period before the interview.

5HTTLPR genotype assays

DNA from both blood and saliva was obtained by standard procedures. The 5-HTTLPR polymorphism at SLC6A4 was genotyped in all samples. Amplification of genomic DNA was performed using 50 ng of DNA, 0.25 μM of each primer (forward: 5′-GGCGTT GCCGCT CTG AAT GCC-3′ and reverse: 5′-CAGGGG AGATCC TGG GAG AGG T-3′), 250 μM each of dATP, dCTP, dGTP and dTTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl and 0.3 units of DNA polymerase in a total volume of 25 μl. Samples were amplified for an initial cycle of 8 min at 95°C followed by 35 cycles each consisting of 30 s at 95°C, 30 s at 62°C and 1 min at 72°C. After amplification, genotypes were resolved by a 2% agarose gel electrophoresis and ethidium bromide staining.

Measures of depression

Six months prevalence of ICD-10 depressive episode (mild, moderate or severe) was ascertained using the depression section of the Composite International Diagnostic Interview (CIDI).[29] The CIDI was administered by trained lay interviewers. We tested our hypothesis by performing two sets of analyses. In the first, we used ICD-10[30] depressive episode of any severity as our depression outcome, and in the second we repeated the analyses only in those with an ICD-10 depressive episode of severe intensity.

Data quality control

Data quality was monitored to ensure that the project yielded data of the highest validity and reliability. The Spanish version of the PREDICT protocol was translated from English and then back translated by professional translators before the coordinating centre in London finally verified no major discrepancy in any back-translation. At a local level, each interview was checked for completion by each interviewer, all of whom had previously undergone a standardized training on administering the CIDI and the risk factor questionnaire, recruitment and interviewing of patients and data management. A Spanish research coordinator made two assessments of each interviewer during the time-1 baseline interviews to monitor the adequacy of the interview and tackle any problems as they arose. Before transferring data to the coordinating centre, data quality control sheets were used and progress reports were submitted every 6 months to allow critical assessment by the PREDICT study steering group during regular project management meetings. Ten per cent of data were double entered which revealed an error rate of only 0.13%.

Statistical analyses

The data were analysed using the STATA 9.0 statistical package.[31] An initial exploratory analysis was performed to study the distribution of both independent and dependent variables. Univariable associations were explored, using parametric or non-parametric significance tests as appropriate. Bivariable risks were estimated using classical stratified analysis. Using a multivariable logistic regression analysis, odds ratios with 95% confidence intervals for depression were calculated across 5HTLPPR genotype categories (s/s vs l/s and/or l/l) and also across the three levels of exposure to previous TLE. Finally, using a logistic regression model, we tested the interaction between the genetic (5HTTLPR genotype) and the environmental (exposure to TLEs) factors, both crudely and then after adjustment for sex, age and family history of psychological problems among first-degree relatives. We calculated probabilities for depression across all combinations of levels of exposure to TLE by 5HTTLPR genotype (s/s vs l/s or l/l). Crude and adjusted probabilities for depression across strata were also calculated. Finally, likelihood ratios tests for both differences of probabilities between such strata and for the genetic by environment interaction were also estimated.

Results

The sample

A total of 737 (80%) out of 922 participants at time-3 gave informed consent to be included in the PREDICT-Gene genetic study and provided a biological sample for genetic testing ($n = 737$). The sample's mean age was 49 years (s.d. = 15.2). Five hundred and twenty-nine participants were women (71.8%) and 208 were men (21.2%). Most participants were married or living with a partner (71%), had primary (60%) or secondary (33.6%) schooling as their highest educational level and were working either in (30%) or away (30.3%) from home, whereas 15.9% were retired and 5.2% were unemployed. 36.6% of the sample had a positive family history of any psychological disorder amongst at least one first-degree relative. Participants who agreed to take part in the genetic analysis did not vary systematically, in terms of sex (female gender: 74 vs 71%, $X^2 = 0.47$, $P = 0.49$), mean age (49.18 vs 50 years, Student's $t = 0.87$, $P = 0.38$), marital status (unmarried 33 vs 29%, $X^2 = 4.37$, $P = 0.49$) or prevalence of ICD-10 depressive episode (35.4 vs 34.7%, $X^2 = 0.021$, $P = 0.88$), from those who refused to give a genetic sample. Nor were there any

significant differences on these variables between participants in the genetic study and those who participated in the initial baseline assessments (mean age 49.18 vs 49.02 years, Student's $t = 0.18$, $P = 0.85$; female gender 74 vs 71.8%, $X^2 = 1.59$, $P = 0.020$; being unmarried 33 vs 31%, $X^2 = 4.37$, $P = 0.49$; prevalence of ICD-10 depressive episode (35.4 vs 33.4%, $X^2 = 0.72$, $P = 0.39$).

Independent variables frequencies

Demographic, genotypic and phenotypic data on the sample are provided in Table 1. Summarizing, one in four participants had not experienced any TLE in the previous 6 months, and of the rest, about half had reported at least one TLE and the other half at least two or more TLEs. Just over a half of participants had the l allele, whereas the rest had the s allele. Approximately half the participants had the s/l genotype, a quarter had the l/l genotype and the remaining quarter had the s/s genotype (see Table 1 for details). Genotype frequencies were in Hardy–Weinberg equilibrium, both in cases and controls.

Associations with depression

The 6-month prevalence of an ICD-10 depressive episode was 35.4% (262) and that of ICD-10 severe depressive episode was 25.4% (183) (see Table 1). Table 2 shows that depression was associated with the 5HTTLPR s/s genotype, as reported in detail elsewhere.[9] In brief, the association between the s/s genotype and depression was independent of age, sex, family history of psychological problems among first-degree relatives and GAD, but these associations were stronger for more severe depressive episodes. Both outcomes of depression were strongly and independently associated with previous exposure to TLE with initial crude associations remaining robust after adjusting for age, gender, marital status, education and family history of psychological problems (Table 2). Conversely, depression was not associated with marital status, professional situation, living arrangement or educational level in this sample.

5HTTLPR genotype interaction with threatening life experiences

The 5HTTLPR polymorphism significantly modified the risk effect for depression conferred by an increasing level of exposure to TLE (Table 3 and Figure 1). The interaction reached a higher level of significance when the TLE effect on depression in s/s genotype carriers was compared with the other two genotypes combined (l/l or l/s) (Table 3) and when only severe depression was considered. On adjustment, age did not modify the results and was hence excluded from the explanatory models. Finally, the interaction was stronger for both depressive outcomes after adjusting for gender, age and family history of psychological problems amongst first-degree relatives (Table 3 and Figure 1).

Table 1 Summarized frequencies of independent variables and depressive outcomes

Socio-demographic variables			
Gender			
Female 529 (71.8%)			
Male 208 (28.2%)			
Mean age			
49.05 years (s.d. 15.21)			
Education			
Illiterate 24 (3.3%)			
Primary 443 (60.1%)			
Secondary or higher 270 (36.6%)			
Marital status			
Married/couple 522 (70.8%)			
Single 126 (17.1%)			
Other 89 (12.1%)			
Profession			
Housekeeping 221 (29.9%)			
Working 223 (30.2%)			
Disabled/retired 220 (29.8%)			
Other 73 (10.1%)			
Living arrangements			
Alone 38 (5.2%)			
Other 699 (94.8%)			
Frequencies of depression outcomes			
ICD-10 depressive episode			
Depressed 262 (35.4%)			
Not depressed 475 (64.6%)			
ICD-10 severe depressive episode			
Depressed 183 (24.8%)			
Not depressed 475 (64.5%)			
Excluded from the analyses 79 (10.7%)			
Independent variables			
5HTTLPR genotypes			
l/l	*l/s*	*s/s*	
192 (26%)	367 (50%)	178 (24%)	
Exposure to threatening experiences			
No TLE	*1 TLE*	*2 TLEs*	
191 (26%)	266 (36%)	280 (38%)	
Family history of psychological problems amongst first-degree relatives			
FH+	*FH−*		
270 (36.6%)	467 (63.4%)		

Abbreviations: FH, family history; s.d., standard deviation; TLE, threatening life events.

Discussion

Summary of results

Our main findings are that the 5HTTLPR s/s genotype and exposure to increasing numbers of TLEs were independently associated with depression, and that

Table 2 Associations between depression and genetic or environmental factors

	ICD-10 depressive episode			*ICD-10 severe depressive episode*		
	Cases	*Controls*	*Adjusted* OR (95% CI),* P	*Cases*	*Controls*	*Adjusted* OR[a] (95% CI),* P
Genotypes						
l/l	64 (24)	129 (27)	1.0 (reference)	42 (23)	129 (27)	1.0 (reference)
l/s	120 (46)	246 (52)	0.8 (0.6–1.4), $P=0.8$	85 (46)	246 (52)	1.0 (0.6–1.6), $P=0.9$
s/s	77 (30)	101 (21)	1.5 (0.9–2.3), $P=0.06$	56 (31)	101 (21)	1.7 (1.0–2.7), $P=0.03$
Homozygous s/s						
l/*	185 (70)	374 (79)	1.0 (reference)	127 (69)	375 (79)	1.0 (reference)
s/s	77 (30)	101 (21)	1.5 (1.1–2.2), $P=0.015$	56 (31)	101 (21)	1.6 (1.1–2.4), $P=0.011$
Alleles						
l	248 (47.5)	503 (52.8)	1.0 (reference)	169 (46)	484 (53)	1.0 (reference)
s	274 (52.5)	449 (47.2)	1.24 (1.0–1.5), $P=0.05$	197 (54)	432 (47)	1.3 (1.0–1.7), $P=0.031$
Family history						
Negative	140 (53)	327 (69)	1.0 (reference)	89 (49)	148 (31)	1.0 (reference)
Positive	122 (47)	148 (31)	1.9 (1.4–2.6), $P=0.0001$	94 (51)	327 (69)	2.1 (1.4–2.6), $P=0.0001$
Threatening life events						
No	50 (19)	141 (30)	1.0 (reference)	31 (17)	141 (30)	1.0 (reference)
1	80 (31)	186 (39)	1.2 (0.8–1.8)	51 (28)	186 (39)	1.2 (0.7–2)
2 or more	132 (50)	148 (31)	2.5 (1.6–3.7), $P=0.0001$[b]	101 (55)	148 (31)	3.1 (1.9–4.9), $P=0.0001$[b]

Abbreviations: CI, confidence interval; OR, odds ratio.
*Adjusted by age, gender, family history of psychological problems and presence of generalized anxiety disorder.
[a]Odds ratio for each increasing level of exposure.
[b]Crude associations that remained robust after adjusting for age, gender, marital status, education and family history of psychological problems.

the 5HTTLPR s/s genotype significantly modified the risk conferred by TLEs for both depressive outcomes. Thus, s/s homozygous participants required minimal exposure to TLE (1 TLE) to acquire a level of risk for depression, whereas l/s or l/l individuals required higher exposure to two or more TLEs. This interaction was more apparent for people with an ICD-10 diagnosis of severe depression and after adjustment for gender, age and family history of psychological problems.

Study design and limitations

A case-control study nested in a cohort study with retrospective environmental and genetic data is an appropriate design to examine the gene–environment interaction hypothesis[15] However, such studies may be limited by selection, recall and/or survivor bias. In this study, these biases were minimized by sampling a representative population of general practice attendees. In addition, no significant differences were found on socio-demographic factors, and the level of depression between the participants included in our genetic analyses with those who refused a genetic specimen or were lost to follow-up at time-3.[9] We did not use the newly reported 5HTTLPR reclassification procedure by additionally genotypying the sample for the so-called A/G variant,[32] which may imply a potential limitation to this study. However, some authors have posed that reclassification of subjects using such new polymorphism seem to render comparable results to the well-established method used by us.[33]

Novelty and interest

We aimed to replicate previous findings in which variation at the 5HTTLPR locus modified the risk effect for depression conferred by previous exposure to stressful life events.[12,18–23] It was important to do this as although, the earliest report on the gene–environment interaction was conducted on an adult sample,[12] most of the other studies were restricted to populations, such as women but not men,[18,19,22] younger people,[18,20,22] twins[21] or people with affective disorders.[34] Our study used consecutive primary-care adult attendees and hence constitutes the first representative population-based replication of the earliest research. To our knowledge, it is also the first study to examine genetic-environment interaction in a homogeneous Spanish population in whom genotype frequencies[7,10] and prevalences of exposure to TLEs[3,28] were similar to most other European populations. Lastly, it is the first study to take account of potential confounders, such as age, gender and family history of psychological problems among first-degree relatives.

Depressive phenotypes

ICD-10 research criteria[30] do not consider the impact of depressive symptoms on daily living activities in arriving at a diagnose of a depressive episode. Consequently, we performed parallel tests using both a broader depressive phenotype (i.e., ICD-10 depressive episode of any severity) and a narrower phenotype (i.e., only ICD-10 severe depressive episode). The results for the gene–environment interaction are more apparent when using the latter construct. This may indicate that ICD-10 severe depressive episode is a more specific depressive phenotype. It may also suggest that there could be a linear tendency for the reported gene–environment interaction to influence increasingly more intense depressive states. The definition of the depressive phenotype is crucial in tests of the gene–environment interactions and has been one of the major limitations of previous research on this topic.[18,22,24,25]

Table 3 5HTTLPR genotype interaction with threatening life experiences

	Adjusted probability (s.e.)*	*Adjusted* OR (95% CI)*, P
ICD-10 depressive episode		
l/l or l/s		
No TLE	0.22 (0.19)	1.0 (reference)
1 TLE	0.23 (0.17)	1.0 (0.6–1.7), $P=0.8$
2*TLE	0.46 (0.14)	3.0 (1.8–4.8), $P=0.001$
s/s		
No TLE	0.30 (0.33)	1.0 (reference)
1 TLE	0.46 (0.25)	2.0 (0.8–4.5), $P=0.10$
2*TLE	0.46 (0.25)	2.0 (0.8–4.5), $P=0.09$
LR test for interaction: LR $X^2=6.4$, $P=0.04$ (adjusted)*		
ICD-10 severe depressive episode		
l/l or l/s		
No TLE	0.14 (0.24)	1.0 (reference)
1 TLE	0.14 (0.21)	0.9 (0.5–1.8), $P=0.9$
2*TLE	0.39 (0.15)	3.9 (2.2–6.7), $P=0.001$
s/s		
No TLE	0.21 (0.38)	1.0 (reference)
1 TLE	0.39 (0.27)	2.4 (0.93–6.0), $P=0.068$
2*TLE	0.39 (0.27)	2.4 (0.9–6.2), $P=0.06$
LR test for interaction: LR $X^2=8.1$, $P=0.017$ (adjusted)*		

Abbreviations: LR, likelihood ratio; TLE, threatening life events.
*Adjusted by gender and family history of psychological problems amongst first-degree relatives.

The gene–environment interaction

Both crude and adjusted gene–environment interactions, on both depressive outcomes, show a non-linear effect of the risk conferred by TLEs for depression as a function of the 5HTTLPR genotype. Hence, among s/s individuals minimal levels of exposure to TLEs (from one onwards) confer a degree of risk for depression that is only reached by non-s/s individuals who have had higher levels of exposure (two or more). Thus, our results show an interaction that follows a step-wise pattern with an abrupt change, when comparing genotypes, at moderate levels (1 TLE) of exposure to TLEs (see Figure 1). We believe this may be partially owing to the intense threatening nature of the stressful life-events measured by the scale we used, where only seriously threatening stressful life events are recorded.[28] Caspi *et al*.[12] reported an interaction of 5HTTLPR genotype with a linear progression of exposure to life events, possibly because their measure of stressful life-events included a wide range of situations, including those with a lower severity and contextual threat than those used in our measure.[35] Our results are most similar to Kendler *et al*.[21] and Wilheim *et al*.,[23] who demonstrated a step-wise pattern for the interaction according to the

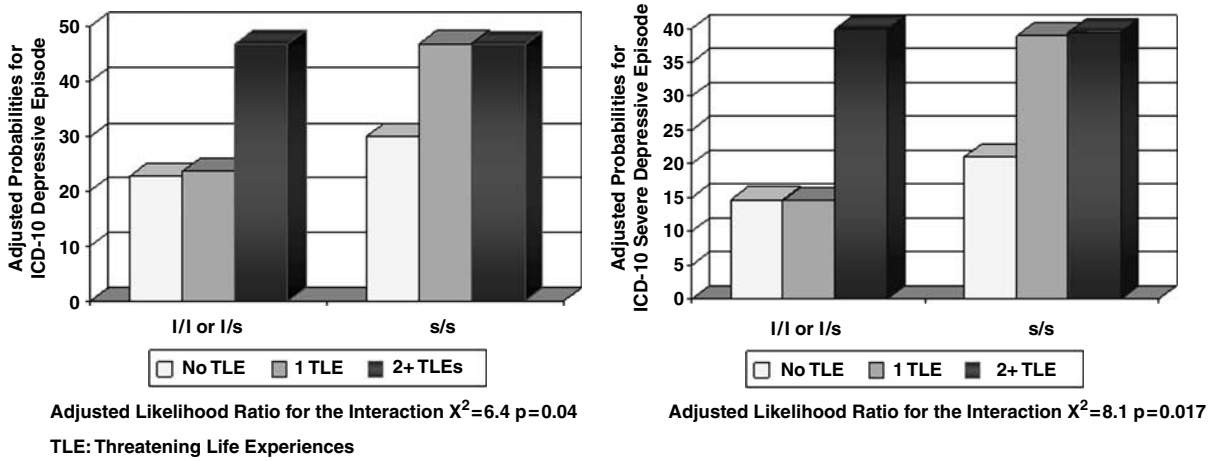

Figure 1 Adjusted s/s genotype by TLEs interaction effect on probabilities for depression.

level of threat conveyed by the stressful experiences. Although two independent, large studies have failed to replicate previous reports of this particular gene–environment interaction,[24,25] their definitions of the depressive phenotypes examined may explain their findings. The study with the largest sample[25] used a self-report, potentially non-specific measure of both depression and life events. Moreover, the study was not designed to measure risk factors for depression, but was based on a cohort developed to investigate cancer and nutritional problems. The second study was also based on a self-report instrument for depression in a study of alcoholism that was adapted to identify cases of DSM-IV depression.[24] On the whole, our independent findings add to other positive studies that support the notion of a true 5HTTLPR by stressful life-events interaction first reported by Caspi *et al.*[12] and replicated by others.[18–23,34]

Accounting for gender, age and family history

Our results show a somewhat better model fit after adjustment for potential confounders, such as gender and family history. Age had little impact. The relationship between gender and this particular gene–environment interaction is puzzling as some studies have reported it as valid for both sexes,[20,21,23,34] whereas others suggest an effect only in women[18,19] or even an inverse effect in men.[22] We found no statistically significant differences in the reported gene–environment interaction when women were compared to men. Family history of psychological problems has been associated with both exposure[36] and outcome,[37] and thus should remain in the model. The independence from family history of our reported gene–environment interaction may suggest that there could be some specific role for the 5HTTLPR genotype (or the serotonin transporter gene) in its modification of the risk effect for depression conferred by previous TLEs. Nevertheless, there is a report for a different candidate gene for depression also interacting with stressful life experiences, although the sample studied was one of affective disorders sufferers with no controls.[34]

In conclusion, our findings add further evidence, from a case-control study nested in a Spanish cohort of adult primary-care attendees, in favour of an effect modification by the 5HTTLPR genotype on the risk of depression conferred by previous exposure to stressful life-events.

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Artículo 3

High-Activity Variants of the uMAOA Polymorphism Increase the Risk for Depression in a Large Primary Care Sample

*Margarita Rivera, Blanca Gutiérrez, **Esther Molina**, Francisco Torres-González, Juan A. Bellón, BertaMoreno-Küstner, Michael King, IrwinNazareth, Luis J. Martínez González, Esther Martúnez-Espıín, María M. Muñoz-García, Emma Motrico, Teresa Martúnez-Cañavate, José A. Lorente, Juan D. Luna, and Jorge A. Cervilla.*

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High-Activity Variants of the uMAOA Polymorphism Increase the Risk for Depression in a Large Primary Care Sample

Margarita Rivera,[1] Blanca Gutiérrez,[1] Esther Molina,[1] Francisco Torres-González,[1] Juan A. Bellón,[2] Berta Moreno-Küstner,[3] Michael King,[4] Irwin Nazareth,[5] Luis J. Martínez-González,[6] Esther Martínez-Espín,[6] María M. Muñoz-García,[1] Emma Motrico,[3] Teresa Martínez-Cañavate,[7] José A. Lorente,[6] Juan D. Luna,[8] and Jorge A. Cervilla[1]*

[1]Sección de Psiquiatría e Instituto de Neurociencias, Universidad de Granada, Granada, Spain

[2]Centro de Atención Primaria "El Palo", Unidad de Investigación de Atención Primaria del Distrito Málaga, grupo SAMSERAP, Departamento de Medicina Preventiva, Universidad de Málaga, Málaga, Spain

[3]Unidad de Investigación de Atención Primaria del Distrito Málaga, grupo SAMSERAP, Departamento de Psicología Social, Universidad de Málaga, Málaga, Spain

[4]Department of Mental Health Sciences, University College London, Royal Free Campus, London, UK

[5]Research Department of Primary Care & Population Health, University College London & MRC General Practice Research Framework, London, UK

[6]Laboratorio de Identificación Genética, Departamento de Medicina Legal, Toxicología y Psiquiatría, Universidad de Granada, Granada, Spain

[7]Coordinadora de programas, Fundación IAVANTE, Granada, Spain

[8]Departamento de Bioestadística, Universidad de Granada, Granada, Spain

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Studies on the association between the functional uMAOA polymorphism and depression have yielded non-conclusive results up till now. One thousand two hundred twenty eight consecutive Spanish primary care attendees, participating in the PREDICT study, agreed to take part in this genetic PREDICT-Gene study. We explored the association between depression and either high-activity uMAOA alleles or genotypes. Depression was diagnosed using the Composite International Diagnostic Interview (CIDI) to establish three different depressive outcomes (ICD-10 Depressive Episode (DE), ICD-10 Severe Depressive Episode (SDE) and DSM-IV Major Depression (MD)). uMAOA genetic variation was determined by PCR amplification and subsequent electrophoresis. Crude and adjusted (gender and/or age) odds ratios, with 95% confidence intervals, were calculated for the associations between allele or genotype frequencies and all three depressive outcomes. We found associations between all three depressive phenotypes and either high-activity alleles or high-activity genotypes in both

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*Correspondence to:
Prof. Jorge A. Cervilla, M.D., M.Sc., MRCpsych, Ph.D., Section of Psychiatry and Institute of Neurosciences, Faculty of Medicine, University of Granada, Avenida de Madrid 11, 18012 Granada, Spain.
E-mail: jacb@ugr.es

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sexes. The associations were statistically significant for females but not for males. Testing the same associations on the entire sample (males and females) also yielded significant associations between depression and either high-activity alleles or high-activity genotype distribution that were independent of age and/or gender (ICD-10 DE: OR = 1.98; 95% CI: 1.42–1.77; $P = 0.00002$; ICD-10-SDE: OR = 2.05; 95% CI: 1.38–3.05; $P = 0.0002$; DSM-IV MD: OR = 1.91; 95% CI: (1.26–2.91); $P = 0.0014$). Our results provide fairly consistent evidence that high-activity variants of the MAOA promoter polymorphism confer a modestly higher risk for depression. © 2008 Wiley-Liss, Inc.

Key words: MAOA; uMAOA polymorphism; depression; association study

INTRODUCTION

Monoamine Oxidase A (MAOA) has a central role in the degradation of monoamines which are involved in the etiology of depression, such as serotonin and noradrenaline [Bach et al., 1988; Berry et al., 1994]. Moreover, MAOA inhibitors, such as tranylcypromine or moclobemide, are well recognized treatments for depression [Nolen, 2003; Frieling and Bleich, 2006; Papakostas and Fava, 2006]. Along the past few years, the MAOA gene has been investigated as a candidate gene for depression, with a particular focus on the uMAOA polymorphism originally described by Sabol et al. [1998]. This is a VNTR polymorphism located in the promoter region of the MAOA gene which consists of a 30 bp repeated sequence that can be present in 3, 3.5, 4, and 5 copies. Experiments with transfected cells and cell cultures of human skin fibroblasts have shown that this is the only polymorphism associated with the transcriptional activity of this gene [Sabol et al., 1998]. In particular, allele 3 seems to be associated with lower MAOA activity whereas alleles 3.5, 4, and 5 are related to higher MAOA activity [Deckert et al., 1999; Denney et al., 1999].

Investigations of the association between uMAOA polymorphism and affective disorders have yielded conflicting results. High-activity variants at the uMAOA promoter polymorphism have been reported to increase the risk for depression [Schulze et al., 2000; Yu et al., 2005] and/or some of its clinical correlates such as suicidal behavior [Ho et al., 2000; Jollant et al., 2007], neuroticism [Eley et al., 2003], impulsiveness [Manuck et al., 2000], or seasonality [Manuck et al., 2000; Eley et al., 2003; Gutierrez et al., 2004]. Furthermore, the genotypes containing only high activity alleles have been found to be associated with major depression in women [Schulze et al., 2000] and with a poorer response to antidepressants [Yu et al., 2005]. However, other studies have failed to replicate such findings [Muramatsu et al., 1997; Furlong et al., 1999; Kunugi et al., 1999; Syagailo et al., 2001; Serretti et al., 2002; Gutierrez et al., 2004; Huang et al., 2004; Christiansen et al., 2007]. In contrast, an association between depressive symptoms and low activity uMAOA alleles (those with a three repeat) has been reported too [Brummett et al., 2007].

In light of the uncertainty emerging from the published research, we aimed to clarify the association between the uMAOA polymorphism and depression in a well-characterized large representative sample of primary care attendees.

METHODS

Study Design

The PREDICT study is a prospective study aiming to identify predictors of depression onset in primary care and its detailed method has been reported elsewhere [King et al., 2006]. The PREDICT-Gene study [Cervilla et al., 2006] explores the association between genetic risk factors and depression in a Spanish sample of primary care patients taking part in the PREDICT study of depression. The present PREDICT-Gene study is a case-control study nested in the PREDICT prospective study. Both the PREDICT Study and the PREDICT-Gene study were approved by all relevant local research ethical committees.

Sample and Power

All consecutive attendees to participating primary care practices in the areas of Málaga and Granada (Spain) were invited to take part in the genetic study. Data collection was completed between discrete periods starting in April 2004 and ending in December 2007. Participants aged over 75, unable to understand Spanish and those with an organic mental disorder and/or any terminal illness were excluded. After informed consent was obtained, a biological sample consisting of 10 cc of blood and/or up to 4 cheek swabs for saliva was collected.

Using EPI Info STATCAL power facility, we estimated the sample size that we would need to test a potential association between uMAOA high activity allele carriers and depression. We performed such calculations on the basis of the following assumptions: (a) reported frequencies for uMAOA high activity allele carriers (77%) in the Spanish population [Gutierrez et al., 2004]; (b) reported prevalences of DSM-IV major depression (20%) in Spanish primary care samples [Aragones et al., 2004]; and, (c) the most conservative effect size reported (OR = 1.74) as described elsewhere [Yu et al., 2005]. Our final sample size of 1,228 subjects (242 DSMIV depressed cases and 980 controls) exceeds the needed sample of 1,185 (237 DSMIV cases and 948 controls) calculated on the above grounds.

Independent Variables

The PREDICT risk factor assessment was shown to have adequate test-retest reliability [King et al., 2006]. Accurate information was gathered on socio-demographic data such as sex, age, education, marital status, living arrangements, and profession. To measure these factors we used either previously validated measures or subjected new measures to reliability testing at the outset of the study as described elsewhere [King et al., 2006].

uMAOA Genotyping and Classifying

Genomic DNA was extracted from both blood and saliva according to standard procedures. The uMAOA polymorphism in the promoter region of the MAOA gene was genotyped in all samples. Polymerase chain reaction (PCR) was performed in a total volume of 15 μl containing 50 ng of DNA, 0.25 μM of each primer (forward: 5′-ACA GCC TGA CCG TGG AGA AG-3′ and reverse: 5′-GAA

CGG ACG CTC CAT TCG GA-3′), 250 μM of each dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl and 0.2 U of Taq DNA polymerase. The samples were preheated to 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 66°C, and final extension at 72°C for 1 min. The polymerase chain reaction products were separated by electrophoresis on a 3% agarose gel and visualized by ethidium bromide staining. For the purpose of this study, and as suggested by previous authors [Deckert et al., 1999; Schulze et al., 2000; Gutierrez et al., 2004], alleles were classified into two groups, that is, low-activity alleles (three copies of the 30-bp repeat sequence) and high-activity alleles (3.5, 4, or 5 repeats). We also divided participants depending on their genotypes, that is, homozygous for low-activity alleles (3/3) versus carriers of high activity alleles (i.e., either homozygous (3.5/3.5; 4/4; 5/5) or heterozygous for high-activity alleles).

Dependent Variables: Measures of Depression

The depression section of the Composite International Diagnostic Interview (CIDI) was used to ascertain the diagnoses of ICD-10 Depressive Episode (mild, moderate or severe) and DSM-IV Major Depression. The CIDI was administered by trained lay-interviewers. We used three different depressive outcomes for this study, that is, ICD-10 Depressive Episode (ICD-10 DE), ICD-10 Severe Depressive Episode (ICD-10 SDE) and DSM-IV Major Depression (DSM-IV MD).

Statistical Analysis

Analyses were performed using the SPSS 15.0 statistical package. We tested all associations using three different depressive outcomes (ICD-10 DE, ICD-10 SDE, and DSM-IV MD). Analyses using ICD-10 SDE as an outcome excluded cases with mild or moderate depression. Provided that uMAOA polymorphism is on the X chromosome, we explored statistically all associations in the whole sample and also among men or women separately. Chi square tests were used to compare genotype and allele frequencies between those with and without depressive outcomes. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using binary logistic regression analyses to compare both allele or genotype frequencies among patients and controls. We successively analyzed the latter associations using all three depressive outcomes, first in women only, then among men only and, finally, in the whole sample. Crude and adjusted (by age and/or sex as appropriate) odds ratios were calculated for all the above regression analyses.

RESULTS

Sample and Frequencies

Out of 1,524 primary care patients who were approached to take part in the study, 1,228 (80.6%) participants provided informed consent to participate in the genetic study (PREDICT-Gene) and provided a biological sample. The frequencies for socio-demographic variables are summarized in Table I. In brief, 884 (72%) were women; the mean age was 50.3 years (SD = 15.2); all 1,228 participants were of Spanish origin; most were educated at least to the level of primary school, were married or living with a partner and were working either in or away from home (Table I). The point prevalence of ICD-10 Depressive Episode was 34.3% (421), that of ICD-10 Severe Depressive Episode was 26% (284) and that of DSM-IV Major Depression was 19.9% (243) (Table I). Participants who agreed to take part in the genetic analysis did not vary systematically from those who refused to give a genetic sample in terms of sex (female gender: 72% vs. 72.7%; $\chi^2 = 0.805$; $P = 0.43$), marital status (married: 69.6% vs. 68.3%; $\chi^2 = 0.598$; $P = 0.32$) or prevalence of depression (34.3% vs. 32.7%; $\chi^2 = 0.52$; $P = 0.28$, as for ICD-10 DE). Nor were there differences on these variables between participants in the genetic study and those who participated in the initial baseline assessments although people who provided a genetic sample were slightly but significantly older.

TABLE I. Demographic and Clinical Characteristics

Variable	Frequencies/mean
Sex (male/female)	344 (28%)/884 (72%)
Mean age	50.3 years (SD 15.2)
Education	
Illiterate	57 (4.6%)
Primary	750 (61%)
Secondary or higher	321 (34.4%)
Marital status	
Married/couple	855 (69.6%)
Single	193 (15.7%)
Widowed	108 (8.8%)
Divorced/separated	72 (5.9%)
Living arrangements	
Alone	95 (7.7%)
Other	1,133 (92.3%)
Profession	
Housekeeping	337 (27.5%)
Working	382 (31.1%)
Disabled/retired	390 (31.8%)
Other	119 (9.6%)
ICD-10 depressive episode	
Depressed	421 (34.3%)
Non depressed	807 (65.7%)
ICD-10 severe depressive episode[a]	
Depressed	284 (26.0%)
Non depressed	807 (74.0%)
DSM-IV major depression[b]	
Depressed	243 (19.9%)
Non depressed	980 (80.1%)

[a]ICD-10 severe depressive episode (mild and moderate cases excluded).
[b]DSM-IV Diagnosis not available for five participants.

Independent Associations Between Depression and uMAOA

Genotype frequencies were in Hardy–Weinberg equilibrium both in patients and controls. uMAOA allele and genotype distributions in patients with depression and controls are shown in Table II. Among females, both high-activity alleles and high-activity genotypes were significantly associated with the three depressive phenotypes but this did not hold true for males (see Table III and

TABLE II. Allele and Genotype Distribution of the uMAOA Polymorphism in Patients With Depression and Controls

	Alleles, n (%)				Genotypes, n (%)									
	3	3.5	4	5	3/3 or 3	3/4	4/4	3/3.5	4/3.5	3.5/3.5 or 3.5	3/5	4/5	5/5 or 5	3.5/5
ICD-10 depressive episode (DE), n = 421														
Male	21 (28.7)	1 (1.4)	50 (68.5)	1 (1.4)	21 (28.7)	—	50 (68.5)	—	—	1 (1.4)	—	—	1 (1.4)	—
Female	208 (29.9)	5 (0.7)	479 (68.8)	4 (0.6)	37 (10.6)	131 (37.6)	173 (49.7)	1 (0.3)	0	2 (0.6)	2 (0.6)	2 (0.6)	0	0
Total	229 (29.8)	6 (0.8)	529 (68.8)	5 (0.6)	58 (13.8)	131 (31.1)	223 (53)	1 (0.2)	0	3 (0.7)	2 (0.5)	2 (0.5)	1 (0.2)	0
ICD-10 severe depressive episode (SDE), n = 284														
Male	13 (29.4)	1 (2.3)	29 (66)	1 (2.3)	13 (29.4)	—	29 (66)	—	—	1 (2.3)	—	—	1 (2.3)	—
Female	150 (31.2)	1 (0.2)	326 (68)	3 (0.6)	25 (10.4)	98 (41)	113 (47)	1 (0.4)	0	0	1 (0.4)	2 (0.8)	0	0
Total	163 (31.1)	2 (0.4)	355 (67.7)	4 (0.8)	38 (13.3)	98 (34.4)	142 (50)	1 (0.4)	0	1 (0.4)	1 (0.4)	2 (0.7)	1 (0.4)	0
Controls, n = 807														
Male	95 (35)	6 (2)	164 (61)	6 (2)	95 (35)	—	164 (61)	—	—	6 (2)	—	—	6 (2)	—
Female	409 (38)	6 (1)	646 (60)	11 (1)	99 (18.5)	207 (38.6)	216 (40.3)	0	0	3 (0.6)	4 (0.7)	7 (1.3)	0	0
Total	504 (38)	12 (1)	810 (60)	17 (1)	194 (24)	207 (26)	380 (47)	0	0	9 (1)	4 (0.5)	7 (0.8)	6 (0.7)	0
DSM-IV major depression (MD), n = 243														
Male	9 (26)	0	24 (71)	1 (3)	9 (26)	—	24 (71)	—	—	0	—	—	1 (3)	—
Female	127 (30)	3 (1)	284 (68)	4 (1)	23 (11)	78 (37)	102 (49)	1 (0.5)	0	1 (0.5)	2 (1)	2 (1)	0	0
Total	136 (30)	3 (1)	308 (68)	5 (1)	32 (13.1)	78 (32.1)	126 (52)	1 (0.4)	0	1 (0.4)	2 (0.8)	2 (0.8)	1 (0.4)	0
Controls, n = 980														
Male	107 (34.5)	7 (2.3)	190 (61.3)	6 (1.9)	107 (34.5)	—	190 (61.3)	—	—	7 (2.3)	—	—	6 (1.9)	—
Female	490 (36.6)	6 (0.4)	833 (62.2)	11 (0.8)	113 (17)	260 (39)	283 (42)	0	0	3 (0.4)	4 (0.6)	7 (1)	0	0
Total	597 (36)	13 (1)	1,023 (62)	17 (1)	220 (22.4)	260 (26.5)	473 (48.4)	0	0	10 (1)	4 (0.4)	7 (0.7)	6 (0.6)	0

TABLE III. Low and High Activity uMAOA Allele and Genotype Frequencies in Patients With Depression Versus Controls

	Alleles				Genotypes			
	Low (%)	High (%)	OR (95% CI)	*P*	Low/low (%)	High/[a] (%)	OR (95% CI)	*P*
ICD-10 DE, n = 421								
Male	21 (29)	52 (71)	1.22 (0.82–1.81)	0.193	21 (29)	52 (71)	1.22 (0.82–1.81)	0.193
Female	208 (30)	488 (70)	1.45 (1.17–1.78)	0.0004	37 (11)	311 (90)	1.74 (1.22–2.47)	0.001
Total	229 (30)	540 (70)	1.42 (1.17–1.72)	0.0003	58 (14)	363 (86)	1.98 (1.42–2.77)	0.00002
ICD-10 SDE, n = 284								
Male	13 (30)	31 (71)	1.19 (0.73–1.93)	0.297	13 (30)	31 (71)	1.19 (0.73–1.93)	0.297
Female	150 (31)	330 (69)	1.36 (1.07–1.72)	0.0088	25 (10)	215 (90)	1.77 (1.18–2.68)	0.003
Total	163 (31)	361 (69)	1.33 (1.07–1.66)	0.0093	38 (13)	246 (87)	2.05 (1.38–3.05)	0.0002
Controls, n = 807								
Male	95 (35)	176 (65)			95 (35)	176 (65)		
Female	409 (38)	663 (62)			99 (19)	437 (82)		
Total	504 (38)	839 (62)			194 (24)	613 (76)		
DSM-IV MD, n = 243								
Male	9 (27)	25 (74)	1.30 (0.73–2.33)	0.229	9 (27)	25 (74)	1.30 (0.73–2.33)	0.229
Female	127 (30)	291 (70)	1.32 (1.04–1.68)	0.0207	23 (11)	186 (89)	1.53 (1.01–2.33)	0.024
Total	136 (30)	316 (70)	1.32 (1.05–1.66)	0.0160	32 (13)	211 (87)	1.91 (1.26–2.91)	0.0014
Controls, n = 980								
Male	107 (35)	203 (66)			107 (35)	203 (65)		
Female	490 (37)	850 (63)			113 (17)	557 (83)		
Total	597 (36)	1,053 (64)			220 (22)	760 (78)		

[a]High/low or high/high.

Fig. 1). Testing those associations on the entire sample (males and females) showed significant associations between depression and high-activity alleles or high-activity genotypes (ICD-10 DE: OR = 1.98; 95% CI: 1.42–1.77; $P = 0.00002$; ICD10-SDE: OR = 2.05; 95% CI: 1.38–3.05; $P = 0.0002$; DSMIV MD: OR = 1.91; 95% CI: (1.26–2.91); $P = 0.0014$). Adjusting all analyses by age and sex in the whole sample, or just by age when women and men were analyzed separately, did not alter the significance of the above reported

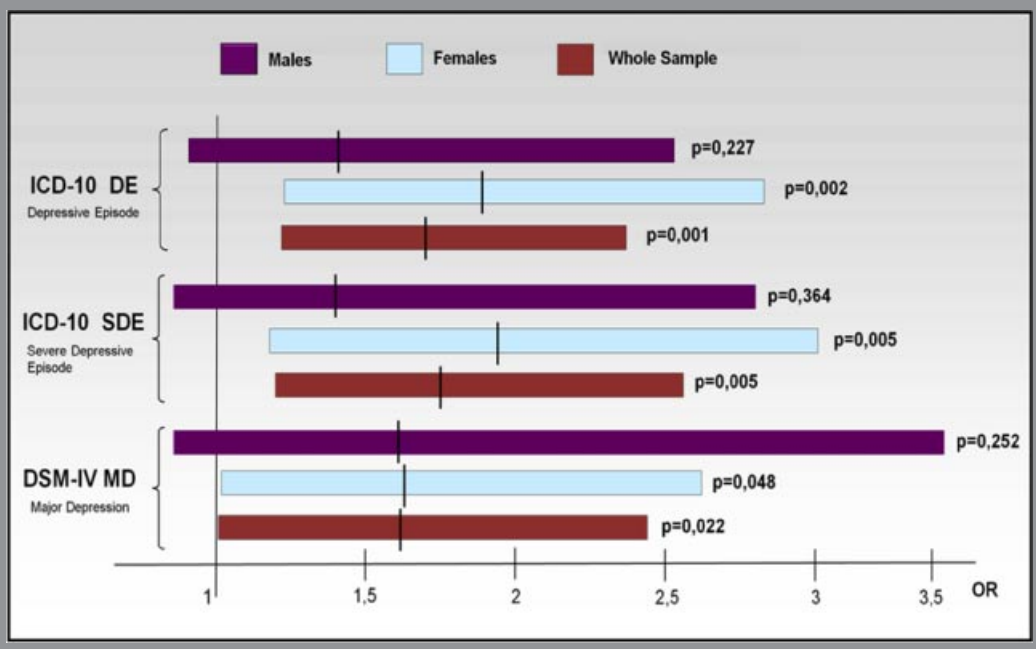

FIG. 1. Adjusted* Odds Ratio (95% CI) for the association between depression and high-activity uMAOA genotypes. *Adjusted by age when using female-only or male-only samples. *Adjusted by age and sex when using the whole sample (both male and female).

TABLE IV. Adjusted Odds Ratios for the Associations Between High Activity uMAOA Genotypes and Depression in Females and/or Males

	β	OR	95% CI	*P*-value
Results adjusted by age in male or female separately				
ICD-10 DE				
Male	0.353	1.423	0.803–2.523	0.227
Female	0.641	1.898	1.260–2.846	0.002
ICD-10 SDE				
Male	0.326	1.385	0.685–2.800	0.364
Female	0.664	1.943	1.216–3.103	0.005
DSM-IV MD				
Male	0.472	1.603	0.715–3.592	0.252
Female	0.484	1.622	1.003–2.622	0.048
Whole sample results adjusted by age and sex				
ICD-10 DE	0.534	1.707	1.228–2.372	0.001
ICD-10 SDE	0.551	1.734	1.177–2.555	0.005
DSM-IV MD	0.481	1.617	1.071–2.441	0.022

associations between uMAOA allele or genotype frequencies and all three depressive outcomes (please, see Tables III and IV and Fig. 1 for detailed results).

DISCUSSION

We have demonstrated that high-activity variants of the uMAOA promoter polymorphism are associated with depression measured using three different valid depressive phenotypes. This association was statistically significant in females but not in males, although among the latter there was also a non-significant trend for an association. To our knowledge, this is the first uMAOA association study conducted on a large representative population sample achieving internal validation of the results by testing the associations across three increasingly restrictive definitions of depression.

The Sample

The sample is one of consecutive primary care attendees who were thoroughly characterized for both demographic and clinical features. To the best of our knowledge, this sample is the largest ever used to test the association between depression and the uMAOA polymorphism. Hence, it provides sufficient statistical power to detect a potential association between a common mental disorder, such as depression, and a postulated small-effect candidate gene for depression, such as MAOA [Furlong et al., 1999; Kunugi et al., 1999]. Negative findings previously reported could be explained by the small sample size or/and a more heterogeneous sampling used in other research [Muramatsu et al., 1997; Furlong et al., 1999; Kunugi et al., 1999; Syagailo et al., 2001; Serretti et al., 2002; Gutierrez et al., 2004; Huang et al., 2004; Christiansen et al., 2007]. Allelic and genotype frequencies reported here are entirely compatible with those expected from previous reports using Caucasian samples [Schulze et al., 2000; Gutierrez et al., 2004]. Similarly, prevalences of depression in our sample are in line with those reported by other primary care studies [Gabarrón Hortal et al., 2002; Aragones et al., 2004]. Patients who agreed to take part in this genetic study were older than those refusing participation. However, our adjustment by age in the analyses did not modify the results. Population stratification is unlikely to influence our results as all 1,228 participants were of Spanish ethnic origin that was datable back at least to their grandparents.

The Association

Our findings are fully consistent with previous reports suggesting that the high-activity uMAOA alleles confer an increased risk for depression, at least among females [Schulze et al., 2000; Yu et al., 2005]. In light of other negative results [Muramatsu et al., 1997; Furlong et al., 1999; Kunugi et al., 1999; Syagailo et al., 2001; Serretti et al., 2002; Gutierrez et al., 2004; Huang et al., 2004; Christiansen et al., 2007] our findings support the notion that carriers of high-activity uMAOA alleles have an increase, yet modest, liability for depression. Further, our results are highly internally consistent as they were: (1) repeatedly tested across three different depressive phenotypes (ICD10-DE, ICD10-SDE, and DSM-IV MD); (2) using first allelic and then genotype frequencies; and (3) over the whole sample or separately among females or males alone. Although the association between uMAOA genetic variation and depression in males did not reach the conventional cut-off for statistical significance, we found a clear trend for the same positive association among males too. Such lack of significance could be explained by the smaller numbers of men in the sample and it is possible that if we had recruited more men the association might have reached statistical significance. Furthermore, when analyzing the associations using the whole sample (including males and females), both high-activity alleles and genotypes do confer an increased risk for depression and this remains unchanged after adjusting for gender in the analysis. On the other hand, a better description of MAOA functionality could have been provided by addition of other potentially functional polymorphisms in the gene. We decided not to do so as the uMAO is the one polymorphism to have more clearly demonstrated to determine an effect on the gene's functionality and expression [Sabol et al., 1998; Deckert et al., 1999]. We also wanted to stick to the most widely reported polymorphism to adhere to our *a priory* hypothesis of a potential excess of negative results due to smaller samples in previous studies. Nevertheless, there are two other silent SNPs (T941G and T1460C) but they do not seem to imply protein structural changes and only one has been demonstrated to convey additional information plausibly leading to MAOA activity changes in vitro only [Hotamisligil and Breakefield, 1991].

From a neurobiological viewpoint, our findings are supported by evidence from neurochemical and neuropharamacological studies. Thus, MAOA high-activity would lead to a higher rate of monoamine degradation which, in turn, would lead to lower intersynaptic monoamine levels what have been traditionally implicated in depression [Yu et al., 2005]. Additionally, MAOA inhibitors are effective in the treatment of depression [Nolen, 2003; Frieling and Bleich, 2006; Papakostas and Fava, 2006]. These and other reports are congruent with our finding that high-activity variants of the uMAOA promoter polymorphism are associated with higher risk for depression. However, there is evidence that the uMAOA polymorphism may also be associated with other psychiatric conditions such as neuroticism, impulsiveness or aggressiveness [Manuck et al., 2000], seasonal pattern in affective disorders

[Gutierrez et al., 2004], antisocial personality [Contini et al., 2006], other personality disorders [Jacob et al., 2005] or depressive symptoms with poorer sleep quality [Brummett et al., 2007]. We postulate that uMAOA can, then, be a non-specific genetic marker for depression that confers a general vulnerability for neurotic-like conditions, clearly in interaction with either other genes and/or environmental factors. Future studies should focus on such potential interactions and also on a systematic review of research on the association between the uMAOA polymorphism and depression.

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ANEXO II

ARTÍCULOS CIENTÍFICOS DEL ESTUDIO PREDICT

A continuación se presentan aquellos artículos publicados en revistas científicas indexadas de impacto en relación a aspectos metodológicos del estudio PREDICT y al algoritmo predictivo de depresión (PREDICT-D).

Artículo 1

Prediction of depression in European general practice attendees: the PREDICT study

Michael King, Scott Weich, Francisco Torres-González, Igor Švab, Heidi- Ingrid Maaroos, Jan Neeleman, Miguel Xavier, Richard Morris, Carl Walker, Juan A Bellón Saameño, Berta Moreno-Küstner, Danica Rotar, Janez Rifel, Anu Aluoja, Ruth Kalda, Mirjam I Geerlings, Idalmiro Carraça, Manuel Caldas de Almeida, Benjamin Vicente, Sandra Saldivia, Pedro Rioseco and Irwin Nazareth.

BMC Public Health 6: 6, 2006

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Study protocol

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Prediction of depression in European general practice attendees: the PREDICT study

Michael King*[1], Scott Weich[2], Francisco Torres-González[3], Igor Švab[4], Heidi-Ingrid Maaroos[5], Jan Neeleman[6], Miguel Xavier[7], Richard Morris[8], Carl Walker[1], Juan A Bellón-Saameño[9], Berta Moreno-Küstner[3], Danica Rotar[4], Janez Rifel[4], Anu Aluoja[5], Ruth Kalda[5], Mirjam I Geerlings[6], Idalmiro Carraça[10], Manuel Caldas de Almeida[11], Benjamin Vicente[12], Sandra Saldivia[12], Pedro Rioseco[12] and Irwin Nazareth[13]

Address: [1]Department of Mental Health Sciences, UCL, London, UK, [2]Division of Health in the Community, University of Warwick, Coventry, UK, [3]Department of Psychiatry, University of Granada, Granada, Spain, [4]Dept. of Family Medicine, University of Ljubljana, Ljubljana, Slovenia, [5]Faculty of Medicine, University of Tartu, Tartu, Estonia5, [6]University Medical Center, Utrecht, Netherlands, [7]Faculdade Ciências Médicas, University of Lisbon, Lisbon, Portugal, [8]Department of Primary Care and Population Sciences, UCL, London, UK, [9]Department of Family and Community Medicine, Malaga, Spain, [10]Encarnação Health Centre, Lisbon, Portugal, [11]Mora Health Centre, Mora, Portugal, [12]Departamento de Psiquiatría y Salud Mental, Universidad de Concepción, Concepción, Chile and [13]Department of Primary Care and Population Sciences, UCL and Scientific Director, Medical Research Council General Practice Research Framework, UCL, London, UK

Email: Michael King* - m.king@medsch.ucl.ac.uk; Scott Weich - S.Weich@warwick.ac.uk; Francisco Torres-González - ftorres@urg.es; Igor Švab - igor.svab@mf.uni-lj.si; Heidi-Ingrid Maaroos - Heidi-Ingrid.Maaroos@ut.ee; Jan Neeleman - J.Neeleman@umcutrecht.nl; Miguel Xavier - xavierm@sapo.pt; Richard Morris - r.morris@pcps.ucl.ac.uk; Carl Walker - c.walker@medsch.ucl.ac.uk; Juan A Bellón-Saameño - jabellon@terra.es; Berta Moreno-Küstner - bertamk@ugr.es; Danica Rotar - Danica.rotar@guest.arnes.si; Janez Rifel - janez.rifel@MF.UNI-LJ.SI; Anu Aluoja - Anu.Aluoja@kliinikum.ee; Ruth Kalda - Ruth.Kalda@ut.ee; Mirjam I Geerlings - M.Geerlings@umcutrecht.nl; Idalmiro Carraça - xavierm@sapo.pt; Manuel Caldas de Almeida - jcaldasalmeida@aol.com; Benjamin Vicente - bvicent@udec.cl; Sandra Saldivia - ssaldivi@udec.cl; Pedro Rioseco - bvicent@udec.cl; Irwin Nazareth - .nazareth@pcpl.ucl.ac.uk

* Corresponding author

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Abstract

Background: Prevention of depression must address multiple risk factors. Estimating overall risk across a range of putative risk factors is fundamental to prevention of depression. However, we lack reliable and valid methods of risk estimation. This protocol paper introduces PREDICT, an international research study to address this risk estimation.

Methods/design: This is a prospective study in which consecutive general practice attendees in six European countries are recruited and followed up after six and 12 months. Prevalence of depression is assessed at baseline and each follow-up point. Consecutive attendees between April 2003 and September 2004 who were aged 18 to 75 were asked to take part. The possibility of a depressive episode was assessed using the Depression Section of the Composite International Diagnostic Interview. A selection of presumed risk factors was based on our previous work and a systematic review of the literature. It was necessary to evaluate the test-retest reliability of a number of risk factor questions that were developed specifically, or adapted, for the PREDICT study. In a separate reliability study conducted between January and November 2003, consecutive

general practice attendees in the six participating European countries completed the risk factor items on two occasions, two weeks apart. The overall response rate at entry to the study was 69%. We exceeded our expected recruitment rate, achieving a total of 10,048 people in all. Reliability coefficients were generally good to excellent.

Discussion: Response rate to follow-up in all countries was uniformly high, which suggests that prediction will be based on almost a full cohort. The results of our reliability analysis are encouraging and suggest that data collected during the course of PREDICT will have a satisfactory level of stability. The development of a multi-factor risk score for depression will lay the foundation for future research on risk reduction in primary care. Our data will also provide the necessary evidence base on which to develop and evaluate interventions to reduce the prevalence of depression.

Background

Depression will rank second to cardiovascular disease as a global cause of disability by 2020 [1]. It occurs in up to a quarter of general practice attendees [2,3], relapse is frequent up to 10 years from first presentation [4-6] and residual disability is common [7]. Prevalence is determined by exposure to risk factors that precipitate or maintain episodes of disorder. The two most consistently identified risk factors are low socio-economic status [8-10] and female sex [11]. Relative poverty and unemployment are associated with longer duration of episodes of depression rather than their onset [10,12]. Socio-economic risk factors that might conceivably be addressed include low income and financial strain [10,13], unemployment [10], work stress [14], social isolation [14,15], and poor housing [9]. Fixed factors such as a family history of depression [12] and personality play a part [16] but it is not yet known whether they act independently of other risk factors.

Prevention of depression must address multiple risk factors [17], include those at low and moderate risk [18] and be acceptable to the target population [19]. However, in contrast to physical disorders such as cardiovascular disease, many mutable risk factors affect the duration of episodes of depression, rather than simply their onset [20]. Estimating overall risk across a range of putative risk factors is fundamental to prevention of depression. However, we lack reliable and valid methods of risk estimation [21].

The PREDICT study is taking place in six European and one Latin American country in order to test the following hypotheses: 1) A reliable and valid multi-factor scale can be developed to determine the risk for the onset and maintenance of depression in primary care attendees; and 2) The overall risk equation derived from data for all countries combined will have similar accuracy in predicting episodes of depression for each country. In this introductory paper, we describe the method, response rates at baseline and first follow-up and the reliability of instruments developed or adapted for the study.

Methods/design

Design

This is a prospective study in which consecutive general practice attendees are recruited and followed up after six and 12 months. Prevalence of depression is assessed at baseline and each follow-up point. The study was approved by the relevant ethical committees in each country.

Setting

Six European and one Latin American centre are participating: 1) 25 general practices in the Medical Research Council's General Practice Research Framework, distributed across the United Kingdom; 2) nine large primary care centres in Andalucía, Southern Spain; 3) 74 general practices distributed nationwide in Slovenia; 4) 23 general practices distributed nationwide in Estonia; 5) seven large general practice centres near Utrecht, The Netherlands; 6) two large primary care centres in urban and rural areas of Portugal that include 25 general practitioners; and 7) 78 general practices in Chile. The general practices taking part extend over urban and rural settings in each country and populations with considerable socio-economic and ethnic variation.

Sample

Consecutive attendees between April 2003 and September 2004 who were aged 18 to 75 were asked to take part. Those over 75 were excluded because prevalence of cognitive impairment increases after that age. Other exclusion criteria were an inability to understand one of the principal languages involved, severe organic mental illness and terminal illness. Participants who gave informed consent subsequently undertook an interview at their home or the general practice within two weeks. Because of local service preferences the recruitment approach was slightly different in each country. In the UK and the Netherlands, researchers approached patients waiting to see the doctor,

while in the other countries the doctors raised the idea of the research first before the researcher was introduced.

Measures of outcome and exposure

Depression

The possibility of a depressive episode was assessed using the Depression Section of the Composite International Diagnostic Interview (CIDI) [22,23], which provides six month and lifetime psychiatric diagnoses according to ICD10 and DSMIV.

Risk factors for the depression

Selection of presumed risk factors was based on our previous work [24,25] and a systematic review of the literature. Where possible, we used published self-report measures of established reliability and validity. In some instances, questions were developed for the study or adapted from available standardised instruments. We addressed risk factors that are intrinsic either to the individual or to the social context, while remaining aware that there is inevitable overlap in such a categorisation. The risk factors in italics were assessed for test-retest reliability (see below).

• Socio-demographic factors.

• A lifetime history of depression (assessed by CIDI at baseline).

• *Controls, demands and rewards for unpaid work using an adapted version of the job content instrument* [26].

• Debt and financial strain [10].

• Consultation rate in the general practice [27].

• Self-rated physical health problems and limiting long-term disability using the Short Form 12, a brief, self-report disability schedule that has application across a number of cultures [28]

• Alcohol misuse using the WHO's AUDIT questionnaire [29]

• *Use of recreational drugs adapted from the relevant sections of the CIDI*.

• Brief questions on cigarette consumption

• For women, questions on menstruation, pregnancy and childbirth from the Patient Health Questionnaire (PHQ) [30].

• *Brief questions on the quality of sexual and emotional relationships adapted from a standardized questionnaire* [31].

• *Problems in people close to participants* [32].

• Childhood experiences of physical, emotional and sexual abuse [33]

• Nature and strength of spiritual beliefs [34].

• *Family psychiatric history: depression in first-degree family members requiring pharmacological or psychological treatment in primary or secondary care. Suicide in first degree relatives* [35].

• Anxiety symptoms using the anxiety section of the PHQ [30].

• One question on whether or not, and at what age, the participant had lost one or both parents by death.

• Household type and composition.

• *The living environment including satisfaction with neighbourhood and perception of safety inside/outside of the home, using questions from the Health Surveys for England* [36].

• Threatening life events in the preceding six months, using a brief validated checklist [37].

• *Experiences of discrimination based on a recent European study* [38].

• Adequacy, availability and sources of social support [39].

Assessment of test-retest reliability

Many of the items in the PREDICT risk factor assessment are either based on previously validated measures, or concern exposures that are likely to be reported with a high degree of reliability (e.g. age, sex, ethnicity and civil status). However, we needed to evaluate the test-retest reliability of a number of risk factor questions (noted in italics above) that were developed specifically, or adapted, for the PREDICT study. In a separate reliability study conducted between January and November 2003, consecutive general practice attendees in the six participating European countries were invited to complete the risk factor items on two occasions, two weeks apart. At the time of retest, we re-contacted participants (using the general practice/health centre letterheads), reminding them of the study. Questionnaires were completed by assisted interviews. Expert opinions regarding the appropriate interval between test and retest vary from an hour to a year, depending on the task; a test-retest interval of between two and 14 days is usual [40]. Two weeks is sufficient time for patients to have forgotten their first responses but for opinions to have remained stable. We did not attempt to

estimate validity of these measures, given that 1) there are many uncertainties in choosing a standard against which to validate patient reports of this type, and 2) patients' reports will form the basis of the eventual risk tool.

Data quality control

Data quality was monitored to ensure that the project yielded data of the highest validity and reliability.

Translation of instruments

We used standardised validated instruments available in the native language of all the participant countries. In those instances where this was not possible we translated standardised instruments from English to the relevant languages. Where we developed our own measures, these were also translated from English into the languages of the participant countries. Each translation was back-translated by professional translators and the penultimate version verified by the co-ordinating centre. No major discrepancy was identified in any of the back-translations.

Data checking

Locally, each interview was checked for completion by the interviewer. Quality assurance focused on the standardised training of researchers in the use of the CIDI and other questionnaires, in the recruitment and interviewing of patients and in data management. Over and above national team meetings a research coordinator made two assessments of each interviewer during the baseline interviews to monitor the interview process, assess adherence to the CIDI, provide structured feedback for improvement and manage other problems as they arose. Structured and standardised data quality control sheets are used to manage data and ensure its transfer to the coordinating centre (UK). Progress reports for each national centre are submitted every six months and critically assessed by the steering group at project management meetings. Each participating country double entered 10% of its data records and accepted a 1% error rate before deciding on full double entry.

Statistical analysis

We calculated test-retest agreement using the kappa statistic for questions with two response options and the intraclass correlation coefficient (ICC) for items with more than two ordinal categories. When both follow-ups are complete we shall 1) be able to identify risk factors for incidence of depression over six and 12 months, from participants who were not depressed at baseline; 2) be able to identify factors for recovery from depression over six and 12 months, from participants who were depressed at baseline; have extra data with which to predict episodes of depression over 6 months, by relating not only data available from baseline and 6 month time points, but also from 6 month to 12 month time points; 3) be able to determine time of onset and offset of episodes with greater precision and reliability over intervals of six, rather than 12 months; and 4) determine how incidence of, and recovery from, depression is associated with changes in risk factors over 6 and 12 months. We shall derive risk factor equations using logistic regression analysis on a randomly chosen 50% sample (training set). We shall then apply the equation for risk to the remaining 50% (test set). Actual occurrence of depression during follow up will be compared with the prediction using relative operating characteristics (ROC) curve analysis. We shall choose the point of the ROC curve corresponding to 70% specificity as a cut-off for estimating sensitivity for subjects in all countries combined (and for participants in each country) in the test set. Confidence intervals for sensitivity in each country will indicate country heterogeneity. If estimated sensitivity in a particular country is significantly worse than overall sensitivity, and this difference is clinically important, new risk factor equations will be derived which include country specific effects. The latter can include an allowance for differences in overall case rate, or varying impacts of certain risk factors. We shall test the new equation until no further reduction in heterogeneity is possible. If after developing the best possible equation, sensitivity is still substantially worse than 70% for any country at the 1% significance level, a new equation will be derived specifically for that country. We believe this is unlikely to prove necessary, as there is no reason to suspect that the model will differ across countries, given our wealth of knowledge about risk factors.

Statistical power and sample size

At the time our sample size was calculated, Chile's participation was not finalised and thus it was estimated on the basis of six participating countries. A DSMIV diagnosis of major depression will provide the primary outcome measure. Our estimate of numbers for the prospective study was based on 1) a specificity and sensitivity of our risk score of at least 70%; 2) an assumption of a case rate of depression of approximately 15% and no major heterogeneity between centres. This requires a sample size of 2193, which we then doubled to allow for development of the risk factor score on one random half of the population and testing on the other, and to allow for an attrition rate of 30%. Thus our target recruitment was 6266 or 1044 in each country. In evaluating test-retest reliability, we calculated item coefficients for all European countries combined. We aimed to recruit at least 200 participants to achieve an intraclass correlation coefficient (ICC) with 95% confidence intervals of ± 0.10, provided the true reliability exceeds 0.58 [40].

Table 1: Response rates

Country	Numbers approached	Numbers refused	Numbers not eligible	Total interviewed at baseline (% of eligible)	Total interviewed 6 months (%of baseline)
UK	3319	1681	313	1325 (44%)	1144 (86%)
Spain	1470	194	6	1270 (87%)	1008 (79%)
Slovenia	1405	276	10	1119 (80%)	1036 (93%)
Estonia	1370	270	6	1094 (80%)	1025 (94%)
The Netherlands	3089	1478	390	1221 (45%)	1162 (95%)
Portugal	1552	369	3	1180 (76%)	1049 (89%)
Chile	3000	82	79	2839 (97%)	2580 (91%)
All countries	**15205**	**4444**	**713**	**10048 (69%)**	**9004 (90%)**

Results

Response rates

The overall response rate at entry to the study was 69%, with the lowest rates in the UK and the Netherlands and the highest in Chile (table 1). We exceeded our expected recruitment rate, achieving a total of 10,048 people in all. Response rates at the six month follow-up point were very high; 12 months follow-up is not yet complete.

Data quality

The baseline error rates for data entry in each country were well below the 1% level of acceptability (table 2).

Test-retest reliability assessment of risk factor questions

285 general practice attendees (152 women and 133 men) completed the questions on two occasions. Numbers in each country ranged from 40 in Slovenia to a maximum of 67 in the Netherlands. Their mean age was 44.6 years (SD 16.0), which was close to the mean age of the eventual study population. Reliability coefficients were generally good to excellent [41,42] (table 3). Questions on unpaid work generated kappa and ICC in the fair to good range (0.59 to 0.70), except for one question concerning how often participants get help and support with unpaid work difficulties. This question also had relatively poor percentage agreement. Five of the six questions on recent discrimination had kappa coefficients in the fair to excellent range. Responses to the sixth, concerning discrimination on the grounds of skin colour, were skewed due to the small number of non-white participants. As a consequence the kappa coefficient was low, but there was very high percentage agreement.

Discussion

Most research into depression in primary care populations has focused on management of current disorders rather than prediction of risk or prevention of future episodes. When our follow-up is complete we shall be able to report on whether a risk assessment is possible in a general practice setting. Our main results to date are 1) that response rates to follow-up at 6 months are high and 2) our instruments have acceptable reliability.

Setting and response rates

Our study is based on general practice attendees and not on a probabilistic sample recruited in the community. However, most people with depression visit their GP, although many will not complain of depression and nor will their mood disorder be recognized [2]. Thus the epidemiology of depression in general practice closely mirrors that seen in the community, with the caveat that prevalence rates are higher in the former [2]. Although

Table 2: Data entry error rates

Country	Total no. of Questionnaires checked (A)	Total min no. of items per participant (B)	Total min no. of items input (A × B)	1% level of input errors (A × B) × 0.01	No of input errors made
UK	131	187	24497	245	39
Spain	127	187	23749	238	32
Slovenia	112	187	20944	209	116
Estonia	110	187	20570	206	63
Netherlands	121	187	22627	226	43
Portugal	125	187	23375	234	22
Chile	2839	187	530,893	5309	2495

Table 3: Reliability analysis by questionnaire

Questionnaire	Analyses	Reliability range of items not removed		Percent agreement range	
		Min	Max	Min	Max
Family History of Psychological Disorder	Kappa and ICC	0.70	1.00	82.35	100
Recreational Drug Use	Kappa	0.64	0.98	88.21	100
Unpaid Work	Kappa and ICC	0.59	0.70	67.14	86.94
Living Environment	ICC	0.59	0.74	72.34	75.80
Discrimination	Kappa	-0.01	1.00	96.38	100
Relationships with Others	ICC	0.64	0.73	64.84	83.67
Difficulties with Persons Close to you	Kappa	0.65	0.84	93.42	96.94

response rates at baseline were lower in the UK and The Netherlands than in other countries, response rate to follow-up in all countries is uniformly high, which suggests that prediction will be based on almost a full cohort. The lower response rate in the UK and the Netherlands may reflect the different recruitment process we undertook in those countries, in which the study was not so obviously endorsed by the GP. There may also be differences in the public's attitudes to research in those two countries, where recruitment is generally lower across a range of research. There were also differences in the geographical distribution of participating general practices in each country, some being more nationally extended than in others. This difference reflected the varying opportunities and networks available to the centres. We shall take account of this variation, particularly urban-rural differences, in our analysis of risk.

Data quality and reliability

Our data monitoring and management has ensured that data quality reaches a high standard across the centres. The results of our reliability analysis are encouraging and suggest that data collected during the course of PREDICT will have a satisfactory level of stability. Reliability increases with sample size and thus we also know that estimates reported here are more conservative than will be the case in the main study. The two week period used in this test-retest evaluation may not be equally appropriate for all questions. For example, answers to questions on unpaid work will have less stability over this time than those to questions on family history of psychological disorder or the living environment, since satisfaction and control at work depend on challenges and interactions that may change daily. A question on how often participants get help and support with unpaid work difficulties exhibited only moderate stability between test and retest and relatively poor percentage agreement. Thus, we shall not it include in the final analysis of prospective data. The question on racial discrimination will be retained as its reliability could not be assessed fairly in this data set.

Significance of the study

Depression accounts for one-fifth of all consultations with GPs [43]. Those affected experience similar levels of excess mortality [44] and reduced quality of life as people with chronic physical disorders [45]. The aim of our study is to break new ground by quantifying the future risk of episodes of depression in primary care settings. The development of a multi-factor risk score for depression will lay the foundation for future research on risk reduction in primary care. Just as in prevention of cardiovascular disease [46], effective interventions for depression will need to address multiple risk factor domains, extend to those at low or moderate risk and be acceptable to the target population. Our data will also provide the necessary evidence base on which to develop and evaluate interventions to reduce the prevalence of depression. In so doing up to 15% of people attending general practitioners will potentially benefit by identification of their risk for episodes of depression, with the consequent reduction of distress and absence from work.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

MK and IN originated the idea for the study, led on its design, obtained funding and coordinated the project and analysis of data. MK led on writing the paper and is the guarantor for the study. SW participated in the design of the study, and read and approved the final manuscript. RM participated in the design of the study and analysis of data for the manuscript. He also read and approved the final manuscript. CW participated in the recruitment of patients and the overall coordination of the project and helped to collect and analyse data for the paper. He also participated in writing the paper.

FT participated in the design of the study design and coordination of the research at the participating centre. JAB

and BM were involved in discussing study design, contributing to Spanish data collection and commenting on the results. All contributed to the paper.

IŠ participated in the design of the study and supervised the study in Slovenia. DRP coordinated the study in Slovenia. JR performed data checking for the Slovenian sample.

HIM participated in the project design and coordination. AA participated in the study design and managed the Estonian data collection. RK participated in the enrolment of GPs and patients and helped to perform Estonian data collection. All read and approved the final manuscript.

JN participated in the study design and coordination of the research in the Dutch context. MIG supervised and participated in the Netherlands data collection and data management and was involved in revising the manuscript.

MX participated in the design of the study, coordinated data collection in Portugal. IC and MCA collected and managed the Portuguese data. All three authors read and approved the manuscript.

BV supervised different stages of the study and contributed to the paper. SS coordinated the field study, performed data management and contributed to the paper. PR assisted in collection and management of data. All authors read and approved the final manuscript.

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Artículo 2

Development and Validation of an International Risk Prediction Algorithm for Episodes of Major Depression in General Practice Attendees. The PredictD Study

Michael King, MD, PhD; Carl Walker, BSc, PhD; Gus Levy, MSc; Christian Bottomley, PhD; Patrick Royston, DSc; Scott Weich, MBBS, DM; Juan Ángel Bellón Saameño, MD, PhD; Berta Moreno, PhD; Igor Svab, MD, PhD; Danica Rotar, MD, MSc; J. Rifel, MD; Heidi-Ingrid Maaroos, MD, PhD; Anu Aluoja, PhD; Ruth Kalda, MD, DrMedSci; Jan Neeleman, MD, PhD; Mirjam I. Geerlings, PhD; Miguel Xavier, MD, PhD; Idalmiro Carraça, MD, MSc; Manuel Gonçalves-Pereira, MD, MSc; Benjamin Vicente, MD, PhD; Sandra Saldivia, PhD; Roberto Melipillan, MSc; Francisco Torres-Gonzalez, MD, PhD; Irwin Nazareth, MBBS,

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ORIGINAL ARTICLE

Development and Validation of an International Risk Prediction Algorithm for Episodes of Major Depression in General Practice Attendees

The PredictD Study

Michael King, MD, PhD; Carl Walker, BSc, PhD; Gus Levy, MSc; Christian Bottomley, PhD; Patrick Royston, DSc; Scott Weich, MBBS, DM; Juan Ángel Bellón-Saameño, MD, PhD; Berta Moreno, PhD; Igor Švab, MD, PhD; Danica Rotar, MD, MSc; J. Rifel, MD; Heidi-Ingrid Maaroos, MD, PhD; Anu Aluoja, PhD; Ruth Kalda, MD, DrMedSci; Jan Neeleman, MD, PhD; Mirjam I. Geerlings, PhD; Miguel Xavier, MD, PhD; Idalmiro Carraça, MD, MSc; Manuel Gonçalves-Pereira, MD, MSc; Benjamin Vicente, MD, PhD; Sandra Saldivia, PhD; Roberto Melipillan, MSc; Francisco Torres-Gonzalez, MD, PhD; Irwin Nazareth, MBBS, PhD

Context: Strategies for prevention of depression are hindered by lack of evidence about the combined predictive effect of known risk factors.

Objectives: To develop a risk algorithm for onset of major depression.

Design: Cohort of adult general practice attendees followed up at 6 and 12 months. We measured 39 known risk factors to construct a risk model for onset of major depression using stepwise logistic regression. We corrected the model for overfitting and tested it in an external population.

Setting: General practices in 6 European countries and in Chile.

Participants: In Europe and Chile, 10 045 attendees were recruited April 2003 to February 2005. The algorithm was developed in 5216 European attendees who were not depressed at recruitment and had follow-up data on depression status. It was tested in 1732 patients in Chile who were not depressed at recruitment.

Main Outcome Measure: *DSM-IV* major depression.

Results: Sixty-six percent of people approached participated, of whom 89.5% participated again at 6 months and 85.9%, at 12 months. Nine of the 10 factors in the risk algorithm were age, sex, educational level achieved, results of lifetime screen for depression, family history of psychological difficulties, physical health and mental health subscale scores on the Short Form 12, unsupported difficulties in paid or unpaid work, and experiences of discrimination. Country was the tenth factor. The algorithm's average C index across countries was 0.790 (95% confidence interval [CI], 0.767-0.813). Effect size for difference in predicted log odds of depression between European attendees who became depressed and those who did not was 1.28 (95% CI, 1.17-1.40). Application of the algorithm in Chilean attendees resulted in a C index of 0.710 (95% CI, 0.670-0.749).

Conclusion: This first risk algorithm for onset of major depression functions as well as similar risk algorithms for cardiovascular events and may be useful in prevention of depression.

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Author Affiliations are listed at the end of this article.

REDUCING THE PREVALENCE of depression is a public health challenge for the 21st century. Depression occurs in up to a quarter of general practice attendees,[1] relapse 10 years from first presentation is frequent,[2] and both residual disability and premature mortality are common.[3] Low socioeconomic status[4,5] and female sex[6] are the 2 most consistently identified risk factors. Socioeconomic risk factors include low income and financial strain,[4] unemployment,[4] work stress,[7] social isolation,[8] and poor housing.[5] Other factors, such as family history of depression, play a part.[9] Additional risk factors identified in adult general practice populations are negative life events, poor physical health, poor marital or other interpersonal relationships, a partner or spouse's poor health, and problems with alcohol.[10] Poor social support, loneliness, and physical disability appear to be particular risks for older adults.[11-13] Estimating overall risk across a range of likely risk factors is essential in efforts to prevent depression. However, effective strategies for prevention are hindered by lack of evidence about the combined effect of known risk factors. Our objectives were

to develop a risk algorithm for the onset of major depression in European general practice attendees and test its predictive power in a non-European setting. We modeled our approach on risk indexes for cardiovascular disease,[14] which provide a percentage risk estimate over a given period.

METHODS

STUDY SETTING AND DESIGN

We undertook a prospective study to develop a quantitative risk prediction algorithm for the onset of major depression over 12 months in general practice attendees. Given the relapsing and remitting nature of major depression, 12 months was considered a useful period for prediction in this setting. The method, described in detail elsewhere,[15] was approved by ethical committees in each country. The study was conducted in 6 European centers: (1) 25 general practices in the Medical Research Council General Practice Research Framework in the United Kingdom; (2) 9 large primary care centers in Andalucía, Spain; (3) 74 general practices nationwide in Slovenia; (4) 23 general practices nationwide in Estonia; (5) 7 large general practice centers near Utrecht, the Netherlands; and 6) 2 large primary care centers in the Lisbon area of Portugal. We assessed the external validity of the risk algorithm in patients attending 78 general practices in Concepción and Talcahuano in the Eighth Region of Chile. General practices covered urban and rural populations with considerable socioeconomic variation.

STUDY PARTICIPANTS

Consecutive attendees aged 18 to 75 years were recruited in Europe between April 2003 and September 2004 and in Chile between October 2003 and February 2005. Exclusion criteria were an inability to understand one of the main languages involved, psychosis, dementia, and incapacitating physical illness. Recruitment differed slightly in each country because of local service preferences. In the United Kingdom and the Netherlands, researchers spoke to patients waiting to see practice staff. In the remaining European countries, physicians introduced the study before contact with researchers. In Chile, attendees were stratified on age and sex according to figures for the populations served by each health center and participants selected randomly within each stratum. Participants gave informed consent and undertook a research evaluation within 2 weeks.

MAJOR DEPRESSION AND KNOWN RISK FACTORS

A *DSM-IV* diagnosis of major depression in the preceding 6 months was made using the depression section of the Composite International Diagnostic Interview (CIDI).[16,17] We selected risk factors to cover all important areas identified in a systematic review of the literature.[18] Where possible, we used standardized self-report measures. Questions adapted from standardized questionnaires or developed for the study were evaluated for test-retest reliability in 285 general practice attendees evenly recruited across the European countries before the main study began.[15] Each instrument or question not available in the relevant languages was translated from English and back-translated by professional translators.[15] The 39 candidate risk factors are numbered, and those subjected to test-retest reliability are italicized.

- (1) Age, (2) sex, (3) occupation, (4) educational level, (5) marital status, (6) employment status, (7) ethnicity, (8) owner-occupier accommodation, (9) living alone or with others, (10) born in country of residence or abroad, (11) satisfaction with living conditions, and (12) long-standing physical illness.
- (13) Lifetime depression was based on affirmative answers to both of the first 2 questions of the CIDI depression section.[19]
- *Stress in paid and unpaid work in the preceding 6 months using questions from the job content instrument.*[20] *Participants were categorized as feeling in control in (14) paid or (15) unpaid work; (16) experiencing difficulties without support in paid or unpaid work; and (17) experiencing distress without feeling respect for their paid or unpaid work.*
- (18) Financial strain using a question used in UK government social surveys.[4]
- Self-rated (19) physical and (20) mental health were assessed by the Short Form 12.[21] The weights used to calculate scores are from version 1.
- (21) Alcohol use in the preceding 6 months using the Alcohol Use Disorders Identification Test.[22] (22) We asked whether participants had ever had an alcohol problem or treatment for same.
- *(23) Whether participants had ever used recreational drugs using adapted sections of the CIDI.*
- *Questions on the quality of (24) sexual and (25) emotional relationships with partners or spouses.*[23]
- (26) Presence of serious physical, psychological, or substance misuse problems, or any serious disability, in people who were in close relationship to participants.
- *(27) Difficulties in getting on with people and maintaining close relationships.*[24]
- Childhood experiences of (28) physical and/or emotional and (29) sexual abuse.[25]
- (30) Holding religious and/or spiritual beliefs.[26]
- *(31) History of serious psychological problems or (32) suicide in first-degree relatives.*[27]
- (33) Anxiety and (34) panic symptoms in the previous 6 months using relevant sections of the Patient Health Questionnaire (PHQ).[28]
- *(35) Satisfaction with the neighborhood and (36) perceived safety inside/outside of the home using questions from the Health Surveys for England.*[29]
- (37) Major life events in the preceding 6 months using the List of Threatening Life Experiences Questionnaire.[30]
- *(38) Experiences of discrimination in the preceding 6 months on grounds of sex, age, ethnicity, appearance, disability, or sexual orientation using questions from a European study.*[31]
- (39) Adequacy of social support from family and friends.[32]

All participants were reevaluated for *DSM-IV* major depression, our main outcome, after 6 and 12 months using the depression section of the CIDI.

STATISTICAL ANALYSIS

All analyses and data imputation were performed using Stata release 9.[33] We included only patients without major depression at baseline. Participants with missing depression diagnoses at any point were excluded as this outcome was central to our risk estimation.

Data Imputation

Missing data in candidate risk factors were imputed using the method of chained equations, implemented in the Stata command ice.[34] We imputed 10 data sets[35] and obtained combined estimates.[36]

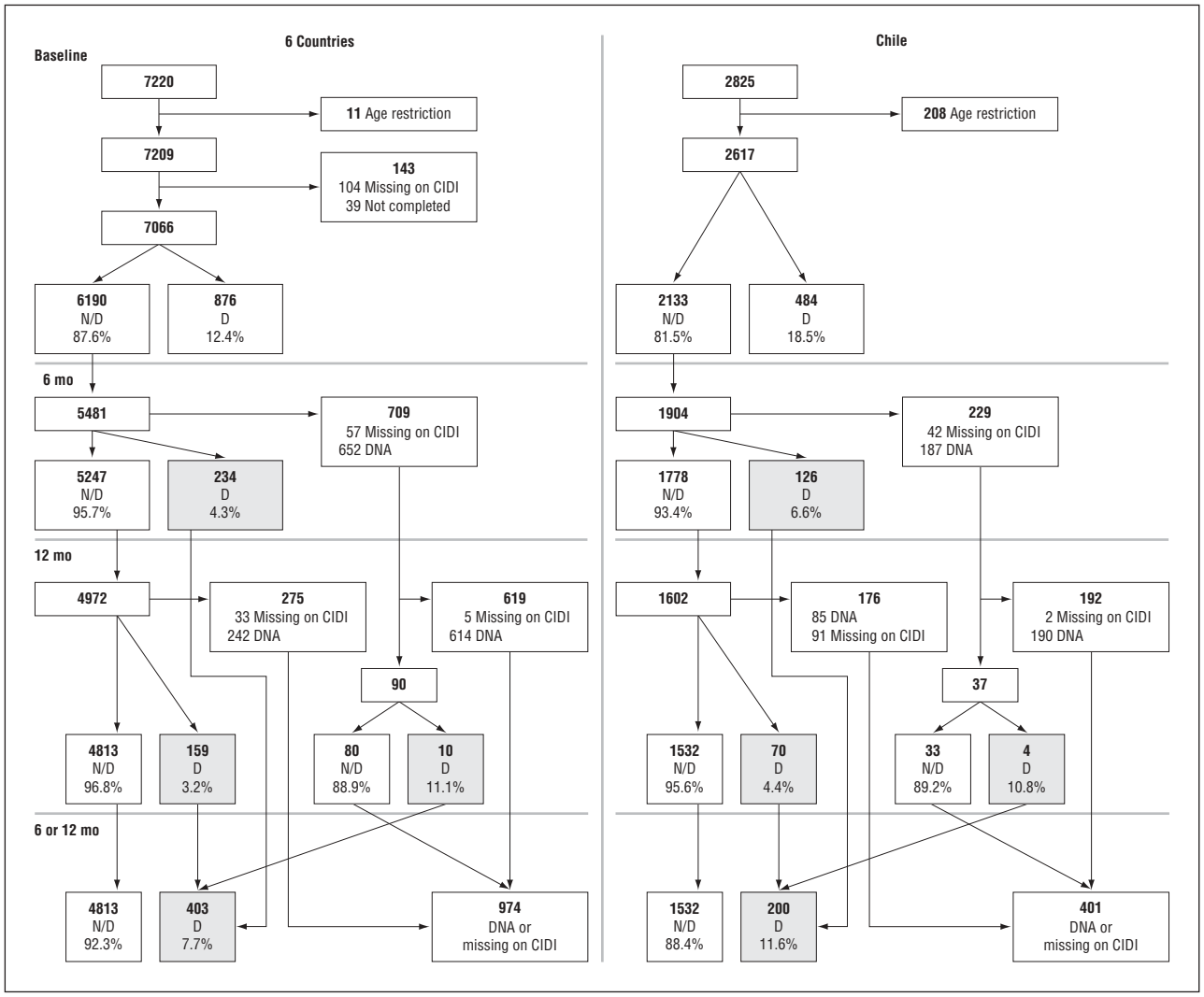

Figure 1. Flow of patients through the study and numbers becoming depressed. CIDI indicates Composite International Diagnostic Interview; DNA, did not attend; N/D, not depressed; and D, depressed.

Model Building

We built a risk model using the 39 risk factors described earlier and country of residence of each participant. We developed this model in the imputed data using stepwise logistic regression with robust standard errors to adjust for general practice clustering. We used a conservative threshold for inclusion of $P < .01$ to produce a stable model and minimize the degree of overfitting. We retained age and sex in all regression models because of their well-known associations with onset of depression.[37,38] We also retained country because of an a priori assumption of clustering within country. Multivariable fractional polynomial analysis was used to assess possible nonlinear effects of continuous predictors. The resulting risk score provides a predicted probability of depression over 12 months.

Internal Validation

We calculated the C index[39] to estimate the discriminative power of the final model in each European country and all European countries combined. We used a calculation proposed by Copas[40] to adjust for overfitting of our prediction model. This involves computing a shrinkage factor that is applied to the model coefficients to provide more accurate predictions when the risk algorithm is applied in new settings. To deal with the overfitting that arises through variable selection, we computed the shrinkage factor based on the initial model including all 39 variables. We assessed the goodness of fit of the final risk model by grouping individuals into deciles of risk and comparing the observed probability of major depression within these groups with the average risk. We calculated effect sizes using the Hedges g[41] for the difference in log odds of predicted probability between patients who were later observed to be depressed and those who were not. Finally, we report the threshold values of risk score, and the associated sensitivity, for a range of specificity that would be practical (minimizing false positives) when using the instrument in a clinical setting

External Validation

We used the C index, Hedges g, and a comparison of predicted vs observed probability of depression to evaluate the performance of the predictD model in the Chilean data.

RESULTS

In the 7 countries, 10 045 people took part (**Figure 1**). Response to recruitment was high in Portugal (76%), Es-

Table 1. Demographic Characteristics and Response to Follow-up

Characteristic	No. (%)							
	All European Countries	United Kingdom	Spain	Slovenia	Portugal	The Netherlands	Estonia	Chile
European	6190 (100)	1131 (18.3)	1006 (16.3)	1048 (16.9)	1005 (16.2)	1077 (17.4)	923 (14.9)	2133
Age, y, mean (SD)	48.9 (15.5)	52.2 (14.7)	50.8 (15.5)	48.8 (14.5)	50.2 (15.4)	48.9 (14.9)	41.6 (16.0)	47 (15.7)
Female	4081 (65.9)	750 (66.3)	689 (68.5)	660 (63)	649 (64.6)	668 (62)	665 (72)	1522 (71.4)
Marital status								
Married or living together	4491 (72.6)	844 (74.6)	708 (70.4)	732 (69.9)	750 (74.6)	827 (76.8)	630 (68.3)	1228 (57.6)
Separated or divorced	421 (6.8)	100 (8.8)	49 (4.9)	56 (5.3)	69 (6.9)	64 (5.9)	83 (9)	179 (8.4)
Single	872 (14.1)	121 (10.7)	181 (18)	152 (14.5)	132 (13.1)	121 (11.2)	165 (17.9)	521 (24.4)
Widowed	383 (6.2)	65 (5.8)	67 (6.7)	105 (10)	53 (5.3)	48 (4.5)	45 (4.9)	205 (9.6)
Missing	23 (0.4)	1 (0.1)	1 (0.1)	3 (0.3)	1 (0.1)	17 (1.6)	0	0
Household status								
Not living alone	5483 (88.6)	981 (86.7)	948 (94.2)	915 (87.3)	929 (92.4)	894 (83)	816 (88.4)	2039 (95.6)
Living alone	707 (11.4)	150 (13.3)	58 (5.8)	133 (12.7)	76 (7.6)	183 (17)	107 (11.6)	94 (4.4)
Missing	0	0	0	0	0	0	0	0
Education								
Higher education	1879 (30.4)	448 (39.6)	135 (13.4)	181 (17.3)	129 (12.8)	458 (42.5)	528 (57.2)	75 (3.5)
Secondary	2038 (32.9)	465 (41.1)	215 (21.4)	385 (36.7)	182 (18.1)	508 (47.2)	283 (30.7)	791 (37.1)
Primary/no education	1767 (28.6)	25 (2.2)	656 (65.2)	235 (22.4)	662 (65.9)	78 (7.2)	111 (12)	998 (46.8)
Trade/other	451 (7.3)	171 (15.1)	0	247 (23.6)	32 (3.2)	0	1 (0.1)	267 (12.5)
Missing	55 (0.9)	22 (1.9)	0	0	0	33 (3.1)	0	2 (0.1)
Employment								
Employed/full-time student	3256 (52.6)	574 (50.8)	349 (34.7)	563 (53.7)	486 (48.4)	602 (55.9)	682 (73.9)	749 (35.1)
Unemployed	300 (4.8)	21 (1.9)	62 (6.2)	53 (5.1)	108 (10.7)	35 (3.2)	21 (2.3)	243 (11.4)
Unable to work	322 (5.2)	86 (7.6)	101 (10)	16 (1.5)	38 (3.8)	48 (4.5)	33 (3.6)	69 (3.2)
Retired/looking after family	2269 (36.7)	450 (39.8)	493 (49)	409 (39)	372 (37)	358 (33.2)	187 (20.3)	1072 (50.3)
Missing	43 (0.7)	0	1 (0.1)	7 (0.7)	1 (0.1)	34 (3.2)	0	0
Professional status								
Yes	1313 (21.2)	309 (27.3)	107 (10.6)	165 (15.7)	96 (9.6)	373 (34.6)	263 (28.5)	29 (1.4)
Missing	143 (2.3)	27 (2.4)	3 (0.3)	3 (0.3)	0	56 (5.2)	54 (5.8)	3 (0.1)
Born in country of residence								
Yes	5655 (91.4)	1054 (93.2)	955 (94.9)	834 (79.6)	973 (96.8)	997 (92.6)	842 (91.2)	2122 (99.5)
Missing	87 (1.4)	3 (0.3)	3 (0.3)	4 (0.4)	0	24 (2.2)	53 (5.7)	4 (0.2)
Ethnicity								
White European	5988 (96.7)	1055 (93.3)	994 (98.8)	1042 (99.4)	992 (98.7)	983 (91.3)	922 (99.9)	0
Missing	72 (1.2)	39 (3.4)	1 (0.1)	2 (0.2)	0	30 (2.8)	0	0
6-mo Response	5538 (89.5)	987 (87.3)	794 (78.9)	963 (91.9)	889 (88.5)	1035 (96.1)	870 (94.3)	1904 (89.3)
12-mo Response	5319 (85.9)	965 (85.3)	731 (72.7)	927 (88.5)	864 (86)	988 (91.7)	844 (91.4)	1748 (82)

tonia (80%), Slovenia (80%), and Chile (97%) but lower in the United Kingdom (44%) and the Netherlands (45%). Ethical considerations prevented the collection of data on nonresponders at baseline. Across all countries, the response to follow-up was at 89.5% at 6 months and 85.9% at 12 months. Women predominated in each country and prevalence of major depression at baseline was 13.9% in women and 8.5% in men. Seven thousand two hundred nine European participants had full CIDI data at recruitment to allow a depression diagnosis, of whom 6190 were not depressed at recruitment (**Table 1**). Of these, 5216 (84.3%) had full CIDI data for a depression diagnosis at 6 and 12 months' follow-up, and of these, 3972 (76.2%) also had full data on all 39 risk factors. Cumulative 12 months' incidence of *DSM-IV* major depression in the European population was 7.7% (United Kingdom, 8.8%; Spain, 15.1%; Slovenia, 4.2%; Portugal, 8.5%; the Netherlands, 5.4%; and Estonia, 5.9%). Missing information was less than 3% for 38 of the 39 risk factors; however, 12.6% of participants had missing data on their emotional relationship with a spouse or partner (risk factor 25 in the "Major Depression and Known Risk Factors" subsection).

DEVELOPMENT OF THE RISK PREDICTION ALGORITHM IN THE EUROPEAN DATA

In our reliability study prior to recruiting the cohort, all risk factors tested (except discrimination on skin color) produced κ coefficients of 0.59 to 1.00 and percentage of agreement of 67% to 100%. The κ coefficient for agreement on discrimination due to skin color was low because of the small number of nonwhite participants.[15]

The risk algorithm was developed on the 5216 European attendees who were not depressed at recruitment and who had data on our main outcome, *DSM-IV* major depression at 6 and 12 months. Nonlinear transformations of continuous variables did not significantly improve the model fit. Seven variables were retained at $P < .01$ and these were included with country, age, and sex in the regression model (**Table 2**). Five variables in the final model concerned past events or patient char-

Table 2. PredictD Model Derived in the Imputed Data Sets

Prognostic Factor	Levels in Factor	Coefficient	SE	Coefficient After Copas Shrinkage	*P* Value
Constant		1.543	0.439	1.155	<.001
Age	Each year	−0.005	0.005	−0.005	.25
Sex	F				
	M	−0.245	0.138	−0.212	.07
Education	Beyond secondary education				
	Secondary education	0.103	0.128	0.089	.42
	Primary or no education	0.472	0.157	0.409	.003
	Trade/other	0.653	0.210	0.566	.002
Difficulties in paid and unpaid work	No difficulties or often supported				
	Difficulties without support	0.423	0.114	0.366	<.001
Physical health	Each point on SF-12 subscale score; possible range, 0-100	−0.034	0.005	−0.030	<.001
Mental health	Each point on SF-12 subscale score; possible range, 0-100	−0.064	0.005	−0.055	<.001
First-degree relative with emotional problem	No				
	Yes	0.456	0.090	0.395	<.001
Discrimination	None				
	In 1 area	0.186	0.220	0.161	.40
	In >1 area	0.850	0.235	0.736	<.001
Lifetime depression	No				
	Yes	0.565	0.131	0.489	<.001
Country	United Kingdom				
	Spain	0.266	0.205	0.230	.20
	Slovenia	−0.841	0.193	−0.729	<.001
	Estonia	−0.540	0.196	−0.467	.006
	The Netherlands	−0.133	0.220	−0.115	.54
	Portugal	−0.195	0.180	−0.169	.28

Abbreviation: SF-12, Short Form 12.

Table 3. C Index Statistics for Each Country[a]

Country	C Index[b] (95% Confidence Interval)
United Kingdom	0.756 (0.705-0.808)
Spain	0.793 (0.746-0.840)
Slovenia	0.833 (0.775-0.891)
Estonia	0.761 (0.690-0.833)
The Netherlands	0.852 (0.799-0.905)
Portugal	0.747 (0.693-0.800)
Mean over all countries	0.790 (0.767-0.813)

[a] The C index is also known as the area under the relative operating characteristic curve of sensitivity against 1 – specificity. A perfect test has a C index of 1.00 while a test that performs no better than chance has a C index of 0.5.[41]

[b] Average C index over 10 imputed data sets.

acteristics (sex, age, education, results of lifetime depression screen, family history of psychological difficulties); 4, current status (Short Form 12 physical health subscale score, Short Form 12 mental health subscale score, unsupported difficulties in paid and/or unpaid work, and discrimination) (Table 2); and 1 concerned country. Examination of the risk model derived in each of the 10 imputed data sets revealed that it was stable in terms of the variables selected. Besides country, age, and sex, 5 variables (results of lifetime depression screen, family history of psychological difficulties, Short Form 12 physical health subscale score, Short Form 12 mental health subscale score, and unsupported difficulties in paid and/or unpaid work) were consistently selected in each of the imputed data sets. Discrimination was selected in 7 data sets and education, in 4 data sets. Three other variables that did not reach the full model were also selected in a number of imputed data sets. These were PHQ panic syndrome (6 sets), childhood sexual abuse (1 set), and PHQ anxiety syndrome (1 set).

We compared a model with interactions between sex and the remaining risk factors to the model with no interactions. A Wald test provided no evidence to suggest that including interaction terms improves the model fit (*P* value = .27; $\chi^2_{16} = 19.06$). There was also no evidence for including interactions with age (*P* value = .21; $\chi^2_{16} = 20.19$).

The average C index across countries for predicted probability of depression at 6 or 12 months in all 6 European countries was 0.790 (**Table 3**). The model was most predictive in the Netherlands (0.852) and least predictive in Portugal (0.747). The effect size for the difference in log odds of predicted probability between attendees in Europe who subsequently became depressed and those who did not was 1.28 (95% confidence interval [CI], 1.17-1.40) (**Table 4**). Again, the model discriminated best in the Netherlands (1.55) and least well in Portugal (0.99). To examine the fit of the model, we divided the European sample into deciles of predicted probability of depression on the predictD score. Within each decile, we plotted mean predicted probability vs observed probability of depression (**Figure 2**A). Figure 2A shows that the incidence of major depression in the highest decile of risk score in Europe was more than 30% in contrast to the overall incidence of 7.7%. Examples of the kinds of participants scoring at increasing levels of predicted

Table 4. Effect Sizes Computed Using Hedges g[a]

Country	Hedges g (95% Confidence Interval)
All European	1.28 (1.17-1.40)
United Kingdom	1.02 (0.78-1.27)
Spain	1.19 (0.97-1.41)
Slovenia	1.40 (1.06-1.75)
Estonia	1.09 (0.76-1.42)
The Netherlands	1.55 (1.25-1.85)
Portugal	0.99 (0.73-1.25)
Chile	0.87 (0.69-1.04)

[a] Predicted probabilities were logarithmically transformed and compared between depressed and nondepressed individuals over the subsequent 12 months. The Hedges g is preferred to the Cohen d where the sizes of each group (depressed/nondepressed) are markedly unequal. The risk score was computed using unshrunk estimates in Europe and shrunk estimates in Chile.

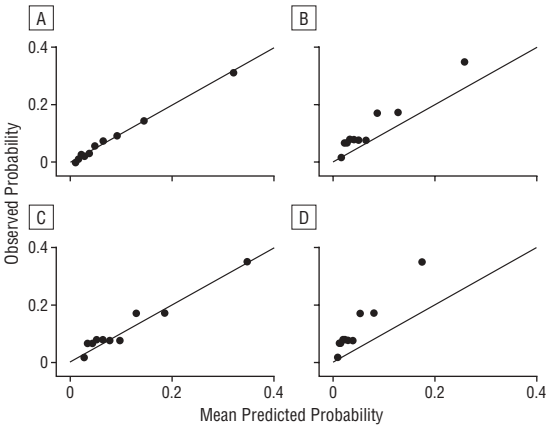

Figure 2. Plots of mean predicted probability against observed probability of depression within deciles of predicted risk. A, Model fitted in the European data using unshrunk coefficients. B-D, European model fitted in the Chilean data using shrunk coefficients. Risk scores are based on the average of the 6 European country coefficients (the UK coefficient is 0) (B) and the coefficients for Spain and Slovenia, respectively (C and D). Each point on the graphs represents a decile of risk.

Risk score (predicted probability of depression) at baseline 5%

A man aged 47 years living in Spain
Primary education
No difficulties or supported in paid and unpaid work
SF-12 mental subscale score 62
SF-12 physical subscale score 44
No personal history of depression
Family history of psychological difficulties
No experiences of discrimination

Risk score 10%

A man aged 55 years living in the Netherlands
Education beyond high school
No difficulties or supported in paid or unpaid work
SF-12 mental subscale score 30
SF-12 physical subscale score 56
No personal history of depression
Family history of psychological difficulties
Experience of discrimination in one area

Risk score 15% (8%)

A man aged 60 years living in the United Kingdom
Education beyond high school
Difficulties and unsupported in paid or unpaid work
SF-12 mental subscale score 56.4
SF-12 physical subscale score 29.7
Personal history of depression
No family history of psychological difficulties
Experience of discrimination in more than one area

Risk score 24% (11%)

A woman aged 62 years living in the United Kingdom
Education to high school
Difficulties and unsupported in paid or unpaid work
SF-12 mental subscale score 45.6
SF-12 physical subscale score 52.8
Personal history of depression
Family history of psychological difficulties
Experience of discrimination in more than one area

Risk score 33% (7%)

A man aged 55 years living in Spain
Education beyond high school
Difficulties and unsupported in paid or unpaid work
SF-12 mental subscale score 49.3
SF-12 physical subscale score 16.2
Personal history of depression
No family history of psychological difficulties
Experience of discrimination in more than one area

Figure 3. Examples of a range of predicted probabilities of depression at baseline. Mean (SD) Short Form 12 (SF-12) mental and physical subscale scores for Europe were 48.5 (10.6) and 44.2 (11.0), respectively. High scores indicate good health/well-being. Scores in parentheses correspond to eliminating discrimination and work difficulties and correcting SF-12 physical and mental health scores to the European mean (see text).

probability of depression on the predictD score algorithm are shown in **Figure 3**. To demonstrate the potential impact of mutable factors on risk, scores in the last 3 examples in Figure 3 were recalculated after mutable risk factors were reduced or eliminated. Estimates of sensitivity and specificity of the risk score in predicting major depression over 12 months are shown in **Table 5**. Questions in the risk algorithm can be tested at http://www.techflora.com/ucl and a risk score obtained. (The questions, responses, coding, and algorithm for the predictD risk tool are available on request.)

EXTERNAL VALIDATION OF THE RISK PREDICTION ALGORITHM IN THE CHILEAN DATA SET

Cumulative 12-month incidence of major depression in Chilean general practice attendees was 11.6%. There were no missing data in Chile on any of the 10 risk factors of the final European model. The model was validated using data on 1732 attendees who were not depressed at recruitment (Figure 1). The Copas shrinkage factor for the European model was 0.866, suggesting a degree of overfitting. We evaluated the prediction algorithm's external validity in the Chilean data using the shrunk regression coefficients derived in the European data and comparing predicted with observed probability. Because country is included in the model, it was necessary to base risk scores in Chile on an assumed country effect. Using the coefficient for Spain gave the best concordance between predicted and observed probability of major depression in Chile (Figure 2C and D) and reflects the prevalence of depression in Chile being more similar to Spain than Slovenia. The C index for the risk algorithm in Chile was 0.710 (95% CI, 0.670-0.749). This lower degree of discrimination can also be seen in the estimates of specificity and sensitivity in Chile (Table 5).

Table 5. Thresholds for Specificity and Sensitivity in Each Setting

	Predicted Probability of Major Depression, PredictD Risk Score	Specificity	Sensitivity	Likelihood, Ratio
Europe	0.106	0.800	0.645	3.22
	0.130	0.850	0.556	3.71
	0.165	0.900	0.464	4.64
Country				
United Kingdom	0.133	0.800	0.506	2.53
United Kingdom	0.154	0.850	0.458	3.06
United Kingdom	0.183	0.900	0.373	3.72
Spain	0.193	0.800	0.667	3.34
Spain	0.231	0.850	0.565	3.76
Spain	0.292	0.899	0.407	4.05
Slovenia	0.063	0.800	0.632	3.17
Slovenia	0.076	0.849	0.579	3.85
Slovenia	0.097	0.900	0.553	5.51
Estonia	0.088	0.801	0.571	2.86
Estonia	0.100	0.850	0.510	3.41
Estonia	0.130	0.900	0.408	4.09
The Netherlands	0.080	0.800	0.769	3.85
The Netherlands	0.096	0.850	0.731	4.87
The Netherlands	0.111	0.900	0.654	6.51
Portugal	0.117	0.800	0.452	2.26
Portugal	0.147	0.850	0.397	2.65
Portugal	0.185	0.899	0.356	3.54
Chile	0.089	0.800	0.525	2.63
Chile	0.109	0.850	0.410	2.73
Chile	0.138	0.900	0.320	3.20

COMMENT

We have developed a risk score from recognized risk factors for major depression over 12 months in 5216 general practice attendees in Europe and validated its use in 1732 attendees in Chile. To our knowledge, this is the first risk algorithm to be developed simultaneously in a number of cultures in one continent for prediction of new episodes of major depression in a general medical setting and validated in another continent. This is arguably the most rigorous test that can be applied to a prediction tool. We emphasize that our study was not about recognition of current depression, nor was it about a search for new risk factors; these are well known. Nor was it about developing a prognostic tool for outcome of depression, which has been achieved recently.[42] Our aim was to determine the key factors in a valid clinical prediction algorithm. Five risk factors are immutable (age, sex, educational level achieved, results of lifetime screen for depression, and family history of depression) and 4 are mutable factors relating to current status (Short Form 12 physical health and mental health subscale scores, unsupported difficulties in paid and/or unpaid work, and experiences of discrimination). The C index provides a standardized way of comparing the discriminative power of tests that use different measurement units in different settings.[43] The predictD risk score compares favorably with a risk index for cardiovascular events developed in 12 European cohorts[44] that reported C indexes between 0.71 and 0.82.

Our calculation of a shrinkage factor provides a measure of overfitting in the European data and allows for its adjustment in predicting risk of depression in new settings. External validation and shrinkage for overfitting are often not undertaken.[45,46] When the algorithm is applied in a country outside of the 6 participating European countries, we recommend that either the average country coefficient be used (Figure 2) or the coefficient for the European country that most closely matches the annual incidence of depression (if known) in the new setting.

Despite the advantages of a cross-national study and an external population in which to validate the risk algorithm, there are limitations to our study. Lower recruitment rates occurred in the United Kingdom and the Netherlands, possibly because the study was not so obviously endorsed by physicians. However, response to follow-up in all countries was high. There were differences in the geographical distribution of general practices in each country, which reflected the varying networks available to the centers. Follow-up was relatively short but in keeping with what would be acceptable for prediction of depression in general practice. People from nonwhite ethnic minorities were relatively underrepresented. Although our risk factors are based on self-report, we used standardized instruments, and nonstandardized questions were tested for reliability. Our data imputation retained power and reduced bias. Although 24% of European participants had missing data on at least 1 risk factor, as we reported, missing data were less than 3.0% on 38 of the 39 factors. Finally, we stress that our study did not aim to provide insights into pathways to depression. Rather, we aimed to develop a predictive tool for the detection of *DSM-IV* major depressive disorders prior to onset. Such an instrument could then be used for prevention of depression in a manner similar to an existing instrument used in cardiovascular prevention in

family practice settings.[14] Some of our risk factors in the predictD algorithm may be mediators on the pathway to depression. For example, childhood experiences of emotional abuse may make depression at an early age more likely, but once it has occurred, this will show up most parsimoniously in the algorithm as lifetime history.

Our study does not address how the risk algorithm for depression might best be implemented in general practice. However, the questions making up the algorithm are brief and easy to complete, and thus it has potential as a clinical tool for prediction of future episodes of depression in this setting (http:www.techflora.com/ucl). Our results expressed by the C index and effect sizes demonstrate a clear difference in risk between participants who became depressed and those who did not do so. In suggesting useful thresholds of sensitivity and specificity (Table 5), we have erred on the side of maximizing specificity at the cost of reduced sensitivity to minimize the workload for family physicians engaging with false positives. We would recommend setting specificity at 80% to 85% (risk score, ≥10.6%) to contain the workload of the physician, albeit at the cost of missing a proportion of future major depressive episodes.

Patients identified as being at risk on screening can be flagged on practice computers to alert physicians when they consult. Recognition of those at risk may be helpful when it leads to watchful waiting or active support, such as restarting treatment in patients with a history of depression. Advising patients on the nature of depression or on brief cognitive behavior strategies they might undertake to reduce their risk could also be envisaged. The application of such strategies to the prevention of depression in primary care would benefit from further evaluation. Four of the 10 factors were open to intervention/change and the impact of such change is shown in Figure 3. Efforts to reduce the incidence of depression might usefully address these factors through a combination of physical, psychological, and medical interventions. However, this implies that the risk model has a causal interpretation, something that our study cannot demonstrate. It also does not mean that when immutable factors predominate in any particular individual there can be no recourse to prevention. The introduction of brief cognitive behavior skills might be a preventive strategy regardless of the risk factors implicated. The same is true for starting or restarting antidepressant medication use.

CONCLUSIONS

This risk algorithm for major depression compares favorably with risk algorithms for prediction of cardiovascular events and may be useful in prevention of depression in general medical settings.

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Author Affiliations: Departments of Mental Health Sciences (Drs King and Walker and Mr Levy) and Primary Care and Population Sciences (Drs Bottomley and Nazareth), University College London, Medical Research Council General Practice Research Framework (Mr Levy and Dr Nazareth), and Medical Research Council Clinical Trials Unit (Dr Royston), London, and Health Sciences Research Institute, University of Warwick, Coventry (Dr Weich), England; Department of Preventive Medicine, El Palo Health Centre, Malaga (Dr Bellón-Saameño), and Department of Psychiatry, University of Granada, Granada (Drs Moreno and Torres-Gonzalez), Spain; Department of Family Medicine, University of Ljubljana, Ljubljana, Slovenia (Drs Švab, Rotar, and Rifel); Faculty of Medicine, University of Tartu, Tartu, Estonia (Drs Maaroos, Aluoja, and Kalda); University Medical Center, Utrecht, the Netherlands (Drs Neeleman and Geerlings); Faculdade Ciências Médicas, University of Lisbon (Drs Xavier and Gonçalves-Pereira), and Encarnação Health Centre (Dr Carraça), Lisbon, Portugal; and Departamento de Psiquiatría y Salud Mental, Universidad de Concepción, Concepción, Chile (Drs Vicente and Saldivia and Mr Melipillan).
Correspondence: Michael King, MD, PhD, Department of Mental Health Sciences, University College London Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, England (m.king@medsch.ucl.ac.uk).
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Correction

Error in Text. In the Original Article by King et al titled "Development and Validation of an International Risk Prediction Algorithm for Episodes of Major Depression in General Practice Attendees: The PredictD Study," published in the December issue of the *Archives* (2008;65[12]:1368-1376), an incorrect URL was given in the "Results" and "Comment" sections for the predictD algorithm. The algorithm can be found at http://www.ucl.ac.uk/predict-depression.

Artículo 3

Predicting the onset and persistence of episodes of depression in primary health care. The predictD-Spain study: Methodology

Juan Ángel Bellón, Berta Moreno-Küstner, Francisco Torres-González, Carmen Montón-Franco, María Josefa GildeGómez-Barragán, Marta Sánchez-Celaya, Miguel Ángel Díaz-Barreiros, Catalina Vicens, Juan de Dios Luna, Jorge ACervilla, Blanca Gutierrez, María Teresa Martínez-Cañavate, Bárbara Oliván Blázquez, Ana Vázquez-Medrano, María Soledad Sánchez-Artiaga, Sebastia March, Emma Motrico, Victor Manuel Ruiz-García, Paulette Renée Brangier-Wainberg, María del Mar Muñoz García, Irwin Nazareth, Michael King for the predictD group.

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Study protocol

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Predicting the onset and persistence of episodes of depression in primary health care. The predictD-Spain study: Methodology

Juan Ángel Bellón*[1], Berta Moreno-Küstner[2], Francisco Torres-González[3], Carmen Montón-Franco[4], María Josefa GildeGómez-Barragán[5], Marta Sánchez-Celaya[6], Miguel Ángel Díaz-Barreiros[7], Catalina Vicens[8], Juan de Dios Luna[9], Jorge A Cervilla[3], Blanca Gutierrez[3], María Teresa Martínez-Cañavate[10], Bárbara Oliván-Blázquez[11], Ana Vázquez-Medrano[5], María Soledad Sánchez-Artiaga[12], Sebastia March[13], Emma Motrico[2], Victor Manuel Ruiz-García[2], Paulette Renée Brangier-Wainberg[14], María del Mar Muñoz-García[3], Irwin Nazareth[15], Michael King[16] for the predictD group

Address: [1]Departamento de Medicina Preventiva, Universidad de Málaga; Unidad de Investigación de Atención Primaria de Málaga (redIAPP, grupo SAMSERAP), Centro de Salud El Palo, Spain, [2]Facultad de Psicología. Universidad de Málaga; Fundación IMABIS; Distrito Sanitario Málaga. Unidad de Investigación de Atención Primaria de Málaga (redIAPP, grupo SAMSERAP), Spain, [3]Departamento de Psiquiatría y Medicina legal, Universidad de Granada; Grupo Andaluz de Investigación en Salud Menta, Granada, Spain, [4]Departamento de Medicina y Psiquiatría, Universidad de Zaragoza; Centro de Salud Casablanca. (redIAPP, grupo Aragón), Zaragoza, Spain, [5]Servicio Riojano de la Salud; Unidad Docente de Medicina Familiar y Comunitaria de La Rioja, Logroño, La Rioja, Spain, [6]Servicio Madrileño de Salud; Área I de Atención Primaria, Unidad Docente de Medicina Familiar y Comunitaria, Madrid, Spain, [7]Servicio Canario de Salud, Gerencia de Atención Primaria de Gran Canaria, Centro de Salud Vecindario, Las Palmas, Spain, [8]Instituto Balear de la Salud; Unidad Docente de Medicina Familiar y Comunitaria de Mallorca, Centro de Salud son Serra-La Vileta, Palma de Mallorca, Illes Balears, Spain, [9]Departamento de Bioestadística (redIAPP, grupo SAMSERAP), Universidad de Granada, Spain, [10]Unidad Docente de Medicina Familiar y Comunitaria de Granada (redIAPP, grupo SAMSERAP), Spain, [11]Instituto Aragonés de Ciencias de la Salud, Unidad de Investigación de Atención Primaria (redIAPP, grupo Aragón), Zaragoza, Spain, [12]Servicio Madrileño de Salud, Área 6 de Atención Primara, Centro de Salud Condes de Barcelona-Boadilla, Madrid, Spain, [13]Instituto Balear de la Salud, Unidad de Investigación de Atención Primaria de Baleares (redIAPP, grupo Baleares), Mallorca, Spain, [14]Servicio Andaluz de Salud, Distrito Sanitario Málaga. Unidad de Investigación de Atención Primaria de Málaga (redIAPP, grupo SAMSERAP), Spain, [15]Medical Research Council General Practice Research Framework, London, UK and [16]Department of Mental Health Sciences, UCL, London, UK

Email: Juan Ángel Bellón* - JABELLON@terra.es; Berta Moreno-Küstner - bertamk@uma.es; Francisco Torres-González - ftorres@ugr.es; Carmen Montón-Franco - carmenmonton@able.es; María Josefa GildeGómez-Barragán - jggomez@riojasalud.es; Marta Sánchez-Celaya - mscelaya@gmail.com; Miguel Ángel Díaz-Barreiros - mdiazba@comlp.es; Catalina Vicens - domcatrin@telefonica.net; Juan de Dios Luna - jdluna@ugr.es; Jorge A Cervilla - jacb@ugr.es; Blanca Gutierrez - blancag@ugr.es; María Teresa Martínez-Cañavate - tmcanavate@samfyc.es; Bárbara Oliván-Blázquez - bolivan.iacs@aragob.es; Ana Vázquez-Medrano - avazquez@riojasalud.es; María Soledad Sánchez-Artiaga - malus209@hotmail.com; Sebastia March - smarch@ibsalut.caib.es; Emma Motrico - emm@uma.es; Victor Manuel Ruiz-García - vmruiz@uma.es; Paulette Renée Brangier-Wainberg - paulette.brangier@gmail.es; María del Mar Muñoz-García - marmg@ugr.es; Irwin Nazareth - i.nazareth@pcps.ucl.ac.uk; Michael King - m.king@medsch.ucl.ac.uk; the predictD group - JABELLON@terra.es

* Corresponding author

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Abstract

Background: The effects of putative risk factors on the onset and/or persistence of depression remain unclear. We aim to develop comprehensive models to predict the onset and persistence of

episodes of depression in primary care. Here we explain the general methodology of the predictD-Spain study and evaluate the reliability of the questionnaires used.

Methods: This is a prospective cohort study. A systematic random sample of general practice attendees aged 18 to 75 has been recruited in seven Spanish provinces. Depression is being measured with the CIDI at baseline, and at 6, 12, 24 and 36 months. A set of individual, environmental, genetic, professional and organizational risk factors are to be assessed at each follow-up point. In a separate reliability study, a proportional random sample of 401 participants completed the test-retest (251 researcher-administered and 150 self-administered) between October 2005 and February 2006. We have also checked 118,398 items for data entry from a random sample of 480 patients stratified by province.

Results: All items and questionnaires had good test-retest reliability for both methods of administration, except for the use of recreational drugs over the previous six months. Cronbach's alphas were good and their factorial analyses coherent for the three scales evaluated (social support from family and friends, dissatisfaction with paid work, and dissatisfaction with unpaid work). There were 191 (0.16%) data entry errors.

Conclusion: The items and questionnaires were reliable and data quality control was excellent. When we eventually obtain our risk index for the onset and persistence of depression, we will be able to determine the individual risk of each patient evaluated in primary health care.

Background

Depression as a public health problem

In 2001 depression was the third leading cause of disease burden in high-income countries [1]. In 2004 the total annual cost of depression in Europe was estimated to be 118 billion euros, or 253 euros per inhabitant [2]. The prevalence of major depression is about 7% in the community [3] and 14% in general practice attendees [4]. Relapse is frequent up to 10 years after the first presentation [5] and residual disability is common [6].

Risk factors for depression

The prevalence of depression is determined by exposure to risk factors that precipitate or maintain episodes of depression. With few exceptions, the prevalence and incidence of depressive disorders are higher in females than males, beginning at mid-puberty and persisting through adult life, although the determinants of gender differences are far from being established [7]. Socio-economic risk factors that might conceivably be addressed include low income and financial strain [8], unemployment [9], work stress [10], social isolation [11] and poor housing [12]. Relative poverty and unemployment are associated with a longer duration of episodes of depression rather than their onset [8], and depressive symptoms are also associated with subsequent unemployment and loss of family income [13]. Fixed factors such as a family history of depression [14] and personality [15] play a part but it is uncertain whether they act independently of other risk factors [16]. Physical health has been related to the onset and persistence of depression [17]. Stressful life events pose a greater risk for depression among women compared to men [18] and, like social support [19], these events also seem to be both a cause and a consequence of depression [20]. Many other candidate risk factors for depression exist, including for example childhood social disadvantage [21], childhood maltreatment [22], cigarette smoking [23], alcohol and drug abuse [24], and anxiety disorders [25].

However, the study of risk factors for depression suffers from limitations: First, it is often difficult to distinguish between their effects on the onset and on the course of depression; second, several risk factors may interact and be either a cause or a consequence of depression; and finally, few studies have controlled for candidate risk factors with comprehensive models, possibly due to the many possible factors involved.

Depression risk indexes

Effective strategies for preventing depression and reducing disease burden are hindered by a dearth of evidence about whether the risk for major depression can be quantified in the same way as other clinical disorders, such as cardiovascular diseases [26].

The predictD study is a pioneering international study whose main objective was to develop a risk index for the onset of episodes of major depression in general practice attendees [26]. The predictD international study recruited and followed-up a large sample of general practice attendees over one year. From 39 potential risk factors for depression, a risk index of 10 risk factors was drawn up with an excellent predictive power and good external validity [27].

The predictD-Spain study

Drawing on our experience as part of the predictD international study [28], the predictD-Spain study aimed to improve certain methodological aspects, extending the follow-up for three years, considering genetic factors in the equation (the predictD-Gene study), and studying professional and organizational factors as contributors to both the onset and persistence of episodes of depression (the predictD-Services study).

A genetic predisposition to depression may be a potential risk factor in the development of depression. Although the neurobiological equivalent of the predisposition remains unclear, the brain's serotonin system appears to play an important mediating role. Individuals with the 5-HTTLPR s/s genotype are more prone to develop depression [29], and this genotype may determine stress coping mechanisms and thereby increase stress vulnerability [30].

A recent systematic review identified only 17 longitudinal studies of depression in primary care, most of which involved small sample sizes or were relatively short-term [31]. The most usual risk factors for persistence of depression in primary care were severity and chronicity of the depressive episode, the presence of suicidal thoughts, poorer self-reported quality of life, lower self-reported social support, experiencing key life events, antidepressant use, lower education level and unemployment. However, whether differences exist in depression outcomes between patients whose depressive disorders are recognized and those whose disorders are unrecognised in primary health care is unclear [32].

In Spain, general practitioneers (GPs) failed to detect 30% of depressed patients [33], while only 30% of those diagnosed received appropiate treatment [34]. The suitable use of antidepressants, medication adherence, and 'case management' between mental health specialists and primary care professionals might be some of the best predictors for the recovery from depression [35]. Thus, the need to control for these factors in predictive models on the persistence of depression in primary care is clear [36].

Each anxiety disorder and panic attack appears to confer an independent risk for the onset of major depression [25] and an association between psychopharmacological treatment for generalized anxiety disorders and a lower risk of depression has been suggested [37]. However, anxiety disorders are not always adequately detected and managed by GPs [38]. Consequently, the detection and treatment of anxiety disorders might also condition the onset of depression.

Several GP factors are related with their ability to detect and manage psychosocial problems: gender, interview training, previous doctor-patient relationship or psychosocial orientation [39-42]. Concerning organizational factors, a recent meta-analysis showed that collaborative care for depression improved the outcome [43]. The most commonly used intervention features in the collaborative care were patient education and self-management, monitoring of depressive symptoms and treatment adherence, decision support for medication management, a patient registry, and mental health supervision of care managers [44]. Finally, one of the best professional-organizational factors associated with the recognition and good management of psychosocial problems in primary care is the length of interviews [42,45].

Accordingly, we aim to develop comprehensive models to predict the onset and persistence of episodes of depression in primary care. As well as individual, genetic, and environtmental risk factors, we are also considering other professional and organizational factors. In this report we explain the general methodology of the study and evaluate the reliability of the questionnaires used.

Methods/Design

Design

This prospective cohort study has recruited a systematic random sample of general practice attendees to be followed up after 6, 12, 24 and 36 months. The prevalence of depression and risk factors for depression are to be assessed at baseline and at each follow-up point. After excluding the patients with depression at baseline, the incidence of depression is to be measured at 6, 12, 24 and 36 months. This project is in compliance with the Helsinki Declaration and the relevant ethics committees in each province have approved the study. In representation of them, from the coordinator centre, the "*Comité de Ética e Investigación Sanitaria del Distrito Sanitario de Atención Primaria de Málaga*" approved the study.

Setting

Seven provinces are participating with 41 health centres and 231 GPs distributed throughout Spain: Malaga and Granada in southern Spain; Zaragoza and La Rioja in northern Spain; Madrid, capital of Spain, situated in the centre; Las Palmas in the Canary Islands; and Majorca in the Balearic Islands. Each health centre, which covers a population of 15,000 to 30,000 inhabitants from a geographically defined area, is staffed by GPs, who see patients over the age of 14 years, and by primary care paediatricians. The GPs in each health centre work as a group, with extensive primary care teams. The Spanish National Health Service provides free medical cover to 100% of the population. The health centres taking part extend over urban and rural settings in each province.

Sample and exclusion criteria

A systematic random sample taken at regular intervals of between 4 and 6 attendees, aged 18 to 75, has been recruited in six Spanish provinces. The 7th province, Malaga, started between October 2003 and February 2004 because it was already participating in the predictD international study [26]. The GPs introduce the study to the selected patients and request permission before contacting the assistant researcher. Patients over 75 years of age have been excluded because the prevalence of cognitive impairment increases after that age. Other exclusion criteria include inability to speak or understand Spanish (foreigners), severe organic mental disease and terminal illness, patients due to be away for more than 3 months during the coming year, and persons (representatives) who attend the surgery on behalf of the person who has the appointment (for example, to collect a prescription or a certificate). Participants who have given informed consent have an interview at the health centre within two weeks. The sample size was computed, using Obuchowski expression [46], to estimate the area under the ROC curve of the index to be obtained. Assuming an area under the ROC curve of 0.80 and a precision of 0.06, and considering an intraclass correlation coefficient of 0.05 with an average of 11 patients per GP in the cluster, the sample size needed was 3,474 patients for an incidence of major depression of about 12% per year.

Outcome measures

Our outcome variable is a depressive disorder. Depression is measured with the 12-month (or modified to 6-month) Depression Section of the Composite International Diagnostic Interview (CIDI) [47-49], which provides psychiatric diagnoses according to ICD10 and DSM-IV.

Risk factors for depression

The selection of risk factors for the onset and persistence of depression was designed to cover all important areas identified in a systematic review of the literature, considering specially those assessed in the predictD international study [26], in addition to other possible professional and organizational risk factors. Where possible, we used published measures with established reliability and international validity, including in Spain. Where this was not possible we translated the measures into Spanish. Each translation was back-translated by professional translators. In some cases, questions were developed for the study or adapted from available standardised instruments. These questions were evaluated for test-retest reliability. Scales without validation data in Spain were also evaluated on their internal consistency and factorial validity.

Individual and environmental risk factors

• Socio-demographic factors: age, marital status, occupation, employment status, ethnicity, nationality, country of birth, educational level, income, owner occupier accommodation, living alone or with others.

• Controls, demands and rewards for unpaid and paid work, using an adapted version of the job content instrument [50].

• Debt and financial strain [9].

• Consultation rate in the general practice through computerized clinical notes [51].

• Physical and mental well-being, assessed by the SF-12 that has application across a number of cultures [52], including Spain [53]; and a question on the presence of long-standing illness, disability or infirmity.

• Alcohol abuse using the WHO AUDIT questionnaire [54], the Spanish validation of which slightly modified the threshold for female hazardous drinkers [55,56].

• Use of recreational drugs (at least once in the past and over the previous six months) adapted from the relevant sections of the CIDI.

• A life-time screen for depression based on the first two questions of the CIDI. People answering yes to both questions screened positive [57].

• Brief questions on cigarette consumption.

• For women, questions on menstruation, pregnancy and childbirth from the Patient Health Questionnaire (PHQ) [58].

• Brief questions on the quality of sexual and emotional relationships with a partner, adapted from a standardized questionnaire [59].

• Presence of serious physical, psychological or substance misuse problems, or any serious disability, in persons who are close friends or relations of participants; and difficulty getting on with people and maintaining close relationships, assessed using questions from a social functioning scale [60].

• Childhood experiences of physical, emotional or sexual abuse [61].

• Nature and strength of spiritual beliefs [62].

• Family psychiatric history in first-degree family members requiring pharmacological or psychological treatment in primary or secondary care, and suicide in first-degree relatives [63].

• Anxiety symptoms using the anxiety section of the PRIME-MD [58]. The Spanish version provides psychiatric diagnoses according to DSM-IV: Panic Attack, Generalised Anxiety Disorder and Other Anxiety Disorders [64].

• One question on whether and when (at what age) the participant had lost one or both parents by death.

• Household type and composition.

• The living environment, including satisfaction with neighbourhood and perception of safety inside/outside the home using questions from the Health Surveys for England [65].

• Recent life-threatening events, using a brief validated checklist [66].

• Experience of discrimination on the grounds of sex, age, ethnicity, appearance, disability or sexual orientation using questions from a recent European study [67].

• Adequacy, availability and sources of social support from family and friends [68].

Genetic risk factors

The participants give their general informed consent and are asked for a new and specific informed consent on genetic tests. We collect saliva and/or blood for genetic testing, with DNA from both blood and saliva obtained by standard procedures. The 5-HTTLPR polymorphism at SLC6A4 is to be genotyped in all samples, as described [29,30].

Professional and organizational risk factors

This group of variables will be gathered from computerised clinical notes, centralised administrative records, and a brief questionnaire to the GPs at 12, 24 and 36 months.

• GP characteristics: age, gender, year of degree in Medicine, postgraduate training and speciality, type of contract, time in the current health centre, list size, mean time per patient during the previous year, satisfaction with relationships and collaborative care between GP and mental health team, social worker, and nurse practitioner, self-perceived comfort with antidepressant use, and a questionnaire on professional satisfaction, perception of workload, and psychosocial orientation [69].

• Health Centre characteristics: size of population attended, number of inhabitants in the city or town, predominant activity in the city or town (agriculture-fishing, industry or services), number and type of professionals in the team, professional-population ratios, type and intensity of relationship with Mental Health team (case management, patient care and shared continued medical education), and "centred variables" (mean or median of the GP characteristics in each health centre).

• Interaction professional-organization-patient variables: number of visits to health centre team, i.e., GP, nurse, and social worker; referrals to the Mental Health team by GPs or direct approaches to mental health specialists by the patient privately; patient's psychosocial and physical problems detected by their GPs; and antidepressants, benzodiazepines or other psychological drugs prescribed (type, dose and duration).

Data checking

Locally, each interview is checked for completion by the interviewer. Quality assurance is focused on the standardised training of researchers in the use of the CIDI and other questionnaires, on the recruitment and interviewing of patients and on data management. Over and above team meetings at the provincial level, a research coordinator assesses each interviewer twice during recruitment to monitor the interview process, verifies adherence to the CIDI and manages other problems as they arise. Progress reports for each province are submitted every two months and examined critically by the steering group at project management meetings. Each participating province double-enters 10% of its data records and a 1% error rate is accepted.

Statistical analysis

When all follow-ups are complete we shall be able to identify risk factors for the incidence (from participants not depressed at baseline) and recovery-persistence (from depressed participants) of depression over 6, 12, 24 and 36 months. The occurrence of major depression (yes/no) will be the dependent variable of multiple logistic regressions from a multilevel analysis that will discern three levels: patient, GP and health centre. The province will be included as a fixed factor since only seven units are involved. When we consider repeated measurements from different times of the follow-up, multilevel analysis will include four levels: time, patient, GP and health centre. A hierarchical model will be used to take into account the distribution of the data at different levels to estimate two types of variability, one due to individuals in the study and another due to the groups in which patients are nested. The candidate risk factors in each level will be included in the model using an entrance value of $P < 0.10$. For each level, the usefulness of including first-degree

interactions in the equation will be considered, and the interactions between levels will also be studied.

Missing data for candidate risk factors will be imputed using multiple imputation by chained equations (MICE), in which each variable is imputed using a regression model conditional on all the others, iteratively cycling through all the variables that contain missing data [70]. We will conduct the analysis in each of 10 imputed datasets and will obtain combined estimates; 10 imputed data sets is a common choice by convention [71]. We will repeat the analysis in just those participants with complete information as a sensitivity analysis. These analyses will be performed by the STATA "ice" command [72].

We will calculate the c-index [73] to estimate the discriminative power of the final model at each time. We will use a calculation proposed by Copas [74] to adjust for overfitting of our prediction models. We will assess the goodness of fit of the final risk model by grouping individuals into deciles of risk and comparing the observed probability of major depression within these groups with the average risk. We will calculate effect sizes using Hedge's g [75] for the difference in log odds of predicted probability between patients who will later be observed to be depressed and those who will not be depressed. All these analyses will be run with STATA release 10.

We have calculated test-retest agreement using the Kappa statistic, which adjusts for chance agreement, for questions with two response options and the Intraclass Correlation Coefficient (ICC) for items with more than two [76]. We have evaluated the internal consistency of the scales through Cronbach's alpha, and explored their subjacent factors through factorial analysis by principal components and varimax rotation. Reliability and validity analyses have been run with SPSS 14.

Results

Reliability

For the test-retest analysis, we selected a random sample of 401 patients stratified by province; 251 completed the predictD-Spain questionnaires as researcher-administered and 150 as self-administered questionnaires between October 2005 and February 2006. The respective distribution by province was: Madrid 41/39, Granada 40/38, La Rioja 49/24, Las Palmas 42/20, Malaga 41/0, Majorca 28/19, and Zaragoza 10/10. The mean number of days between test and retest was 11.0 (95% CI, 10.2–11.8; standard deviation = 7.5).

Additional file 1 shows reliability coefficients for all items and questionnaires evaluated. Most of the coefficients were good or excellent. Use of recreational drugs over the previous six months had poor agreement for the two methods of questionnaire administration. One item on the perception of safety inside the home had a coefficient of 0.80 (excellent) for researcher-administered and 0.37 (poor) for self-administered questionnaires, and another on cigarette consumption also had poor agreement (researcher-administered = 0.85 and self-administered = 0.40), although only for the self-administered way.

We evaluated the internal consistency of three scales: dissatisfaction with unpaid work, dissatisfaction with paid work and social support from family and friends. The respective Cronbach's alphas were good (Additional file 1).

Validity

Factorial analysis of the scale dealing with social support from family and friends found one factor that explained 58.7% of the variance. Factorial analysis of the scale dealing with dissatisfaction with paid work found three factors that explained 81.6% of the variance: F1 "feeling in control" (3 items = 28.3%), F2 "experiencing difficulty without support" (2 items = 26.4%), and F3 "experiencing distress without being respected" (2 items = 26.9%). Factorial analysis of the scale dealing with dissatisfaction with unpaid work differed slightly from the scale dealing with paid work. It found two factors that explained 77.3% of the variance: F1 "feeling in control without difficulties and with gratitude" (5 items = 50.4%) and F2 "experiencing distress without support and being respected" (2 items = 26.9%).

Error rates for data entry

The baseline error rates for data entry in each province are well below the 1% level of acceptability. We have checked 118,398 items from a random sample of 480 patients stratified by province and found only 191 errors (0.16%).

Discussion

The questionnaires used showed good reliability and the factorial validity of the three scales tested was coherent. Quality control of data was excellent.

Reliability and validity analysis

The results of reliability analyses were good or excellent for practically all the questionnaires and items. This suggests that data stability over time is satisfactory. We were interested in testing the questionnaires using both methods of administration, researcher-administered and self-administered, since, for cultural reasons; questionnaires administered to primary care attendees in Spain are almost always researcher-administered. Moreover, we also wanted to test whether self-administered questionnaires have different degrees of reliability as a result of a different socio-cultural patient profile (higher level of education, income and social class), the variability introduced by

interviewers, or other circumstances. In the end, the test-retest reliability was similar for most items with both methods of administration, although we did not measure inter-interviewer reliability. Nevertheless, several differences were interesting. The perception of safety inside the home had an excellent coefficient for researcher-administered questionnaires and a poor coefficient for self-administered questionnaires. Perhaps not all the patients interpreted this question the same way or maybe some were less sincere. We think that it was probably a "hot" question, perhaps interpreted as domestic violence, and, when the interviewers were not directly present; the patient may have felt less need to be sincere. It might also be that the question was a bit ambiguous. Fewer doubts concerned the absence of reliability for the item on the use of any recreational drugs over the previous 6 months. It contrasted with the good reliability of the same question in reference to the use of recreational drugs at least once. From the viewpoint of social desirability it is coherent, as it is considered better to try a drug than to consume it more often. A similar reasoning could be attributed to the item on cigarette consumption per day, although it is only a hypothesis. However, the predictD-International study obtained good coefficients for both time references on drug consumption [26]. In any case, we have decided not to include the use of recreational drugs over the previous six months in the final analysis of risk factors. The items on perception of safety inside the home and consumption of cigarettes per day will also be excluded from the final analyses.

Factorial analysis and Cronbach's alpha of the scale dealing with support from family and friends showed that its use as a single scale is appropriate. This is a conceptual difference with those questionnaires that separate support from family and support from friends [77]. The 7 items that we used on dissatisfaction with paid and dissatisfaction with unpaid work were adapted from the "Job Content Questionnaire" (JCQ), based on Karasek's demand-control-support model [78]. Our factorial analysis of paid work identified three factors that coincide with those obtained by the JCQ in different countries (job control, social support and psychological demands). However, factorial analysis of unpaid work changed. This was to be expected, as most unpaid work was probably related with looking after the family or the home, though we are unaware of any background to this question in the literature. Whatever the case, both Cronbach's alphas justified the use of each scale as a single scale.

Significance and practical implications of the study

These comprehensive models for the prediction of depression will facilitate the understanding of its causes, specifying the contribution of each risk factor and the importance of patient, environmental, genetic, professional and organizational factors. When we eventually obtain our risk index for the onset of depression, we will be able to determine the individual risk of each patient evaluated in primary health care. We hope to produce a simple prediction tool similar to the cardiovascular risk index [79]. Additionally, we plan to develop another risk index for recovery from depression, thus laying the foundation for future research on risk reduction in primary care.

Abbreviations

GPs: General Practitioners; ICD10: International Classification of Diseases, version 10; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th edition; WHO: World Health Organization; AUDIT: Alcohol Use Disorders Identification Test; PRIME-MD: Primary Care Evaluation of Mental Disorders.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JAB is guarantor for the predictD-Spain study. JAB, BM-K, FT-G, and CM-F obtained funding for implementing the study in Spain. The predictD-Spain study was designed and based on an idea by MK, IN, and the predictD-Europe core group. JAB coordinated the predictD-Spain study. BM-K, FT-G, CM-F, MJGdG-B, MS-C, MAD-B, and CV coordinated the study in each Spanish province. JAC and BG, based on the predictD-Spain and predictD-International study, designed the sub-study predictD-Gene and obtained funding for it. MTM-C, BO-B, AV-M, MSS-A, SM-LL, EM-M, VMR-G, PRB-W collaborated implementing the study in each province. JDL collaborated in the design, and JDL and JAB analysed the data. JAB drafted the paper and all authors agreed the final version.

Additional material

Additional file 1

Table 1. Reliability analysis by predictD – Questionnaires.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2458-8-256-S1.doc]

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