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GENOME-WIDE ASSOCIATION STUDY (GWAS) FOR DEPRESSION IN AN ANDALUSIAN EPIDEMIOLOGICAL SAMPLE. RELATIONSHIP BETWEEN BODY MASS INDEX, PHYSICAL ACTIVITY AND DEPRESSION.

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GENOME-WIDE ASSOCIATION STUDY (GWAS) FOR DEPRESSION IN AN ANDALUSIAN EPIDEMIOLOGICAL SAMPLE. RELATIONSHIP BETWEEN BODY MASS INDEX, PHYSICAL ACTIVITY AND DEPRESSION

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El doctorando **Juan Antonio Zarza Rebollo** ha realizado la presente Tesis Doctoral Internacional como beneficiario de una ayuda para contratos predoctorales para la formación de doctores, en el Subprograma Estatal de Formación, del Programa Estatal de Promoción del Talento y su Empleabilidad, en el marco del Plan Estatal de Investigación Científica y Técnica y de Innovación 2013-2016, con referencia BES-2017-082698, concedida por el Ministerio de Economía, Industria y Competitividad (MINECO), por Resolución del 1 de junio de 2017, de la Presidencia de la Agencia Estatal de Investigación, por la que se conceden ayudas para contratos predoctorales para la formación de doctores, convocatoria 2017).

ABSTRACT

Abstract

Depression is a highly prevalent mental disorder with devastating consequences in the general population, posing a public health concern worldwide. The increased mortality associated with depression is largely related to its frequent comorbidity with physical illnesses and other mental disorders, aggravating patients' prognosis and complicating their treatment. Furthermore, depression is a genetically complex disorder, with multiple common genetic variants involved in its aetiology, and it is the aggregation of risk alleles that confers a certain genetic predisposition. The general aim of this Doctoral Thesis is to investigate the genetic differences between individuals with depression and controls in an adult epidemiological sample, representative of the general Andalusian population (the PISMA-ep study), and to identify potential interactions between the genetic background of an individual and environmental factors related to physical health.

Chapter I aims to conduct a genome-wide association study (GWAS) for depression in an subsample of an epidemiological cohort representative of the general adult population of Andalusia from the PISMA-ep study. Although no variable was found to reach statistical significance at the genome-wide level, 9 genetic risk variants were found in the "grey zone" and were analysed. The construction of a polygenic risk score (PRS) allowed us to establish an association between depression prevalence in the PISMA-ep subsample and a weighted set of genetic variants that were identified in the largest depression GWAS meta-analysis to date.

In Chapters II and III, the focus shifts to the relationship between depression and physical health, focusing on the role of body mass index (BMI) and physical activity, respectively. Chapter II aims to investigate the relationship between depression and BMI as an indicator of physical health, because of its relationship with obesity. To this end, firstly, a systematic review of the most studied polymorphism of the *FTO* gene (rs9939609, classically associated with an increase in BMI) was carried out to elucidate the potential relationship between this genetic variant, depression and BMI, although we highlighted the need for more methodologically homogeneous studies to obtain conclusive results. Subsequently, a new unweighted genetic risk score (GRS) was constructed from the sum of risk alleles from variants of 30 candidate genes for depression in a Spanish epidemiological cohort (PredictD-CCRT). In addition to being associated with depression, we found an improvement in the predictive ability of the model when non-genetic risk factors were included, and in particular an interaction between BMI and the GRS was observed.

Chapter III aims to explore the relationship between physical activity and depression. To begin, we conducted a systematic review of *BDNF* and its relationship with depression and physical activity, both of its most studied polymorphism (rs6265, Val66Met) and of the levels of the protein. Despite the need for consensus that was highlighted, the results suggested a transient increase in the protein as an acute effect of physical activity, as well as a greater antidepressant effect due to exercise reported in carriers of the risk allele (Met) of the variant studied. In the following study, we sought to analyse whether this *BDNF* polymorphism was related to depression and physical activity in the PISMA-ep study. In both the total sample and in women,

we observed the effect described in the systematic review, i.e. a decrease in the prevalence of depression in people carrying the risk allele, which became more pronounced as the reported weekly hours of physical activity increased.

Chapter IV integrates the insights from the previous chapters by constructing a PRS for depression in a phenotypically characterised PISMA-ep subsample comprising BMI and physical activity information. Using the summary statistics of the largest GWAS meta-analysis to date, it was observed that a higher polygenic risk was associated with a higher prevalence of depression in the subsample analysed. Furthermore, the addition of non-genetic variables, such as not being physically active or higher BMI, improved the predictive ability of the model and its association with the prevalence of depression.

The results described in this Doctoral Thesis contribute to the knowledge about the genetic architecture of depression, by means of a GWAS study and PRS constructions. Furthermore, they provide evidence that, for a better prediction of depression risk, incorporating variables related to physical health such as BMI and physical exercise would be particularly valuable.

Resumen

La depresión es un trastorno mental altamente prevalente, con consecuencias devastadoras en la población general, suponiendo un desafío en materia de salud pública a nivel mundial. El aumento de mortalidad asociado a la depresión está en gran parte relacionado con su frecuente comorbilidad con enfermedades físicas y otros trastornos mentales, agravando el pronóstico de los pacientes y complicando su tratamiento. Por otro lado, la depresión es un trastorno genéticamente complejo, con múltiples variantes genéticas comunes involucradas en su etiología, y es la agregación de los alelos de riesgo la que confiere una cierta predisposición genética. El objetivo general de esta tesis es investigar las diferencias genéticas entre casos con depresión y controles en una muestra epidemiológica adulta, representativa de la población general andaluza, e identificar potenciales interacciones entre la variabilidad genética de un individuo y condicionantes de su entorno relativos a la salud física.

En el Capítulo I se tiene como objetivo llevar a cabo un estudio de asociación del genoma completo (GWAS, *genome wide association study*) para depresión en una submuestra proveniente del estudio epidemiológico PISMA-ep, representativo de la población general adulta andaluza. Pese a no encontrar ninguna variable que alcanzase la significancia estadística a nivel de genoma completo, 9 variantes genéticas de riesgo se encontraron en la "zona gris" y fueron analizadas. La construcción de una puntuación de riesgo poligénico (PRS, *polygenic risk score*) nos permitió establecer una asociación entre prevalencia de depresión en la submuestra de la cohorte PISMA-ep y un conjunto ponderado de variantes genéticas que fueron identificadas en el metaanálisis de GWAS más extenso realizado en depresión hasta la fecha.

En los Capítulos II y III, el foco se traslada a la relación entre la depresión y la salud física, centrándonos en el papel del índice de masa corporal (IMC) y de la actividad física, respectivamente. En el Capítulo II se plantea el objetivo de investigar la relación entre depresión e IMC como indicador de la salud física, por su relación con la obesidad. Para ello, en primer lugar, se llevó a cabo una revisión sistemática sobre el polimorfismo más estudiado del gen *FTO*, rs9939609, clásicamente asociado a un incremento en el IMC, para elucidar la posible relación existente entre esta variante, depresión e IMC, aunque se destacó la necesidad de estudios metodológicamente más homogéneos para obtener resultados concluyentes. Seguidamente, se construyó una nueva puntuación de riesgo genético no ponderado a partir de la suma de 30 alelos de riesgo de variantes genéticas en genes candidatos para depresión, en una cohorte epidemiológica española (PredictD-CCRT). Además de asociarse a depresión, encontramos una mejora en la habilidad predictiva del modelo cuando se incluían factores de riesgo no genéticos, y en particular se observó una interacción entre el IMC y la puntuación de riesgo genético.

El Capítulo III plantea como objetivo profundizar en la relación entre actividad física y depresión. Se comenzó con una revisión sistemática sobre BDNF y su relación con depresión y actividad física tanto de su polimorfismo más estudiado (rs6265, Val66Met) como de los niveles de la proteína. Pese a la necesidad de consenso que se observó, los resultados sugirieron un aumento transitorio de la proteína como efecto agudo de la actividad física, así como un mayor efecto antidepresivo a causa del ejercicio reportado en portadores del alelo de riesgo (Met) de la

variante estudiada. En el siguiente estudio, tratamos de analizar si este polimorfismo se relacionaba con depresión y actividad física en la cohorte del estudio PISMA-ep. Tanto en la muestra completa como en mujeres, observamos el efecto descrito en la revisión sistemática, es decir, una disminución de la prevalencia de depresión en las personas portadoras del alelo de riesgo, que se acrecentaba conforme aumentaban las horas semanales reportadas de actividad física.

El Capítulo IV integra las perspectivas de los capítulos previos, construyendo un PRS para depresión en una submuestra del estudio PISMA-ep caracterizada a nivel fenotípico, conteniendo información de IMC y actividad física. Empleando los resultados del metaanálisis de GWAS más extenso hasta la fecha, se observó que un mayor riesgo poligénico estaba asociado con una mayor prevalencia de depresión en la submuestra analizada. Además, la adición de variables no genéticas, como el no practicar actividad física o un mayor IMC, mejoraron la habilidad predictiva del modelo y su asociación con la prevalencia de depresión.

Los resultados descritos en esta Tesis Doctoral contribuyen a ampliar los conocimientos acerca de la arquitectura genética de la depresión, por medio de un estudio GWAS y construcciones de PRS. Además, aportan evidencia de que, para una mejor predicción del riesgo de depresión, es importante incorporar variables relativas a la salud física tales como el IMC y la práctica de ejercicio físico.

INTRODUCTION

INTRODUCTION

1. Depression

1.1. General description and epidemiology

Depressed, sad, empty, melancholic, anxious, euphoric, manic, cheerful... are a few examples of the adjectives used to describe a person's mood. Mood is defined as a sentimental or emotional tone that influences someone's behaviour and perception of the world. Depression, also referred to as major depressive disorder (MDD) or clinical depression — terms used indistinctly hereafter— is encompassed within mood disorders, a major category of psychiatric disorders. It has long been described as a heterogeneous condition with multiple different signs and symptoms including sadness, apathy, hopelessness, lack of motivation, loss of affection or feelings, psychomotor inhibition (i.e., slowness), changes in appetite or sleep and alterations of cognitive functions, among others. All these signs and symptoms may result in a deterioration of the person's quality of life at interpersonal, occupational and social levels.

Depression is a major public health problem, and one of the most common mental disorders, affecting 322 million people worldwide in 2017[1]. The World Health Organization (WHO) estimated its global prevalence at 4.4% in 2015, with the risk being higher in adulthood and in women and estimating that one in five people will suffer from depression during their lifetime[1]. Interestingly, WHO classified major depression as the third leading cause of morbidity burden worldwide and is expected to occupy first place by 2030[2]. Although the first episode of depression usually appears from adolescence to the mid-40s, almost 40% of patients usually experience it before their twenties[3,4]. Interestingly, the prevalence of depression is almost twice as high in women, and in both sexes increases in adulthood[4–6]. This gender gap appears to be determined by psychological, biological, and environmental factors[7]. **Figure 1** shows the prevalence of depression, classified by age and sex, both worldwide and in Spain.

Concerning the course of the disorder, for most patients it is composed of acute episodes, with absence of symptoms between them. However, depression is quite unpredictable and the number of episodes, duration and severity are variable[8,9]. Since depression is recurrent throughout life, the term *cure* is used to refer to those patients who are asymptomatic. The probability of recovery decreases with increasing number of episodes, age[10], and comorbidities, and the prognosis is less favourable the older the patient is, with 27% of patients developing chronic depressive disorder[11,12].

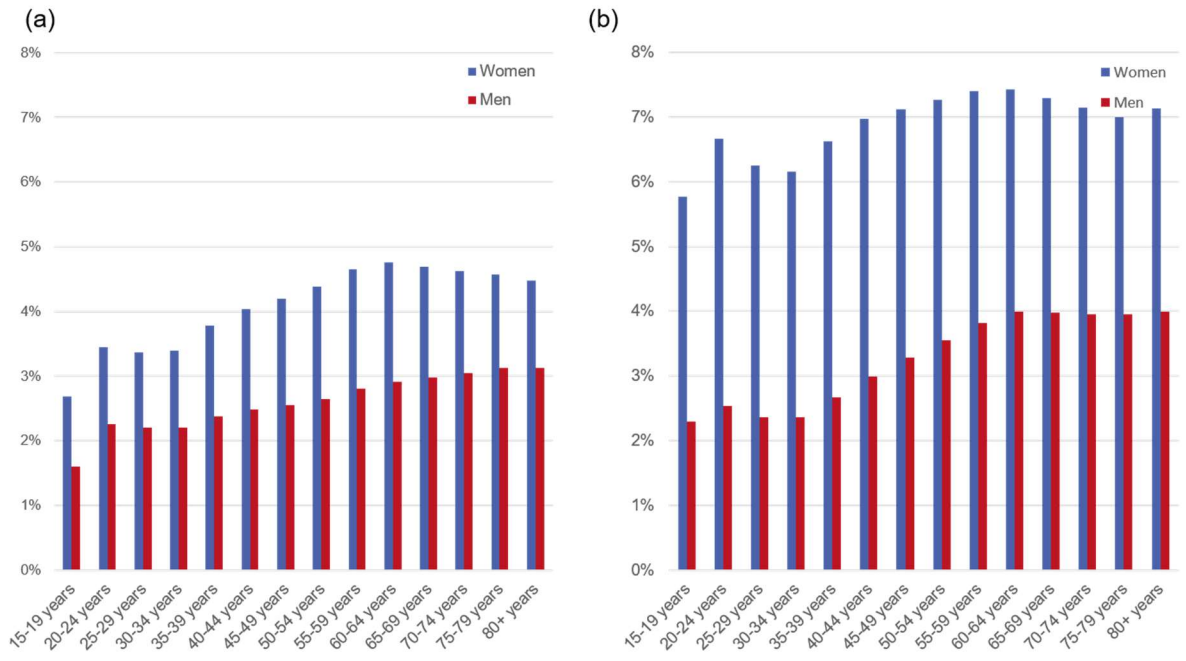

Figure 1. Prevalence of depression, by age and sex (%), (a) worldwide and (b) in Spain. Source: Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Results. Seattle, United States: Institute for Health Metrics and Evaluation (IHME), 2020. Available from https://vizhub.healthdata.org/gbd-results/.

Although depression is the most common mental disorder, the severity of its symptoms and consequences cannot be underestimated. This psychiatric disorder is associated with a higher mortality, being leading cause for disability worldwide and a major contributor to suicide. The most immediate clinical concern related to depression is its strong relation to suicide attempt and completed suicide[13]. Patients with depression have a 1.8-fold increase overall mortality and a loss of an estimated 10.6 life years in men and 7.2 years in women[14]. Partly, this is due to the increased risk of suicide in this population, which has been estimated in almost 20-fold higher risk in patients with depression than in the general population[14].

Even though there are no solid conclusions yet, several studies have revealed that depression is not only a consequence of socioeconomic and cultural factors, but a complex disorder in which biological factors, such as genetics, may play a relevant role in its onset and progression[15–17].

1.2. Symptoms and diagnosis

The diversity of symptoms that a person with depression can display makes it a highly complex disorder to address clinically. Mainly two institutions have developed the current diagnostic tools used in clinical practice: the World Health Organisation has developed the International Statistical Classification of Diseases and Related Health Problems (currently in

its 11th revision, ICD-11)[18], and the American Psychiatric Association has published the Diagnostic and Statistical Manual of Mental Disorders, currently in its fifth version, DSM-5[19], although the previous version DSM-IV-TR is still widely employed[20].

In the ICD-11, for the diagnosis of depression individuals must meet, in a minimum of two weeks, two symptoms: depressed mood and loss of interest or joy in previously pleasurable activities. In order to determine the severity of the episode, the recent version has modified their criteria. Whereas in ICD-10 there were seven items, the sum of which would define severity; in ICD-11 the focus is on the intensity of symptoms and how they interfere in the person's life. In this classification, we observe single episodes and recurrent depressive disorder, with seven subtypes in each, as depicted in **Table 1**.

Table 1. Categories of depressive disorders according to the ICD-11 criteria. Source: data from the International Statistical Classification of Diseases and Related Health Problems (11th ed.; ICD-11; World Health Organization, 2019).

ICD-11 classification of depressive disorders	
6A70 Single episode depressive disorder	**6A71 Recurrent depressive disorder**
6A70.0 Single episode depressive disorder, mild	6A71.0 Recurrent depressive disorder, current episode mild
6A70.1 Single episode depressive disorder, moderate, without psychotic symptoms	6A71.1 Recurrent depressive disorder, current episode moderate, without psychotic symptoms
6A70.2 Single episode depressive disorder, moderate, with psychotic symptoms	6A71.2 Recurrent depressive disorder, current episode moderate, with psychotic symptoms
6A70.3 Single episode depressive disorder, severe, without psychotic symptoms	6A71.3 Recurrent depressive disorder, current episode severe, without psychotic symptoms
6A70.4 Single episode depressive disorder, severe, with psychotic symptoms	6A71.4 Recurrent depressive disorder, current episode severe, with psychotic symptoms
6A70.5 Single episode depressive disorder, unspecified severity	6A71.5 Recurrent depressive disorder, current episode, unspecified severity
6A70.6 Single episode depressive disorder, currently in partial remission	6A71.6 Recurrent depressive disorder, currently in partial remission
6A70.7 Single episode depressive disorder, currently in full remission	6A71.7 Recurrent depressive disorder, currently in full remission

Following DSM-5 criteria, diagnosis of depression requires the appearance of (at least) five of nine symptoms, and be present for a two-weeks period or more, representing a change from the previous functioning. At least one of the symptoms has to be either (1) depressed mood or (2) anhedonia (decreased interest in activities an individual used to enjoy, and reduced ability to feel pleasure or joy). It should be considered that symptoms clearly attributable to another medical circumstance should not be included. These symptoms are:

1) Depressed mood during the majority of the day, almost daily, obtained from the subjective report of the individual (e.g. feeling sad, hopeless) or others about the individual (e.g., appears sad or tearful). In children and adolescents, it can be related to an irritable mood.
2) Loss of interest or pleasure in the majority of activities during the majority of the day, almost every day, as self-reported or by observation of others.
3) Significant changes in weight (more than 5% of body mass in a month), or in appetite almost daily. In children, the absence of the expected weight gain should be considered.
4) Alterations of the sleeping patterns (insomnia or hypersomnia) almost daily.
5) Psychomotor alterations (agitation or retardation) almost daily. This must be observed by others, not being enough subjective feelings.
6) Fatigue or decreased energy, almost daily.
7) Feeling worthless, or guilty, in an excessive or inappropriate manner (which could be delusional thinking), almost daily (not just feeling guilty or self-reproduction for being sick).
8) Decreased ability to think clearly or focus, or difficulty in taking decisions, almost daily (self-reported or observed by others).
9) Recurrent thoughts of death (different from merely fear of dying), repetitive suicidal ideation in a not planned manner, suicide attempt, or having defined a suicide plan.

To consider it an episode of major depression, these symptoms have to cause clinically significant distress, or have a notable impairment in functional areas (e.g., social or occupational). Moreover, this episode must not be attributable to the physiological effects of any substance or medical condition.

Once a major depressive episode has been diagnosed, a diagnosis code is applied, in order to define the severity of the episode and its current status, as it is described in **Table 2**.

Table 2. Categories of depressive disorders according to the DSM-5 criteria. Source: data from the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM-5; American Psychiatric Association, 2013).

* To be considered recurrent, there must be a minimum interval of two consecutive months between episodes during which the criteria for a major depressive episode are not met.
** It should be indicated when psychotic features are present, independently of the severity.

DSM-5 classification of depressive disorders		
Severity/course specifier	**Single episode**	**Recurrent episode***
Mild	296.21 (F32.0)	296.31 (F33.0)
Moderate	296.22 (F32.1)	296.32 (F33.1)
Severe	296.23 (F32.2)	296.33 (F33.2)
With psychotic features**	296.24 (F32.3)	296.34 (F33.3)
In partial remission	296.25 (F32.4)	296.35 (F33.41)
In full remission	296.26 (F32.5)	296.36 (F33.42)
Not specified	296.20 (F32.9)	296.30 (F33.9)

Therefore, when registering a diagnosis of major depressive disorder, the following concepts must be specified, in this order: major depressive disorder, single or recurrent episode, severity/psychotic/course specifier, and then any of the following applicable specifiers: with anxious distress, with mixed features, with melancholic features, with atypical features, with mood-congruent psychotic features, with mood-incongruent psychotic features, with catatonia, with peripartum onset, or with seasonal pattern (recurrent episode only). For any of these specifiers, a number of symptoms are listed for consideration. Depending on the number of symptoms the patient presents, the severity will also be determined.

As aforementioned, it is to be considered that symptoms of depression are commonly identifiable in a sort of medical and psychiatric conditions in which they overlap[21]. This heterogeneity hampers not only diagnosis, but also how the treatment is approached. Regarding diagnosis, a wrong classification of a certain medical condition -i.e., a misdiagnosis- can have detrimental effects in depression and psychiatric disorders, namely an inappropriate treatment or approach, or the stigma attached to a psychiatric diagnosis[22,23]. In consequence, a correct differential diagnosis takes on crucial relevance for the proper diagnosis and classification of depression.

1.3. Aetiology and risk factors

When attempting to identify the causes of depression, much of the difficulty stems from an impairment common to mental disorders: we ignore much more of the brain than we know about it. There is therefore a complexity arising from this lack of knowledge of the abnormal behaviour of the brain in psychiatric disorders.

As could be noted in the previous section, there has been observed a substantial diversity in the presentation of depression between individuals, in terms of age of onset, chronicity and

severity of symptoms, relapses, or consequent functional deterioration[24,25]. This clinical heterogeneity also remains when approaching the causes of depression, encompassing a range of factors, explanations and theoretical frameworks that have been proposed over the years to define its aetiology.

1.3.1. Psychosocial factors

Any person, under certain circumstances, can develop depression, regardless of their **premorbid personality traits**. Nonetheless, some kinds of premorbid personality have been associated with a certain degree of predisposition to depression. In this respect, a personality requiring external approval for self-approval (sociotropy) will be at increased risk of depression following an adverse life situation[26]. Other personality traits, such as perfectionism or neuroticism –understood as emotional instability– have largely been associated with predisposition to depression[27].

Given the greater risk of depression in women merely by virtue of being a woman –approximately twice as likely, irrespective of country or culture–, **gender** should be considered as a risk factor. This was first reported almost half a century ago by Weissman and Klerman[28], and has been replicated multiple times in different studies. There are a number of neurobiological and psychosocial reasons underlying this difference. An important component of risk derives from gender roles and socio-cultural norms, with behavioural models such as learned helplessness, and multiple psychosocial stressors that are more prevalent in women than in men. Related to this, women are also at greater risk of physical and sexual abuse, and in general, to stressful life events. Finally, differences in hormonal functioning and events such as giving birth are proposed as reasons for women's increased risk of developing depression[29,30].

Prevalence of depression varies depending on the **age**. As can be observed in **Figure 2**, different nature of risk factors for developing a depressive episode can be attributed to every moment in the lifespan[31]. The distribution and accumulation of risk factors are in line with epidemiological studies reporting that the middle age –around 45 years– is the moment at lowest risk of developing depression[32]. According to this, prevalence is higher in younger adults and also in the elderly, which is highly associated to a diverse nature of losses, worries and fears associated with age groups –e.g., jobs, marriage and general wellness in young adults, and physical decay, comorbidities, and status and personal losses in older adults. Generally, an early age-of-onset is associated with an increased illness burden[33].

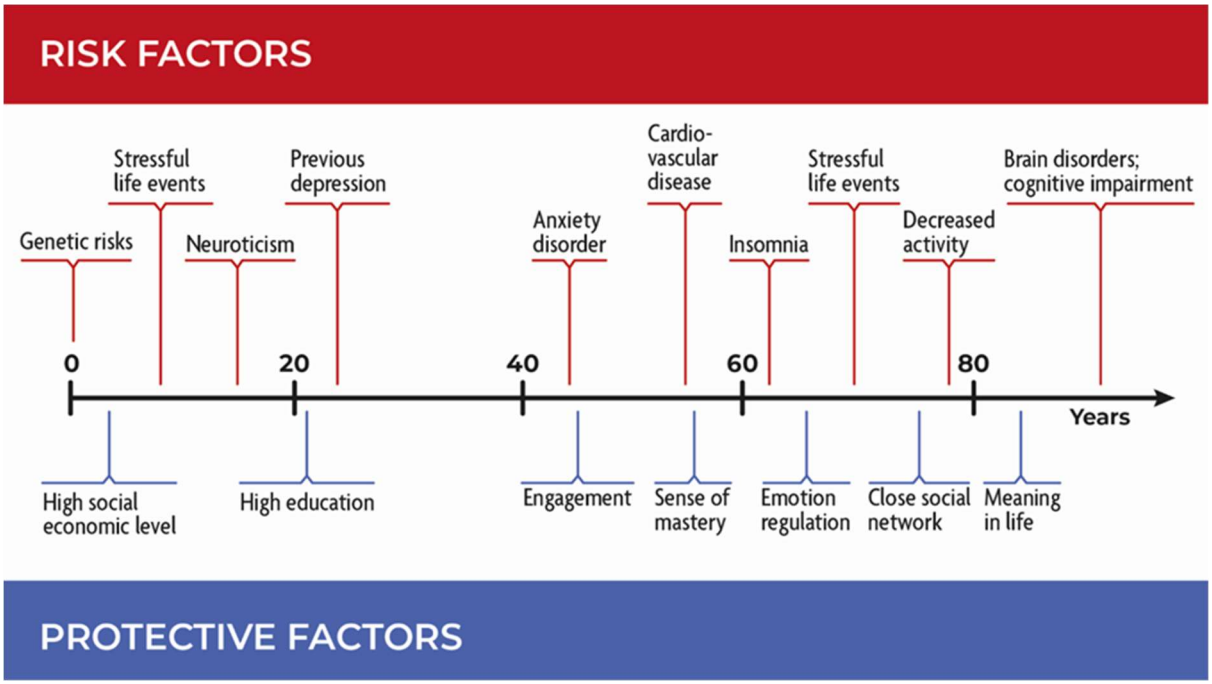

Figure 2. Risk and protective factors for late-life depression in a lifespan perspective. Adapted from Fiske et al., 2009.

We can find different sociodemographic factors that may play a role in the prevalence of depression. For instance, **marital status** is an important aspect to be considered in the epidemiology of depression, given the increased prevalence of depression in marital disruption. This relationship is bidirectional, broadly considered, depression has a deleterious effect on marriage, and also marriage disruptions are a risk factor for depression[34]. On the other hand, **education** has a protective effect against depression, being generally greater for women than for men, for whites than for blacks and for people with a background of limited socio-economic resources[35]. This newly mentioned **socio-economic status** has been described as another protective factor[36], as is **work status**, particularly that losing or not having a job increases the propensity to develop depression[37]. **Urbanicity** is another related factor, pointing towards the risk factor of living in a city, for mental illnesses[38], and also for depression[39,40], due to inherent characteristics, such as pollution, noise, criminality, or the difficulty of establishing social circles.

Furthermore, there are environmental factors that can predispose or even precipitate an episode of depression. **Predisposing environmental factors** are considered those that took place before adulthood and can increase the risk for depression during adulthood, such as losing a progenitor, parents divorce, or suffering abuse[41]. On the contrary, **precipitating environmental factors** include stressful life events, which can lead to a depressive episode themselves[42].

1.3.2. Biological factors

As already mentioned, depression is a complex and heterogeneous disorder in which different factors are involved. Since there is a variety of psychosocial and biological stressors, aspects

such as the onset of the disorder, episodes, symptoms, progression and treatment may differ between individuals. Furthermore, extrapolating results from animal models to humans has been proven to be quite challenging[43]. Therefore, although knowledge of the pathophysiology of this disease has advanced greatly in recent years, there is no single model or mechanism that can explain all the elements involved. In fact, theories based on different biological pathways have led to the development of antidepressants during the second half of the last century.

Monoamine hypothesis

Since the discovery in the mid-20th century that reserpine could induce major depression while decreasing the amount of monoamines, the knowledge of the role of monoamine neurotransmitters in the pathogenesis of the disorder has gained interest. Some examples of monoamine neurotransmitters are dopamine, noradrenaline and serotonin. Several in vivo and post mortem studies have supported this theory. Following this hypothesis, antidepressants that inhibit monoamine oxidase (MAOIs) or serotonin agonists, among others, have been developed[44,45]. However, the clinical variability of the episodes and the long period it takes for the drugs to take effect are not explained by this model[46] .

Hypothalamic–pituitary–adrenal axis changes

The hypothalamic-pituitary-adrenal (HPA) axis is a highly relevant neuroendocrine system which affects multiple body processes[47,48]. One of the most relevant biological findings related to this axis is the increase in plasma cortisol levels as a consequence of increased stress, as well as a feedback inhibition mediated by glucocorticoid receptors. Impairments of this system have been associated with disturbances at a cognitive level[49]. Although attempts are being made to develop drugs to re-establish the function of the HPA axis, glucocorticoid receptor antagonists have not been successful in clinical trials[50–52].

Inflammation

The cognitive alterations observed in depression have also been associated with circulating levels of cytokines, which may act directly on astrocytes and microglia or through signals mediated by afferent pathways, e.g., the vagus nerve[53,54]. For instance, people with autoimmune diseases or serious infections are more likely to suffer from depression. There are several studies that support this theory. Some authors have observed that the administration of cytokines, e.g., interferon alpha, triggers depressive symptoms[55,56]. Furthermore, other studies relate high levels of interleukin 6 in childhood with a greater probability of suffering depression in their early adulthood[57]. Likewise, in brains of patients with depression analysed post mortem, activation of microglia has been reported and neuroinflammation has been observed[58]. These findings have driven the use of nonsteroidal anti-inflammatory drugs in the treatment of major depression[59,60].

Neuroplasticity and neurogenesis

The discoveries of neurogenesis in the adult brain —the generation of new neurons through pluripotent stem cells—, and neuroplasticity —neuronal level growth and adaptability—

have been noteworthy discoveries of this century, and are highly implicated in depression[61,62]. Both processes are related with the previously described mechanisms of inflammation and the HPA axis impairment, causing alterations in the processes of neurogenesis and neuroplasticity[63]. Therefore, under this precept, the regulators of the latter processes become even more significant. In this respect, the brain-derived neurotrophic factor (BDNF), a neurotrophin present in certain brain regions and the peripheral nervous system, has been a main subject of study. During development, BDNF contributes to neuronal growth and differentiation[64], whereas in the adult brain BDNF regulates synaptic transmission and neuroplasticity[65,66]. A great body of evidence has shown diminished peripheral levels of this protein in depression, and have been observed to be restored following antidepressant treatments, e.g., pharmacological or psychological[67,68].

1.3.3. Genetic factors

Early epidemiological studies pointed to an increased predisposition to develop depression if other members of the family were previously affected. This is the premise to consider that a disorder may be caused by genetic factors, i.e., the risk for the disorder in genetically related individuals should be similar[69]. An early meta-analysis of family-based studies showed evidence of a familial aggregation of depression, with a 2.84-fold higher relative risk for developing depression in first-degree relatives of patients with depression[70]. On this basis, further efforts were aimed at distinguishing between the proportion of contribution of genetic factors and environmental factors, since families typically share the two kinds. Two types of studies have been conducted to define and characterise this heritable proportion of depression: twin studies and adoption studies.

Twin studies have classically compared monozygotic and dizygotic twins to define heritability, i.e., the proportion of the observed variation that can be attributed to genotypic changes (additive genetic effects), when we understand a certain phenotype as a result of a combination between genotype and environment (Phenotype = Genotype + Environment)[71]. An initial meta-analysis estimated heritability of depression at 37%, with a 95% CI of 31–42%[70]. Further twin studies estimated a higher heritability in women than in men[72,73], and a more recent meta-analysis found higher heritability in recurrent depression than in single episodes[74]. **Adoption studies** aim to distinguish those aetiological factors of a genetic nature from environmental factors, by comparing between adoptive –which typically only share an environmental context– and non-adoptive families. In the already presented meta-analysis of Sullivan et al., (2000) conflicting results were obtained, probably due to the small sample size[70].

Considering the question of the difference between sexes in the heritability of depression—which might explain differences in prevalence—, the meta-analysis by Sullivan at the beginning of the century did not find evidence of sex differences in heritability for this disorder[70]. More recent results, in line with the genetic complexity of depression, support this hypothesis of the majority of the genetic risk being shared in men and women[73].

Candidate genes studies have been present since the late 70s, focusing on polymorphisms in genes participating in the relevant pathways from the different theories involved in the pathophysiology of depression, as well as genes potentially involved in drug responses[67]. In this aspect, studies analysing polymorphisms in genes involved in the neurotransmission of serotonin, dopamine and noradrenaline have been prolific, guided by the monoamine theory. Polymorphisms in genes coding for the serotonin transporter (*SLC6A4*, also referred to as *SERT* or *5-HTT*)[75], serotonin receptors (*HTR1A*)[76], dopamine receptors (for instance, *DRD3* or *DRD4*)[77,78], or genes for enzymes, as monoamine oxidases (MAOA)[79], catechol-o-methyltransferase (*COMT*)[80], or tyrosine hydroxylase (*TH*)[81], were particularly successful in their associations with higher risk for depression at the end of the last century and the beginning of the current one. Even though these studies were of great relevance at the time and served to guide pharmacological approaches, results over the years have been conflicting and hardly replicable. In a large meta-analysis conducted in 2008 on polymorphisms in candidate genes involved in monoaminergic transmission, only *SLC6A4* and *SLC6A3* maintained statistical significance[82]. A high number of studies have established associations between depression and candidate genes related to stress and the HPA axis, particularly in genes encoding targets of glucocorticoids, e.g., cortisol, secreted during stress. Although there are plenty of individual studies on the matter, reporting polymorphisms associated with depression, different meta-analyses do not report statistically significant results for any polymorphism related to these pathways[82,83].

Another large body of scientific literature has focused on neurogenesis and neuroplasticity, with a particular interest in the previously described *BDNF* gene. While several studies have independently reported an association between the *BDNF* rs6265 polymorphism (Val66Met), the most extensive meta-analysis did not find a statistically significant association[84]. Nonetheless, interactions between the *BDNF* Val66Met SNP and other polymorphisms or with environmental factors may have a greater relevance than the association of depression with the polymorphism itself[85,86].

Despite the interest in candidate genes persists —although most of the associations have disappeared in meta-analyses or more methodologically robust analyses—, a recent large-scale study (with sample sizes between 62,138 and 443,264) outlined that most of the associations classically reported are likely to be false positives[87]. In this study, 18 candidate genes associated with depression in 10 or more studies were analysed in a large cohort from the UK Biobank and the Psychiatric Genomics Consortium (PGC). No polymorphism remained statistically significant in polymorphism-level analyses, while at gene-level only DRD2 had a statistically significant association with depression.

A noteworthy improvement in methodological technologies made it possible to conduct **genome-wide association studies (GWAS)**, a research approach used to identify genetic variants associated with a particular trait, by analysing hundreds of thousands of genetic variants across the whole genome in large groups of individuals. Comparing these variants

between individuals who have the trait to those who do not, it is possible to detect variants that are common in the particular trait or disease, providing insights into its genetic basis. GWAS have been instrumental during the past decade in identifying genetic risk factors for genetically complex disorders, and have the advantage of not requiring further previous hypothesis than assuming that there are variants conferring risk or protection for a particular trait or disorder, which was groundbreaking in a field dominated by candidate genes studies.

However, the majority of GWAS studies in depression did not reach enough sample size to find genome-wide significant loci associated with the disorder (p value threshold of $<5 \times 10^{-8}$). Some of the first GWAS identified suggestive loci, such as occurred with the gene PCLO in 2009[88], were further replicated in more recent studies[89,90], whereas others —even the first signal to reach genome-wide statistical significance, in the *SLC6A15* gene[91]— failed to be replicated in larger samples.

Since their foundation in 2007, the Psychiatric Genomics Consortium (PGC) has had a critical impact in driving the evolution of GWAS outcomes in depression —among other psychiatric disorders[92]. Once the relevance of increasing the sample size to increase statistical power was identified, efforts were focused on this aspect. Although the first GWAS mega-analysis performed by the PGC did not report any genome-wide statistically significant locus[93], the last years have been highly rewarding in terms of novel findings in GWAS in depression. In 2016, a large-scale GWAS (75,607 self-reported cases with depression and 231,747 controls) was performed in a cohort of 23andMe, with a self-reported diagnosis of depression, identifying 15 genome-wide significant loci[94]. One year later, in 2017, another locus was identified in a meta-analysis of GWAS of two cohorts (246,363 cases and 561,190 controls)[95]. These studies reflected the new position in favour of increasing the sample size, using a more diffuse diagnosis of depression, also referred to as *broad depression*. Under the umbrella of broad depression diagnosis, the greatest successes in this field were achieved in 2018. In this year, a GWAS conducted in the UK Biobank, with a sample size of 322,580 participants resulted in 17 novel independent SNPs from 15 genetic loci significantly associated with broad depression[96]. In the same year, the PGC MDD Working group published a meta-analysis of GWAS from 7 large cohorts, with a total sample size of 135,458 cases with depression and 344,901 controls, finding 44 novel loci associated with depression[89]. In 2019, another meta-analysis of GWAS data that included 246,363 cases of depression and 561,190 controls reported 102 independent variants, finding that 87 of them were replicated in an independent sample[90]. In 2020, the milestone of a million participants was overreached, in a large meta-analysis that included individuals of European (340,591 cases and 813,676 controls) and African ancestry (25,843 cases and 33,757 controls), in which 223 independently significant SNPs at 178 genomic risk loci were found for European ancestry participants[97].

The heritability observed in these three last meta-analyses was of $h^2 = 10.2\%$ in Howard *et al.* (2018) —for the broad depression phenotype—, $h^2 = 8.7\%$ in Wray *et al.* (2018), $h^2 = 8.9\%$ in Howard *et al.* (2019), and $h^2 = 11.3\%$ in Levey *et al.* (2020). Comparing these values with the heritability of 35-40% described in early twin studies reveals the missing heritability issue

existing towards the genetic architecture of depression[98]. One of the main points to pursue is to reduce phenotypic heterogeneity within the case cohorts, since there have been observed differences in the genetic architecture between this broad definition of the disorder and clinically assessed depression[99]. Novel findings suggest that only a fraction of the genetic architecture is shared among subtypes, and that some subtypes, generally with more severe manifestations, e.g., depression with atypical-like symptoms, postpartum depression, recurrent depression, with severe impairment or with severe symptoms, have an increased heritability in comparison with the broad depression phenotype[100].

2. Relationship between depression and comorbid physical conditions

Besides the immediate impact on the lives of people with depression, in terms of quality of life and disability, having depression represents a dramatic decrease in life expectancy. As it has been reported in longitudinal studies, affected men live on average 15.3 years less, while women live on average 12.5 years less[101]. There are multiple causes associated with this lower life expectancy –for instance, the elevated risk of suicide–, although there is a high proportion of deaths caused by physical health conditions. Remarkably, depression has been associated in large meta-analyses with an increased risk of stroke[102], metabolic syndrome[103], and diabetes mellitus[104], among other physical conditions, and psychiatric disorders. In the same way, metabolic syndrome has been identified as a risk factor for depression, with a bidirectional association suggesting a pathophysiological overlap[105]. As stated above, the comorbidity of depression with chronic physical conditions is associated with a worse prognosis and is, partly, a factor involved in the increased mortality observed in depression[106]. One of the most relevant physical conditions comorbid with depression is obesity, which commonly results in significant aggravation of depression.

2.1. Obesity

2.1.1. General overview

Overweight and obesity are broadly defined as an excessive or abnormal fat accumulation, which can be detrimental to health. Body mass index (BMI) is employed in adults to assess overweight and obesity, following the formula: weight in kilograms divided by square of the height in metres. Thus, according to WHO criteria, a normal BMI range would be considered between 18.5 – 24.9 kg/m^2, overweight between 25 – 29.9 kg/m^2 and obese if BMI is higher than 30 kg/m^2.

Obesity is the most prevalent form of malnutrition in the vast majority of the world, currently acquiring pandemic dimensions. While at the beginning of the century obesity was a problem mainly in more developed countries, this pandemic is now present and growing in low- and middle-income countries. Thereby, overall prevalence of obesity has soared in recent decades, being three times higher in 2016 than in 1975. In absolute terms, the NCD Risk Factor Collaboration reported in 2016 that the total population with obesity was 671 million (390 million women and 281 million men, resulting in 12% of the global adult population), increasing to nearly 2 billions adults when the threshold for overweight was considered (almost 20% of the global adult population)[107]. Predictions for 2030 estimate that over 1 billion people will have obesity, with a higher prevalence in women (1 of 5 is the estimate for women, whereas 1 of 7 for men)[108].

The fat accumulation that characterises obesity is usually caused by a positive energy imbalance, i.e., there is more energy consumption than expenditure, over a prolonged and chronic period. There are a number of risk factors that can lead to this outcome. Beyond diet

and physical exercise, factors such as lifestyle, psychosocial, environmental and genetics can contribute to triggering this condition[109].

2.1.2. Genetic risk factors

When considering the influence of genetics for obesity, it is required to differentiate between two types of obesity: severe, monogenic obesity, and the common phenotype of polygenic obesity.

On the one hand, **monogenic obesity** is characterised by a severe phenotype, with endocrine disorders and aberrant feeding behaviours. It is typically rare, early-onset, and, in contrast to the small contribution of environmental factors, genetics plays an important role. Particularly, rare mutations and chromosomal deletions are usually involved in monogenic obesity. On the contrary, **polymorphic obesity** leads to the well-known phenotype of common obesity, which arises from an interaction between an obesogenic environment and a genetic risk profile conferred by the accumulation of hundreds of small-effect polymorphisms.

Early candidate gene studies identified variants linked to monogenic severe obesity, which were associated with an early-onset obesity. These were located in genes encoding components implicated in pathways of particular relevance, such as the leptin receptor (leptin receptor gene *[LEPR]*[110]), or the melanocortin pathway (for instance, melanocortin 4 receptor gene *[MC4R]*[111,112], proprotein convertase subtilisin/kexin type 1 gene *[PCSK1]*[113] and proopiomelanocortin gene *[POMC]*[114]).

During the following years, until 2007, hundreds of variants in these genes and others later identified in monogenic obesity were tested for their association in polygenic obesity, with little success. Only a few exceptions, including variants in the *MC4R, PCSK1* and brain-derived neurotrophic factor *(BDNF)* genes were found to be associated with polymorphic obesity, being later confirmed in the initial GWAS that were performed in BMI and obesity[115,116]. As in other genetically complex traits, the development of genome-wide association studies represented a major breakthrough in the search for candidate genes for the common phenotype of obesity. In 2007, four different GWAS reported an association between SNPs in the first intron of the fat mass and obesity associated gene *(FTO)* and different obesity-related traits[117–120]. *FTO* remains as the strongest and most robust signal for BMI, validated across diverse ancestries, although more than 80 GWAS have identified over a thousand independent loci associated with BMI increase and obesity related traits[121].

Two recent GWAS have been significant milestones. In 2015, the Genetic Investigation of ANthropometric Traits consortium (GIANT) reported a meta-analysis with more than 300,000 individuals, identifying 97 loci for BMI (56 of them were novel variants)[122]. These loci were found to be located near genes with enriched expression in the central nervous system, suggesting that processes such as hypothalamic control of energy intake had a main role on

BMI. In 2018, a meta-analysis of GWAS included nearly 700,000 individuals, identifying 751 SNPs in loci not previously reported associated with BMI (941 considering also previously associated signals)[123]. Here, BMI-associated genes were particularly enriched among genes involved in the development of the central nervous system and neurogenesis.

Missing heritability is also present in BMI and obesity related traits. Although simulations suggest that SNPs should account for approximately 30% of the variance in BMI[124], the 97 loci reported in Locke et al. (2015) only explained 2.7% of the variance[122], whereas in Yengo et al. (2018) a 6% of the variation is explained by the 941 SNPs[123]. This shows evidence that a great proportion of variants implied remain to be discovered.

2.1.3. The bidirectional relationship between depression and obesity

Both depression and obesity are major public health problems, with increasing prevalence and reaching the category of pandemics. Both conditions tend to co-occur, and several lines suggest that they are not independent. Multiple meta-analyses of cross-sectional studies have reported increased association with depression or depressive symptoms in participants with obesity[125]. Also meta-analyses performed on longitudinal studies found an association between the risk for developing depression in participants with obesity, and vice versa, becoming obese after developing depression[125].

It seems clear that their simultaneous presence leads to a series of adverse physiological adaptations that are related in a vicious circle, reinforcing and aggravating these conditions. In particular, the *atypical* subtype of depression is characterised with features related to weight gaining, as increased appetite or increased sleep. It is remarkable that having depression and obesity at the same time has a number of consequences that worsen the prognosis of the patient. It becomes harder to treat than when separately, indeed being associated with a more chronic course[126], and a worse response to antidepressants[127]. Furthermore, as a consequence of this comorbidity, the risk for several physical and mental conditions becomes highly increased.

A variety of factors of different nature take place in this relationship. Psychosocial factors as internalisation of weight-related negative stereotypes[128], low weight-related self-efficacy[129], or negative self-body image[130,131] are factors commonly prevalent in people with obesity which increase the risk for depression. There are also diverse biological mechanisms that have been linked with the comorbidity between depression and obesity. **Figure 3** attempts to illustrate the multitude of mechanisms that are implicated in the bidirectional relationship between obesity and depression, how environmental factors, such as stress, diet and lifestyle affects these pathways, and their potential interaction with the individual's genetic background.

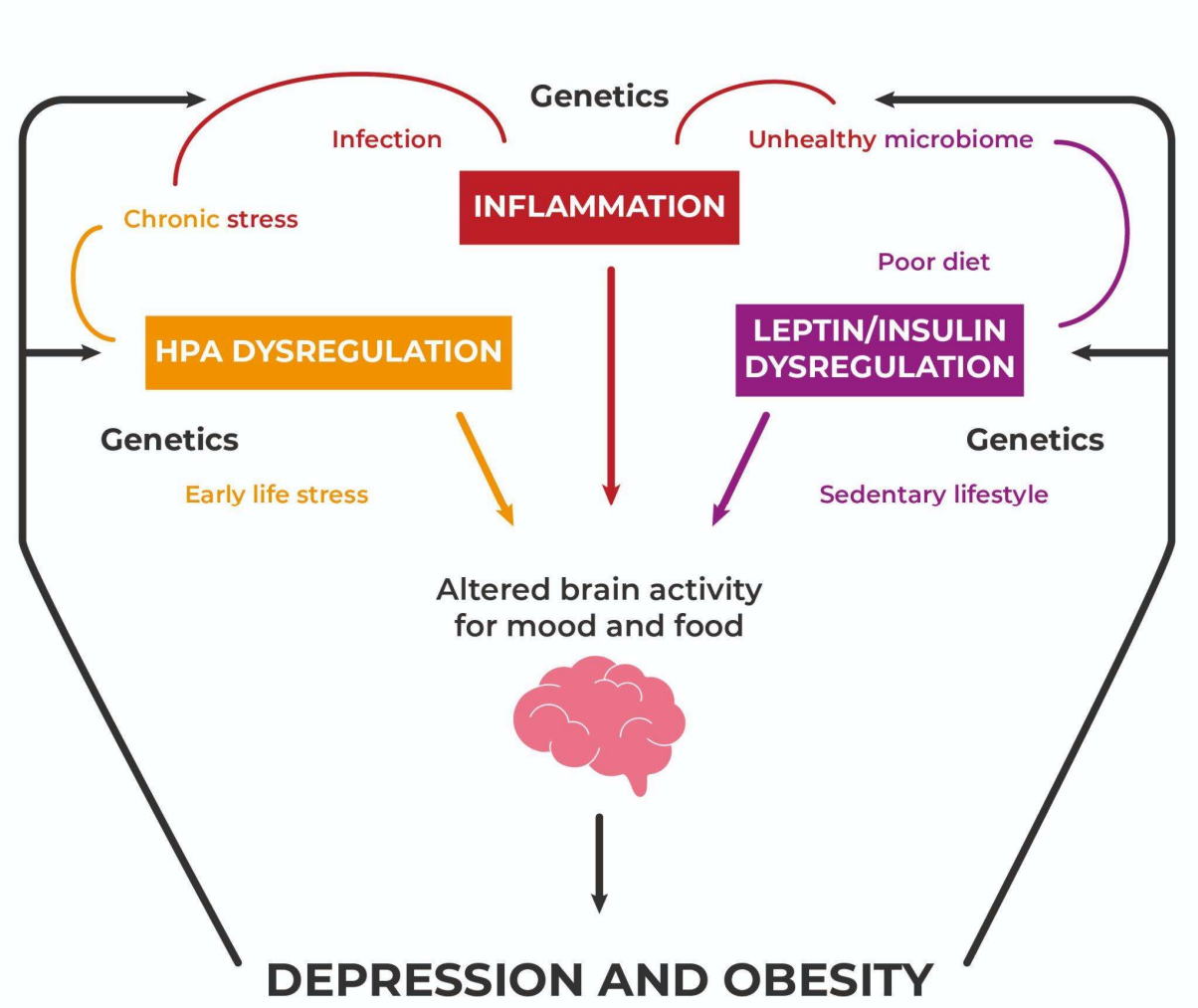

Figure 3. Diagram of shared biological pathways underlying depression and obesity. Adapted from Milaneschi et al., 2019.

As depicted in **Figure 3**, alterations in the mechanisms involved in **energy homeostasis**, and, in particular, dysregulations in the pathways of leptin and insulin, have been proposed as a pivotal link regarding this comorbidity. The role of leptin regulating energy homeostasis is well known, triggering a cascade of signals that end integrating physiological and behavioural processes which suppress food intake, and promote energy expenditure[132]. Insulin is key regulating glucose metabolism, and its impaired functioning ultimately leads to type 2 diabetes. Whereas leptin has an impact on mood, and leptin resistance has therefore been proposed as a risk factor for depression[133], the dysregulation of insulin has been postulated to have a role in depression and dementia due to the association of insulin resistance with cerebral metabolic decline in certain brain regions, which leads to neuronal damage in the hippocampus and medial prefrontal cortex, and has been related to impairment in memory and executive function[134].

Besides, the **hypothalamic-pituitary-adrenal (HPA) axis** regulates the production of glucocorticoids, by stimulating forward and feedback inhibition loops. The activity of the HPA axis is an indication of the stress response, and its dysfunction is a mediator of further pathological consequences. Its overactivity has been well described and associated with depression in neuroendocrine studies[135]. A key consequence of the HPA axis overactivation is an excessive cortisol production. This is also a risk factor for obesity. Indeed, HPA axis is hyperactivated in nearly half of obese adults[136], and there are several mechanisms involved in this association: a) increasing appetite, in particular of caloric food; b) increasing adipogenesis and visceral fat hypertrophy; and c) suppressing brown adipose tissue-related thermogenesis, thus reducing energy expenditure[137].

Also the inflammatory process is related to both conditions. **Chronic low-grade inflammation** is one of the hallmarks of obesity, and is related with the HPA axis. Since proinflammatory cytokines are released by white adipose tissue as a consequence of an inflammatory response, this immune activation also affects multiple depression-related pathways. For instance, cytokines can impact the well-established monoaminergic neurotransmission[56], or can hyperactivate the HPA axis, by hindering its negative feedback, as a consequence of the disruption of the glucocorticoid receptor, cortisol's target[138].

2.1.4. Genetics on the relationship between depression and obesity

Genetics also represents a major factor regarding the bidirectional relationship between depression and obesity. Both conditions have been described to be influenced by genetics, with around 30–40% of heritability explained by additive genetic effects[70,139]. Considering BMI and obesity-related traits, the gene prioritisation of the two most recent GWAS report, as described above, that among genes near to BMI-associated SNPs there is an over-representation of genes closely linked with central nervous system development and neurogenesis, and a differential expression in brain[122,123]. Moreover, there was found a significant enrichment for brain cells when the cell types involved in the polygenic contribution to BMI heritability was studied[140], reinforcing the idea of an involvement of common brain regions in energy homeostasis and depression.

On the other hand, GWAS on depression have recently reported more than a hundred of independent loci associated with different depressive phenotypes. Significant genetic signals located in or near to genes previously associated with BMI, such as the neuronal growth regulator 1 gene (*NEGR1*), or olfactomedin 4 (*OLFM4*) provide further support for this relationship.

More complex interactions have been observed between genotypes, depression and obesity, such as the moderator effect of the *FTO* rs9939609 polymorphism, by which the main BMI-increasing effect is more pronounced in those carriers of the risk allele that have depression[141]. Further analyses performed in these recent GWAS, in order to find genetic correlations of depression with multiple traits, found significant positive correlations

between depression and multiple traits, including obesity and BMI-related measurements, such as body fat, waist circumference or waist-to-hip ratio. In Wray, et al. (2018), the genetic correlation between depression and each trait was found between 0.09 – 0.20 [89], whereas in Howard, et al. (2019) genetic correlations remained significant although the goodness of the correlation slightly dropped to 0.07 – 0.17 [90]. These findings support the potential contribution of a common genetic background to the comorbidity of depression and obesity.

Moreover, data from the latest GWAS on depression, which examined subgroups with atypical features -in particular, increased appetite and weight during an active episode- found a genetic overlap between this subgroup and immunometabolic traits[142]. In essence, they found that a subgroup of cases with depression characterised by increased appetite/weight symptoms were associated with a higher polygenic risk score for circulating leptin, increased BMI, and C-reactive protein, a classic inflammation marker. This reflects that also these shared mechanisms seem to have a common genetic base.

Two final remarks towards the bidirectional relationship between depression and obesity should be considered. First, the use of antidepressants in overweight and obese patients with depression might not be optimal, since their use has been associated with higher risks for diverse complications, namely cardiovascular disorders and diabetes, compared to those patients not receiving antidepressants[143]. Indeed, also in the general population, different antidepressant drugs have been related with increased risks for diverse outcomes. Therefore it is strongly recommended to consider their individualised prescription, considering and balancing benefits and potential risks[144].

Finally, as is revealed in the evidence presented above, the alterations in lifestyle are related to the aforementioned biological pathways. For instance, an impaired sleeping pattern is related to increased cortisol, indicators of inflammation and leptin, and reduced insulin sensitivity, relevant pathways involved in both depression and obesity, and also risk factors for both conditions[145,146]. Sedentary lifestyle, spending less time in physical activity, eating more high caloric palatable food, and sleeping less hours are commonly found in patients with depression, together with other risk factors such as smoking or alcohol abuse, and are common risk factors for obesity[147,148].

2.2. Physical activity

2.2.1. General overview

Physical activity is defined by the WHO as any bodily movement produced by skeletal muscle resulting in energy expenditure[149], using the definition provided by Caspersen nearly half a century ago[150]. This definition allows for the inclusion of movement in essentially all circumstances, whether in leisure time, commuting to work or any place, or during a person's work.

Exercise refers to a specific category of physical activity which is planned, structured, repetitive, and performed with the aim of improving or maintaining one or more components of physical fitness[150]. **Physical fitness** is the ability that the body has to perform both physical activity and exercise, and is closely related to health[150]. Components of physical fitness are divided into those related to health (cardiovascular and muscular endurance, muscular strength, flexibility and body composition), and to athletic skills (agility, balance, coordination, speed, power and reaction time).

The relevance of bodily motion for the maintaining or regaining of health has been described by the most ancient bearers of medical wisdom, such as Hippocrates (460 – c.370 BC) and Galen (129 – c. 216 AC)[151,152]. Nowadays, the benefits that the regular practice of physical activity exerts, delaying all-cause mortality, and improving overall health, physical function and fitness are globally unanimous, not depending on age and morbidity status[153–156].

Therefore, the effect of physical activity has extensively been the focus of randomised controlled trials for a wide range of applications. Two types have predominated among the exercise interventions: aerobic and resistance exercise. **Aerobic exercise** includes activities performed sustainedly or using intervals, which increase heart and respiratory rates, thereby improving the efficiency of the cardiovascular and respiratory systems[155]. Common examples of aerobic exercise are walking or running, cycling, or swimming. **Resistance exercise** involves movements exerted sustained or intermittently against a certain resistance or force, aiming to improve muscular strength or endurance[155]. Common examples of resistance exercise are weights-based equipment or body-weight training.

Generally, the intensity of aerobic physical activity can be divided into light, moderate and vigorous. The metabolic equivalent of task (MET) is the physiological measure employed for measuring intensity[149]. Seating at rest expends the energy equivalent of one MET. The equivalences of each category of intensity are summarised in **Table 3**, including examples of activities.

The WHO guidelines for physical activity include, for adults, 5 days/week of moderate-intensity activity for a minimum of 150 minutes/week or 3 days/week of vigorous activity for more than 75 minutes/week, and at least two days/week of resistance exercise training[149]. The risks of sedentary behaviour are also advised, and therefore recommended to limit sedentary time. However, since the beginning of the century, insufficient physical activity has increased in high-income countries (from 31.6% to 36.8%), where sedentary behaviour and the proportion of physical activity is twice as high in high-income countries. Globally, in 2016, 23% of adult men and 32% of adult women did not meet these global recommendations[157], and no improvements have been found since the beginning of the century either globally.

Table 3. Equivalence in METs of the different levels of aerobic physical activity intensity, including examples.

Intensity of aerobic physical activity	**METs**	**Examples**
Sedentary behaviour	1.5 or lower	Watching TV, driving a car, lying, sitting
Light-intensity	1.6 –2.9	Slow walking, light housework (cooking, ironing)
Moderate-intensity	3.0 – 5.9	Brisk walking, light biking, most manual labour
Vigorous-intensity	6 or more	Running, biking for exercise, swimming, sports (soccer, tennis, basketball)

2.2.2. Physical activity and mental health

Although it has been classically understood differently from the original intention, the iconic quote "*mens sana in corpore sano*", from the Roman poet Juvenal, has been widely employed to illustrate this relationship[158]. While Juvenal intended to explain in his Satires that the only prayer-worthy objectives were physical and mental wellness –rather than banal pursuits such as power or fame– it is nowadays known that physical activity would bring a person closer to both aims.

The effect of physical activity on fitness and health has mostly been referred to physical health, where the effects remain more than evident. In this respect, it is important to note that people living with mental illness –a vulnerable population for diverse physical conditions– also are benefited from the effects of physical activity, e.g., increasing cardiorespiratory fitness, improving glucose metabolism or lowering blood pressure, overall reducing cardiometabolic risk, among others[159–161].

However, the link between physical activity and mental health has long been present and extends far beyond the most obvious physical benefits. Moreover, the COVID-19 pandemic has turned this relationship into a trending subject. Indeed, a recent systematic review on how physical activity affected mental health during the COVID-19 pandemic remarks that higher physical activity was associated with a better mental health –i.e., less depressive, anxiety and stress symptoms– independently of age[162]. Nonetheless, although the importance of physical activity on mental health was highlighted during the pandemic, there is a wealth of knowledge regarding this relationship. In sum, current literature points towards three different types of mechanisms by which physical activity exerts its beneficial effects on mental health: (1) neurological pathways, (2) physical/hedonic effects of physical activity itself, and (3) a reinforcement of positive behavioural mechanisms for aiming positive changes[163].

When talking about the relationship between physical activity and mental health, we have to draw a clear distinction between cross-sectional studies and longitudinal studies. The former address the prevalence of certain conditions in a cohort with a set of characteristics collected at a given point in time, whereas the latter are designed to permit a follow-up of the same cohort. Therefore, establishing causal relationships is feasible only in longitudinal studies. Moreover, randomised controlled trials are a subtype of longitudinal studies that provide a higher validity and bias control, therefore making easier to draw relationships between the intervention and the outcome.

The current evidence from **cross-sectional studies** describes a strong association between sedentary lifestyle and insufficient physical activity with an increased risk for poorer mental health. The largest up-to-date cross-sectional study, which included 1.2 million US adults pointed out that participants who exercised self-reported 1.49 fewer days of mental health burden –including stress, depression, or problems with emotions– than participants who did not exercise[164]. Here, the associations were consistent across socio-demographic variables –e.g. age, sex, ethnicity– and had higher variations depending on exercise variables –e.g., frequency or intensity. An U-shaped relationship was observed between exercise duration and intensity and lower mental health burden, with exercise durations of around 45 min, and between 3 and 5 days per week leading to the greatest reduction of poor mental health days. Interestingly, BMI was found to be responsible for a 4% worse burden.

Results from a community population sample, which assessed psychological distress using the 12-item General Health Questionnaire, suggested the association between risk of psychological distress and sedentary time[165]. Sedentary time and physical activity were both self-reported (n = 11658) and objectively measured with an accelerometer (n = 1947) during a week, and categorised into tertiles. An association between sedentary time and risk of psychological distress was found in both measurements, although the greatest association was found in the highest tertile of both self-reported and accelerometry-measured sedentary time. Associations between physical activity and risk of psychological distress did not match between both measurements. While an association between self-reported moderate-to-vigorous physical activity and lower risk of psychological distress was reported, in accelerometry measurements only light-intensity activity replicated this association.

Findings from a cross-sectional study conducted in a representative sample of the Canadian adult population (n = 8150) disclosed different patterns in the interplay between self-reported mental health, sedentary time and different physical activity modalities: light-intensity physical activity (LIPA), moderate to vigorous physical activity (MVPA), the combination of LIPA and MVPA, and daily steps[166]. Physical activity and sedentary behaviour were objectively reported using accelerometers during a week. All modalities of physical activities were associated with mental health in non-linear dose-response patterns. The most remarkable results extracted from these analyses suggest that, for MVPA, effects on mental health start from the first minute, increasing up to 50 minutes/day, and, for daily steps, increasing benefits were observed until 5000 – 16000 steps, and beyond that amount, the effect was

detrimental. Analysing the combination of physical activity and sedentary time showed a reduction of the positive physical activity-mental health association as sedentary time increases. Therefore, these results highlight the benefits of even a small amount of physical activity and daily steps, improving when sedentary time –which should be considered a risk factor for poorer mental health– is avoided. This effect has been shown in previous evidence, reporting that the associations between MVPA and self-reported psychological distress, depressive symptoms and anxiety symptoms, were dependent on sedentary behaviour[167].

This latter concept of sedentary time as a risk factor for poorer mental health, even in presence of physical activity, modulating its positive effect, has been repeatedly observed and could be of great relevance given current lifestyle trends[167–169]. As a consequence, potential differences of the effect of physical activity on mental health depending on sedentary behaviour could be expectable.

On the other hand, **prospective studies** suggest a protective effect of physical activity against developing diverse mental health disorders. Physical activity has been robustly associated with prevention of depression and anxiety in longitudinal studies, as reported in recent meta-analyses[170–172]. This relationship between depression and anxiety and physical activity has been reported to be bidirectional, with an association described as a vicious circle[173]. Meta-analyses of randomised controlled trials of exercise interventions on depression have reported an improvement in depressed patients allocated to an exercise intervention[174], with similar findings in trials only including resistance exercise[175]. Moreover, in a review of randomised controlled trials studying the effect of interventions with exercise in sedentary adult participants suffering a chronic illness, reduced anxiety symptoms in the intervention groups, when compared to a control no-treatment condition[176]. Even though the same effect direction has been observed in a recent meta-analysis on schizophrenia and psychotic related disorders, it does not maintain statistical significance after adjusting for covariates[177]. Results from randomised controlled trials have reported similar evidence, with therapeutic benefits compared with no interventions or conventional control conditions in schizophrenia and non-affective psychotic disorders, although without reaching statistical significance[178].

Additionally, the benefits of physical activity would extend to reducing the risk of cardiovascular and metabolic diseases, which are usually comorbid with depression, as described above. Nonetheless, a recent meta-analysis including 69 case-control studies noted that cases with severe mental illness –schizophrenia, bipolar disorder or depression– were more prone to sedentary behaviours and less likely to meet the aforementioned guidelines recommendations than their mentally-healthy counterparts[160]. Therefore, it is necessary to remember that in people with severe mental illness, multiple challenges of various nature hinder adherence to programmes and regular physical activity[178] –particularly of three types: physical, psychological and socio-environmental barriers[179]. Thus, encouraging and helping patients to overcome these barriers has a key role to play.

2.2.3. Physical activity in depression

When considering the relevance of physical activity on depression in particular, we must understand that this has been a rather long-lasting journey. Centuries ago, Robert Burton dedicated the chapter *Exercise rectified of Body and Mind* from his medical textbook *The Anatomy of Melancholy* (1621) to highlighting the capacity of physical activity to alleviate melancholy, proposing it as one of its "cures" [180].

Different cross-sectional studies have found a higher prevalence of sedentary behaviours in people with depression, rather than physically active behaviours. The extensive study by Chekroud and colleagues was previously described and included 1.2 million US adults. Here, when only participants with a previous diagnosis of depression were considered, those who exercised reported 3.75 days (34.5%) of poor mental health less than their sedentary counterparts (Wilcoxon test = 1.61×10^9, $p < 2.2 \times 10^{-16}$), similar to the outcome observed in the general population[164].

When comparing between the aerobic and resistance exercise it is important to highlight that aerobic exercise requires less equipment and experience, and dosing variables are easier to control, being this also able to be extrapolated to application in everyday life. This has been proposed to be a key reason for the observed predominance of aerobic exercise interventions in randomised controlled trials[181].

In the meta-analyses of prospective studies by Pearce and colleagues, a dose-response between physical activity and depression was observed, with lower risk of depression in participants exercising following WHO guidelines (25% lower risk of depression [95% CI = 18 - 32%])[182]. Exercising at half of the recommended amount reduced the risk in 18% (95% CI = 12 - 23%), providing evidence of the inverse dose-response relationship between exercise and depression. However, in higher levels of exercise, the authors reported less benefits and higher uncertainty.

This is in line with results by Dishman et. al, who reported in a meta-analysis of 111 studies a steeper decrease in the risk of depression after moderate-to-vigorous physical activity than following light physical activity –although both types met public health guidelines[183]. Therefore, it appears reasonable to expect that, as exposed in a review by Ross and colleagues, the efficiency of the intervention is increased when the exercise doses meet the current health guidelines and recommendations[181].

Despite the above-described role of physical activity on depression, there are mechanisms underlying this relationship which remain to be fully understood. Results suggesting a bidirectional relationship support both the "protective" and the "inhibitory" hypotheses. According to the "protective hypothesis", the decrease of depressive symptoms as a consequence of physical activity is driven through its biological (increased neuroplasticity, angiogenesis, lowered inflammation and cortisol levels, anti-oxidative effects) and

psychological effects (self-esteem, social support)[184]. In contrast, the "inhibition hypothesis" maintains that depressive symptoms, e.g., anhedonia, lack of energy, or social withdrawal, negatively influence a regular physical activity behaviour[185].

2.2.4. Genetics on the relationship between physical activity and depression

Physical activity is a genetically complex trait, with different dimensions that may be influenced by genetic inheritance. A recent study using data from the UK Biobank performed GWAS in different physical activity traits, obtaining different sets of independent loci for each trait: self-reported MVPA (9 loci), self-report practice of 3 or more days of vigorous physical activity (5 loci), self-report of practising strenuous sports for more than 2-3 days/week for more than 15-30 min (6 loci), and two measurements using accelerometry (2 and 1 loci, respectively)[186]. The sample in the self-reported analyses was of approximately 377,000 participants, whereas the accelerometry analysis was performed in a sample of n ≈ 91,000. In another recently published GWAS, a total of 7 independent SNPs were identified and associated with accelerometer-measured physical activity –two of them already known– and sedentary behaviour, in a UK Biobank cohort of around 91,000 participants[187]. In the most up-to-date GWAS meta-analysis, which includes 51 studies and data from more than 700.000 individuals, 104 independent SNPs in 99 loci were associated with self-reported MVPA and sedentary behaviour (leisure screen time and/or sedentary behaviour at work)[188].

The relationship between physical activity and depression has been assessed by different approaches involving the existing genetic information from the most recent large-scale GWAS. A polygenic risk score (PRS) generated using data from the mega-GWAS of depression published in 2019 was associated with depression in a UK Biobank cohort[189]. Here, the practice of physical activity showed a protective effect at every level of genetic vulnerability, i.e., physical activity reduced the risk for developing depression even for individuals with a high PRS. A recent two-sample Mendelian randomization (MR) study performed for depression and physical activity in 611,583 participants provided evidence of a protective causal association between objectively measured physical activity –using an accelerometer– and depression[190]. In contrast, there was no statistically significant evidence of a relationship between depression and self-reported physical activity.

Considering specific biological targets, there has classically been a great interest in the function of neurotrophins, where BDNF holds a prominent position. As aforementioned, BDNF has classically raised considerable interest, given its function in the adult brain, regarding neuroplasticity and synaptic transmission. Apart from the previously described relationship between depression and the *BDNF* rs6265 polymorphism —leading to conflicting results—, studies analysing changes in protein levels have been performed to analyse the link between an adequate BDNF function and viability of neurons in brain circuits involved in mood disorders[191]. For instance, lower serum BDNF concentrations have been observed in chronic depression patients compared to their healthy counterparts, as reported

in a 2-year longitudinal study in 1751 individuals[192]. Here participants included patients with incident, remitted and persistent depression, as well as controls, and significant decreases of BDNF were observed in persistent and remitted depression. Results from a meta-analysis suggest that these low levels of serum BDNF which are observed during depression return to normal concentrations during remissions[68]. Results in mice have reported that depression-like symptomatology is reduced following peripheral BDNF administration, together with an increase in neurogenesis[193].

Moreover, physical activity has also been closely associated with modulation of BDNF levels, inducing a transient increase in peripheral BDNF concentrations following an acute bout of exercise. This has been observed in the general population[194,195], and in patients with depression[196–199]. Given the positive effects of physical activity on neuroplasticity and BDNF levels, BDNF has been suggested as a potential mediator of the therapeutic and preventive benefits of exercise on depression[200].

Further research is required for a better understanding on the relationship existing between depression and physical health. It seems plausible that the genetic background of individuals may interact or have a certain relationship with parameters related with physical health. For this reason, the present Doctoral Thesis aims to study the genetic differences between cases with depression and controls, and then to assess the potential relationship between genetics and physical health.

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AIMS

AIMS

The overall aim of this Doctoral Thesis is to investigate the genetic differences between cases with depression and controls in an epidemiological sample representative of the Andalusian general population (the PISMA-ep study), as well as to identify the potential interaction between the genetic background and the environment, in terms of physical health. The present International Doctoral Thesis is composed of six studies, divided into four different parts: **Chapter I** focuses on the study of the genetic variants associated with depression in the PISMA-ep cohort; **Chapter II** focuses on the relationship between depression and obesity; **Chapter III** focuses on the relationship between depression and physical activity; and **Chapter IV** focuses on the relationship between depression, physical activity and the potential implication of genomics.

Chapter I

General objective 1: To identify the genetic variants associated with depression through the analysis of a GWAS in the PISMA-ep epidemiological study.

- Specific objective 1.1: To conduct a GWAS in the epidemiological sample of the PISMA-ep study, assessing the genetic variants associated with depression, and to evaluate in the PISMA-ep cohort a polygenic risk score (PRS) using base data from the largest up-to-date GWAS on depression (**Study 1**).

Chapter II

General objective 2: To investigate the relationship between depression and BMI, as an obesity-related indicator of physical health, in a cohort representative of the general population.

- Specific objective 2.1: To conduct a systematic review of the existing literature on the role of the *FTO* rs9939609 polymorphism in the relationship between depression and BMI (**Study 2**).

- Specific objective 2.2: To construct and evaluate a genetic risk score (GRS) using candidate SNPs in the PredictD-CCRT cohort, assessing the predictive effect of the inclusion of BMI in predictive models (**Study 3**).

Chapter III

General objective 3: To investigate the relationship between depression and physical activity in a cohort representative of the general population.

- Specific objective 3.1: To conduct a systematic review of the existing literature on the role of both the BDNF protein and its most relevant polymorphism (rs6265, Val66Met) on the relationship between physical activity and depression (**Study 4**).

- Specific objective 3.2: To assess the potential interaction existing between the*BDNF* Val66Met polymorphism), depression and physical activity in the PISMA-ep sample (**Study 5**).

Chapter IV

General objective 4: To investigate the genomics of depression in the PISMA-ep study and to assess its relationship with obesity and physical activity as parameters related to physical health.

- Specific objective 4.1: To generate a polygenic risk score (PRS) using base data from the largest up-to-date and assess its predictive ability on a subsample of the PISMA-ep study. Analyse the effect on the predictive ability of including BMI and physical activity in the predictive models (**Study 6**).

METHODOLOGICAL OVERVIEW OF THE STUDIES INCLUDED

Methodological overview of the studies included

The present Doctoral Thesis employed genomics data derived from two ambitious epidemiological research studies representative of the general population, previously conducted by our research group and collaborators in Spain, as well as summary statistics provided from the Psychiatric Genomics Consortium latest GWAS.

1. PISMA-ep study

The PISMA-ep study is a cross-sectional study, based on a broad community-dwelling sample, representative of the adult population from the Southern Spanish community Andalusia. The PISMA-ep study aimed to establish the prevalence of the most common mental disorders in Andalusia and to detect their potential risk factors (e.g., social, psychological, genetics, etc) in this representative sample, as well as to create a base cohort for potential follow-ups or prospective studies. The study interviews took place between 2013 and 2014. Inclusion criteria for being eligible were: being between the ages of 18 and 75 years old, and having resided for at least one year in Andalusia. Participants were excluded if they could not complete the interview due to illness, if they could not speak Spanish fluently, if they presented severe cognitive impairment or intellectual disability, or if they were institutionalized (usually residing in an institution, e.g., hospital, prison, etc.).

Following standard stratification levels of the population, the participants were selected using a simple random method of assignment, and further interviewed following a "door-knocking" approach. Age and gender quotas were considered throughout the selection process. From the 5,496 households that were initially selected, 3,892 (70.8%) were substituted due to absence of response or not including any eligible participant, whereas 989 (16.3%) refused to participate. Thus, 4,507 (83.7%) completed their participation, and 4,286 of them (78%) provided a biological sample (saliva) for the genetic analysis.

This study was approved by the Research Ethic Committee of the University of Granada. All participants provided written informed consent, and all procedures of the study complied with the ethical standards, i.e., the Helsinki Declaration of 2008, and relevant national and institutional committees on human experimentation. A full description of the PISMA-ep protocol is available elsewhere [1].

1.1. GRANADΣP. A pilot study in the province of Granada

The GRANADΣP was the pilot study for the PISMA-ep study. It was a cross-sectional study based on a community-dwelling adult population living in the province of Granada, Southern Spain. The same protocol as in the PISMA-ep study was followed, although performed in the province of Granada. Therefore, the aim of the GRANADΣP study was to estimate prevalence and correlated factors of common mental disorders in the province of Granada.

Analogous inclusion and exclusion criteria were used for determining the eligibility of participants. A target sample of 1176 participants was determined and contacted to participate. Of these, 367 participants refused their participation and a total of 54 participants were excluded and further substituted with individuals matched for sex, age and location. A total sample of 809 participants were finally included in the study [2].

2. PredictD-CCRT Study

The PredictD-CCRT study is a multicentre, cluster-randomized controlled trial, which was conducted in 7 Spanish cities, with 70 primary care centers participating. Since the Spanish National Health System provides coverage with free medical service to more than 95% of the population, there was a representative sample from the three Andalusian cities participating (Málaga, Jaén and Granada). Cluster assignment was performed by primary care centers, and a 18 months follow-up was conducted. The main aim of this study is to evaluate and compare the performance of a preventive intervention on depression incidence, based on an assessment of the risk and profile of each individual. A detailed characterization for clinical, psychological, sociodemographic, anthropometric, lifestyle, and other environmental variables were performed, and those agreeing to participate in the genetic study gave specific informed consent and provided a biological sample. A total of 2,123 participants were included in the PredictD-CCRT study.

In each participating city, a relevant ethics committee approved this study, which was in compliance with the Helsinki Declaration. For the selection of participants, in each health center, an assistant researcher selected participants using a systematic random sampling from the family physician's appointment lists. Participants would be further excluded if they were not between 18 and 75 years old, if they were unable to understand or speak Spanish, if they presented a severe mental disorder, cognitive impairment, or terminal illness, if they planned to be out of the city for more than 4 months during the follow-up period, or if a representative attended the appointment instead of the patient. A full description of the PredictD-CCRT protocol is available elsewhere [3].

3. Characterization of the sample

All individuals were assessed for sociodemographic, clinical, anthropometric and physical activity variables using validated questionnaires and clinimetric instruments. The main variables analysed were the following:

3.1. Depression

In the GRANADΣP and PISMA-ep studies, the Mini-International Neuropsychiatric Interview (MINI) was employed to ascertain the diagnosis of mental disorders. The MINI is composed of modules, corresponding to different diagnostic categories from Axis I psychiatric disorders compatible with DSM-IV and CIE-10 criteria. All interviewers were previously adequately

trained in order to conduct the interviews with knowledge of interviewing techniques, protocol scales and inventories.

In the PredictD-CCRT study, depression was ascertained using that particular section of the Composite International Diagnostic Interview (CIDI). The CIDI is a structured interview designed to provide current diagnosis of mental disorders according to DSM-IV and ICD-10 criteria. It has been developed by the WHO [4], and validated in different cultures [5,6], allowing the measurement of prevalence, severity and burden of mental disorders. Here, interviewers providing the CIDI were specifically trained for that aim, and were independent from those family physicians that would provide assistance during the intervention.

3.2. BMI and obesity

In PISMA-ep, GRANADΣP and PredictD-CCRT studies, self-reported measurements of height and weight were obtained for every participant. Body mass index was calculated as weight (in kilograms) divided by height (in square meters): weight [kg] / height [m²]. Obesity categorization was obtained applying international cut-off reference points reported by the WHO: underweight (BMI < 18.5 kg/m²), normal weight (BMI 18.5–24.99 kg/m²), overweight (BMI 25.0–29.99 kg/m²) and obesity (BMI ≥ 30 kg/m²) [7].

3.3. Physical activity

In the PISMA-ep study, four questions were included in a questionnaire in order to gather this information about the participants: a) a dichotomous question to report whether the participant performed physical activity or not; b) in case of a previous affirmative answer, the nature of the physical activity, having to choose between 1) leisure, 2) work, 3) housework; c) the number of hours of physical activity performed per week, and the intensity that the participant considered this activity was, to be chosen between 1) light, 2) moderate and 3) vigorous. This information was not obtained for the GRANADΣP study nor the PredictD-CCRT.

4. Genomic procedures

Participants from PISMA-ep, GRANADΣP, and PredictD-CCRT studies who gave specific informed consent, participated in the genetic study. Biological samples were obtained using Oragene® saliva DNA collection kit (OG-500; DNA Genotek Inc.), and DNA was further extracted following the Oragene® Saliva Collection Kit protocol.

4.1. Genotyping of candidate genes

In the samples of PISMA-ep and GRANADΣP, two key SNPs were genotyped: rs6265, the *BDNF* Val66Met polymorphism, and the *FTO* SNP rs9939609.

In the PredictD-CCRT, 56 candidate SNPs mapping genes involved in depression were genotyped. These SNPs were selected after performing an extensive review of the literature, of the existing GWAS and case-control studies.

4.2. GWAS genotyping

A total of 288 cases with depression from PISMA-ep and GRANAD□P studies, together with 1427 controls from PISMA-ep, were genotyped using the Illumina Infinium PsychArray-24 BeadChip (Illumina, San Diego, CA, USA), which was developed in collaboration with the Psychiatric Genomics Consortium.

4.3. Data from the Psychiatric Genomics Consortium

Summary statistics from the largest GWAS on depression at the moment [8], were downloaded from the PGC website (https://pgc.unc.edu/for-researchers/download-results/). These data were used as base data for generating polygenic risk scores, further tested on the PISMA-ep cohort.

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RESULTS

RESULTS

CHAPTER I
Study 1

CHAPTER I: Genetics of depression in the PISMA-ep epidemiological sample

Study 1: GWAS and PRS of depression in the PISMA-ep sample

1. Introduction

Depression is the main cause of disability worldwide[1]. It is also the most prevalent mental disorder in the U.S. and in Europe —together with anxiety disorders–, with a prevalence of 5.2% in Spain in 2015[1,2]. Depression is a highly disabling mental disorder and can manifest itself along with a wide range of symptoms, making it a particularly heterogeneous disorder [3].

Depression has been described as a multifactorial and polygenic, genetically complex trait, therefore being influenced by a large number of genetic variants, each of small effect[4]. The emergence of genome-wide association studies (GWAS), in the beginning of the past decade, brought substantial progress in the understanding of the genetic architecture of depression. However, the initial GWAS performed in this disorder did not succeed in identifying variants significant at a genome-wide level[4–8], which led to considering new approaches, once different obstacles -e.g., high prevalence, heterogeneity or a moderate heritability- were identified[9,10]. The recent efforts by different scientific communities, such as the *Psychiatric Genomics Consortium*, for increasing sample sizes, as well as the use of a broader definition of depression to optimise the sample to be analysed, have led to the discovery of an appreciable number of significant loci[11–13]. Nonetheless, the following steps have been suggested to be aimed towards a narrower definition of the depressive phenotype, while maximising sample size[14,15]. This occurs as a consequence of observing the risk of identifying associated loci that might not be specific to the disorder, but common with different psychiatric disorders[16].

In this study we perform, for the first time in a Spanish sample, a GWAS for depression, analysing a cohort from the PISMA-ep study, which is representative of the general Andalusian population and allows the comparison between clinically ascertained cases with depression -diagnosed following a structured interview- and controls. Moreover, a polygenic risk score (PRS) analysis was performed, in order to assess the validity of the largest up-to-date genome-wide association available results for predicting clinical depression.

2. Methods

Study population

The sample included in the current study is part of the PISMA-ep cohort, a cross-sectional study, based on a broad community-dwelling sample, representative of the adult general population from the Southern Spanish community of Andalusia[17], and its pilot study, GRANADΣP, which was carried out in the province of Granada with the same inclusion and exclusion criteria as the PISMA-ep[18]. Since the main aim of these studies was to estimate the prevalence of the most frequent psychiatric disorders, a DSM-IV diagnosis of major

depression was ascertained by a trained team of psychologists which employed the Spanish version of the Mini-International Neuropsychiatric Interview (MINI). A more complete description of the samples is available in the *Methodological overview of the studies included* section.

Genotyping
A total of 288 depression cases and 1427 controls were genotyped using the Illumina Infinium PsychArray-24 BeadChip (Illumina, San Diego, CA, USA), which was developed in collaboration with the Psychiatric Genomics Consortium and is commonly employed for large-scale genetic studies researching for psychiatric predisposition and risk. This array includes ~593,260 fixed markers, including SNPs from arrays developed for identifying putative functional exonic variants (Exome-24 BeadChip) and informative genome-wide tag SNPs (Infinium Core-24 BeadChip), and 50,000 additional markers associated with common psychiatric disorders. The genotyping of the controls was performed at the Stanley Center for Psychiatric Research at Broad Institute (USA), whereas cases were genotyped in the Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO).

GWAS quality control (QC), principal component analysis (PCA) and imputation
The merge of cases and controls, first QC, first PCA and imputation was also performed at the Stanley Center for Psychiatric Research at Broad Institute (USA), using the PGC RICOPILI pipeline[19]. Initially, merging the genotypic information from cases (571,389 SNPs) and controls (588,628 SNPs), which were genotyped separately, led to an exclusion of nearly 40% of the variants (339,626 SNPs remaining), the majority of them for being monomorphic sites. An initial PCA of the merged sample was employed to identify and exclude 61 outliers (11 cases and 50 controls). The first QC, after removing SNPs with a missingness >5%, consisted in excluding a total of 29 IDs (11 cases and 18 controls) for any of these criteria: per-sample call rate <98%, excessive heterozygosity (inbreeding coefficient >20%), or sex mismatch. Then, 7,935 SNPs were removed due to any of the following filters: per-sample call rate <98%, being invariant SNPs, Hardy-Weinberg disequilibrium (p-value $< 1 \times 10^{-6}$ in controls and $< 1 \times 10^{-10}$ in cases), difference in call rate between cases and controls >1%; minor allele frequency (MAF) <2%. Since there were 43 genome-wide significant SNPs in the pre-imputation association test, a stricter threshold on MAF (<1%) and missingness (>1%) was applied, excluding 61,223 SNPs in this step. A total of 266 cases, 1359 controls (n = 1,625) and 270,468 SNPs for subsequent analyses remained after QC.

Then, the presence of relatives was assessed, estimating the identity-by-descent (IBD) with PLINK v1.90[20,21], and removing an individual of each pair of duplicates (pi-hat > 1.99) or relatives (pi-hat > 0.45), giving priority to the cases among each pair, and in case they belong to the same group, keeping the one with the highest call rate. In this step, 144 samples were discarded (7 cases and 137 controls).

In order to identify ancestry outliers, a reduced number of quality-filtered independent variants (101,025 SNPs) were selected from our sample. We employed PLINK software to perform a second PCA, calculating the first 20 PCs per individual and plotting PC1 and PC2 using R version 4.2.2[22] in the RStudio software[23]. A total of samples (10 cases and 19 controls) that were >4 standard deviations from the centroid of the plotted cluster were considered outliers.

Later, genotypes were imputed at the Stanley Center for Psychiatric Research at Broad Institute, using the Minimac3 imputation service[24], and the first release of the Haplotype Reference Consortium (HRC) panel[25]. A total of 6,338,441 SNPs were available after imputation.

A final QC was performed before the association study, in order to remove SNPs that after the imputation had a MAF < 1%, a call rate <98% or were deviated from Hardy-Weinberg equilibrium (p-value < 1×10^{-6}). Here, 54,557 variants were removed due to missing genotype data (low call rate), 4 variants due to Hardy-Weinberg disequilibrium, and 1,553,900 variants had a MAF lower than 1%.

In the final association analysis, 249 cases and 1203 controls were included (n = 1452), with a total of 4,729,950 genomic variants.

Genome-wide association study (GWAS)
A logistic regression model of additive effects was employed to conduct the genome-wide association study using PLINK, adding the two first PCs as covariates. Summary statistics were then uploaded in FUMA v1.4.1[26], with 1×10^{-5} as the p-value threshold to consider independent significant SNPs. These were considered lead SNPs if pairwise SNPs had $r^2 < 0.1$. A maximum distance was set at 250 kb between LD blocks to be merged into a single genomic locus. To compute r^2 in LD analyses, the European population in 1000 Genome Project Phase 3 was considered the reference panel[27].

Four repositories were considered for eQTL datasets (GTEx v8[28], PsychENCODE[29], CommonMind Consortium (CMC)[30] and EyeGEx[31]), and genes were mapped to Ensembl gene IDs. LocusZoom was the visualisation tool further employed to have an insight into the highlighted regions of the genome-wide association analysis top-hits, plotting SNP association values, LD structure of a certain risk loci and surrounding genes[32].

Gene and gene-set analyses were performed in addition to single-marker-based GWAS using MAGMA (v1.08)[33]. In this aspect, FUMA uses input GWAS summary statistics to compute gene-based p-values (gene analysis) using the MAGMA tool. The gene-based p-value is computed for protein-coding genes by mapping SNPs to genes if SNPs are located within the genes, using a SNP-wise model and the Bonferroni correction to correct for multiple testing.

Manhattan plots and quantile-quantile (Q-Q) plots for summary statistics and gene-based association tests were generated with FUMA. In the Manhattan plot of summary statistics, in case of overlapping points, the plotted data points were randomly selected. In the gene-based Manhattan plot, the red dashed line was set at P = 0.05 / 18,128 (number of tested protein coding genes) = 2.758×10^{-6}.

Polygenic risk score

With the objective of performing a PRS using the target data of the PISMA-ep sample, the summary statistics from the GWAS mega-analysis of Howard et al., 2019 was employed as base data[12]. Preliminary QCs were followed as reported in Choi et al., 2020[34]. Base data already had, as QC for their original GWAS, an 'info' score (informative of the imputation quality for each SNP) > 0.8. Using PLINK, a MAF > 1% filter was employed, and duplicate, ambiguous or mismatching SNPs were removed. From 8,483,301 variants initially observed, 424,528 were excluded due to MAF threshold and 1,244,172 were ambiguous variants, remaining a total of 6,814,601 SNPs.

A second QC on the base data was performed in a further step. In the QC for the target data, carried out using PLINK, SNPs with MAF < 1%, in Hardy-Weinberg disequilibrium (p-value < 1×10^{-6}), with a missingness in a fraction > 1% of subjects, or individuals with low genotyping rate (> 1% missing), were removed.

In this QC step for the target data, 1,053,004 SNPs were removed due to MAF threshold, whereas 12 variants were removed for Hardy-Weinberg disequilibrium, and 1,660,229 variants were removed due to missing genotype data. 8 individuals were excluded for having a high rate of genotype missingness. From the original number of 1,625 individuals and 6,338,411 variants loaded, a total of 3,625,166 variants and 1,617 individuals (266 cases and 1,351 controls) remained.

Then, using PLINK, samples with extreme heterozygosity (indicating DNA contamination or high levels of inbreeding) were removed, excluding in this step 26 individuals (1,591 individuals remaining, 259 cases and 1,332 controls). Subsequently, relatives were removed, using the same criteria as for the QC prior to the GWAS, resulting in a final sample of 252 cases and 1,197 controls, with a total of 1,449 individuals constituting the target sample.

Prior to the PRS calculation, SNPs from target data were pruned, using a window size of 200 kb, with a step size of 50 kb and a LD r^2 threshold of < 0.25, filtering out 3,453,482 SNPs whereas the final number of SNPs considered for the PRS calculation in the target sample was 171,684 SNPs.

The PRSice-2 software, a PRS program that implements the standard C+T PRS method, was employed to run the PRS analysis[35]. To create independent SNP adjusting LD, clumping was applied, using a LD r^2 threshold of < 0.1 and a 500 kb sliding window (PRSice clumped variants that were within 500kb to both ends of an index SNP).

PRS was calculated assuming an additive model and as recommended when analysing limited sample sizes, we used the standardised calculation of PRS[34,36]. PRS were calculated using the following p-value thresholds: 0.001, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and a full model including every SNP (p-value = 1). We used Nagelkerke's R^2 to assess the proportion of phenotypic variance explained. PRS were divided into quintiles, and the ORs for depression in each quintile were calculated considering the first quintile as the reference group. Using PRSice-2, no covariates were included for the PRS calculation. The logistic regression model was further completed in R to include age, sex and the two first PCs as covariates.

3. Results

Genome-wide association study (GWAS)

We performed a genome-wide association analysis in an epidemiological sample representative of the Andalusian population, from the PISMA-ep and GranadΣp studies, comprising a total of 249 cases with depression and 1203 controls (n = 1452). We analysed the effects of 4,729,950 genetic variants on depression, of which none achieved genome-wide statistical significance ($p \leq 5 \times 10^{-8}$), and 220 SNPs reached a suggestive statistical significance threshold ($p \leq 5 \times 10^{-5}$) in the summary statistics. We did not observe genomic inflation after correcting for the first two principal components (PCs), obtaining a genomic inflation factor (λ) of 1.01353.

Figure 4.a represents the Manhattan plot obtained from the GWAS summary statistics, with the resulting quantile–quantile plot being represented in Figure 4.b. In this analysis we identified 9 independent SNPs that reached the threshold of $p \leq 1 \times 10^{-5}$, whose data are available in Table 4. A total of 430 SNPs were in linkage disequilibrium (LD) ($r^2 > 0.6$) with one of these 9 lead SNPs. Of these candidate SNPs, only 267 existed in the summary statistics file, and were therefore the considered definitive candidate SNPs.

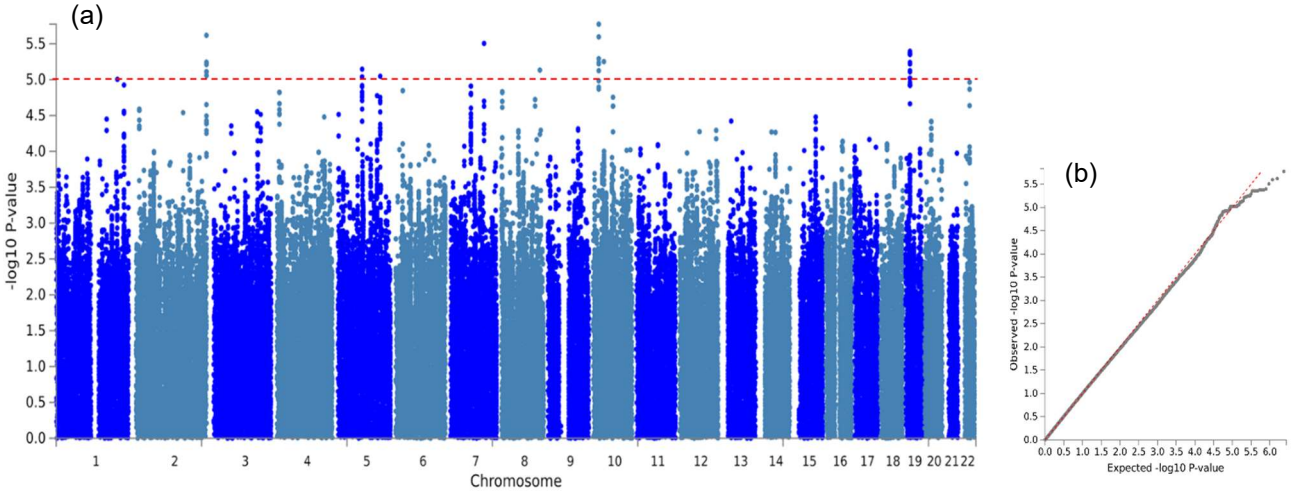
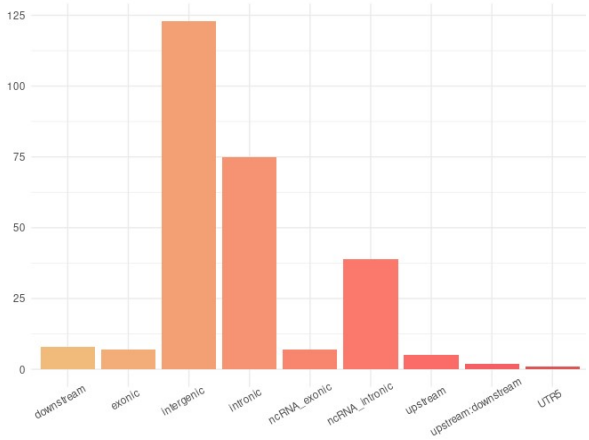


Figure 4. a) Manhattan plot of the resulting -log10 values of the analysed SNPs for their association with depression in a PISMA-ep subsample of n = 1452 (249 cases with depression and 1203 controls). The red dashed line is set at $p = 1 \times 10^{-5}$. b) Q-Q plot of the GWAS summary statistics.

Table 4. Top genomic risk loci found in the summary statistics from the GWAS analysis. 'nSNPs' indicates the number of candidate variants in the locus, both tagged and non-tagged SNPs in the GWAS. The 'nGWASSNPs' column specifies the subset of those that are present in our GWAS summary statistics.

rsID	SNP ID	chr	position	OR	SE	p-value	nSNPs	nGWASSNPs
rs660900	1:210480014:C:T	1	intergenic	4.822	0.356	9.89×10^{-6}	2	1
rs35923607	2:242477025:A:G	2	intergenic	1.783	0.123	2.41×10^{-6}	25	19
rs7716262	5:83302657:G:T	5	intronic (*EDIL3*)	2.995	0.244	7.14×10^{-6}	25	20
rs11540216	5:145506100:A:G	5	exonic (*LARS*)	2.111	0.168	8.95×10^{-6}	115	26
rs62475204	7:115832911:C:T	7	intergenic	1.690	0.113	3.12×10^{-6}	9	8
rs77228953	8:132714713:A:T	8	intergenic	4.121	0.316	7.36×10^{-6}	3	1
rs7916522	10:19768204:C:T	10	intronic (*MALRD1*)	0.583	0.113	1.68×10^{-6}	29	11
rs75288559	10:37390433:C:T	10	intergenic	2.061	0.159	5.60×10^{-6}	53	43
rs11669240	19:15808025:A:G	19	downstream (*CYP4F12*)	1.631	0.106	4.02×10^{-6}	169	138

Results from the positional mapping revealed that the great majority of these 267 candidate SNPs were intergenic or intronic, as can be observed in Figure 5. Whereas only 7 variants were exonic, 88.76% (n = 237) of the variants are related to intergenic or intronic regions.



downstream	8
exonic	7
intergenic	123
intronic	75
ncRNA_exonic	7
ncRNA_intronic	39
upstream	5
upstream:downstream	2
UTR5	1

Figure 5. Positional mapping of the 267 candidate SNPs, based on ANNOVAR annotations (maximum distance of 10kb).

The 9 top-SNPs reported in Table 4 subsequently underwent a fine-mapping analysis. In this preliminary analysis, we could observe the surroundings of the top-hits, as shown in Figure 6. These 9 loci span across multiple genes, harbouring –occured for 6 out of the 9 top-hits– or being in LD with different eQTLs. The lead SNP **rs7916522** is an expression quantitative trait locus (eQTL) variant for the plexin domain–containing 2 *(PLXDC2)* gene (data source: Eye Genotype Expression (EyeGEx) Database[31]; $p_{eQTL} = 3.01 \times 10^{-7}$) and the MAM And LDL Receptor Class A Domain Containing 1 *(MALRD1)* gene ($p_{eQTL} = 2.00 \times 10^{-5}$ in Brain anterior cingulate cortex and $p_{eQTL} = 1.48 \times 10^{-7}$ in Esophagus-Muscularis according to GTEx/v8 database, and $FDR_{eQTL} = 0.009$ in CommonMind Consortium database). Also the lead SNP **rs35923607** is an eQTL for the High Density Lipoprotein Binding Protein (*HDLBP*) gene (data source: GTEx/v8; $p_{eQTL} = 7.44 \times 10^{-5}$ in Adipose-Visceral and $p_{eQTL} = 2.99 \times 10^{-5}$ in Nerve-Tibial). The SNP **rs62475204** is an eQTL for two genes according to GTEX/v8: Testin LIM Domain Protein (*TES*) gene ($p_{eQTL} = 1.87 \times 10^{-5}$ in Adrenal gland; $p_{eQTL} = 3.10 \times 10^{-5}$ in Lung; $p_{eQTL} = 4.33 \times 10^{-5}$ in Testis and $p_{eQTL} = 1.64 \times 10^{-11}$ in Nerve-Tibial) and Caveolin 2 (*CAV2*) gene ($p_{eQTL} = 5.13 \times 10^{-5}$ in Pancreas and $p_{eQTL} = 1.33 \times 10^{-6}$ in Testis). The SNP **rs11669240**, which is located downstream the *CYP4F12* gene, has an influence on the expression of different genes from the Cytochrome P450 Family 4 Subfamily F Members (especially *CYP4F12*, but also *CYP4F2*, *CYP4F3* and *CYP4F11*) in a variety of tissues, according to data from GTEX/v8, EyeGEx and PsycENCODE (Table 5), and also is an eQTL for the Olfactory Receptor Family 10 Subfamily H Member 5 (*OR10H5*) gene, according to the latter database ($p_{eQTL} = 1.44 \times 10^{-7}$). The variant **rs75288559** has been reported to affect the expression of the Zinc Finger Protein 248 gene (*ZNF248*) gene ($p_{eQTL} = 5.63 \times 10^{-5}$ in Colon-Transverse, according to GTEX/v8). Finally, the SNP **rs11540216** is an exonic variant in the Leucyl-TRNA Synthetase *(LARS)* gene, and acts as an eQTL for this gene ($p_{eQTL} = 1.29 \times 10^{-5}$ in Heart-Atrial appendage, according to GTEX/v8), and for the RNA Binding Motif Protein 27 (*RBM27*) gene ($FDR_{eQTL} = 0.049$ in CommonMind Consortium database).

Table 5. Risk increasing allele refers to the direction of the effect found in this GWAS: an allele would be risk increasing if OR > 1, otherwise being the risk increasing allele the alternative allele. Aligned direction depends on the direction of the tested allele's effect and its concordance with the risk increasing allele: it can be either "+" (risk increasing allele increases gene expression) or "-" (risk increasing allele decreases gene expression).

SNP ID	Database	Tissue	Tested allele	p-value	Beta	FDR	Risk incr. allele	Aligned direction	Symbol
rs35923607	GTEx/v8	Adipose_Visceral_Omentum	G	7.44E-05	0.141	5.62E-20	G	+	*HDLBP*
	GTEx/v8	Nerve_Tibial	G	2.99E-05	0.168	1.81E-37	G	+	*HDLBP*
rs7916522	EyeGEx	EyeGEx	T	5.99E-08	-0.385	3.85E-05	C	+	*PLXDC2*
	GTEx/v8	Brain_Anterior_cingulate_cortex_BA24	T	2.00E-05	0.425	3.32E-03	C	-	*MALRD1*
	CMC	CMC_SVA_cis	T	-	-	9.00E-03	C	-	*MALRD1*
	GTEx/v8	Esophagus_Muscularis	T	1.48E-07	-0.293	1.31E-16	C	+	*MALRD1*
rs62475204	GTEx/v8	Adrenal_Gland	C	1.87E-05	-0.339	2.40E-16	C	-	*TES*
	GTEx/v8	Lung	C	3.10E-05	-0.134	2.56E-06	C	-	*TES*
	GTEx/v8	Nerve_Tibial	C	1.64E-11	-0.333	3.93E-31	C	-	*TES*
	GTEx/v8	Testis	C	4.33E-05	-0.141	1.66E-17	C	-	*TES*
	GTEx/v8	Pancreas	C	5.13E-05	-0.349	1.25E-56	C	-	*CAV2*
	GTEx/v8	Testis	C	1.33E-06	0.300	4.79E-63	C	+	*CAV2*
rs11669240	GTEx/v8	Adipose_Subcutaneous	G	6.21E-22	0.357	4.42E-24	G	+	*CYP4F12*
	GTEx/v8	Adipose_Visceral_Omentum	G	9.05E-14	0.272	1.60E-17	G	+	*CYP4F12*
	GTEx/v8	Whole_Blood	G	1.42E-08	0.247	3.73E-25	G	+	*CYP4F12*
	GTEx/v8	Artery_Coronary	G	8.73E-06	0.227	1.96E-03	G	+	*CYP4F12*
	GTEx/v8	Artery_Tibial	G	4.99E-05	0.135	1.01E-05	G	+	*CYP4F12*
	GTEx/v8	Breast_Mammary_Tissue	G	1.94E-13	0.284	4.49E-12	G	+	*CYP4F12*
	GTEx/v8	Esophagus_Mucosa	G	6.72E-05	0.168	4.12E-22	G	+	*CYP4F12*
	GTEx/v8	Heart_Atrial_Appendage	G	2.77E-08	0.238	2.08E-06	G	+	*CYP4F12*
	GTEx/v8	Liver	G	1.19E-10	0.319	1.25E-07	G	+	*CYP4F12*

	GTEx/v8	Muscle_Skeletal	G	7.42E-15	0.315	2.30E-17	G	+	*CYP4F12*
	GTEx/v8	Nerve_Tibial	G	5.76E-05	0.125	1.36E-08	G	+	*CYP4F12*
	GTEx/v8	Skin_Not_Sun_Exposed_Suprapubic	G	5.21E-12	0.212	1.20E-25	G	+	*CYP4F12*
	GTEx/v8	Skin_Sun_Exposed_Lower_leg	G	1.48E-13	0.232	4.18E-52	G	+	*CYP4F12*
	GTEx/v8	Skin_Not_Sun_Exposed_Suprapubic	G	9.38E-05	-0.172	1.14E-20	G	-	*CYP4F2*
	GTEx/v8	Testis	G	1.36E-09	0.476	4.03E-16	G	+	*CYP4F3*
	EyeGEx	EyeGEx	G	4.20E-05	-0.327	3.07E-02	G	-	*CYP4F11*
	PsychENCODE	PsychENCODE_eQTLs	G	4.29E-04	0.071	2.91E-02	G	+	*CYP4F11*
	PsychENCODE	PsychENCODE_eQTLs	G	1.44E-07	-0.150	2.24E-05	G	-	*OR10H5*
	PsychENCODE	PsychENCODE_eQTLs	G	1.27E-05	0.144	1.35E-03	G	+	*CYP4F12*
	PsychENCODE	PsychENCODE_eQTLs	G	9.82E-07	0.155	1.32E-04	G	+	*CYP4F3*
rs75288559	GTEx/v8	Colon_Transverse	T	5.63E-05	-0.169	1.86E-14	T	-	*ZNF248*
rs11540216	GTEx/v8	Heart_Atrial_Appendage	A	1.29E-05	-0.253	9.09E-27	A	-	*LARS*
	CMC	CMC_SVA_cis	A	-	-	4.90E-02	A	+	*RBM27*

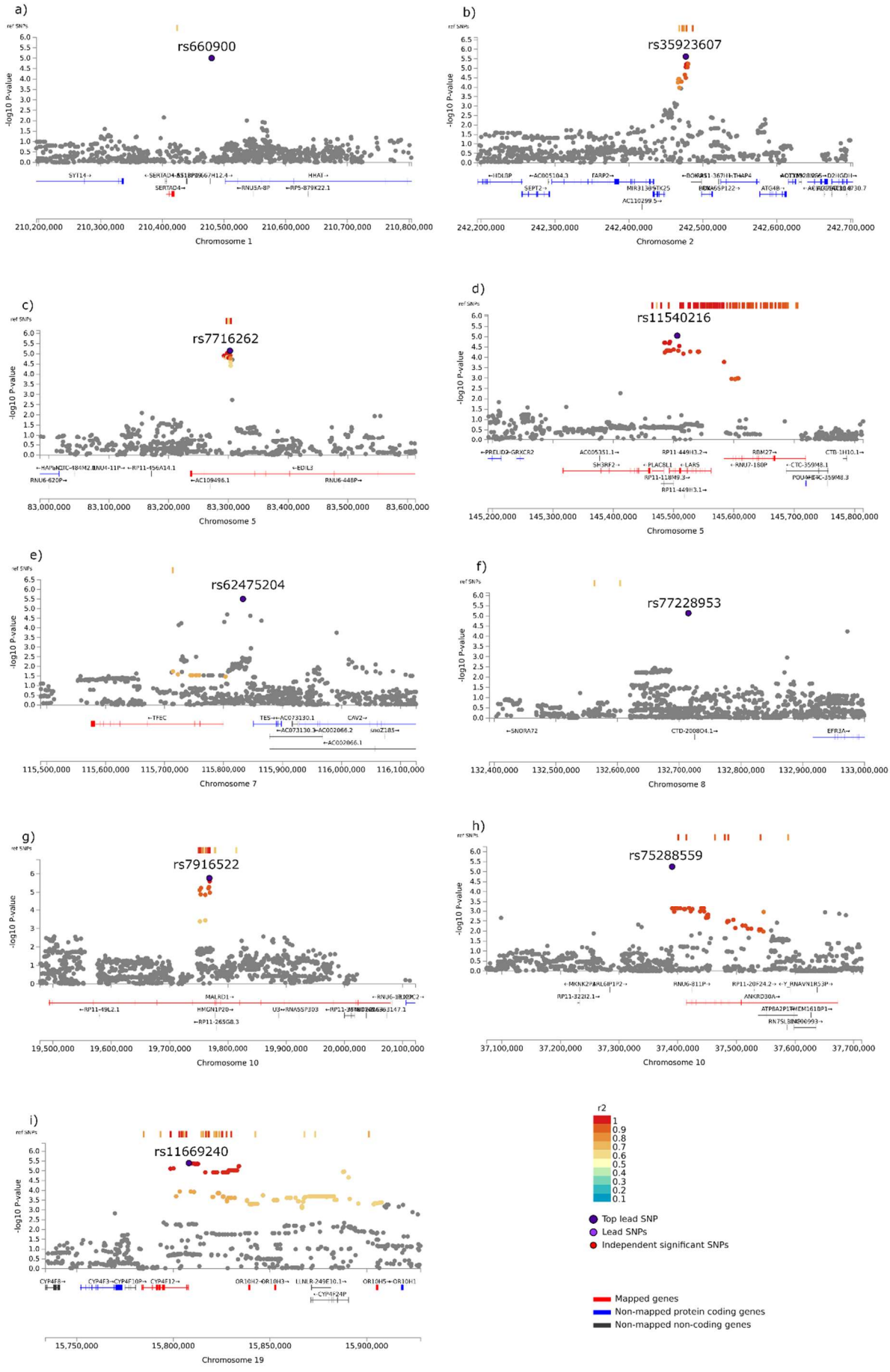

a)
rs660900
-log10 P-value
Chromosome 1
b)
rs35923607
-log10 P-value
Chromosome 2
c)
rs7716262
-log10 P-value
Chromosome 5
d)
rs11540216
-log10 P-value
Chromosome 5
e)
rs62475204
-log10 P-value
Chromosome 7
f)
rs77228953
-log10 P-value
Chromosome 8
g)
rs7916522
-log10 P-value
Chromosome 10
h)
rs75288559
-log10 P-value
Chromosome 10
i)
rs11669240
-log10 P-value
Chromosome 19
r2
Top lead SNP
Lead SNPs
Independent significant SNPs
Mapped genes
Non-mapped protein coding genes
Non-mapped non-coding genes

Figure 6. LocusZoom plot for the regions surrounding the 9 top-hits. From a) to i), the LocusZoom plots show approximately 50-300 kb upstream and downstream the SNP of interest, using an LD panel and reference genome from the EUR population in the 1000 Genome Project Phase 3, defining this length according to the interest of the region, based on a preliminary visual analysis. SNPs are colour-coded as a function of their r^2 to the lead SNP in the locus, as follows: red ($r^2 > 0.8$), orange ($r^2 > 0.6$), or grey (SNPs that are not in LD with the lead SNP –with $r^2 \leq 0.6$– or with missing LD information).

A second Manhattan plot, resulting from the gene-based association test is available in Figure 7. This gene-wise analysis did not provide either any genome-wide significant result. The top-10 most significant genes are represented in Table 6. We observed interesting genes among those most strongly associated with the cases with depression. Among the most significant genes we found *LINGO1*, described in neural processes and disorders[37,38]; other genes whose transcripts are overexpressed in the brain, as occurs with *CA14* or *GSX1* (according to the GTEx/v8 database); *OR10H2* and *OR10H5*, genes that are related to G protein-coupled serotonin receptor activity and its signalling pathway, according to gene-ontology (GO:4993)[39]; or *TFAP2D*, a gene with a variant that has been reported in a GWAS related to antidepressants response (citalopram/escitalopram)[40].

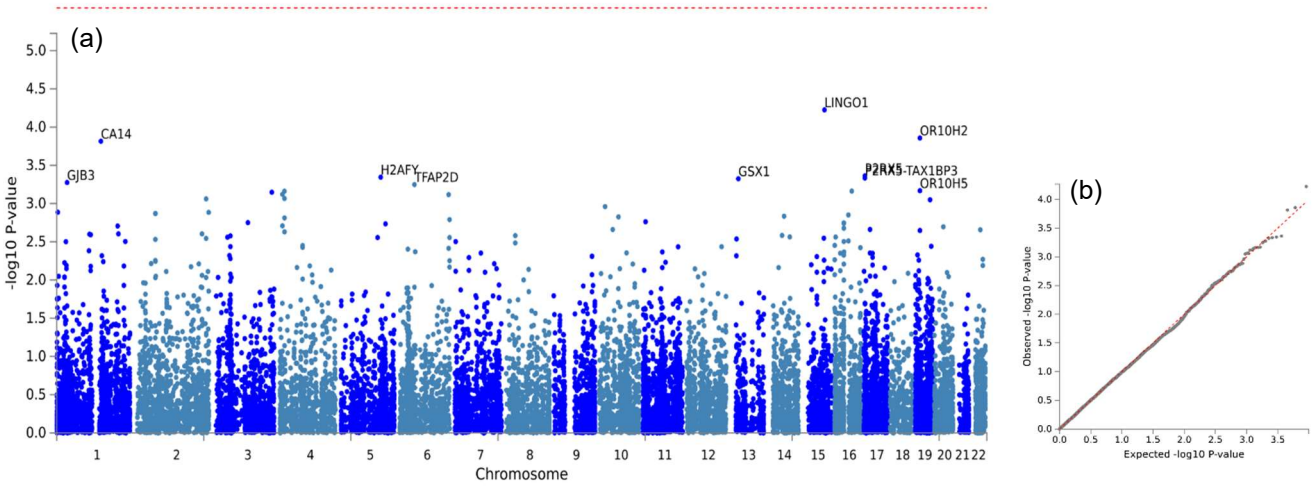


Figure 7. a) Gene-based Manhattan plot, indicating the 10 most strongly associated genes. The red dashed line is set at $p = 2.758 \times 10^{-6}$, resulting from the Bonferroni correction of the 18,128 protein coding genes that were mapped. b) Q-Q plot of the gene-based test, computed by MAGMA v1.08.

Table 6. Most represented genes obtained from the gene-based analysis, obtained using MAGMA v1.08. The column 'nSNPs' indicates the number of variants annotated to the specific gene found in the data and maintained after QCs, whereas the 'nPARAM' column indicates the number of principal components extracted from these SNPs.

gene	symbol	chr	start	stop	nSNPS	nPARAM	z-score	p-value
ENSG00000169783	*LINGO1*	15	77905369	78113242	513	29	3.8478	5.96×10^{-5}
ENSG00000171942	*OR10H2*	19	15838834	15839862	2	1	3.6349	1.39×10^{-4}
ENSG00000118298	*CA14*	1	150229554	150237478	1	1	3.6097	1.53×10^{-4}

ENSG00000083454	*P2RX5*	17	3575493	3599698	36	4	3.3294	4.35×10^{-4}
ENSG00000113648	*H2AFY*	5	134669590	134735604	42	3	3.3184	4.53×10^{-4}
ENSG00000257950	*P2RX5-TAX1BP3*	17	3566357	3599488	46	6	3.3116	4.64×10^{-4}
ENSG00000169840	*GSX1*	13	28366780	28368905	1	1	3.3054	4.74×10^{-4}
ENSG00000188910	*GJB3*	1	35246790	35251970	10	4	3.2731	5.32×10^{-4}
ENSG00000008197	*TFAP2D*	6	50681541	50740701	69	6	3.256	5.65×10^{-4}
ENSG00000172519	*OR10H5*	19	15904761	15905892	11	4	3.2031	6.80×10^{-4}

Polygenic risk score (PRS)

A polygenic risk score was calculated using summary statistics from Howard, et al (2019)[12], the largest up-to-date genome-wide association study performed in depression. A total of 1449 participants (252 cases with depression and 1197 controls) from the PISMA-ep and GranadΣp studies were included in the analysis. For calculating the PRS, no covariates were included. Here, the most explanatory PRS ($R^2 = 0.0115$) was obtained using a p-value threshold of 0.2445, including a total of 31,916 SNPs. The comparison of the different results obtained using different p-value thresholds is represented in Figure 8.

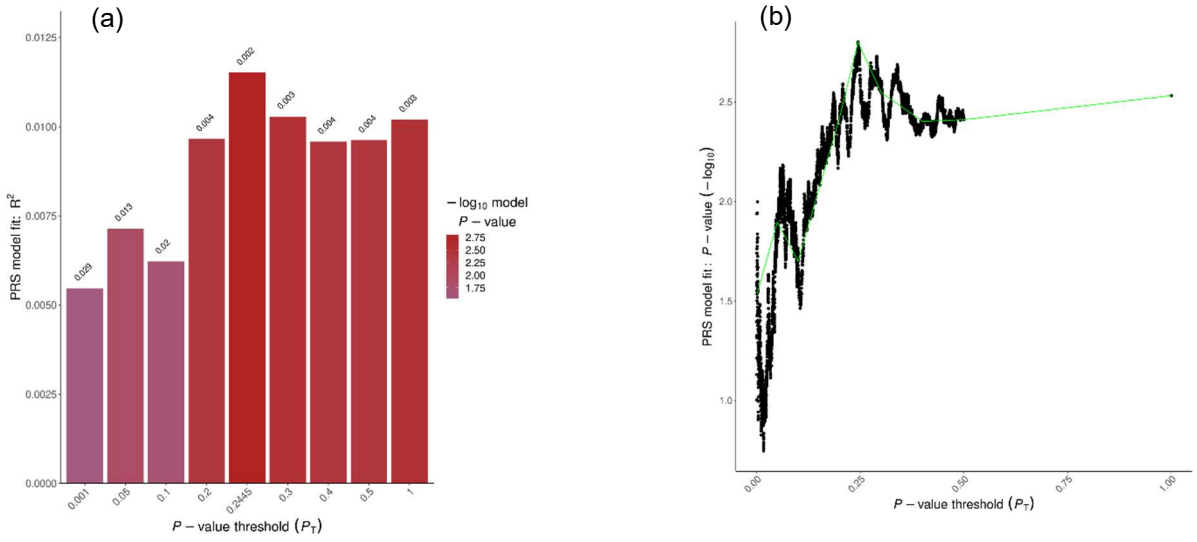


Figure 8. Representation of the model fit of the resulting PRS using different p-value thresholds, as a a) bar plot and b) high-resolution plot.

As can be observed in Figure 9a and 9b, we found a higher prevalence of depression as the PRS increased. A logistic model including only PRS as an independent variable to assess risk of depression provided an OR = 1.238 ($p = 3.112 \times 10^{-3}$). When the model was further adjusted by age, sex and the two first principal components, the association with depression remained statistically significant (OR = 1.229, $p = 5.95 \times 10^{-3}$), as represented in Figure 9. The results of the ANOVA comparison between both models also provided evidence that the full model provided a more parsimonious fit (deviance = 7.65; $p = 5.70 \times 10^{-3}$).

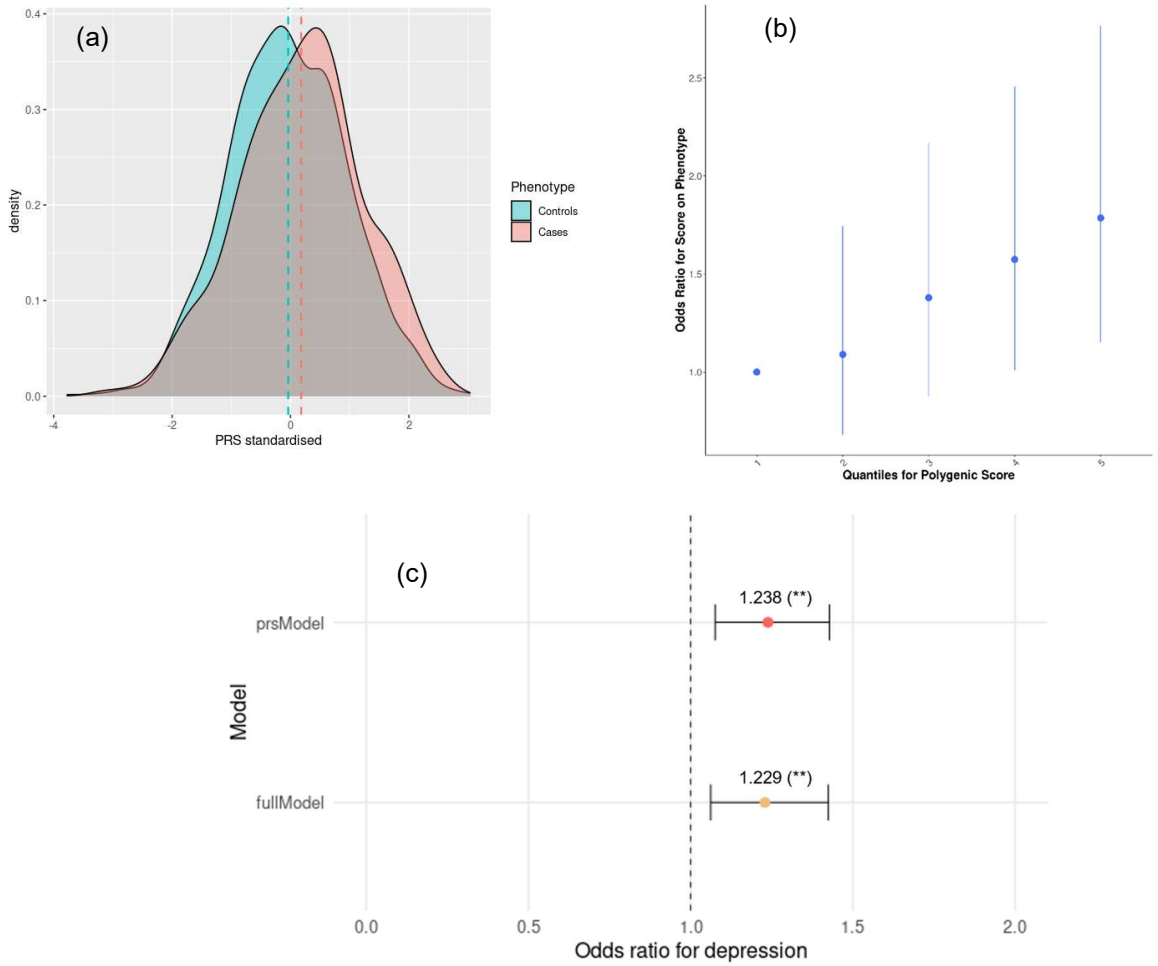


Figure 9. a) Density plot representing the cumulative population obtaining a certain PRS. b) OR for depression depending on PRS quintiles. c) Representation of the odds ratio for depression in the two different models employed in the logistic association. These plots were constructed using the most explanatory PRS.

The obtained Nagelkerke's R^2 –calculated as the difference between the Nagelkerke's R^2 of the full model and the same of the null model– was 0.0086, indicating that approximately ~0.8% of the variability of depression is explained by the constructed PRS.

4. Discussion

In this study, we first present, a genome-wide association analysis performed in an epidemiological sample, representative of the general population of Andalusia, which encountered 249 cases with clinically ascertained MDD and 1203 controls, and reported no genome-wide significant loci, although 9 suggestively significant independent SNPs ($p \leq 1 \times 10^{-5}$) were identified. Secondly, we performed a PRS in a sample from the same studies, using the summary statistics from Howard, et al (2019)[12] as the base data.

The results presented in this chapter are highly valuable, since they represent the first results of GWAS studies on depression carried out in Spain. Although these are preliminary results that would require further research, our initial findings did not identify any genome-wide-significant polymorphism. Nonetheless, this outcome does not come as a surprise: as presented in the introduction, a whole decade of initial 'failing' GWAS and multiple lessons learnt guided the community of 'depression genomics' towards the recent mega-analyses identifying a considerable number of SNPs[11,12]. In this respect, part of the value of this study consists in the –already performed– inclusion of this cohort in the PGC, which would imply that a Spanish population will be represented for the first time in the next mega-analysis. This contribution will help to follow the steps that have been recently described in the scientific literature, taking into account the results obtained in mega-analyses of GWAS with a less strict phenotypic characterisation. In this regard, considering cohorts where the assignment as cases depended on the self-reporting of either depressive symptoms according to questionnaires or the diagnosis of depression–in some cases constituting more than half of the sample[11,12]–, while resulting in a considerable number of significant SNPs, has been suggested to result in variants appearing statistically significant that were not expressly related to depression but to more general issues[16,41]. In contrast, GWAS with a more homogeneous phenotype have achieved less significant results. For instance, although more than 5000 cases were included, only two genome-wide significant SNPs were identified in Han Chinese women with recurrent severe MDD[8], whereas no significant association have been found in GWAS with European samples when a clinical diagnosis of depression or a diagnostic interview was the only ascertainment[4,42]. In the latter studies the number of cases were 5,763 and 9,240, respectively. Consequently, with our sample, although being more genetically homogeneous because it belongs entirely to a Spanish region, all precedents indicated that no significant loci would be found.

Regarding our GWAS results, these should be considered as a starting point, which deserve a more in-depth analysis in order to be able to functionally extract and understand the results obtained. Although no variant reached genome-wide significance, as stated above, there were 9 independent SNPs that met the threshold of $p \leq 1 \times 10^{-5}$, therefore being considered in the 'grey-zone' and consequently susceptible to be included in further in-depth analyses.

Aiming to provide an initial functional analysis, we identified some of the genes affected by the top-hits as eQTLs previously reported or with a potentially interesting relationship[43]. Therefore, here we reported that rs7916522 is an eQTL for the gene *MALRD1,* a gene with a variant that has previously been identified as the only genome-wide significant SNP in a GWAS for early age at onset of depression[44]. The most promising variant at first sight could be considered rs11669240. Both this variant and different close SNPs that were in LD consisted of a great number of eQTLs for interesting genes. *OR10H5* has been linked, through gene ontology, to the G protein-coupled serotonin receptor signalling pathway, which are closely related and drug targets for depression –among other psychiatric disorders[45,46]. Also this SNP is an eQTL for different genes that are members of the cytochrome P450 4F (CYP4F) subfamily (*CYP4F2*, *CYP4F3*, *CYP4F11*, and in particular *CYP4F12*, the gene where this SNP is

located downstream to). Although the main functions of this family are the metabolism of drugs and xenobiotics and maintaining lipid homeostasis[47,48], their implication in the latter may play a significant role on the inactivation of inflammatory responses, participating in the metabolism of mediators as arachidonic acid, eicosanoids and leukotrienes. Both neuroinflammation and low grade chronic inflammation are processes related to the development of depression that are dependent on the balance of these molecules[49,50], and therefore could be modulated with changes in the expression of these enzymes catalyst of their metabolism. However, our results suggest that the variant rs11669240 is an eQTL that induces the expression of *CYP4F* genes in different tissues, which would have an anti-inflammatory effect, according to results enhancing and decreasing their activity in mice[51]. This would be an interesting aspect to investigate when an adequate sample size allows an stratified analysis of the genetic background of different subgroups of depression, since there are subgroups characterised with inflammation symptoms and elevated markers –being this usually more associated to the atypical depression subtype[52,53].

The exonic variant rs11540216, located in the *LARS* gene, results in a synonymous change of nucleotide that does not have any molecular consequence. Neither this SNP nor the genes involved in an eQTL relationship (*LARS* and *RBM27*) have been associated with depression or with any process that could be related to its aetiology. Similarly, scarce information is available for rs62475204, an intergenic variant which is eQTL for *TES* and *CAV2*, neither of them related to depression. The intergenic SNP rs75288559 has not ever been reported in previous studies, although according to GTEx/v8 is an eQTL variant for *ZNF248*. Therefore, a variant in LD with the lead SNP of a GWAS meta-analysis for a treatment-resistant depression phenotype, also was an eQTL for *ZNF248*[40].

Considering the gene-based analyses, our cohort with a modest sample size did not reach genome-wide significant results. However, we observed among the most significant genes some protein-coding genes expressed in the brain (for instance, *CA14* or *GSX1*), potential targets due to their relationship with the serotonin receptor activity (*OR10H2* and *OR10H5*), or related to neural disorders (*LINGO1*)[37,38], or depression in a certain manner (*TFAP2D*)[40]. Although none of these genes were replicated in the two main GWAS mega-analyses in broad depression up-to-date, their gene-wise analyses identified significant genes and gene-sets also involved in potential targets related to different processes such as neurotransmission of different molecules or synaptic structure and activity[11,12].

Our results also suggest that a PRS constructed using the summary statistics from a GWAS in a broad phenotype of depression, when applied in our sample –clinically interviewed and diagnosed with depression– shows a higher prevalence of depression in participants with a higher score. Although a little percentage of the variability of depression would be explained by the adjusted PRS (approximately 0.8%), we hypothesise that, with a more homogeneous cohort as a base data, differences in the genetic architecture between the base data and the target data would be less, and therefore genetic tools like this would be more effective. In this respect, PRS have demonstrated their utility for prediction and risk discrimination, optimised

when combined with other risk factors, improving previous predictive models in depression and other medical conditions[11,54–57]. It is also remarkable that in different studies, the predictive ability of PRS has been observed to be valid not only in research case-control cohorts but also in samples representative of the general population[58,59]. Therefore, the prediction of genetic risk through PRS would be useful to take advantage of screening frequencies and early detection or personalised lifestyle recommendations, based on their predicted risk[60].

We are conscious of the important limitation of this study regarding the sample size of our cohort. As aforementioned, a limited sample size has been the main barrier for obtaining successful GWAS in depression during the past decade and for that reason our results were similar to what would be expected. A major effort will have to be made in order to obtain a larger sample and gain statistical power. However, it is worth mentioning that despite the limited sample size, the main strength of our cohort is the homogeneity and thoroughness of the diagnosis of depression, described in the methodological scientific manuscript of the PISMA-ep study[17]. In our opinion, great efforts should be made to achieve larger samples clinically ascertained and meticulously characterised in order to be able to understand the genetic cornerstones of stratified subtypes of depression.

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RESULTS

CHAPTER II
Study 2

CHAPTER II: Genetics of the relationship between depression and physical health: Depression and obesity

Study 2: Systematic review of the role of the *FTO* rs9939609 SNP in the relationship between depression and obesity

1. Introduction

Depression and obesity are major global health problems. They are main causes of disease burden and disability, leading to severe implications not only in public health and economy, but also at the personal level[1–4]. Independently, both conditions are highly prevalent and risk factors for chronic physical conditions such as type 2 diabetes, cardiovascular disease and hypertension[5–7], among others. Furthermore, these two conditions are frequently comorbid, leading to a more severe impact on individuals´ general health[8–10]. Even though obesity and a higher body mass index (BMI) have been reported to be associated with a higher risk of developing depression[11–13], the direction of the association between these disorders has not been completely elucidated yet[14,15]. Evidence from epidemiological studies indicates that depression and obesity have a strong bidirectional relationship, i.e., BMI increases the risk for developing depression, and vice versa, individuals with depression have an increased risk of high BMI, both in adults[16,17], and in adolescents[18]. Nevertheless, the causes leading to this comorbidity remain largely unknown and several mechanisms have been proposed.

These mechanisms can be common to both conditions or be present in a first condition and lead to an increased susceptibility to develop the second. For instance, psychological pathways, such as stigma or low self-esteem, are prone to trigger a vicious cycle involving both conditions[19,20]. Several biological mechanisms have been suggested to be involved in this relationship, including physiological, genetic and molecular pathways. These mechanisms include the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis activity. It consists mainly of the neuroendocrine system being responsible of secretion and regulation of cortisol in humans, modulating body processes implicated in both depression and obesity[21,22]. Inflammation processes have also been described to be involved in both conditions and may play a role in their co-occurrence[23,24]. Moreover, neuroendocrine mechanisms, e.g. the leptin-melanocortin pathway, with a well-established role in obesity, have recently been proposed to be involved in depression[25,26] too. Remarkably, the heterogeneity of depression entangles this relationship, e.g., immunometabolic dysregulations and environmental factors are likely to have an impact in differences found in the development of depression and also in treatment responses[27]. Furthermore, there are two major clinical subtypes of depression, melancholic and atypical. Mainly, the atypical subtype is characterised by lethargy, fatigue, excessive sleepiness, mood reactivity, hyperphagia and weight gain, resulting in a higher risk of obesity. On the other hand, melancholic depression is characterised by anhedonia, pronounced feelings of worthlessness, nonreactive mood, psychomotor disturbances, diurnal mood variation, impaired cognitive abilities,

insomnia and weight loss[27,28]. This heterogeneity opens the way to multiple possible mechanisms involved in this comorbidity (see Milaneschi, et al [29] for a review).

Within biological mechanisms, genetic risk factors have also been proposed as a potential factor involved in the comorbidity between depression and obesity[30–32]. Concerning obesity, multiple genome-wide association studies (GWAS) have investigated the association between different polymorphisms with obesity or increased BMI, resulting in multiple loci reported to be associated with both conditions[33–36]. A recent meta-analysis including 339,224 individuals has found 97 polymorphisms associated with body mass index (BMI) and obesity[32]. Another novel combined GWAS meta-analysis of approximately 700,000 participants of European ancestry identified 941 near-independent significant SNPs for BMI, at 549 polygenic loci[37]. Both studies, following pathway enrichment analyses, highlight the role of genes involved in the development of the central nervous system[37], and pathways related to its function, such as synaptic function or neurotransmitter signalling[32]. On the other hand, in the last years GWAS studies have led to a rapid increase in the number of loci known to influence the risk for depression. Recent GWAS meta-analyses including 480,359 and 807,553 individuals have identified 44 and 102 independent single-nucleotide polymorphisms (SNPs), respectively, associated with depression[38,39]. Particularly, among the 44 SNPs described by Wray and colleagues associated with depression, there were multiple SNPs located in genes related to BMI and obesity, such as *NEGR1* and *OLFM4*[38].

Besides, it has been revealed the existence of an overlap of common genetic variants between both disorders. It is estimated that up to 12% of the genetic component of depression is shared with obesity[38]. In addition, these genes belong to important interrelated signalling pathways involved in the aetiology of both conditions, e.g., signalling of dopamine and serotonin receptors, leptin, AMPK, axonal guidance and corticotropin-releasing hormone, among others[40].

Within the genes related to obesity and BMI, the fat mass- and obesity-associated (*FTO*) gene has one of the strongest links with these conditions in the human population. It was the first gene associated with an increase in BMI in two independent GWAS from European populations[35,36]. Its effect in BMI and obesity has been further confirmed in many independent studies, as well as in large GWAS studies (see Fawcett, et al[41] for a review). These results, however, have been reported to be less or not significant in other ancestries[42–44]. The SNPs found in the first intron of the *FTO* gene have been reported to increase BMI (by 0.39 kg/m^2 for each allele), and the risk of obesity (by 1.20-fold)[45].

Among the SNPs identified in the *FTO* gene, it is worth mentioning the rs9939609, the most studied polymorphism in this gene. The presence of the risk 'A' allele of this polymorphism, located in the first intron, has been reported to be associated with increased odds of obesity and body weight gain[34,36]. Moreover, this allele has also been associated with processes related to BMI increase, such as energy intake increase[46], or

reduction of satiety[47]. Although multiple pathways have been hypothesised, the mechanisms underlying a direct relationship of this polymorphism on BMI and obesity are still unknown (see Loos, et al[48] for a review).

On the other hand, this polymorphism has also been studied in depression independently of BMI or obesity, although scarcely. To the best of our knowledge, only two studies carried out in Asian populations have evaluated this association to date [49,50]. In 2015, Du et al, performed a case-control study including 738 depression cases and 1,098 controls and did not find any association between the *FTO* polymorphism and depression. Shortly after, a meta-analysis including the previous cohort and a total of 6,531 cases and 12,359, also found no evidence of association. However, the *FTO* gene is highly expressed in the brain, making it possible to hypothesise about its role in the development of depression[35]. Besides, this gene has been related to brain atrophy [51], a characteristic classically associated to both high BMI and depression[52,53], thus could exert a plausible direct or indirect effect in the brain. Furthermore, its role catalysing the demethylation of N6-methyladenosine (m^6A)[54] has been recently described, which has also been proposed to have an important role in the nervous system and associated diseases[55]. This demethylation role has also been linked to relevant brain mechanisms such as neurogenesis[56] or dopaminergic circuitry[57]. For instance, in humans there was found an alteration in the levels of m6A following a glucocorticoid stimulation, suggesting a role of this epitranscriptomic regulation in stress response and stress-related psychiatric conditions[58]. On the other hand, recent studies investigating in *Fto* knockout mice investigating depression and anxiety and associated behaviours have led to inconclusive results[58–60]. Besides, not only depression but also different neuropsychiatric disorders, e.g., Alzheimer's disease, Parkinson's disease, epilepsy and anxiety, have been investigated in relation to this novel function of *FTO*, which indicates a potential relevance of this gene in these diseases[61].

The aim of this work is to perform a systematic review of the scientific literature examining the relationship between the *FTO* gene, BMI or obesity and depression, in order to assess the possible role of this gene on the relationship between these disorders.

2. Methods

Search strategy and study selection

All procedures were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines[62]. Databases used for identifying studies of interest were PubMed (MEDLINE), Web of Science, Scopus and PsycINFO. The search was performed during the months of October to November 2020. We searched papers published from 2012 (when the first paper on the topic was published) to November 2020.

The search strategy was: *FTO* AND (BMI OR obes*) AND (depress* OR "mental disorders" OR "psychiatric disorders").

Studies were eligible for inclusion if they met the following criteria: (1) original articles using observational design (cross-sectional or longitudinal) or reviews of observational published studies; (2) studies that analysed the relationship between BMI or obesity, *FTO* gene and diagnostic of depression assessed following the International Statistical Classification of Diseases and Related Health Problems (ICD-10 or previous versions) or the Diagnostic and Statistical Manual of Mental Disorders (DSM-5 or previous versions) criteria[63,64]), (3) studies performed in humans from 18 years old published form 2012 (when the first paper on the topic was published) to 2020, (4) in English or Spanish. Finally, we selected those documents that were considered as potential sources of evidence by having an optimal methodological quality, which was assessed by the Scottish Intercollegiate Guidelines Network (SIGN) checklist[65]. The 11 questions present in this checklist aim to identify the main features that should be present in a well-designed case-control study. The document was rejected when it failed to address or report on more than 2 of the eleven questions addressed in the checklist as is advised in the mentioned tool.

First, we selected articles reviewing their titles and abstracts. Further, we fully read those papers selected and also the ones whose eligibility was not clear after reading the abstract. Two reviewers assessed the eligibility of the studies independently. If there was disagreement between the reviewers, this was resolved by consensus.

Data extraction

We extracted data from the eligible manuscripts into a spreadsheet including document´s reference, authors, year of publication, sample size and characteristics (cases and controls, when applicable), statistical analysis performed and results (odds ratios or beta coefficients, confidence intervals or standard errors and p-values).

3. Results

The initial search performed in the different databases identified 115 studies, of which 40 full-texts articles remained after screening for the inclusion criteria and removing duplicates. In total, 5 studies were included in the qualitative synthesis. The main aims of these studies were distinguishable. Two of them focused on the relationship between *FTO* gene and BMI, considering depressive status as a covariate. The remaining three studies investigated the effect of *FTO* on depression, including BMI as a covariate. The reasons for not including the remaining 35 articles were: not considering depression or assessing it without a diagnosis following the International Statistical Classification of Diseases and Related Health Problems (ICD-10 or previous versions) or the Diagnostic and Statistical Manual of Mental Disorders (DSM-5 or previous versions) criteria[63,64]. Also, the studies that excluded participants with depression, did not consider the *FTO* gene, were methodological articles or assessed other variables not related, were excluded from the systematic review. **Figure 10** shows the PRISMA flowchart with the studies selection for the systematic review.

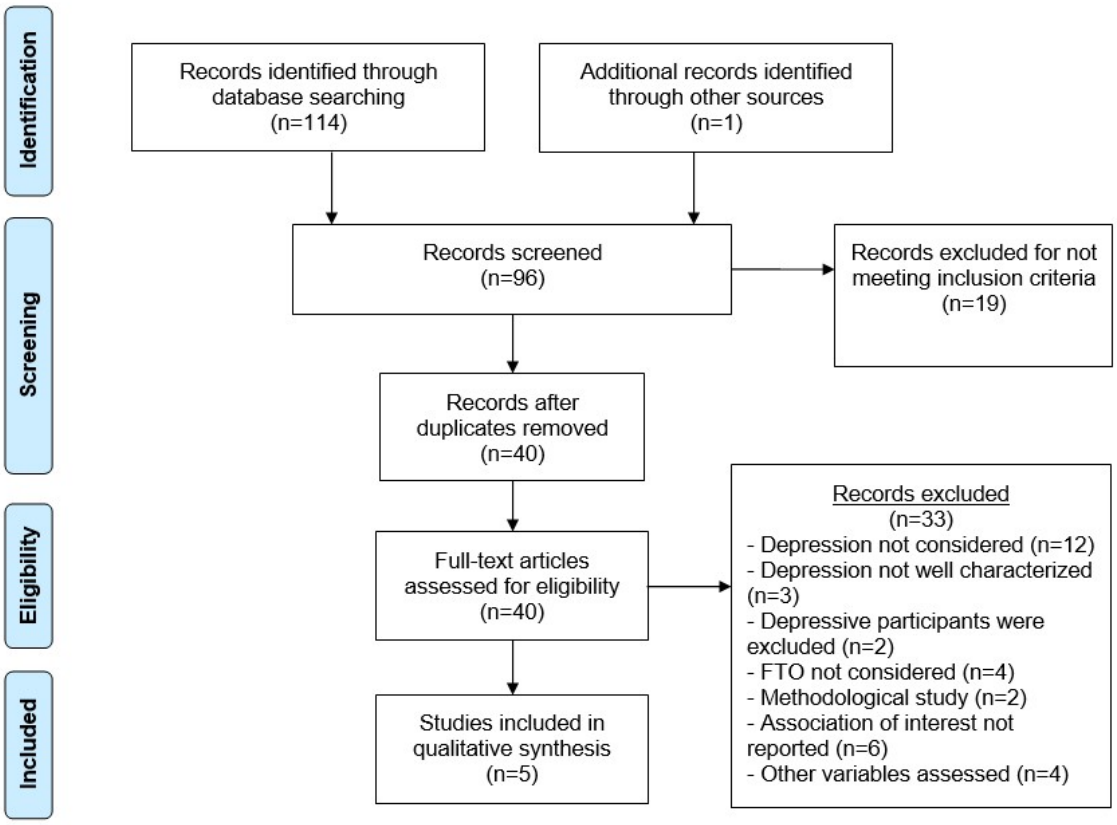

Figure 10. PRISMA flowchart showing studies selection for the systematic review. PubMed (MEDLINE), Web of Science, Scopus and PsycINFO databases were searched to identify studies relating *FTO* polymorphisms with depression and BMI or obesity.

The most relevant information regarding the methodology and results of the documents included in the present systematic review is detailed in **Tables 7a and 7b**.

Table 7a. Description of the studies included in the systematic review, focusing on the relationship between *FTO* gene and BMI. β: beta coefficient; s.e.: standard error; P: p-value.

* In Rivera et al., 2012, rs9939609 was only assessed in the UK subsample from the Radiant cohort.

Reference	Sample				Results					
	Cohort		Cases	Controls	Association with BMI			Interaction (rs9939609, BMI & depression)		
					β	s.e.	*P*	β	s.e.	*P*
Rivera et al. 2012	Discovery	Radiant (UK subsample)*	1361	813	-0.006	-	0.0149	-0.01	-	0.0047
	Replication	PsyCoLaus	1296	1690	-	-	0.0058	-	-	0.0444
	Meta-analysis		3738	2499	-	-	-	-0.01	0.03	0.001
Rivera et al., 2017	Discovery	Extended Radiant	2442	809	0.08	0.02	0.001	0.18	0.06	0.002
		PsyCoLaus	1296	1698	0.07	0.02	0.006	0.12	0.05	0.034
	Replication	GSK	821	856	0.04	0.03	0.193	-0.09	0.07	0.168
		MARS	575	541	0.06	0.04	0.119	0.26	0.15	0.083
		NESDA/NTR	1768	2895	0.09	0.02	1.2×10^{-5}	0.19	0.04	3.2×10^{-6}
	Meta-analysis: *fixed effects model*		6902	6799	0.07	0.01	1.3×10^{-12}	0.13	0.03	3.1×10^{-7}
	Meta-analysis: *random effects model*		6902	6799	-	-	-	0.12	0.05	0.027
	Meta-analysis: *Han/Eskin model*		6902	6799	-	-	-	0.12	0.05	6.91×10^{-8}

Table 7b. Description of the studies included in the systematic review, focusing on the relationship between *FTO* gene and depression. OR: odds ratio; CI: confidence interval; P: p-value.

* In Hung et al., 2014, prediction of the risk, as well as its dispersion, is measured with the beta coefficient.

Reference	Sample				Results		
	Cohort		Cases	Controls	Association with depression		
					OR	CI	P
Samaan et al., 2013	Discovery	EpiDREAM	3187	14020	0.92	0.87 – 0.98	0.0076
	Replication	INTERHEART	1359	813	0.89	0.78 – 1.00	0.05
		DeCC	719	5401	0.91	0.79 – 1.04	0.18
		CoLaus	1296	1698	0.98	0.88 – 1.09	0.75
	Meta-analysis		6561	21932	0.92	0.89 – 0.97	$3x10^{-4}$
Milaneschi et al., 2014	Discovery	NESDA + NTR (2470 controls)	255 (severe typical)	2806	1.01	0.83 – 1.23	0.93
			687 (moderate intensity)		1.10	0.97 – 1.24	0.14
			256 (severe atypical)		1.34	1.11 – 1.61	0.003
			1544 (all cases)		1.07	0.98 – 1.18	0.107
Hung et al., 2014	Discovery	Radiant	2430	792	-0.03*	-0.18–0.13*	0.73

Effect of the FTO gene on BMI

In 2012, Rivera and colleagues investigated the genetic influence of the *FTO* gene, analysing a total of 88 SNPs spanning the gene after applying stringent quality control criteria for missing genotypes, departure from Hardy–Weinberg equilibrium and low minor allele frequency[66]. They included a total of 2,442 individuals with major depressive disorder (740 men and 1,702 women; avg. age ± s.e.: 45.25 ± 12.15 years old) from the Radiant study, which was sourced from three studies: Depression Case-Control (DeCC) study, Depression Network (DeNT) study and Genome-Based Therapeutic Drugs for Depression (GENDEP) study. They were excluded if they or a first-degree relative reported a history of bipolar disorder, schizophrenia, mania or hypomania, or if in their cases there was an association between depression and alcohol, substance misuse, medical illness or medication. Controls were 809 individuals without any psychiatric disorder, neither in their first-degree relatives (313 men and 496 women; avg. age ± s.e.: 39.9 ± 13.71 years old). They included a replication sample consisting of a cohort from the PsyCoLaus study, with a total of 3,738 cases with depression and 2,499 controls. Both cases and controls only included participants of white European ancestry. This sample included 1,296 cases with major depressive disorder (431 men and 862 women; avg. age ± s.e.: 49.69 ± 8.68 years old) and 1,698 controls (974 men and 724 women; avg. age ± s.e.: 50.59 ± 8.94 years old) without a diagnosis of depression. Participants of this study were residents of Lausanne (Switzerland), of Caucasian ancestry. They found an association between the rs9939609 *FTO* polymorphism and BMI, in the whole sample, including depression cases and controls (β = -0.006, P = 0.0149). When they analysed cases with depression and controls separately, the results showed that the SNPs were only associated with BMI in the depression cases group and not in the controls. Finally, they found a significant interaction effect between genotype and depression in relation to BMI, i.e., depression moderated the effect of *FTO* on BMI (β= -0.01, P = 0.0047). Thus, the individuals with depression carrying the *FTO* risk allele had a higher average BMI than their psychiatrically healthy counterparts. This study reported for the first time that having depression moderates the effect of the *FTO* gene on BMI, suggesting the implication of this gene in the mechanism underlying the association between depression and obesity. These results were further replicated and confirmed in a larger sample from the PsyCoLaus study (β= -0.01, s.e. = 0.03, P = 0.001).

Later, in 2017 Rivera et al. further investigated the effect of the *FTO* polymorphism rs9939609 in BMI in three new cohorts (GSK, MARS and NESDA/NTR) of individuals with depression and controls without any psychiatric disorder, and performed a meta-analysis[67]. In this study, their discovery sample consisted in the previously described Radiant and PsyCoLaus cohorts, whereas their results were replicated in the three new cohorts. The GSK study included 821 MDD cases (277 men and 544 women; avg. age ± s.e.: 50.94 ± 13.74 years old) and 856 controls (278 men and 578 women; avg. age ± s.e.:51.92 ± 13.26 years old). The MARS cohort included 575 MDD cases (271 men and 304 women; avg. age ± s.e.: 48.09 ± 13.95 years old) and 541 controls (243 men and 298 women; avg. age ± s.e.: 47.42 ± 13.50 years old). Both GSK and MARS individuals were

white European individuals from Munich (Germany). The NESDA/NTR included a total of 1,768 MDD cases (555 men and 1,213 women; avg. age ± s.e.: 42.68 ± 12.41 years old) and 2,895 controls (1,120 men and 1,775 women; avg. age ± s.e.: 42.83 ± 14.96 years old). Participants from the NESDA and NTR studies, both based in the Netherlands, were of western European ancestry. In the replication cohorts, they found a significant interaction between *FTO*, BMI and depression with fixed effects meta-analysis ($\beta = 0.12$, s.e. = 0.03, $P = 2.7 \times 10^{-4}$) and Han/Eskin random effects method ($\beta = 0.1$, s.e. = 0.11, $P = 1.41 \times 10^{-7}$). When they combined the discovery cohorts with the new cohorts in a meta-analysis including 6,902 cases and 6,799 controls, random effects meta-analysis also supported the interaction ($\beta = 0.12$, s.e. = 0.05, P = 0.027), being highly significant when the Han/Eskin method was used ($\beta = 0.12$, s.e. = 0.05, $P = 6.9 \times 10^{-8}$). This corresponded to a BMI increase of 2.2% for cases with depression, for each risk allele, additional to the effect of *FTO* itself on BMI.

Effect of the FTO gene on depression

In 2013, Samaan and colleagues conducted a case-control study and a meta-analysis, including 6,561 depression cases and 21,932 controls from four different cohorts (EpiDREAM, INTERHEART, DeCC, CoLaus)[68]. In this study they aimed to investigate the association between the *FTO* variant rs9939609 and depression. The discovery sample was selected from the EpiDREAM study, which included 3,187 individuals with depression (775 men and 2412 women; avg. age ± s.e.: 51.08 ± 10.55 years old) and 14,020 controls (5,933 men and 8,087 women; avg. age ± s.e.: 53.00 ± 11.53 years old). Here, five ethnic groups were included (South Asian, European, African, Latin American and Native North American). At the discovery stage, they found that the rs9939609 'A' risk variant was associated with depression (OR = 0.92, 95%CI = [0.87–0.98], P = 0.0076) and reduced the risk of depression by 6% for each copy of this allele, independently of BMI. With the aim of replicating their results, they examined three cohorts. INTERHEART included 719 cases (511 men and 208 women; avg. age ± s.e.: 56.38 ± 12.48 years old) and 5,401controls (4,139 men and 1,262 women; avg. age ± s.e.: 58.07 ± 11.97 years old), from four ethnicities (South Asian, Chinese, European and Latin American). The DeCC study consisted in a total sample of 1,359 cases and 813 controls, all of them of European ancestry (717 men and 1455 women; avg. age ± s.e.: 43.90 ± 13.05 years old). The sample from CoLaus was the same that was previously described, with a cohort from Lausanne (Switzerland), which included 1,296 cases and 1,698 controls. When they examined this association in the replication cohorts, they did not find any association between the SNP and depression in the three samples (INTERHEART: OR = 0.89, 95%CI = [0.78–1.00], P = 0.05; DeCC: OR = 0.91, 95%CI = [0.79–1.04], P = 0.18; CoLaus: OR = 0.98, 95%CI = [0.88–1.09], P = 0.75). The overall results from a meta-analysis including the four cohorts showed an association between the rs9939609 polymorphism and depression (OR = 0.92, 95%CI = [0.89–0.97], $P = 3 \times 10^{-4}$).

Shortly after, Milaneschi and colleagues performed the same association analyses between the *FTO* rs9939609 polymorphism and depression as in the paper of Samaan et al[69]. They included individuals from two cohorts consisting of 1,544 depression cases from the NESDA study (493 men and 1051 women; avg. age ± s.e.: 42.5 ± 12.3 years old) and 2,806 controls from the NESDA and the NTR studies (1,092 men and 1,714 women; avg. age ± s.e.: 43.00 ± 15.00 years old), both cases and controls of European ancestry. In addition, they also explored the association between rs9939609 and three clinical depression subtypes: severe typical, moderate severity and severe atypical. Atypical depression features increased appetite, hypersomnia and weight gain, in contrast to typical or melancholic depression. They also found an association between the rs9939609 'A' risk variant and BMI (OR = 1.492, 95%CI = [1.363–1.632], P = 1.56×10^{-5}) and between BMI and depression (OR = 1.08, 95%CI = [1.07–1.10], P = $5.47\text{x}10^{-24}$). In contrast to the results obtained by the study of Samaan et al, the *FTO* variant was no longer significantly associated with depression after additional adjustment for BMI. However, when they considered depression subtypes, they found statistically significant differences in a multinomial logistic regression in the severe atypical subtype, before and after correcting the analyses for BMI (adjusting for age, sex and principal components: OR = 1.42, 95%CI = 1.18–1.71, P = 1.84×10^{-4}; adjusting for the previous covariates + BMI: OR = 1.34, 95%CI = 1.11–1.61, P = 0.003).

In 2014, Hung and colleagues using a different methodological approach, performed a Mendelian randomisation study to test the causal relationship between obesity and depression. Mendelian randomisation is a novel approach which makes possible to define a causal role between a potential cause and an outcome, by using genetic variants reliably associated with the potential cause as instrumental variables. They studied the effect of another variant of the *FTO* gene, rs3751812, which is in complete linkage disequilibrium with the rs9939609 polymorphism, as an instrumental variable of BMI, on its relationship with depression[70]. Here, they used the *FTO* genotype, due to its robust association with BMI, in an additive model to assess its relationship with BMI and depression in the previously described Radiant study, including 2,430 individuals with depression (735 men and 1695 women; avg. age ± s.e.: 45.2 ± 12.2 years old) and 792 controls without psychiatric disorders (308 men and 484 women; avg. age ± s.e.: 39.9 ± 13.7 years old). The *FTO* genotype was found to be associated with BMI after adjusting for different covariates (age, gender, depression status and principal components of ancestry) (B = 0.048, P = 0.011 for one risk allele in rs3751812; B = 0.062, P = 0.001 for two risk alleles in rs3751812). Moreover, BMI was associated with depression after a probit regression analysis (coefficient = 0.05, 95%CI = [0.04–0.06], P<0.001). However, the results of the prediction of depression risk using this SNP as an instrumental variable for BMI (coefficient = -0.03, 95%CI = [-0.18–0.13], P = 0.73), showed that this association was not due to the effect of the *FTO* genotype on BMI.

4. Discussion

The main findings of this systematic review did not reveal a clear effect of the *FTO* gene in the relationship between depression and obesity. In this review, we distinguish two categories of studies: those where the effect of *FTO* was investigated on BMI, and those where the effect of *FTO* was assessed on depression. A total of five studies were included in the final qualitative analysis.

In this respect, Rivera and colleagues reported in 2009, for the first time, an interaction between *FTO* gene, depression and BMI[66], which suggested that *FTO* is involved in the mechanism underlying the largely reported association between depression and obesity. Their results were replicated in 2017 in a large meta-analysis including 13,701 individuals from five different cohorts, showing that depression increases the effect of *FTO* gene on BMI and point to a genetic mechanism by which individuals who suffer from depression are at increased risk for obesity and higher BMI.

The results presented by Samaan and colleagues in 2013 investigated the presence of a link between the *FTO* rs9939609 polymorphism and the risk of depression[68]. They found an inverse association between the risk 'A' allele and major depression in the discovery sample, which was not significant in any of the three replication cohorts. However, when they performed a meta-analysis, the results showed a significant association between *FTO* and depression. It is worth mentioning that in the discovery sample of this study, depression was assessed with a case report form which included the following two questions: whether they had experienced a variety of symptoms that fulfil Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) associated to depression in the past 12 months and whether they lasted a minimum of 2 weeks. In contrast, in the replication cohorts reported in their manuscript, depression was clinically ascertained following DSM-IV or ICD-10 criteria using validated diagnostic tools, e.g., the Composite International Diagnostic Interview (CIDI)[71], the Schedules for Clinical Assessment in Neuropsychiatry (SCAN)[72], the Diagnostic Interview for Genetic Studies (DIGS)[73], and the Dimensions of the Hamilton-Depression-Scale (HAM-D)[74] . The differences found in the results from their discovery sample and the replication cohorts could be due to the different methods used for the diagnosis of depression, thus pointing towards the need of an appropriate diagnosis method for depression, following well-established criteria.

Soon after, Milaneschi and colleagues tried to replicate Samaan´s results studying the effect of this genetic variant on the risk of depression[69], and going a step further classifying depression cases into three subtypes: severe typical, moderate severity and severe atypical. These profiles are usually associated with divergent metabolic functioning, being the patients with atypical depression those characterised with a higher rate of obesity and increased appetite[75,76]. They did not find the previously reported protective effect of the risk allele of the rs9939609 polymorphism, found by Samaan and colleagues[68]. However, they found a statistically significant risk effect in the

severe atypical subtype which was independent of BMI. Their results show the importance of including depression subtypes in genetic association studies as they can contribute to the variability of results. It is probable that the conclusions about the association between *FTO* and depression may be different when considering the heterogeneity of depression[69].

In the Mendelian randomization study performed by Hung and colleagues, although the regression analysis found that increased BMI was strongly associated with depression, the genetic instrumental variable analysis did not support the hypothesis that increased BMI raises the risk of developing depression using the polymorphism studied in *FTO* gene[70].

There is an extensive literature of studies investigating the association between the *FTO* gene and BMI or obesity, with evidence of an increase in BMI and obesity risk associated with SNPs in the first intron of the *FTO* gene[45]. Much less information is available concerning the involvement of this gene in depression, only a couple of studies in Asian populations, which found no significant associations between this gene and depression[49,50]. Even though the latest study performed a meta-analysis with a large sample size, several considerations that could explain the negative results should be considered, such as allelic frequencies in different ethnicities, sex differences in depression risk, and differences among clinical subtypes of depression. In contrast, there are hardly any studies that investigate the association between *FTO* gene and both depression and obesity or BMI concurrently, even though the relationship between depression and obesity has been largely studied. Moreover, the evidence of the presence of this gene in the brain makes plausible the hypothesis that there is an implication of *FTO* in both conditions[35]. Its expression in human brain areas such as hypothalamus, adrenal glands and pituitary, confers this gene a potential role in the previously mentioned HPA axis, an important shared mechanism between the origin of depression and body weight regulation[35].

As it has been previously described, multiple mechanisms are implicated in the highly reported association between depression and obesity. These include from psychological to physiological mechanisms. Furthermore, upcoming studies analysing the expression of the *FTO* gene in the brain and its role in the demethylation will contribute to unravelling the underlying molecular pathways involved in these disorders. For instance, the function of *FTO* in the brain may affect further than its sole relationship with depression. Thus, recent approaches are trying to assess the existing link between the *FTO* gene and different neuropsychiatric disorders, i.e., depression, Alzheimer's disease, Parkinson's disease, epilepsy and anxiety[61]. These are promising studies based on murine models, which even though are not able to be inferred to humans, could shed light on the role of the *FTO* gene on the comorbidity between obesity and depression[58–60]. Finally, the findings in recent years have been crucial to providing a deeper insight into the genetic architecture of depression. On the one hand, two massive GWAS have led to

the identification of more than a hundred independent loci associated with depression[38,39]. Interestingly, multiple of these reported SNPs are located in genes associated with BMI and obesity, e.g. *NEGR1* and *OLFM4*[38]. Even though the rs9939609 was included in these two mega-analyses, its association with depression was not significant considering GWAS thresholds (in Wray[38], OR=1.02480, SE=0.008, p=0.002197, in Howard[39], LogOR=0.0109, SE=0.0044, p=0.01295;). Unfortunately, the full discovery sample was not available in any of the two studies. Summary statistics correspond to 59,851 cases and 113,154 controls in Wray et al[37], and 170,756 cases and 329,443 controls in the study performed by Howard et al[38]. Data from the full discovery sample was only available for the 10K most significant variants in each study, and the rs9939609 polymorphism was not included among them. On the other hand, research in obesity and BMI has led to a prolific amount of candidate SNPs associated with these conditions[32,37]. Although genetic studies performed in both conditions independently have recently provided important results, research on this comorbidity needs to be promoted.

We consider that further research investigating the genetic relationship between depression and obesity or BMI should include a more detailed clinical characterization of the sample. In line with the current studies, the heterogeneity of depression is probably leading to a wide spectrum of subtypes and endophenotypes, in a model consistent with the watershed theoretical framework described by Cannon and Keller[77] – rather than clinical binary subtypes[27,78]. The definition of more similar clinical subtypes or endophenotypes of depression may help to disentangle its relationship with obesity or BMI. In this respect, a more accurate characterization of depression would be required in order to approach the pursued personalised or precision medicine for the treatment of depression[79].

Overall, an interesting result of this systematic review is that there is a marked imbalance between the number of papers investigating the role of *FTO* gene on BMI in contrast to the studies analysing *FTO* on depression. There is strong evidence of the involvement of *FTO* in obesity and BMI and its potential role in depression, along with its recently described implication in the central nervous system and high expression in the brain. Therefore, there is a need for further research that deepens knowledge of this gene on depression, and particularly in the coexistence of both conditions, with the aim of shedding some light on the genetic basis of this comorbidity.

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RESULTS

CHAPTER II
Study 3

CHAPTER II: Genetics of the relationship between depression and physical health: Depression and obesity

Study 3: Genetic risk score (GRS) for depression and its relationship with BMI

1. Introduction

Depression and obesity are common conditions that tend to co-exist. Depression is the most common psychiatric disorder with more than 300 million people suffering from it. At the same time, the prevalence of obesity is a serious worldwide issue and one of the major health challenges of the 21st century (1). The co-occurrence of both conditions has been designated as one of the most important contributors to the worldwide disability burden, further leading to major personal and public health implications as well as generating an enormous economic and social cost (2,3). Given the high prevalence of both disorders and their consequences, understanding the nature of their relationship is a pressing clinical problem.

There is evidence that people with depression are more likely to be obese compared to psychiatrically-healthy controls (4). Conversely, people with obesity are also more prone to develop depression than normal-weight subjects so that the association between both conditions is bidirectional (5). Several longitudinal meta-analyses have robustly evidenced this phenomenon, showing how obesity longitudinally increases the risk of developing depression, and viceversa (6–9). The mechanisms underlying this association remain unclear nonetheless (6). Behavioral, sociocultural, psychological and biological factors have been proposed as a plausible explanation (3). Regarding behavioural and sociocultural factors, obesity would lead to depression due to stigma, interpersonal distress and changes in body image; while depression would lead to obesity as a result of physical inactivity, alcohol abuse and emotional eating (1,6,10,11). Interestingly, several biological dysregulations have been further described to derive from such behavioral alterations in both depression and obesity (3). On the other hand, it might happen that depression and obesity share some molecular disturbances, strongly connected by alterations in the systems involved in homeostatic adjustments and the brain circuitries that integrate mood regulatory responses (e.g. the hypothalamic–pituitary–adrenal (HPA) axis, immuno-inflammatory activation, neuroendocrine regulators of energy metabolism, or the microbiome) (5,6,12–14).

Family-based and twin studies have proven a strong heritable component for both depression and obesity, with heritability estimates of ~35% and ~40% for depression and body mass index (BMI) respectively (15–17). Although in both cases rare genetic variants and other chromosomal aberrations represent the bulk of the genetic load, genome-wide association studies (GWAS) have also identified a great number of associated single nucleotide polymorphisms (SNPs). These SNPs only represent a small fraction of the genetic susceptibility to these diseases nonetheless. While GWAS studies on BMI already identified hundreds of associated SNPs more than a decade ago (18–21), GWAS performed on depression have had notable difficulties for identifying associated variants (22). Indeed, it has not been

until quite recently that two depression GWAS meta-analyses identified 44 (23) and 102 (24) independent and significant loci associated with the disorder. Besides each individual genetic characterization, a shared genetic susceptibility profile has also been revealed for both conditions, which could be another influencing factor for the bidirectional depression–obesity relationship. Particularly, it has been estimated that up to 12% of the genetic component for depression could be shared with obesity (3,25). This promising finding has led to innovative approaches aiming to unveil the molecular mechanisms underlying the depression–obesity relationship (26–29).

Although initial expectations for GWASs on depression were high, mentioned SNPs individually account for only small proportions of reported heritability. Consequently, the practice of utilizing individual SNPs to predict depression is now considered a limited approach and other innovative perspectives have emerged to take advantage of available GWAS insights (30). On this matter, several genomic studies have proposed to study multiple common SNPs collectively to improve the estimation of disease predisposition (31). Based on the construction of genetic risk scores (GRSs), that include multiple genetic variants at the same time, these approaches have recently gathered considerable interest (32), and have proven utility identifying groups of individuals who could benefit from the knowledge of their probabilistic susceptibility to disease. In brief, a GRS is usually calculated as a sum of the number of risk alleles carried by an individual, where the risk alleles are defined by the SNPs and their measured effects as detected by GWAS in a particular trait (33). Although some authors have previously evaluated the performance of GRSs to discriminate depression (23), no study to date has investigated the utility of GRSs for depression prediction in people with obesity accounting for BMI information. On this matter, and given the strong relationship between obesity and depression, it could be possible that the inclusion of BMI information into the model (along with the GRS) elicits an improvement in predictive ability. Previous results from our group have already proved the hypothesis but in the opposite direction; a GRS for obesity improved its performance when the model included information about the depression status of each patient (34).

Therefore, in the present study we aimed: i) To investigate whether a GRS combining a number of well-defined SNPs associated with depression might have utility for depression prediction in individuals with obesity, ii) To evaluate whether the predictive ability of the model improves when obesity information is considered as a covariate, and iii) To obtain a general picture of the cellular and molecular pathways mapped by those SNPs included into the GRS.

2. Methods

Study population

The sample consisted of 2,123 community-based individuals (136 depression cases, 1,987 controls) from the PredictD-CCRT study; a Cluster, Controlled, Randomized Trial (CCRT). The PredictD-CCRT study was a national, multicentre randomized controlled trial, which had two

parallel groups: cluster assignment by primary care centre, and a follow-up of 18 months. The aim of this study was to assess the performance of a preventive intervention on the depression incidence, taking into account the level and profile of risk of depression of each individual. The PredictD-CCRT was conducted in 70 primary care centres from 7 Spanish cities. The Spanish National Health Service covers over 95% of the population, providing free medical service, which ensured a representative sample from the south of Spain. Participants were assessed for clinical, psychological, sociodemographic, anthropometric, lifestyle, and other environmental variables. Individuals who also agreed to participate in genetic studies gave specific informed consent and provided a biological sample. This study was approved in each participating city by the corresponding ethic committee, and it was conducted in compliance with the Helsinki Declaration. The PredictD protocol, effectiveness and cost-effectiveness analyses are fully described and available elsewhere (35–38). Briefly, patients belonging to the recruiting centers were selected using a systematic random sampling, each 4–6 patients, from the family physician's appointment lists at random starting points for each day. Family physicians further checked whether the selected patients met any of the following exclusion criteria: age under 18 or over 75 years; inability to understand or speak Spanish; severe mental disorder (psychosis, bipolar, personality disorder…); cognitive impairment; terminal illness; the patient is scheduled to be out of the city more than four months during the 18 months of the follow-up; and persons who attend the primary care centre on behalf of the person that initially has the appointment (35).

Characterization of depression

The psychiatric interview section was conducted by trained interviewers, independently from physicians. These research assistants completed a 20-hours training by accredited instructors, in order to guarantee standardization. The section of depression of the Composite International Diagnostic Interview (CIDI) was used for the assessment of depression. The CIDI (39,40) which is a structured interview was used for the diagnosis of depression according to the DSM-IV criteria.

Characterization of BMI and obesity

Height and weight data from each individual were used to calculate body mass index (BMI) using the formula: weight in kilograms divided by height in square meters (kg/m^2). International cut-off reference points were applied for obesity categorization (BMI <25: normal weight, BMI >= 25: overweight, and BMI >30: obesity). Underweight individuals (BMI <= 18.5) were excluded from analyses.

SNP selection

An extensive review of the literature was performed by the research team. Medline and Scopus databases were explored using relevant terms in the field of depression-associated genes (e.g., "depressive disorder", "major depressive disorder", "major depression", "MDD", "candidate gene", "SNP", "polymorphism", "loci''). SNPs were initially selected based on two criteria: i) SNPs from candidate genes reported in case-control studies on depression and replicated in more than one independent study, or in loci having a significant potential role

in depression (i.e., loci involved in well-established pathways associated with depression: the hypothalamic–pituitary–adrenal (HPA) axis (41) and the serotonergic system (42)) (n=25); ii) SNPs associated with depression from GWAS or meta-analyses establishing a p-value cut-off of $p \leq 7x10^{-6}$ (n=47). The information obtained from each approach was then combined and compared to the list of SNPs available from Illumina technology (San Diego, California), so that a definitive list of candidate variants was obtained: 6 and 10 SNPs initially selected from candidate gene studies and GWAS, respectively, were discarded in this step. Finally, 56 SNPs were selected for downstream analyses: i) 19 SNPs from candidate gene association studies (43–55) and ii) 37 SNPs associated with depression in GWAS or meta-analyses (56–68).

Genotyping

A saliva sample was obtained from each participant using the Oragene DNA saliva collection kit (OG-500; DNA Genotek Inc.). DNA extraction was performed using standard procedures. DNA concentration was measured by absorbance measure using the Infinite® M200 PRO multimode reader (Tecan, Research Triangle Park, NC). Genotyping was performed using the TaqMan® OpenArray™ Genotyping System (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Raw data was analysed with the TaqManGenotyper v1.2 software (Thermo Fisher Scientific). SNPs showing a linkage disequilibrium (LD) value of $R^2 > 0.8$ in pairwise unphased correlations were removed from the selection. For all candidate markers, we further evaluated call-rate, Hardy-Weinberg equilibrium (HWE) and minor allele frequency (MAF). MAFs of all SNPs were >= 0.05 and similar to those reported for Iberian populations in Spain in phase 3 of the 1000 Genomes Project. To account for the presence of genotyping errors, all SNPs with less than a 95% call rate were excluded from the analyses. In relation to HWE, the Wigginton's exact test was applied only in controls at an alpha level of 0.05. After all quality control checks, the selected 56 markers were available for downstream analyses. A complete workflow detailing the whole SNP selection procedure can be found in Figure 11.

Genetic risk score construction (GRS)

To explore whether common variants with small risk effects on depression predict depression occurrence in our sample, a GRS was constructed following an unweighted approach, as implemented in the *PredictAbel* R package (78). In this approach, a GRS is calculated for each individual based on the number of risk alleles for depression, without accounting for each SNP effect size. The motivation underlying this choice is the fact that most of the selected markers came from individual genetic studies, where no robust beta values were available. The 56 SNPs initially selected from the literature, were assessed for association in our sample applying univariate regression analyses on depression. Although most of these SNPs were not significantly associated with depression in our sample, all effect sizes and direction of associations were compared to those reported in the literature for each selected SNP. With the aim of constructing a robust genetic risk score, we only selected those SNPs showing concordant associations (in terms of effect size direction) between the association signal reported in the literature and the association signal reported in our sample. As a result, 30 SNPs from the 56 markers were selected for the construction of the GRS. The rationale behind

this procedure is the fact that many of the selected SNPs might not be significant in our population while still being associated with depression according to other studies. For these SNPs that individually do not show statistical association, constructing a GRS is of special interest since we can combine their small effects so joined, they report a significant relationship with depression. SNPs in which the minor allele was reported as a protective marker (instead of a risk variant) were flipped in order to compute the risk score. Since the GRS cannot be estimated if any of the markers present a non-callable genotype for a certain individual, subjects showing a call-rate less than 100% for any of the 30 candidate SNPs were removed from the analyses. As a result, 1,650 individuals (104 depression cases and 1,546 controls) were considered for the GRS construction.

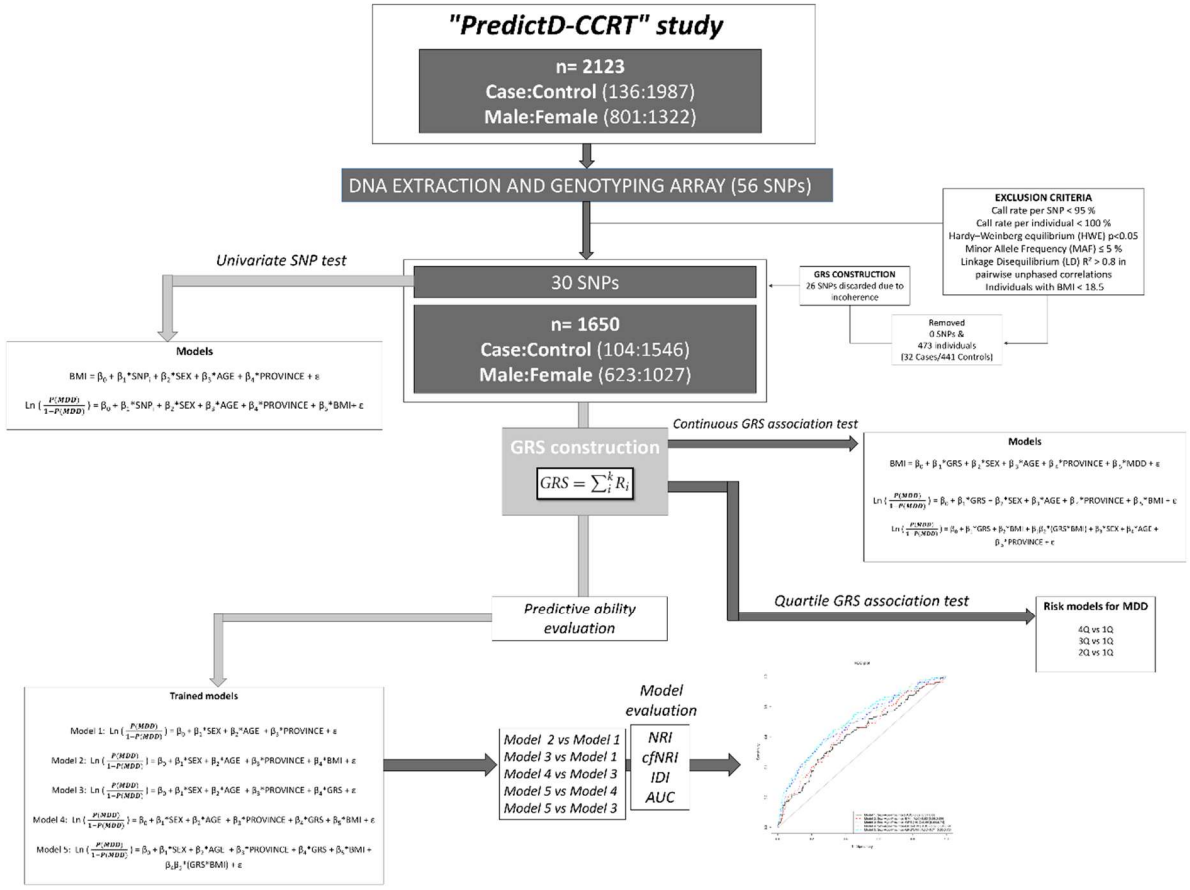

Figure 11. Complete workflow detailing the study design and statistical analyses performed: quality control process, association analysis and construction and evaluation of predictive models. Abbreviations: AUC, Area under the receiver operating characteristic curve; cfNRI, the category-free net reclassification improvement; HWE, Hardy–Weinberg equilibrium; IDI, Integrated discrimination improvement; LD, linkage disequilibrium; MAF, minor allele frequency; MDD, major depressive disorder; NRI, Net reclassification improvement; SNP, single nucleotide polymorphism.

Statistical analysis

A complete workflow detailing the study design and statistical analyses performed can be found in Figure 11. Differences between cases and controls for main clinical characteristics were analyzed using the Student's t Test, the Welch's Test or the U Mann Whitney test for quantitative variables. The Pearson's Chi-squared test was used for investigating group differences in categorical variables instead. Cohen's *d* and Cramér's *V* were calculated to assess effect sizes in quantitative and qualitative variables, respectively.

Binary logistic regression models were employed to test the effect of each individual SNP on depression under an additive genetic model of inheritance (thereafter named in the paper as univariate SNP analyses). We performed a post-hoc power analysis based on a Z test for logistic regression using G-Power software. Power estimation was conceived for a logistic regression model including the GRS and the rest of adjusting covariates. Under the assumption of a normal distribution for the GRS in our sample, the power of our logistic regression in the N=1,650 sample, was estimated 99.99 % (e.g., there is a 99.99% chance of correctly rejecting the null hypothesis that a particular value of the GRS is not associated with the value of the outcome variable (depression), with 1,650 participants). Multiple linear regressions were used for the univariate SNP analyses on BMI. Regression models were evaluated by model control investigating linearity of effects on outcome(s), consistency with a normal distribution and variance homogeneity. Continuous variables were tested for normality using the Shapiro–Wilk test and transformed when necessary by means of the natural log or the rank-based inverse normal transformation. All regression models employed are detailed in Figure 11. Given the number of genetic markers analysed, we considered false discovery rates (FDRs) calculated as in Benjamini and Hochberg to correct for multiple hypothesis testing in univariate SNP analyses (79). Regarding GRS analyses, logistic regression models were applied to test whether higher genetic risk scores were observed for depression-cases than controls. Logistic regression models were also applied for comparing participants presenting a high-risk genetic profile (Q2, Q3 or Q4) *vs.* those belonging to the reference quartile (Q1). Multiple linear regression was employed to investigate association between continuous GRS and BMI. Model deviance of logistic regressions (D^2) was calculated to assess the amount of outcome variability explained by each group of variables. All tested models in our work were properly adjusted by confounders such as sex, age, province (geographical location) or BMI whenever necessary (Figure 11).

To assess the predictive ability of the constructed GRS, five different predictive models were trained and evaluated in our sample (see trained models in Figure 11). The area under the receiver operating characteristic (ROC) curve (AUC) was calculated for each model and all possible comparisons between constructed models were tested for statistical significance in terms of prediction improvement. Besides AUC, three recently proposed statistical metrics were also adopted to quantify the added predictive value of each model with respect to its immediate prior. These statistical metrics were the integrated discrimination improvement (IDI), the net reclassification improvement (NRI) and the category-free NRI (cfNRI). All of them have been previously described (80). Since no established risk categories exist in depression, the NRI was applied according to standard risk categories (low (<5%), medium (5% to <25%), or high (≥25%)) (80). For this reason, the cfNRI and the IDI were preferred

estimators than NRI in our study. Discrimination plots, predictiveness curves, prior posterior risk curves and risk distribution plots were obtained for each trained model (data not shown but available upon request). All predictive assessments were conducted using the *PredictABEL* and the *pROC* R packages (78,81). All statistical analyses were performed in R environment, version 3.4.5 (R Project for Statistical Computing).

Besides constructing our models in the whole population (interpretable models), we also implemented a sampling validation procedure. Particularly, K-fold cross validation is a great method in case the classes are not equally balanced in a dataset. The use of this sort of validation consists of dividing the dataset in k groups or folds of samples (of equal sizes, if possible). Thus, the learning process is done with k – 1 folds (training), and the evaluation of model's performance is done with the fold left out (testing). This iterative method helps to create models using different folds, and evaluate the model's performance through different metrics (with the fold left out). In particular, we opted for a 5-fold cross-validation. To create the folds we used the function createFolds() from the caret package. To calculate the rest of the metrics we used the reclassification() function from the predictABLE package.

3. Results

Demographic characteristics

A complete workflow detailing the adopted study design and the statistical analysis conducted can be found in Figure 11. General characteristics of the study population by experimental condition are summarized in Table 8. After excluding subjects with any missing genotypes or BMI under 18.5, a total of 1,650 participants were finally included in our analysis (104 depression cases and 1,546 controls). A significantly higher BMI was found in depression cases than in control (P=0.02; Cohen's *d*=0.24). Furthermore, we found statistically significant differences in the case-control proportion between the different provinces of recruitment (P=0.008; Cramér's *V*=0.103), suggesting that the geographical location of participants could be an important confounding variable to adjust genetics models for. The mean age of the participants was slightly higher in the group with depression than in controls, although this difference was not statistically significant (P=0.19; Cohen's *d*=0.13). Regarding sex, 62.24% of the total sample were females with no sex differences observed between cases and controls (P=0.64; Cramér's *V*=0.12).

Genetic association analyses on Depression

Five SNPs from the 56 candidate genetic variants showed a significant association with depression status in the univariate analyses (data available under request). While the reference minor alleles of SNPs rs6537837 and rs242939 (mapping the *GNAI3* and *CRHR1* genes, respectively) were reported as protective markers for depression, the reference minor alleles for the rs349475, rs310501 and rs1800532 (mapping the genes *LINC02223;CDH18*, *VCAN;HAPLN1* and *TPH1*) were identified as risk markers for depression. Univariate SNP analyses for depression were adjusted for all pertinent confounders as illustrated in Figure 11. Although none of these results remained statistically significant after strict multiple-hypothesis correction by FDR (alpha=0.05), all associations were nominally confirmed using

Fisher's exact tests. Surprisingly, all significant SNPs were identified as intronic or intergenic variants.

From these analyses, 30 SNPs were carefully selected (as they showed similar directional effect compared to the literature findings) and incorporated into an unweighted GRS. The GRS was tested for association with depression status as described in the method section and in Figure 11. The density distribution plot of the constructed GRS in our population is presented in Figure 12. The mean (and standard deviation) of the GRS in the whole sample was 20.82 (2.97), being 22.38 (2.91) in depression cases and 20.71 (2.94) in controls, being this difference statistically significant (P=1,02 $x10^{-7}$; Cohen's *d*=0.57) (Table 8). Interestingly, a logistic regression model adjusted for sex, age, province and BMI revealed a strong risk association between the GRS and the depression status, so that the odds of being depressed were estimated to increase by factor 1.35 for each additional risk allele in the GRS (OR=1.35; CI 95%= [1.13, 1.27]; P=1.35$x10^{-7}$). The depression variability attributable to the genetic component in the model was estimated at 4.17%. When comparing individuals presenting the highest risk scores (Q4) to those belonging to the first quartile (Q1) a stronger association was evidenced (OR=4.19; CI 95%= [2.3, 7.62]; P=2.76$x10^{-6}$). When comparing individuals in the third quartile (Q3) to those belonging to the first quartile (Q1), the association was quantified with an OR=2.37 (CI 95%= [1.25, 4.46]; P=0.008). The remaining comparison (Q2-*vs*-Q1) reported a non-significant result otherwise (OR=1.73; CI 95%= [0.92, 3.26]; P=0.08). When modelling BMI on depression in a model adjusted for sex, age and province, no significant association was reported (OR=1.2; CI 95 %= [0.98, 1.46]; P=0.09). On the other hand, the most intriguing result was the interaction found between the GRS and BMI (further adjusted for age, sex and province), with depression (OR= 1.14; CI 95 %= [1.07-1.20]; P=1$x10^{-4}$). The direction and magnitude of this interaction can be observed in Figure 13, and suggest the existence of a gene-environment interaction phenomena by which BMI increases the genetics-conferred risk of depression in high-susceptibility individuals.

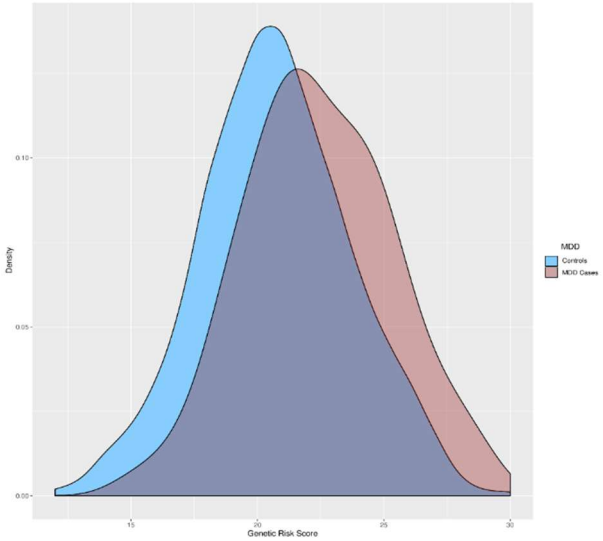

Figure 12. Density distribution plot of the constructed GRS in our population. Abbreviations: MDD, major depressive disorder.

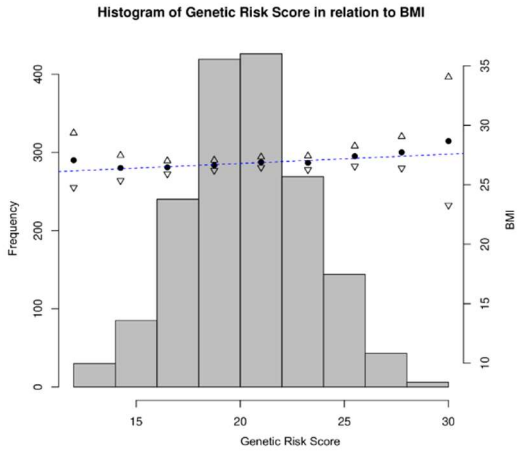

Figure 13. Graphical representation of the direction and magnitude of the GRS*BMI interaction. Abbreviations: BMI, body mass index, MDD, major depressive disorder; SD, standard deviation.

Table 8. Demographic characteristics of the study population by experimental condition.

		MDD-cases	Controls	Effect size	p-value
		n=104	n=1546		
Age (years)		53.51 (14.99)	51.57 (14.50)	0.132	0.187
Sex (male/female)		37/67	586/960	0.12	0.636
BMI (kg/m²)		27.99 (5.24)	26.80 (4.52)	0.244	**0.026**
GRS		22.38 (2.91)	20.71 (2.94)	0.571	**1.02 x10^{-7}**
Province				0.103	**0.008**
	Barcelona	9	132		
	Bilbao	7	181		
	Granada	16	238		
	Jaen	23	230		
	Malaga	25	211		
	Valladolid	14	293		
	Zaragoza	10	261		

Abbreviations: BMI, body mass index; GRS, genetic risk score. Data are expressed as mean (standard deviation). p-values of the categorical variables Sex and Province were obtained after performing a χ^2 test. Effect sizes were reported as Cohen's d and Cramer's V for quantitative and qualitative variables, respectively.

Prediction of depression

To demonstrate the validity of the GRS for the prediction of depression, five logistic regression models were constructed, trained and evaluated in our sample. These models comprised a model with classical demographic information only (age, sex and province), named model 1, and other four additional models that further included (alone or combined) BMI or GRS information (see Figure 11 for more details). The predictive ability of each model was evaluated using AUC. Statistical significance and magnitude of improvements in predictions between models was further assessed by means of the metrics NRI, cfNRI and IDI, as described in the *Methods* section. Results for all models and performed comparisons are presented in Figure 14 and Table 9. The lowest predictive ability corresponded to the model 1, incorporating classical demographic information only (AUC=0.62, 95% CI= [0.57, 0.68]) (Figure 14). The inclusion of the GRS into this model (model 3) reported an increase in the AUC up to 0.69 (95% CI= [0.64, 0.74]) and ($P=1x10^{-5}$ for cfNRI and IDI). Instead, the inclusion of BMI into the classical demographic model (model 2) did not provoke any improvement in the AUC of model 1 (Table 9). A model combining both the GRS and BMI as marginal terms alongside the classical demographic variables (model 4) barely improved the AUC reported for the model 3 (with the GRS as a marginal term). Surprisingly, a model incorporating an interaction term between the GRS and the BMI (model 5) achieved the higher predictive ability for depression (AUC=0.71, 95%CI= [0.65, 0.76]). The significance of this improvement of model 5 with regard to both model 3 and 4 was estimated at P=0.009 for IDI and NRI. Presented results correspond to the model constructed in the whole sample. Additionally, we cross-validated our findings employing a 5-fold CV procedure. Our main conclusions remained, although the AUC of all models slightly decreased (data available under request).

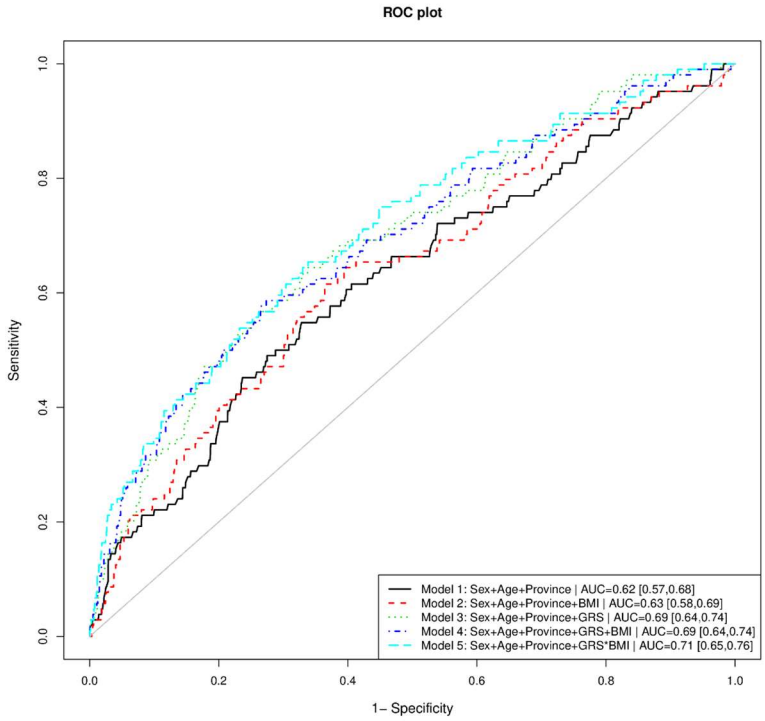

Figure 14. Evaluation of the predictive ability of the constructed predictive model using AUC. Abbreviations: AUC, Area under the receiver operating characteristic curve; GRS, genetic risk score.

Table 9. Statistics for model improvement with the addition of genetic and non-genetic risk factors for MDD. Model 1 (Sex+Age+Province); Model 2 (Sex+Age+Province+BMI); Model 3 (Sex+Age+Province+GRS), Model 4 (Sex+Age+Province+GRS+BMI) and Model 5 (Sex+Age+Province+GRS*BMI). Abbreviations: NRI, net reclassification improvement; cfNRI, category-free NRI; IDI, integrated discrimination improvement; AUC, area under the curve of the receiver operator characteristic curve. The 95% confidence intervals are shown in parentheses.

	Initial model: Model 1 Final model: Model 2	**Initial model: Model 1 Final model: Model 3**	**Initial model: Model 3 Final model: Model 4**	**Initial model: Model 3 Final model: Model 5**	**Initial model: Model 4 Final model: Model 5**
NRI	-0.03 (-0.08,0.009)	0.09 (-0.001,0.17)	-6e-04 (-0.04,0.04)	0.11 (0.02,0.19)	0.11 (0.02,0.19)
NRI *P*-value	0.12	0.05	0.98	**0.01**	**0.009**
cfNRI	0.19 (-0.006,0.39)	0.43 (0.24,0.63)	0.16 (-0.04,0.36)	0.24 (0.04,0.43)	0.17 (-0.03,0.37)
cfNRI *P*-value	0.06	**1e-05**	0.11	**0.01**	0.09
IDI	0.002 (-6e-04,0.005)	0.02 (0.01,0.03)	0.003 (5e-04,0.006)	0.02 (0.007,0.03)	0.01 (0.005,0.02)
IDI *P*-value	0.12	**1e-05**	**0.02**	**0.001**	**0.001**

Genetic pleiotropy on BMI

Given previous evidence of a shared genetic-risk profile between depression and BMI, we investigated whether an association could exist between candidate SNPs and BMI in our sample. For that purpose, both univariate SNP and GRS-based analyses were performed with BMI as outcome variable (see Figure 11 for more details regarding adjusting covariates). As a result, no significant association was reported between the GRS and BMI (OR=1.2, 95% CI= [0.98-1.46], and P=0.09). In univariate-SNP analyses, only the rs12457996 (mapping the *SYT4* gene) showed a significant association being the C allele associated with a lower BMI in our sample (P=0.03). The association did not remain statistically significant after multiple-testing correction.

4. Discussion

In this study, we constructed an un-weighted GRS, including 30 depression-associated genetic risk variants from previous GWAS and candidate gene studies on depression (Figure 11) (57–63). As a first goal, we aimed to investigate whether the constructed GRS was associated with depression as well as if it was able to predict depression with enough precision and accuracy. Given the strong connection between depression and obesity, we also aimed to elucidate whether the predictive ability of the GRS improved with the inclusion of BMI information for each individual. As a result, we found that the GRS is strongly associated with depression status and that it presents a not negligible depression predictive ability by itself. Remarkably, we showed how the interaction of BMI information with the GRS improves the predictive ability of the genetic component, deriving to a predictive ability of certain clinical relevance (AUC=0.71). This result goes in line with recent approaches in which a close relationship between both conditions has been described (26–28) and complements our previous study in which we demonstrated the opposite relation (34).

We found that higher scores from the constructed GRS are strongly associated with a greater prevalence of depression in our sample ($P=1.35x10^{-7}$) (Figure 12). In these analyses, the genetic component represented by the GRS accounted for 4.17% of the depression heritability. For the accomplishment of all these analyses, an unweighted GRS approach was employed, instead of a weighted GRS, due to the lack of GWAS or genetic meta-analyses providing robust estimates for the effects of the SNPs of interest in the literature (56–67). Despite not using a weighted approach, our unweighted GRS demonstrated a strong association with depression, which is in line with previous reports on the clinical utility of GRS un-weighted approaches (34,82,83).

The GRS showed a stronger association with depression than individual SNPs. Thus, it is quite probable that common genetic variants tested here represent only a small and cumulative contribution to the whole genetic susceptibility profile of depression (23,24,84), which is a commonly observed phenomenon in the genetic architecture of many complex diseases (85). In our study, the finding of SNPs eliciting small and cumulative risk effects on depression was further supported by the fact that the greater and more significant differences were observed

for the comparisons between individuals presenting a considerable number of risk variants (Q4) and individual from the bottom reference quartile (Q1), which are individuals barely presenting risk alleles (OR=4.19; CI 95%= [2.3, 7.62]; P=2.76x10^{-6}).

Both alone and in combination with classic demographic information, the GRS has demonstrated a good performance for the prediction of depression status in our sample. Contrary to the GRS, the addition of BMI information alone to the basic model did not show an improvement of its performance (although an increase in the AUC was reported, it did not reach statistical significance). The further inclusion of BMI information as an interaction term (along with the GRS) elicited a significant improvement in the clinical prediction of depression (P-value IDI=0.001 and P-value NRI=0.009) (Table 9). Particularly, BMI was identified as a trigger-like risk factor for depression acting in a concerted way with the GRS component (Figure 13). These findings, therefore, support the existence of a link between obesity and depression and reinforce the theory that the relationship is bidirectional.

There are certainly some limitations that should be mentioned. Some of the main drawbacks from this study include a high unbalanced design between depression cases and control as well as the absence of analysed SNPs from the recently published meta-analysis list (23,24). Therefore, generated hypotheses here would require more detailed characterization in bigger and independent cohorts.

In summary, we found that a GRS based on 30 depression associated risk loci was significantly associated with depression. Although GRS on its own explained only a small amount of variance of depression, a significant novel feature of this study is that including non-genetic risk factors such as BMI together with a GRS came close to the conventional threshold for clinical utility used in ROC analysis and improves the prediction of depression. This has potential clinical implications as well as implications for future research directions in exploring the links between depression and obesity-associated disorders. While it is likely that future genome-wide studies with very large samples will detect variants other than the common ones, it seems probable that a combination of non-genetic information will still be needed to optimize the prediction of obesity.

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RESULTS

CHAPTER III
Study 4

CHAPTER III: Genetics of the relationship between depression and physical health: Depression and physical activity.

Study 4: Systematic review of the relationship between *BDNF*, physical activity and depression

1. Introduction

Major depressive disorder (MDD) or depression is one of the most common mental disorders globally, affecting around more than 300 million people (WHO, 2020). The molecular mechanisms that lead to depression are currently unknown, although different factors that could be associated with this disorder have been proposed. Some scientific evidence suggests that oxidative stress plays an important role in MDD, as higher levels are found in depressed patients (Maes et al., 2011; Maletic et al., 2007). In addition, a dysfunction in the hypothalamic-pituitary-adrenal (HPA) axis (Hasler, 2010), a dysregulation in the transmission of serotonin (Ogilvie et al., 1996; Owens & Nemeroff, 1994; Schildkraut, 1965), and an increase in the release of proinflammatory cytokines (Dowlati et al., 2010), have also been suggested to be involved in the pathophysiology of depression. Furthermore, some studies have observed an increased cellular dysfunction in brain cortical and limbic areas in depressed patients (Guilloux et al., 2012; Sen et al., 2008), which are highly related to decreased neurotrophic activity (Duman & Monteggia, 2006). This lower neurotrophic activity has been reported to be associated with a reduced number of cells in the prefrontal cortex (Rajkowska, 2000), in the amygdala (Bowley et al., 2002; Hamidi et al., 2004), and with a decrease in hippocampus volume (Campbell et al., 2004; Videbech & Ravnkilde, 2004). All together, these neurobiological alterations are commonly expressed in neuroplasticity loss. Neuroplasticity is a key brain attribute in learning and memory processes (Arnone et al., 2013), and several studies show that it could be influenced by the brain-derived neurotrophic factor (BDNF) (Bus et al., 2015). BDNF belongs to the family of neurotrophins, which are brain-synthesised proteins that contribute to the survival, growth and maintenance of neurons, and take part in a variety of learning and memory related functions. BDNF is mainly found in the hippocampus and the brain cortex, with an important role in the regulation of activity-dependent neuronal plasticity (Goldstein & Young, 2013).

BDNF protein induces dendritic spines formation and promotes cellular growth and the survival of serotonergic neurons (Y. Lu et al., 2008; Messaoudi et al., 2002), which has been involved in the pathophysiology of depression and synaptic plasticity (Kang & Schuman, 1996). Moreover, changes in BDNF have been associated with age-related memory loss, depression and hippocampus atrophy (Erickson et al., 2012; Guilloux et al., 2012; Molendijk et al., 2011; Sen et al., 2008).

A functional polymorphism in the *BDNF* gene, causing the substitution of Valine amino acid to Methionine in codon 66 from the prodomain of the peptide BDNF, disrupts BDNF release and intracellular trafficking. This polymorphism has been associated with an increased risk of suffering depression (Egan et al., 2003) (Cardoner et al., 2013; Pei et al., 2012; Phillips, 2017). Similarly, different studies have found a reduction in serum and plasmatic BDNF levels in depressed patients (Lee et al., 2007; Yoshida et al., 2012; Birkenhäger et al., 2012; Kim et al., 2007). It has been observed that the normalisation of BDNF levels occurs as a response to various treatments, such as antidepressants (Sheldrick et al., 2017; Matrisciano et al., 2009) or physical activity (Engesser-Cesar et al., 2007). Furthermore, depressed patients have been reported to have decreased BDNF levels and function in hippocampus and medial prefrontal cortex (Autry & Monteggia, 2012). These changes have been described leading to the dysfunction of astrocytes and microglia cells in depressive circuits (Phillips, 2017).

Currently, antidepressant medication is the first line of treatment for depression (Holsboer et al., 1995), specially selective serotonin reuptake inhibitors (SSRIs), although the remission rate is only 60% (Kennard et al., 2009). Moreover, side effects of these drugs, as well as the poor adherence associated to antidepressant therapies (Sansone & Sansone, 2012), have caused an increased interest in alternative treatments, such as personalised medicine (Sinyor et al., 2010), and physical activity (Malhi et al., 2015).

Physical activity is the development of activities that require an energetic expense which involve body movements produced by skeletal muscles (Bherer et al., 2013). It has been demonstrated that regular exercise usually changes the mood through various mechanisms, e.g., an increase in self-efficacy, motivation and energy and a better psychosocial functioning (Ross et al., 2019). Regular exercise also involves positive neurobiological adaptations such as an increase in neurogenesis in the hippocampus, monoamine transmission and synaptic growth (Dishman et al., 2006; van Praag et al., 1999). Therefore, it is suggested that the regular practice of physical activity may be implied in the observed improvement of depressive symptoms, memory and other cognitive functions (Cotman & Berchtold, 2002; Liu-Ambrose & Donaldson, 2008); although the molecular mechanisms underlying this improvement have not been fully described yet (Coelho et al., 2012; Cotman & Berchtold, 2002; Laske et al., 2010). Furthermore, there is evidence that interventions with physical exercise can decrease the risk of suffering depression (Abu-Omar et al., 2004; Motl et al., 2004), ease recovery (Kvam et al., 2016; Schuch et al., 2016) and reduce the incidence of relapse (Babyak et al., 2000; Hoffman et al., 2011).

The study of the neurobiological pathways involved in the reduction of depressive symptoms by exercise is required to optimise the efficacy of this potential therapeutic strategy (Dinoff et al., 2018). One of the hypotheses currently under research is the involvement of BDNF, since physical activity affects the production of this neurotrophin and improves neuroplasticity (Coelho et al., 2012; Cotman & Berchtold, 2002; Laske et al., 2010). It has been shown that physical exercise increases BDNF levels in the hippocampus and in other brain regions. (Phillips, 2017). Furthermore, benefits of exercise include an improvement on the release and

function of BDNF in synapsis, which promotes the integrity of dendritic spines, avoids the hippocampus atrophy, reduces the astrocytic dysfunction and activates different cellular pathways involved in neuronal plasticity (Duman & Monteggia, 2006; Krishnan & Nestler, 2008; Patterson, 2015). For these reasons, it has been proposed that consequences of practising regular exercise allow for the homeostatic processes involved in maintenance, repair and reorganisation of the circuits that are damaged in depressed patients (Phillips, 2017).

The relationship between depression, physical activity and BDNF emerges as an interesting potential interaction to be explored in the development of an effective therapy for MDD. Moreover, a role of physical activity on the prevention of depression has already been described (Mammen & Faulkner, 2013; Schuch et al., 2018; X. Wang et al., 2019). The therapeutic potential is of high value due to the easy manipulation and long-term monitoring that physical activity allows (Erickson et al., 2012). Nevertheless, the molecular mechanisms involved in this relationship remain unclear.

Considering all the above, our aim was to carry out a systematic review of the scientific literature about the potential role of BDNF, both *BDNF* genetic variability and protein levels, in the relationship between physical exercise and depression incidence, prevalence or improvement of depressive symptoms.

2. Methods

Search strategy and study selection

A comprehensive systematic search was conducted from February to March 2022 in PubMed, Scopus, Web of Science and PsychInfo databases to identify eligible references. The combination of controlled descriptors previously selected using MESH thesaurus, along with the boolean operators "AND" and "OR" led to the following search equation that was used across all databases: (BDNF OR "level* of protein") AND (gene* OR "polymorphism" OR "SNP" OR "Single Nucleotide Polymorphism") AND ("physical exercise" OR "exercise" OR "physical activity") AND (depress* OR "MDD" OR "unipolar disorder").

The search strategy and selection of eligible documents was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement (Page et al., 2021). Two authors screened all titles and abstracts.

Selection criteria

Studies that fulfilled the following criteria were eligible for inclusion: (1) original articles using experimental or observational design (cross-sectional or longitudinal), reviews and/or meta analyses of published studies; (2) studies that analysed the relationship between exercise, depression and BDNF in general adult population; (3) published in the last 10 years; (4) and in English or Spanish.

Two authors independently selected the documents, reviewed full-texts, extracted the data and assessed the risk of bias and quality of evidence. Any conflicts that arose from the review of studies were discussed and resolved between them.

Quality assessment and risk of bias

To assess the methodological quality and risk of bias of the studies, we used the Scottish Intercollegiate Guideline Network (SIGN) checklists for case-control studies, randomized controlled trials and systematic reviews and meta-analyses, accordingly (SIGN, 2019). Two authors independently evaluated and graded the studies, resolving disagreements in a consensus meeting. Studies were considered of "high quality" if little or no risk of bias was identified, "acceptable" if they presented some flaws unlikely to alter conclusions, or "low quality", if few or no criteria were fulfilled.

Data extraction

We created two independent spreadsheets to depict the relevant information from the selected manuscripts. The first one included manuscripts studying *BDNF* Val66Met polymorphism; the second one included records focused on BDNF protein levels. Both spreadsheets contained document's reference, article type, epidemiological design, objectives, type of sample (sample size and main characteristics), principal variables explored (exercise, depression and *BDNF* Val66Met polymorphism or BDNF protein levels); techniques and measurements, and main results.

3. Results

Figure 15 shows the search and selection process based on the PRISMA statement. Tables 10a and 10b summarise the most relevant information regarding methodology and results of the selected studies included in this systematic review.

Characteristics of the selected studies

Among 537 records included in our initial search, a total of 18 scientific articles met the inclusion criteria stated above (Figure 1). For this review, the studies were classified in two groups: the first group focuses on the *BDNF* gene (n = 6), and the second one evaluates the BDNF protein (n = 13). Also, the latter group was divided into manuscripts that evaluated the change in BDNF levels after an acute exercise intervention (n = 4), and manuscripts that assessed this change after chronic exercise intervention (n = 9). One article included results of both *BDNF* gene and protein, thus it was included in both sections. Two of the 18 articles included in this manuscript were systematic reviews and meta-analyses.

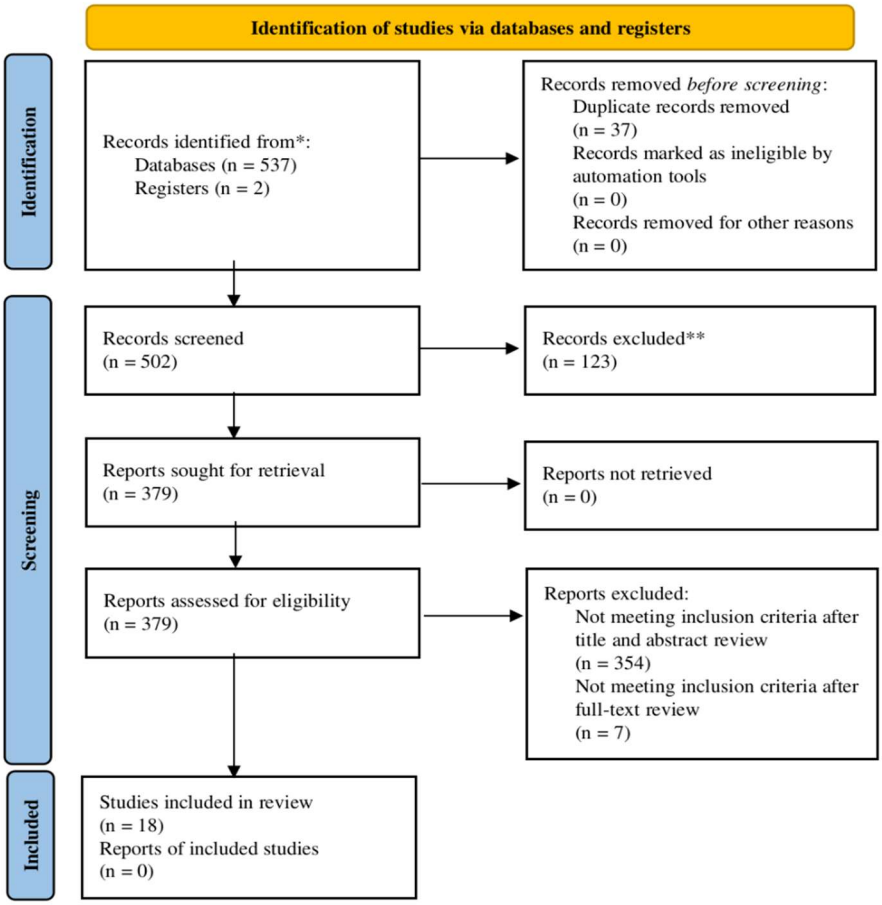

Figure 10. PRISMA flow diagram of the search and selection process

Considering the study design, from the six studies analysing the *BDNF* gene, four were observational case-control studies with a cross-sectional design, whereas two studies were randomised controlled trials (Table 10a). The four studies assessing the acute effect of exercise on the BDNF protein followed pre-experimental designs (Table 10b). From the nine studies analysing the chronic effect of exercise on the BDNF protein, we found two systematic reviews and meta-analyses, and seven randomised controlled trials (Table 10b).
Most of the selected studies were designed in order to minimise the risk of bias, obtaining at least an acceptable overall score in the SIGN checklist. Of note, the four pre-experimental studies were included despite a lower methodological quality since they represent the only scientific evidence evaluating the acute effect of exercise on BDNF levels.

Characteristics of the samples
The population of the selected scientific articles were young and medium-age adults in 10 studies, while 5 articles performed the study in elderly people (older than 60 years), and another one included participants from 18 to 75 years old. The population of 3 out of the 18 studies was composed exclusively by women (Laske et al., 2010; Meyer et al., 2016; Pereira et al., 2013).

Within the experimental studies, six works were conducted integrally on cases with depression. Among these, two studies included outpatients (Krogh et al., 2014; Toups et al., 2011), and three of them included inpatients (Kerling et al., 2017; Salehi et al., 2016; Schuch et al., 2014). Three other experimental studies were conducted on community-dwelling samples (Dotson et al., 2016; Pereira et al., 2013; Rahman et al., 2017), although one of them consisted of sedentary participants (Dotson et al., 2016). Among the pre-experimental studies, two of them only included cases with depression (Kallies et al., 2019; Meyer et al., 2016), and the remaining ones assessed cases versus controls (Laske et al., 2010; Ross et al., 2019). Two observational studies were representative of the general population (Gujral et al., 2014; Zarza-Rebollo et al., 2022), whereas one of them compared athletes versus controls (Haslacher et al., 2015), and the remaining one was composed of military veterans (Pitts et al., 2020).

Studies exploring the BDNF Val66Met polymorphism

A total of 6 documents evaluated the effect of the *BDNF* Val66Met polymorphism on depression and physical activity. Four of them were observational studies, whereas two other studies were experimental studies (randomised controlled trials).

Gujral *et al.* (2014) conducted an observational study to assess the effect of *BDNF* Val66Met polymorphism in the association between physical activity and depressive symptoms in 1,072 middle-aged adults. The authors found that there were more depressive symptoms in Met allele carriers than in Val allele homozygous ($p = 0.03$), even though the relationship was only significant in men; while physical activity was associated with less depressive symptoms only in women ($p = 0.01$). Nonetheless, the moderation effect of the Val66Met polymorphism on the interaction between physical activity and depressive symptoms in middle-aged adults was not significant (Gujral et al., 2014).

In 2015, Haslacher *et al.* investigated whether intensive endurance sports mitigated the genetic vulnerability to depression in a cohort of elderly marathon athletes (> 60 years old) (58 controls and 55 athletes). Athletes must have participated in at least one competition in the 3 years prior to the study, and performed physical training for more than 2 hours per week. Beck Depression Inventory (BDI) (Beck et al., 1961) and Geriatric Depression Scale (GDS) (Yesavage & Sheikh, 1986) were used to assess the depressive state of the participants. The results showed a statistically significant interaction between the group (athletes vs controls) and *BDNF* genotypes in depressive symptoms (BDI: $p = 0.027$, GDS: $p = 0.013$). Moreover, among *BDNF* Val/Val participants, only controls showed a higher risk of getting a score of ≥ 10 in the BDI (RR= 3.537; 95% CI = 1.276-9.802), while this effect did not appear in Met allele carriers. They concluded that physical exercise positively influences the effect of *BDNF* on mood and that this effect was greater in Val/Val allele homozygous, suggesting a counteracting effect of physical exercise in the genetic susceptibility to depression (Haslacher et al., 2015).

More recently, Pitts *et al.* (2020) aimed to evaluate whether the *BDNF* Val66Met polymorphism and physical activity had a role in the effect of depression on cognitive

functioning in 1,386 USA veteran soldiers. Two groups were established: those who practised any exercise (median of 3 days per week), and those who did not. The Patient Health Questionnaire-2 (PHQ-2) was used to assess the depressive symptoms, and the Medical Outcomes Study Cognitive Functioning Scale (MOS-CFS), and Cogstate Brief Battery (CBB) were used for assessing different cognitive functioning aspects, subjective and objective, respectively. They reported a statistically significant interaction between depression, the Val66Met genotype and physical activity. They observed better score results for all the MOS-CFS measures ($p < 0.003$), the CBB measures of visual learning ($p < 0.001$) and working memory ($p < 0.001$) in depressed veterans that carried the Met allele and exercised in comparison to Met allele carriers nonexercisers (Pitts et al., 2020).

In a recently published research, Zarza-Rebollo *et al.* (2022) aimed to investigate the existing relationship between the Val66Met polymorphism, physical activity and depression in a cross-sectional study. Their sample consisted of a total of 3,123 participants representative of the general population, with ages between 18 and 75 years old, including 209 cases with depression and 2,914 controls. The Spanish version of the Mini-International Neuropsychiatric Interview (MINI) interview was used to diagnose major depression, following Diagnostic and Statistical Manual of Mental Disorders (Fourth edition) (DSM-IV) criteria. The results showed an interaction effect between the Val66Met polymorphism and the number of hours of self-reported physical activity on the risk of depression. This effect was observed in the total sample (OR = 0.95, 95%CI = 0.90–0.99, p = 0.027) and was strengthened in women (OR = 0.93, 95%CI = 0.87–0.98, p = 0.019). The results suggested that participants carrying the Met allele decreased their risk of depression as more hours of physical activity were reported, compared to Val/Val individuals (Zarza-Rebollo et al., 2022).

Regarding the experimental approaches, Dotson *et al.* (2016) examined the impact of the *BDNF* Val66Met polymorphism and sex in depressive symptoms after an intervention with physical activity in 365 sedentary adults. The intervention consisted of a 12-month program in specialised centres, whereas the control group attended instruction sessions on health education. Depressive symptoms were analysed at baseline and at 12-months. The results showed the most pronounced decrease in somatic symptoms in men that participated in the physical activity intervention, with a preferential benefit on Met allele male-carriers ($p = 0.043$). Furthermore, the impact of physical activity in depressive symptoms was marginally dependent on the *BDNF* Val66Met genotype and sex ($p = 0.079$) (Dotson et al., 2016).

The experimental study performed by Rahman et al., (2017) aimed to investigate the predictive ability of the Val66Met polymorphism in the improvement of depressive symptoms due to physical exercise, considering the influence of childhood adversity. The study sample was composed of 547 adults from the general population with mild-to-moderate depression, determined with a score of ≥ 10 on the Patient Health Questionnaire (PHQ-9). The depressive symptoms were assessed before and after the intervention. The experimental group was randomly divided into three intensity levels and was recommended to exercise in a gym 3 sessions per week during 12 weeks. Among Met allele carriers, they found a greater

response to the intervention with physical exercise in those patients that did not report childhood adversity ($p < 0.05$) in comparison to Val/Val participants (Rahman et al., 2017).

Genotyping techniques

The *BDNF* Val66Met polymorphism was genotyped by TaqMan genotyping method in most of the studies (Dotson et al., 2016; Haslacher et al., 2015; Rahman et al., 2017; Zarza-Rebollo et al., 2022). However, Pitts and colleagues (2020) used the PsychChip genotyping array (Pitts et al., 2020), and Gujral *et al.* (2014) used an amplification and fluorescence detection method described elsewhere (Gujral et al., 2014).

Studies analysing the levels of BDNF protein

Acute exercise interventions

This section includes four articles whose main objective was to assess the effect of single bouts of physical exercise. All studies followed an experimental design, determining BDNF protein levels before and after performing the requested exercise bout.

Laske *et al.*, (2010) conducted a study to investigate the effects of exercise in BDNF serum concentration. A total of 55 women older than 50 years participated in the study: 35 of them were diagnosed with MDD (20 were treated with SSRI antidepressants during the 3 months prior to the study), and 20 were control women. BDNF serum concentrations were measured before and after an incremental exercise test in a treadmill. These concentrations did not significantly differ between MDD patients with medication and those who did not have any treatment. Furthermore, compared to controls, MDD cases showed significantly lower BDNF levels before the exercise test ($p = 0.001$), whereas no significant differences were observed after performing exercise ($p = 0.233$). Following a unique short-term exercise, a statistically significant increase in BDNF serum levels was found in MDD patients ($p < 0.001$). After a 30-minute break following exercise, both groups showed a significant reduction of BDNF serum levels, even below the levels prior to the exercise.

Later, Ross *et al.* (2019) analysed the effect of different intensity aerobic exercise in BDNF serum levels in a sample of 26 individuals: 13 MDD cases and 13 controls. A total of three exercise sessions were performed. Sessions consisted of 15 minutes of work with different objectives: 1) low intensity cycling at 35% heart rate reserve (LO); 2) high intensity cycling at 70% heart rate reserve (HI); or 3) remaining seated as a control group (CON). BDNF concentration was measured before, immediately after exercise, and every 15 minutes post-exercise for 1 hour. The results showed that BDNF serum levels were significantly higher immediately after exercise in HI compared to LO ($p = 0.003$) and to CON ($p = 0.027$). Furthermore, BDNF levels after exercise were significantly higher than levels measured before exercise in HI ($p < 0.001$) and LO ($p = 0.019$) conditions. BDNF levels at 15 minutes after exercise in HI and LO conditions were not significantly different from the values obtained before exercise, and no differences were found at any time later (Ross et al., 2019).

The group of Meyer *et al.* (2016) analysed the response in serum BDNF to exercise bouts in 24 female MDD patients between 20 and 60 years old. Three 30-minute sessions of exercise in a cycle ergometer at different prescribed intensities (light, moderate or hard) were performed, one session per week. A control session consisting of a resting session in the cycle ergometer was included. Blood was drawn from the participants before exercise and 10 minutes later, while they completed a Profile of Mood States (POMS) and a BDI-II questionnaire to assess depressive symptoms at those moments and 30 minutes after each exercise session. Results in POMS suggested an improvement in the depressive mood after exercise. In addition, there was a significant increase in serum BDNF ($p = 0.006$), independent of the intensity of the exercise. Nevertheless, the changes in BDNF serum levels were not significantly correlated with changes in the POMS questionnaire, nor 10 or 30 minutes after exercise ($p > 0.05$). This correlation was not present with changes in BDI-II either. The 14 participants who had antidepressants showed lower BDNF protein serum levels post-exercise than the participants without medication ($p = 0.015$), but similar mood changes were observed (Meyer et al., 2016).

Later, Kallies *et al.* (2019) analysed changes in BDNF serum levels before and after an incremental exercise test, in a sample of 30 adult MDD patients. These participants, previously diagnosed in a larger study (Heinzel et al., 2018), were from 18 to 65 years old and did not exercise for more than 90 minutes per week. The change in plasma volume and the number of platelets was considered in the analyses. The incremental exercise test took place in a cycle ergometer, with periodical progressions until the participant was physically unable to continue. The results indicated a significant increase of serum BDNF induced by exercise ($p < 0.001$) after adjustment by plasma volume shift and platelet count. Furthermore, they found a significant interaction effect between the change in BDNF serum levels and the number of platelets ($p = 0.001$), showing a higher increase of these levels in participants who had a smaller amount of platelets (Kallies et al., 2019).

Chronic exercise interventions

A total of eight manuscripts analysing the effect of chronic exercise intervention -extended for various weeks- have been included in this section, with the aim of assessing differences between before and after a long-lasting intervention. Seven studies were randomised controlled trials and two works were systematic review and meta-analysis.

Toups *et al.* (2011) conducted a study to evaluate the change in BDNF levels after an intervention with exercise in 126 MDD adult patients, partially responders to a 2 to 6-months treatment with SSRIs. The intervention lasted 12 weeks and 70 participants completed the study. Subjects were randomly divided into two groups: high and low energy expenditure. The high energy expenditure group performed supervised physical activity aiming to burn 16 kilocalories per kilogram of body weight per week, whereas 4 kilocalories per kilogram of body weight per week was the target for the low energy expenditure group. Results showed that basal BDNF serum concentration was stable and did not correlate with the energetic expenditure ($p = 0.15$) or the improvement of the clinician rated version of the Inventory of Depression Symptomatology (IDS-C) score ($p = 0.89$) in the entire sample. However, subjects

with higher baseline BDNF levels improved their IDS-C score with exercise in a shorter period of time and independently of the group of energy expenditure ($p = 0.003$) (Toups et al., 2011).

The study conducted by Rahman *et al.* (2017), previously mentioned in the *Influence of the BDNF Val66Met polymorphism* section, also measured the concentrations of serum proBDNF and mature BDNF. They did not find any effect of the intervention with physical exercise in these concentrations. The intervention consisted of a 12-weeks program of physical exercise in which 547 participants with mild-to-moderate depression attended 3 gym sessions per week. They found a statistically significant difference between *BDNF* Val66Met Met allele carriers and Val/Val participants in their baseline levels of mature BDNF, which were higher in the Met allele carriers ($p = 0.019$). Besides, they analogously reported higher levels of mature BDNF in Met allele carriers at baseline in those participants that used antidepressants ($p = 0.04$), but this effect was not observed in participants who did not take antidepressants. There were no differences between before and after the intervention with physical exercise after comparing mBDNF or proBDNF (Rahman et al., 2017).

Pereira *et al.* (2013) investigated the effect of two standardised exercise programmes in BDNF plasma levels and depressive symptoms in 451 inactive women (65-89 years old). Participants were divided into two groups to follow a supervised protocol of muscle strength exercises (SE) or aerobic exercises (AE) (1-hour, 3 times a week for 10 weeks). The Geriatric Depression Scale (GDS) was used to evaluate depressive symptoms. A significant difference in BDNF levels was found between the groups SE and AE ($p = 0.009$), but the difference between before and after intervention was only present in the SE group ($p = 0.008$). There was a significant difference between pre- and post-intervention in GDS scores in both groups ($p = 0.001$), suggesting that the effects of these exercise protocols were comparable regarding depressive symptoms ($p = 0.185$) (Pereira et al., 2013).

Following a similar strategy, Salehi *et al.* (2016) also evaluated the changes in BDNF plasma levels after different types of intervention in 60 patients with MDD. Participants were young adults and had a BDI score higher than 30 and a Hamilton Depression Rating Scale (HDRS) higher than 25. Depending on the intervention, participants were randomly assigned to one of the three following groups: electroconvulsive therapy (ECT), aerobic exercise training (AET), or the combination of both interventions (ECT+AET). The intervention lasted 4 weeks and was combined with a standard SSRI treatment. Here, BDNF plasma levels increased with time in all groups, being the increase higher in the ECT+AET condition and the lowest in the AET condition. Moreover, the BDI and HDRS scores significantly decreased between pre- and post- intervention, and were stronger in the combined intervention than in the ECT and AET conditions. No association between the increase in plasma BDNF and the improvement in the symptoms was found. Furthermore, they reported a significant association between the treatment condition and the remission rate ($p < 0.001$), with the highest remission rate observed in the combined intervention (more than half of the patients showed complete remission) (Salehi et al., 2016).

Krogh and colleagues (Krogh et al., 2014) conducted a randomised clinical trial to assess the effect of exercise intervention on the hippocampus volume and serum levels of BDNF, VEGF and IGF-1 proteins in 79 MDD patients. Patients were diagnosed using the Danish version of the MINI interview and had a score higher than 12 in the HDRS. Participants did not receive antidepressant medication for the 2 previous months to the study and were randomly assigned in two groups. Experimental intervention (41 individuals) consisted of 45-minute sessions of exercising on stationary bikes at 80% of their maximum heart rate, whereas control intervention (38 individuals) consisted of low impact exercise for the same time interval. The intervention lasted 3 months and participants were advised to participate in 3 sessions per week. The results showed that the hippocampus volume and the serum concentrations of BDNF, VEGF and TFG-1 did not vary between groups, although they found a significant association between the change in the hippocampus volume and the depressive symptoms ($p = 0.03$) (Krogh et al., 2014).

In 2014, Schuch et al. (2014) evaluated the effect of the combination of exercise to a pre-established treatment in BDNF serum levels of 26 MDD patients. MDD diagnosis was assessed with the MINI and depressive symptoms were assessed using the HDRS (all participants must have a score ≥ 25). Participants were splitted into two groups: 11 patients maintained their usual treatment, and 15 of them added aerobic exercise sessions to the treatment. The intervention consisted of 3 weekly sessions for 3 weeks where they could choose the intensity and the modality according to their preferences. The results showed a significant association between the BDNF levels and the time ($p < 0.001$), but not between the groups and the time ($p = 0.13$). They concluded that the combination of exercise with the usual treatment does not have any effect on BDNF serum levels in MDD patients (Schuch et al., 2014).

Later, Kerling *et al.* (2017) analysed the effect of additional exercise in BDNF serum levels in 42 patients diagnosed with MDD according to the DSM-IV criteria. The control group maintained their treatment with no changes throughout the intervention, whereas the experimental group added an intervention with exercise. This intervention consisted of 45-minute sessions (3 per week) of moderate intensity for 6 weeks. A significant effect was found between time and group regarding BDNF serum levels ($p = 0.030$), with an increase of BDNF concentrations in the experimental group in comparison with controls. Nonetheless, the differences in BDNF serum levels before and after the intervention were not statistically significant in any group (Kerling et al., 2017).

Finally, Dinoff et al. (2018) conducted a systematic review and meta-analysis which included 6 studies with a total of 176 individuals. Their aim was to investigate whether chronic exercise interventions had, consequently, an increase in BDNF blood concentration in MDD patients diagnosed using DSM guidelines. They also assessed whether the effect was dependent on individuals' variables (age or sex) or the parameters of the intervention (exercise duration or intensity). They considered interventions where intensity of the exercise was $\geq 50\%$ of the maximum oxygen absorption. The meta-analysis including the 6 studies showed that BDNF concentrations were not significantly higher (neither in serum nor in plasma) after chronic

exercise intervention, although a trend towards association was observed (p = 0.09) (Dinoff et al., 2018).

Similarly, in the review and meta-analysis by Kurebayashi & Otaki (2018), the authors explored the effect of physical exercise on BDNF levels in MDD patients, in order to establish or reject this effect as a potential mechanism by which the exercise improves depressive symptoms. The review included 5 experimental studies with a total of 199 patients with severe depressive symptoms. The intervention consisted of chronic aerobic exercise. The results of the meta-analysis showed that there was no significant effect of physical exercise in BDNF levels (Z = 0.32, p = 0.75). Thus, they suggested that, given the benefits of exercise on MDD, this effect may occur through a different mechanism than that involving BDNF (Kurebayashi & Otaki, 2018).

BDNF serum/plasma levels quantification techniques

The quantification of BDNF serum concentration was performed with an ELISA assay in all the included studies. The majority of them used the Quantikine Human BDNF Immunoassay kit (R&D Systems) (Kallies et al., 2019; Kerling et al., 2017; Krogh et al., 2014; Laske et al., 2010; Meyer et al., 2016; Rahman et al., 2017; Ross et al., 2019; Toups et al., 2011), although Schuch *et al.* (2014) used an ELISA Sandwich assay commercial kit from Chemicon (USA) (Schuch et al., 2014). It is worth mentioning that Kerling *et al.* (2017) analysed several forms of serum BDNF, such as free BDNF, BDNF connected to Trk, pro-BDNF and mature-BDNF (Kerling et al., 2017). Regarding the measurement of BDNF plasma concentration, also an ELISA assay (R&D Systems) was the preferred technique (Pereira et al., 2013; Salehi et al., 2016).

Other techniques that were used as a complement to the evaluation of BDNF were an hemogram with platelet count (Sysmex XE-2100, Sysmex Corp.) (Kallies et al., 2019), and magnetic resonance imaging (MRI) (Siemens 3.0T system using MPR sequence with 3D gradient T1-weighted) to measure the hippocampus volume (Krogh et al., 2014).

Table 10a. Summary of eligible studies analysing *BDNF* Val66Met polymorphism.

Reference	Study design	Objectives	Sample characteristics	Study variables		Techniques and measurement	Summary of findings
				Physical activity	Depression		
Gujral, S, et al. (2014). The BDNF Val66Met polymorphism does not moderate the effect of self-reported physical activity on depressive symptoms in midlife.	Observational study. Cases and controls.	To assess the effect of the *BDNF* Val66Met polymorphism in the association between physical activity and depressive symptoms in adults.	N = 1072 non-Hispanic Caucasians (525 males/547 females; avg. age = 44.7 years old). 378 Met allele carriers; 694 Val/Val.	PPAQ (self-assessed) Estimation of average energy expended per week.	CES-D	Amplification conditions reported by Cheng and Yeh (2005) (Cheng and Yeh, 2005). Detection by fluorescence polarisation (Chen et al., 1999).	In men, greater depressive symptoms were observed in Met allele carriers compared to those who were homozygous ValVal ($p = 0.03$). In women, physical activity was associated with fewer depressive symptoms ($p = 0.01$). The *BDNF* Val66Met genotype did not moderate the effect of physical activity on depressive symptoms ($p = 0.94$).
Haslacher, H, et al. (2015). Physical exercise counteracts genetic susceptibility to depression.	Observational study. Cases and controls.	To evaluate the relationship between resistance sports and the attenuation of the genetic susceptibility to depression in elderly marathon athletes.	N = 55 athletes/58 controls, >60 years old. Inclusion criteria: ≥ 1 participation in established competitions in the three years prior to the study, and ≥ 2 hours of physical activity per week.	Participants were endurance sports athletes (marathon runners and endurance cyclists).	BDI and GDS	Val66Met polymorphism genotyping (TaqMan).	A statistically significant interaction was found between groups (athletes vs controls) and genotypes (BDI: $p = 0.027$; GDS: $p = 0.013$). An increased relative risk of 3.54 (95%CI = 1.276-9.802) of obtaining a BDI score ≥10 was found among ValVal homozygous, although only in controls. This effect was not found in Met allele carriers.
Dotson, VM, et al. (2016). Genetic moderators of the impact of physical activity on depressive symptoms.	Experimental study. Randomized controlled trial	To assess the relationship of the *BDNF* Val66Met polymorphism and depressive symptoms in elderly adults after a physical activity intervention. To evaluate the effects of this intervention on depressive symptoms.	N = 365 adults (70 – 89 years old). Inclusion criteria: sedentary, able to walk 400 m in < 15 min.	The intervention consisted in: 12 months with sessions of mainly 40 min of moderate-intensity walking, modulating the intensity and location of the sessions. The control group received an intervention about ageing health.	CES-D	Val66Met polymorphism genotyping (TaqMan).	After a physical activity intervention, a greater decrease in somatic symptoms was observed only in men, with a higher benefit in Met allele carriers, compared with ValVal homozygous and women ($p = 0.043$).

Rahman, MS, et al. (2017). BDNF Val66Met and childhood adversity on response to physical exercise and internet- based cognitive behavioural therapy in depressed Swedish adults.	Experimental study. Randomized controlled trial.	To assess the relationship between the *BDNF* Val66Met polymorphism and treatment response of an intervention with either physical activity or ICBT. To assess the interaction between *BDNF* Val66Met and childhood adversity in response to treatment.	N = 547 Swedish adults with mild-to-moderate depression. Randomized controlled trial with physical exercise, ICBT and treatment as usual, during 12 weeks.	Participants in the intervention group were allocated in randomly assigned groups (low, medium and high intensity). Assistance was recommended to be 3 weekly 60-minute sessions in gyms.	Inclusion criteria: PHQ-9 score > 10. Depressive symptoms: MADRS and social attachment ability (5 questions from ISSI subscale)	Val66Met polymorphism genotyping (TaqMan).	In participants that had not reported an exposure to childhood adversity, Met allele carriers were observed to have a greater response to the intervention with physical exercise ($p < 0.05$) compared to ValVal homozygous.
Pitts, BL, et al. (2020). Depression and cognitive dysfunction in older U.S. military veterans: Moderating effects of BDNF Val66Met polymorphism and physical exercise.	Observational study. Cases and controls.	To evaluate the effect of depression in cognitive functions in US veterans. To assess the role of the *BDNF* Val66Met polymorphism and physical exercise (or their interaction) in this effect.	N = 1386 US veterans of European-American descent (avg. age = 63 years old).	Participants were divided into two groups: those who self-reported no exercise, or those who reported practising any exercise (≥1 days per week, median of 3 days per week).	PHQ-2. Cognitive functioning: Medical Outcomes Study Cognitive Functioning Scale (MOS-CFS), and Cogstate Brief Battery (CBB) for psychomotor speed, attention, visual learning and working memory.	PsychChip array.	Among Met allele carriers that were depressed at the moment of the study, those who exercised obtained better results than those who did not exercise, in all the MOS-CFS measures ($p > 0.003$ in all p's), and CBB measures of visual learning ($p > 0.001$) and working memory ($p > 0.001$).
Zarza-Rebollo, JA, et al. (2022). Interaction Effect between Physical Activity and the *BDNF* Val66Met Polymorphism on Depression in Women from the PISMA-ep Study.	Observational study. Cases and controls.	To analyse the effect of the *BDNF* Val66Met genotype in the relationship between physical activity and depression.	N= 209 cases and 2914 controls from the general population, with ages between 18-75 (avg. age = 43.18 years old)	Self-reported number of hours per week of physical activity.	MINI (DSM-IV criteria)	Val66Met polymorphism genotyping (TaqMan).	A lower prevalence of depression was observed in Met allele carriers with a higher reported number of hours of physical activity, compared to ValVal homozygous. An interaction effect was observed in the total sample (OR = 0.95, 95%CI = 0.90–0.99, p = 0.027) and in women (OR = 0.93, 95%CI = 0.87–0.98, p = 0.019).

Abbreviations:
ICBT: Internet-based Cognitive Behavioural Therapy
PPAQ: Paffenbarger Physical Activity Questionnaire
CES-D: Center for Epidemiology Scale for Depression
BDI: Beck Depression Inventory
GDS: Geriatric Depression Scale
PHQ-9: Patient Health Questionnaire 9-Item Depression Scale
MADRS: Montgomery Åsberg Depression Rating Scale
ISIS: Interview Schedule for Social Interaction
PHQ-2: Patient Health Questionnaire 2-Item Depression Screener
MINI: Mini-International Neuropsychiatric Interview
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders Version Four.

References:

Chen, X., Levine, L. and Kwok, P., (1999). Fluorescence Polarization in Homogeneous Nucleic Acid Analysis. *Genome Research*, 9(5), pp.492-498. doi: 10.1101/gr.9.5.492
Cheng, Q. and Yeh, H., 2005. PLC$_\gamma$ signaling underlies BDNF potentiation of Purkinje cell responses to GABA. *Journal of Neuroscience Research*, 79(5), pp.616-627. doi: 10.1002/jnr.20397

Table 10b. Summary of eligible studies analysing the BDNF protein.

Reference	Study design	Objectives	Sample characteristics	Study variables		Techniques and measurement	Summary of findings
				Physical activity	Depression		
ACUTE EFFECT OF PHYSICAL ACTIVITY							
Laske, C, et al. (2010). Exercise-induced normalization of decreased BDNF serum concentration in elderly women with remitted major depression.	Experimental study with cases and controls	To assess the association between acute exercise and serum BDNF in MDD female patients.	N = 35 MDD cases (21 with SSRI treatment during prior 3 months) and 20 controls. Only women, >50 years old.	An incremental exercise test (on a treadmill) was conducted, starting with a speed of 3 km/h and inclination of 0%, increasing linearly every 3 minutes. The test concluded when the participant was physically unable to continue.	HDRS. Participants were considered cases when their score was ≥ 18.	ELISA (R&D Systems).	In cases, lower BDNF levels before exercise were found compared to controls ($p = 0.001$), although this difference was not statistically significant after exercise ($p = 0.233$). After acute exercise, greater BDNF level increases were observed in cases, compared to controls ($p = 0.046$). After 30 min of rest following exercise, serum BDNF decreased below baseline levels. In cases, an inverse correlation trend was found between baseline BDNF concentration and change after exercise ($r = -0.325$, $p = 0.056$), but not in controls ($r = -0.337$, $p = 0.146$).
Meyer, JD, et al. (2016). Relationships between serum BDNF and the antidepressant effect of acute exercise in depressed women.	Experimental study.	To evaluate the response of serum BDNF concentration to acute exercise in MDD female patients, comparing intensity levels. Relationship between antidepressant use, pre-exercise psychological health and BDNF.	N = 24 women (20 - 60 years old) with depression. Able to perform physical activity. Participants had no psychiatric treatment regimen, or with a stable regimen for 8 weeks prior to the study.	3 sessions of 30 minutes of exercise in a cycle ergometer at one of the prescribed intensities, including a 5-minute warm-up and a 5-minute cool-down. Sessions were separated by one week. A control session consisted of a rest session of the same time in the cycle ergometer.	MDD diagnosis was confirmed using MINI. Before exercise, 10 and 30 minutes after exercise, subjects completed the POMS rating scale.	ELISA (R&D Systems).	An acute improvement in depressive mood was found after exercise. Also, there was a significant increase of BDNF ($p = 0.006$), independent of the exercise intensity. Changes in BDNF levels did not correlate to changes in POMS scores (10 min nor 30 min post-exercise). There was a significant difference in post-exercise BDNF between participants taking medication and those not taking medication ($p = 0.015$), although no difference was observed in mood changes.
Kallies, G, et al. (2019). Serum brain-derived neurotrophic factor (BDNF) at rest and after acute aerobic exercise in major depressive disorder.	Experimental study	To evaluate the effect of incremental aerobic exercise on BDNF level in MDD patients, considering changes in plasma volume and platelets count.	N = 30 MDD cases (7 with a single depressive episode) (18 - 65 years old). All were sedentary (<90 minutes per week).	An incremental exercise test on a cycle ergometer was conducted, starting at 25 W and increasing 25 W every 2 minutes. The test concluded when the participant was physically unable to continue.	MDD diagnosis was assessed for a larger project. Severity of depressive symptoms was assessed using BDI.	ELISA (Promega Inc.). Haemogram including platelet count (Sysmex XE-2100, Sysmex Corp.)	A statistically significant BDNF increase after exercise was found ($p < 0.001$) adjusting for change in plasma volume and platelet count. The interaction between change in BDNF and platelet count ($p = 0.001$) suggested a greater increase in BDNF in participants with lower number of platelets.
Ross, RE, et al. (2019). High-Intensity Aerobic Exercise Acutely Increases Brain-derived Neurotrophic Factor.	Experimental study with cases and controls.	To evaluate the effect of different intensities of aerobic exercise on the level of BDNF and serum cortisol in depressed patients and controls.	N = 13 MDD cases and 13 controls (18 - 50 years old). Participants were able and safe to perform physical activity.	3 sessions of 15 minutes. Different procedures were performed by three groups: low intensity cycling (35% of heart rate reserve), high intensity cycling (70% of heart rate reserve), or sitting (control condition).	MDD diagnosis was confirmed using MINI, and severity of depressive symptoms using MADRS (considering a score > 10 for cases).	ELISA (R&D Systems).	No effect of the group was observed when post-exercise BDNF response was assessed ($p = 0.73$). BDNF significantly increased immediately after high intensity exercise compared to low ($p = 0.003$) and control condition ($p = 0.027$), whereas no differences were found between low intensity and control condition. Comparing pre- and immediately after exercise, BDNF significantly increased in the high ($p < 0.001$) and low intensity ($p = 0.019$) conditions. 15 min after exercise (or any time further) BDNF was not significantly different from the baseline levels in any condition.
CHRONIC EFFECT OF PHYSICAL ACTIVITY							
Toups, MSP, et al. (2011). Effects of serum Brain Derived Neurotrophic Factor on exercise augmentation treatment of depression.	Experimental study. Randomized controlled trial.	To analyse the changes in BDNF levels after exercise training in MDD patients. To assess the relationship between baseline BDNF levels and treatment response.	N = 126 adults (18–70 years old). Only 70 with blood samples completed the study. All participants were being treated with SSRIs for 2 to 6 months (with partial or no response).	Participants were divided into two groups: high energy expenditure and low energy expenditure. The intervention consisted of 12 weeks of education and personalised training with an exercise regimen appropriate to the dose.	Participants were partial or non-responders to the prior SSRI treatment (in case their HDRS score was ≥ 14), with MDD diagnosis confirmation with the SCID. The severity of depressive symptoms was assessed using IDS-C and HDRS.	ELISA (R&D Systems).	Baseline serum BDNF concentration did not significantly vary between before and after completing the intervention, and was not correlated with energy expenditure ($p = 0.15$) or improvement in IDS-C score ($p = 0.89$). Subjects with higher baseline BDNF concentration reported faster improvements in their IDS-C score with statistical significance ($p = 0.003$).

Pereira, DS, et al. (2013). Effects of Physical Exercise on Plasma Levels of Brain- Derived Neurotrophic Factor and Depressive Symptoms in Elderly Women – A Randomized Clinical Trial.	Experimental study. Randomized controlled trial.	To assess the effect of 2 standardised exercise programs (muscular strength exercises (SE) and aerobic exercises (AE)) on BDNF plasma levels and depressive symptoms in elderly women.	N = 451 elderly sedentary women (65–89 years old).	Participants were divided into two groups, with two exercise programs: SE and AE. The protocols include 3 one-hour sessions a week, for a total of 30 supervised sessions in 10 weeks.	Geriatric Depression Scale (GDS).	ELISA (R&D Systems)	Statistically significant differences were reported in plasma BDNF levels between SE and AE groups ($p = 0.009$). Pre- and post-intervention differences in BDNF levels were only observed within the SE group ($p = 0.008$). The difference between pre- and post-intervention in GDS score was significantly different in both exercise protocols ($p = 0.001$).
Krogh, J, et al. (2014). The effect of exercise on hippocampal volume and neurotrophines in patients with major depression-A randomized clinical trial	Experimental study. Randomized controlled trial.	To evaluate the changes in hippocampal volume and serum levels of neurotrophins (BDNF, VEGF and IGF-1) after an exercise intervention, compared to a control condition, in patients with depression.	N = 79 sedentary MDD patients (38 in control condition, 41 in aerobic exercise condition). Inclusion criteria: 18 to 60 years old, <1h of exercise per week, no current psychotherapeutic or antidepressant treatment in the past 2 months.	Two groups were differenciated: (1) Experimental group: exercise bikes at 80% of their maximum heart rate. (2) Control group: stretching, low impact exercise. Interventions consisted of 3 sessions/week for 3 months.	The Danish version of the MINI was used for DSM-IV diagnosis, and HDRS score > 12 was the threshold to consider cases with MDD.	Magnetic resonance imaging (MRI) (Siemens 3.0T). ELISA (R&D Systems).	No differences in BDNF were found between groups, in any of the reported measures, nor in any group during the intervention. In both conditions, average maximum oxygen consumption increased after the intervention ($p = 0.03$). The volume of the hippocampus and the amount of serum BDNF did not vary between groups. Average attendance was 1 weekly session.
Schuch FB, et al. (2014). The effects of exercise on oxidative stress (TBARS) and BDNF in severely depressed inpatients.	Experimental study. Randomized controlled trial.	The evaluate the effects of adding exercise to the treatment of patients with depression on the serum levels of BDNF.	N = 26 MDD patients (15 in exercise plus treatment as usual condition, 11 in control group). Participants were 18 to 60 years old.	The intervention consisted of supervised aerobic exercise 3 times a week (16.5 kcal/kg/week, with a median of 9 sessions (3 weeks)). The modality and intensity was free to choose until completing the indicated kcal/kg. All the sessions were composed of: warm-up, main part and cool-down.	MDD diagnosis was ascertained using the MINI, with DSM-IV criteria. Considered cases with score ≥ 25 in HDRS.	ELISA (Chemicon, USA).	A significant association was found between time and BDNF concentrations ($p < 0.001$), whereas they did not find an interaction between group and time ($p = 0.13$), for BDNF levels. Serum BDNF levels were not modified after the addition of exercise to the usual treatment.
Salehi, I, et al. (2016). Electroconvulsive therapy (ECT) and aerobic exercise training (AET) increased plasma BDNF and ameliorated depressive symptoms in patients suffering from major depressive disorder.	Experimental study. Randomized controlled trial.	To analyse the differential effects of electroconvulsive therapy (ECT), aerobic exercise (AET) and their combination in depressive symptoms and in plasma BDNF, in MDD patients.	N = 60 MDD patients from 25 to 40 years old. Participants were randomly assigned in three groups: ECT, ECT+AET and AET. All patients maintained their standard SSRI medications.	In the AET condition sessions consisted of 45 minutes of treadmill (3 sessions per week during 4 weeks).	MDD diagnosis was confirmed using DSM-IV criteria, score ≥30 in BDI, score ≥ 25 in HDRS.	ELISA (R&D Systems).	Scores in BDI and HDRS significantly decreased between pre- and post-intervention in the three conditions, although not varying between groups. Plasma levels of BDNF increased with time in the three conditions. A significant association between the treatment condition and remission observed ($p < 0.001$) was found, with the highest remission rate being observed in the combined treatment, and the lowest in the ECT condition.
Kerling, A, et al. (2017). Exercise increases serum brain-derived neurotrophic factor in patients with major depressive disorder.	Experimental study. Randomized controlled trial	To assess the effect of an exercise intervention on serum BDNF levels in MDD patients, added to their usual treatment.	N = 42 MDD patients from 18 to 60 years old. 22 were assigned to the exercise group, and 20 to the control group. Their medication did not change during the intervention.	The intervention consisted of 3 weekly 45-min training sessions of moderate intensity for a total of 6 weeks. These sessions were 25 min on a cycle ergometer and 20 min on machines of their choice. The control exercise consisted of walking, ball games and stretching for 20 min.	MDD diagnosis was confirmed using the SCID. MADRS was employed to assess the severity of depressive symptoms.	Immunoassay (Quantikine HS R&D Systems)	BDNF increased in the exercise condition and decreased in controls, finding a significant time x group effect regarding serum BDNF concentrations ($p = 0.03$). No significant relationship was observed between relative change of MADRS and BDNF concentrations, before and after the intervention.

Rahman, MS, et al. (2017). BDNF Val66Met and childhood adversity on response to physical exercise and internet- based cognitive behavioural therapy in depressed Swedish adults.	Experimental study. Randomized controlled trial.	To analyse the association between serum mature-BDNF or pro-BDNF levels and the response to treatment with physical exercise.	N = 547 Swedish adults with mild-to-moderate depression.	Three groups participated in three interventions: physical exercise, ICBT, or treatment as usual, during a 12-weeks intervention. Among the participants following the physical activity intervention, 3 intensities were randomly assigned: low, medium and high. Assistance to 3 weekly 60-min sessions in gyms was recommended.	Inclusion criteria were having a PHQ-9 score ≥ 10. Depressive symptoms were assessed using MADRS and evaluating social attachment ability (5 questions from ISSI subscale)	Mature BDNF and proBDNF quantification (ELISA).	Met allele carriers had higher baseline concentrations of mature BDNF than Val/Val participants in the total sample (p = 0.019) and in antidepressant users (p = 0.04). No differences were found in antidepressant non-users, nor comparing between baseline and post-intervention levels.
Dinoff, A, et al. (2018). The effect of exercise on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF) in major depressive disorder: A meta-analysis.	Systematic review and meta-analysis	To review and meta-analyse the effect of exercise intervention on BDNF concentrations in MDD patients, and to assess the role of sex, age, intensity or duration of exercise.	6 original articles included, with a total of 176 participants with MDD.	Inclusion criteria included: chronic exercise intervention (prolonged over various weeks), with intensity ≥50% of maximum oxygen uptake.	MDD cases included should have been diagnosed following DSM criteria.	-	Six studies that met the inclusion criteria. BDNF concentrations were not significantly higher (neither in serum nor in plasma) after the chronic exercise intervention (p = 0.09) in the meta-analysis.
Kurebayashi, Y & Otaki, J (2018). Does physical exercise increase brain-derived neurotrophic factor in major depressive disorder? A meta-analysis.	Systematic review and meta-analysis	To review and meta-analyse the effect of physical exercise on BDNF levels in MDD patients.	5 original articles included, with a total of 199 MDD patients with severe symptoms.	Inclusion criteria included: chronic aerobic exercise intervention.	All subjects were MDD patients. Depressive symptoms were assessed by BDI or HDRS.	-	The meta-analysis showed no significant effect of physical exercise on BDNF levels (p = 0.75).

Abbreviations:
SSRI: Selective serotonin reuptake inhibitors
HDRS: Hamilton Depression Rating Scale
MINI: Mini-International Neuropsychiatric Interview
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders Version Four.
POMS: Profile of Moods States
BDI: Beck Depression Inventory
SCID: Structured Clinical Interview for DSM-IV Axis I Disorders
IDS-C: Clinician rated version of Inventory of Depression Symptomatology

4. Discussion

Here, we have performed a systematic review of the scientific literature about the role of BDNF (*BDNF* Val66Met polymorphism and/or protein level) in the potential relationship between physical exercise and the prevalence or incidence of depression, or the improvement of depressive symptoms.

The role of BDNF Val66Met polymorphism
Regarding the *BDNF* Val66Met polymorphism, a total of 6 studies were selected, which mainly included advanced aged people.

The results of four out of the six studies included in this section suggested a greater impact of physical activity on depression depending on the *BDNF* Val66Met genotype. In particular, physically active participants or individuals following an intervention with physical activity that carried the Met allele showed lower risk of depression and greater effects on the improvement of depressive symptoms compared to Val/Val homozygous individuals. Opposite to these findings, the other two studies included in this section did not find a statistically significant interaction between the *BDNF* polymorphism, physical activity and depression, although one of the works argued an insufficient sample that might have hindered obtaining significant results (Gujral et al., 2014).

It is worth mentioning that the six studies included in this section were not easily comparable due to the heterogeneity found in different aspects between them. First, there were two longitudinal studies (Dotson et al., 2016; Rahman et al., 2017), whereas four of them were cross-sectional studies. Second, the inclusion criteria and sample size also varied, (e.g., two of the articles included adults of all ages (Rahman et al., 2017; Zarza-Rebollo et al., 2022), one study included middle-aged adults (Gujral et al., 2014), and three studies analysed an older sample (Dotson et al., 2016; Haslacher et al., 2015; Pitts et al., 2020). Within the latter group, one sample consisted of veteran soldiers (Pitts et al., 2020), another compared athletes and controls (Haslacher et al., 2015), and one study included advanced aged adults from the general population (Dotson et al., 2016). Furthermore, they differed on how the included variables were considered. In this sense, the variable “depression” or “depressive symptoms” was assessed using different methods and scales, e.g., CES-D (Dotson et al., 2016; Gujral et al., 2014), BDI and GDS (Haslacher et al., 2015), MADRS (Rahman et al., 2017), PHQ-2 (Pitts et al., 2020; Zarza-Rebollo et al., 2022) or following DSM-IV criteria using the MINI interview (Zarza-Rebollo et al., 2022). Moreover, there were also differences on how the variable “physical activity” was measured. Whereas only one cross-sectional study compared athletes with a control group (Haslacher et al., 2015), the remaining three cross-sectional studies compare self-evaluated physical activity, using different parameters and scales (Gujral et al., 2014; Pitts et al., 2020; Zarza-Rebollo et al., 2022). These differences were also apparent in the interventions performed in the two longitudinal studies (Dotson et al., 2016; Rahman et al., 2017). In addition, Rahman and colleagues considered childhood adversity as an additional

parameter suggesting that this event may play an important role in the response to the physical exercise treatment in depressed patients (Rahman et al., 2017).

Regarding the effect of the *BDNF* Val66Met polymorphism, the association between the Met allele and the risk of depression has largely been addressed. The first study assessing the effect of this polymorphism in humans found a deficient intracellular trafficking and secretion derived from the Met allele, potentially causing impaired episodic memory and reduced hippocampal volume (Egan et al., 2003). Subsequent studies achieved diverse findings, both in agreement (Miyajima et al., 2008; L. Wang et al., 2012), and in disagreement with these prior results (Benjamin et al., 2010; Harris et al., 2006). Therefore, the Val66Met polymorphism has become a contentious study field without a conclusive role established on the susceptibility that conferred to different aspects of cognition and psychiatric disorders (Notaras et al., 2015). Focusing on depression, a meta-analysis of 14 studies did not find any association between this polymorphism and depression in the total sample, neither after stratifying according to ethnicity (Verhagen et al., 2010). However, this meta-analysis reported a significant risk effect of the Met allele in men after sex stratification. Given the conflicting results regarding the association between the *BDNF* Val66Met polymorphism and depression, the observations obtained from the present systematic review could be explained by an interaction effect between this polymorphism and physical activity, although the direction of this effect remains unclear.

It would be of great interest to investigate whether different types of physical activity lead to this effect or whether particular benefits occur with different types of interventions. Including sex as a relevant factor for the interaction effect would also provide more conclusive results. Future research should also clearly state the characteristics of the interventions, as a lack of a detailed description on the procedure followed was a generalised weak point. Overall, further research into the type of physical activity may help to understand the physiological mechanisms that link the improvement observed in active participants carrying the Met allele and the association with depression.

The role of BDNF protein levels

Acute exercise intervention

Four articles assessing the effect of an acute exercise intervention on the relationship between BDNF levels, depression and physical activity were included in this section. The results of the four studies pointed to a significant change of BDNF levels before and after physical activity in depressed patients, although two of the studies found that these concentrations were restored after a short period of time (Laske et al., 2010; Ross et al., 2019). This finding is consistent with the main results from a recent meta-analysis evaluating the acute effect of physical activity on BDNF concentration (Szuhany et al., 2015). Here, despite the heterogeneity of the meta-analysed samples, the authors observed an increase in BDNF levels following a single bout of physical activity. Regarding this increase in BDNF concentrations, it is remarkable that Kallies and colleagues (2019) only reported this effect

when an adjustment with changes in platelets count and plasma volume was considered (Kallies et al., 2019). Interestingly, in the two studies comparing cases with depression and controls, no differences were found depending on this condition (Kallies et al., 2019; Ross et al., 2019). Moreover, three of the studies did not assess the effect of acute exercise or BDNF levels alterations on depressive symptoms (Kallies et al., 2019; Laske et al., 2010; Ross et al., 2019). Only one study determined an improvement in depressive mood after exercise, although the changes in these symptoms were not correlated with changes in BDNF levels (Meyer et al., 2016).

Although the sample sizes of these studies were similar, several sources of heterogeneity were found between them. As an example, concerning the study cohorts, one of the studies included exclusively elderly women (Laske et al., 2010), whereas another study only considered middle-aged women (Meyer et al., 2016). This fact hinders the comparison with the studies by Kallies *et al.* (2019) and Ross *et al.* (2019) that included samples of middle-aged men and women (Kallies et al., 2019; Ross et al., 2019). Moreover, two of the four studies exclusively included patients with depression, thus case-control comparison was not assessed (Kallies et al., 2019; Meyer et al., 2016). The structure of the interventions with exercise also differed between the studies: two studies performed incremental exercise tests -although they differed in logistic aspects- (Kallies et al., 2019; Laske et al., 2010), whereas the other two works reported different sessions of physical activity at certain defined intensities (Meyer et al., 2016; Ross et al., 2019). Thus, future studies considering a more standardised approach would ease our current understanding of the observed effect.

Chronic exercise intervention

Seven original works performing a prolonged exercise intervention and evaluating the relationship between BDNF levels, depression and chronic physical activity were included. Similar to what was described in the previous sections, we found a high heterogeneity in different aspects that made it impossible to meta-analyse the studies.

We have included in this section two systematic reviews with meta-analyses, and seven original articles. Among the original articles, there were two studies including a sample from the general population, which did not include MDD cases but evaluated depressive symptoms instead (Pereira et al., 2013; Rahman et al., 2017). In contrast, the remaining five articles included cases with MDD diagnosis. These five studies were randomised controlled trials, and their samples were entirely composed of depression cases. Consequently, due to the complexity of this approach, sample sizes were limited, and smaller than the observed in the previous section.

The effect of the chronic exercise intervention on depressive symptoms, as a variable, would be a key outcome to be considered. Unfortunately, the effect of the intervention on this variable was only reported in three studies. Of note, the three of them found a positive effect of the intervention, decreasing depressive symptomatology (Kerling et al., 2017; Salehi et al., 2016; Toups et al., 2011). These three studies did not associate this improvement to

differences in BDNF levels, suggesting that the improvements in depression observed were due to independent mechanisms other than BDNF levels. Despite that, the effect of single bouts of physical activity on the increase of BDNF levels was consistent among the four studies analysed in the *Acute exercise intervention* section (Kallies et al., 2019; Laske et al., 2010; Meyer et al., 2016; Ross et al., 2019). Therefore, given this well-established link, which is in agreement with prior literature (Szuhany et al., 2015), it could be hypothesised that the long-term improvement of exercise on depressive symptoms could be, at least, partly mediated by the effect of a repeated exposure to acute increases in BDNF levels (Meyer et al., 2016). In this regard, the well-established role of BDNF on neuroplasticity and neurogenesis (Costa et al., 2022; Leal et al., 2017), the effect of physical activity on this processes (Erickson et al., 2011), and the growing evidence of the potential implication of neuroplasticity and neurotrophic factors in depression (Liu et al., 2017) would support the potential interaction between depression, physical activity and BDNF. Other mechanisms, such as the anti-inflammatory effect that physical activity exerts in the human body could also be involved (Gleeson et al., 2011; Rethorst et al., 2013).

From the interventions, only a randomised controlled trial performed on the general population obtained both improvements in depressive symptoms and increased BDNF levels following the intervention (Pereira et al., 2013). However, this result was only observed in the strength exercise branch, and was not observed in the aerobic exercise group, in opposition to the results obtained in a large meta-analysis (Knaepen et al., 2010).

It is important to note that the measurement of peripheral BDNF concentrations may not be the proper strategy for detecting changes and availability of BDNF in the brain, according to previous research (Elfving et al., 2010) (although there are studies finding a positive correlation between both parameters (Klein et al., 2011)). In this regard, it should be considered that a significant variation has been observed among different commercial assays for the measurement of BDNF levels (Polacchini et al., 2015). Therefore the reliability of the comparisons between studies could be jeopardised. Remarkably, different commercial assays are able to recognise both pro-BDNF and mature BDNF, or have a higher specificity for the mature form of BDNF. Considering that the physiological effects of both forms are broadly opposite, the fraction measured by each kit would be of critical interest (B. Lu et al., 2005). Besides, it would be important to consider the effect that age has on BDNF levels, since a decrease in plasma and serum concentrations of this protein throughout life has been reported (Erickson et al., 2010; Lommatzsch et al., 2005). Therefore, the wide age ranges included in some of the included studies could be a confounding factor and a probable cause for the heterogeneity observed in the results. BDNF levels are also likely to vary between genders (Lommatzsch et al., 2005), as well as to have a lower increase in women after physical activity (Szuhany et al., 2015), and to fluctuate in women even within the course of a day (Pluchino et al., 2009).

Antidepressants have also been observed to increase peripheral BDNF levels. Thus, how they were considered across the analysed cohorts could be of relevance for understanding the

results (Sheldrick et al., 2017; Zhou et al., 2017). Whereas Krogh *et al.* (2011) excluded participants with recent antidepressant treatment, in Salehi *et al.* (2016) all patients were treated, and in the remaining studies the use of them was allowed (Krogh et al., 2014; Salehi et al., 2016). Apart from the heterogeneity provided by their use, it would be logical to consider that they might be moderating or masking the effect of physical activity on BDNF concentrations.

As an additional source of heterogeneity, important differences were also observed in the physical activity interventions performed across studies, as well as in the procedure followed for the control branch. In this regard, the use of light-intensity physical activity for the control branch in sedentary MDD cases -as in Toups *et al.* (2011) or Krogh *et al.* (2014)-, might lead to underestimating the effect of the intervention, as it has been observed with other placebo interventions (Josefsson et al., 2014; Krogh et al., 2014; Toups et al., 2011).

Finally, it is necessary to highlight the differences found on the management of depression diagnosis since clinician-rated instruments and self-assessed questionnaires have been demonstrated to not be equivalent (Cuijpers et al., 2010). Whereas most of the studies followed DSM criteria using MINI or clinically validated interviews, two studies employed self-assessed questionnaires (Pereira et al., 2013; Rahman et al., 2017).

As a final remark, we included two systematic reviews and meta-analyses, both assessing chronic exercise interventions. The novelty of these studies, together with its publication in the same year, evidences both the growing interest in this research field and the paucity of studies analysing the effect of physical activity on depression and BDNF. The conclusions of both systematic reviews and meta-analyses are in line with the limitations stated in the previous sections, highlighting the potential bias caused by antidepressants, the variability of the interventions, sex and age of the participants, along the limited sample sizes.

General considerations

To the best of our knowledge, this is the first systematic review covering the analysis of the relationship between BDNF (Val66Met polymorphism and BDNF protein levels), depression, and physical activity. The study of the Val66Met genotype on this relationship allowed a more integrative perspective, providing a comprehensive vision of the state of the art in this research field. This first approach pointed towards a greater antidepressant effect of physical activity in Met allele carriers in four out of the six studies. On the contrary, we observed a considerable disparity in the results regarding the existence of a relationship between BDNF levels, depression and physical activity, with an important heterogeneity across studies in relevant parameters (e.g., type of intervention, the use of a control group in physical activity interventions, sample size, and the scales for assessing depression). The heterogeneity observed across studies highlights that standardisation towards these different methodological aspects should be considered in future research in order to obtain more comparable and conclusive results. Other important recommendations include considering

the potential confounder variables, such as the use of antidepressant medication, sex and age of the cohort, or the BDNF measurement assays.

One of the most robust results was the acute increase of BDNF levels following a single bout of physical activity, described in the *Acute exercise intervention* section. Together with the observed trend found in most of the studies assessing the Val66Met polymorphism, it could be hypothesised that the greater benefit found in Met allele carriers from physical activity could be related to this acute increase of BDNF. Therefore, physical activity could contribute to mitigate the previously reported detrimental effect of the Met allele on cognition and depressive symptoms.

Conclusions

A considerable number of studies addressing the potential role of BDNF -both the genetic variability of the Val66Met polymorphism and the protein levels- in the interaction between depression and physical exercise were found, highlighting the current interest of this research field. There is cumulative evidence supporting the involvement of BDNF in the molecular mechanisms behind the association of physical exercise and the improvement of depression. However, this systematic review pointed to a high heterogeneity between studies in important methodological aspects, and potential sources of bias, that must be considered in future research in order to obtain more robust conclusions. Overall, we still consider the recommendation to practise physical activity for the treatment of depression and depressive symptoms to be evident and effective. However, a better understanding of the different variables of exercise (type of activity, duration or intensity), and their role in the improvement of depression would be necessary, as well as a deeper knowledge of the physiopathology of depression, to identify the optimal way to properly implement this therapeutic strategy.

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RESULTS

CHAPTER III
Study 5

CHAPTER III: Genetics of the relationship between depression and physical health: Depression and physical activity.

Study 5: Interaction effect between physical activity and the BDNF Val66Met Polymorphism on depression in women from the PISMA-ep study.

1. Introduction

Depression is a major public health problem, affecting more than 264 million people worldwide, with a higher prevalence in women. It is a leading cause of disease burden and years of disability [1,2], and is also associated with excess mortality [3]. Even though novel pharmacological choices have arisen in the past decades, there still remains a lack of efficiency in the pharmacological treatment. In this aspect, there is a rate of non-response to the first election of up to two thirds of the patients, and between 15-33% in multiple interventions [4]. Therefore, the use of antidepressants has prompted a long-standing debate regarding their effectiveness compared to placebo [5,6]. In an extensive meta-analysis of published and unpublished clinical trials, a small drug-placebo difference directly related to the initial severity of depression was reported [7].

Consequently, novel approaches, such as physical activity, have emerged as potential therapeutic strategies, with a promising effect both decreasing the symptomatology of depression [7,8], and in its prevention [9–11]. Remarkably, among other variables, it is plausible that sex may have a differential role in the impact of physical activity on depression, according to different studies. In this regard, it has been reported that physical activity leads to more extensive benefits on executive processes in women than in men in a healthy population [12]. These differences remain unclear when a population with depression is considered. Some studies have reported greater differences in the relationship between physical activity and the improvement of depressive symptoms in men [13,14], whereas others have reported similar results in women [15–17]. Nonetheless, recent studies with extensive samples and meta-analyses did not find that these differences between sexes were statistically significant [18–20].

One of the mechanisms suggested to be involved in the relationship between depression and the practice of physical activity is the brain-derived neurotrophic factor (*BDNF*), a neurotrophin related to key brain processes at molecular and functional stages, such as growth and survival of neurons [21], learning and memory [22]. The practice of physical activity has been associated with an increase of BDNF and genetic expression profiles promoting brain plasticity in animal models [23], with similar results in humans (for a review, see [24]). In this respect, current literature points towards a transient increase in peripheral BDNF concentrations after acute aerobic exercise, but not after strength exercise [25], and also an increase in resting concentrations of this neurotrophin after interventions with aerobic - but not resistance- physical activity [26]. Nonetheless, a recent meta-analysis of exercise

interventions in depression was not able to extract any conclusive results from the available literature, highlighting the diversity of the studies included in the systematic review [27].

The Val66Met polymorphism, also known as rs6265, is a functional polymorphism within the promoter of the *BDNF* gene. The Met allele of this polymorphism has been associated with impaired regulation of secretion and intracellular trafficking of the BDNF protein in hippocampal neurons [28]. This risk allele has also been associated with modifying the protein function of BDNF in humans [29,30], and although it has been extensively studied in relation to depression and physical activity, the results remain inconclusive. The most recent meta-analyses investigating the association between depression and the Val66Met polymorphism found that this polymorphism does not confer risk for depression [31–33]. The effect of physical activity on serum BDNF has also been reported to depend on the Val66Met genotype, being that the difference in serum BDNF is significantly higher after physical activity exclusively in Val/Val participants [34,35]. However, this association could not be replicated in a subsequent study [36]. A more recent systematic review included studies assessing the association of this genotype with the effect of physical activity in diverse cognitive domains, concluding that the available evidence is too limited to draw conclusions [37]. Moreover, the association of this polymorphism as a mediator of the effect of physical activity on depressive symptoms has been assessed in several studies, also showing conflicting results. In a cross-sectional study comparing genetic susceptibility for depression between athletes and non-athletes, the Val/Val genotype was associated with a higher risk of reporting depressive symptoms only in non-athletes [38]. A recent observational study shows that physical activity moderates the association between depression and cognitive function, obtaining better results in different tasks in Met allele carriers [39]. In contrast, another observational study did not find a relationship between the Val66Met genotype, physical activity and depressive symptoms [16]. Studies that included an intervention with physical activity in patients with depression, have reported a greater decrease in depressive symptoms after the intervention, only in men with the Met allele of the Val66Met polymorphism [40], or in Met allele carriers who did not inform exposure to childhood adversity [41]. In adolescent women, a protective effect against depressive symptoms was found with higher levels of physical activity only in Met allele carriers [42]. Given the inconclusive results shown in the literature, we aim to investigate the involvement of the *BDNF* Val66Met polymorphism with depression and physical activity in a large sample of community-dwelling adults.

2. Methods

Study design

The PISMA-ep is a cross-sectional epidemiological study performed in a representative cohort of community-dwelling adults aiming to analyse the prevalence of psychiatric disorders and their correlates in Andalusia (south of Spain). The three main objectives of the PISMA-ep were: 1) to estimate the prevalence of common mental disorders in Andalusia, 2) to explore the associations existing between social, psychological and genetic

factors with mental disorders, and 3) to gather data from an extensive cohort that could be used as the basis for further prospective studies. A more detailed description of the methodology and procedure of this study has been published elsewhere [43].

Sample

Randomly selected adults aged 18-75 years old living in all 8 provinces of Andalusia for at least a year were asked to participate in the PSIMA-ep study. We undertook a multistage sampling using different standard stratification levels utilizing a door-knocking approach. We excluded those individuals with illness that precluded the completion of the interview, not speaking Spanish fluently, suffering from severe cognitive impairment or intellectual disability, and usually residing in an institution.

Measures

Neuropsychiatric measures: The DSM-IV diagnosis of major depression was ascertained using the Spanish version of the Mini-International Neuropsychiatric Interview (MINI) [44,45]. The MINI is a brief diagnostic structured interview that provides Axis I DSM-IV and ICD-10 compatible diagnoses for 16 mental disorders, including major depression. The MINI has obtained satisfactory psychometric properties, with good rates of validity and reliability on community-based populations [46,47]. This interview was conducted by a team of fully trained psychologists.

Anthropometric measures: For each participant, self-reported height and weight were obtained to calculate their body mass index (BMI) using the formula: weight in kilograms divided by height in meters squared (kg/m^2). Participants were grouped into four categories, following WHO criteria [48]: Underweight (BMI <18.5 kg/m^2), Normal weight (BMI 18.5–24.99 kg/m^2), Overweight (BMI 25.0–29.99 kg/m^2) and Obesity (BMI ≥ 30 kg/m^2).

Physical activity: This information was gathered from a questionnaire including 3 questions about whether the participant practiced any physical activity, the number of hours per week of physical activity and the intensity of the activity. The intensity was classified based on the Metabolic Equivalents of Task or METs (2 METs = two times the amount of oxygen consumed at rest) as light (< 3 METs), moderate (3 – 5 METs) or vigorous (≥ 6 METs).

Genotyping analysis: A biological sample was obtained from each participant with an Oragene® saliva DNA (OG-500; DNA Genotek Inc.) collection kit. The Oragene® saliva collection kit protocol was used for DNA extraction. The original DNA samples were prepared to be stored at −80°C in matrix plaque format. DNA quantification was measured using the Infinite® M200 PRO Multimode Microplate Reader (Tecan, Research Triangle Park, NC). Genotyping of the *BDNF* Val66Met polymorphism was assessed using TaqMan® StepOnePlusTM Real-Time PCR System (Applied Biosystems, Foster City, California, USA) following the manufacturer's instructions. The system software was used to analyse raw data.

Statistical analyses

All statistical analyses were performed using R (version 4.0.3) [49]. The R package 'HardyWeinberg' was used to test Hardy-Weinberg equilibrium (HWE) and distribution of genotypes, both in the entire sample and in depression cases and controls, using Pearson's Chi-squared tests [50,51].

We performed descriptive exploratory analyses to survey how dependent and independent variables were distributed and then explored univariable associations, considering parametric or non-parametric significance tests when required.

Logistic regression models were performed to explore the associations between: (1) physical activity variables (a. whether the participant practiced physical activity, b. number of hours of physical activity and c. intensity of the activity) and depression and (2) the Val66Met genotype and depression. A dominant genetic model was assumed, due to the limited sample size. Finally, we assessed the interaction between the genetic (Val66Met genotype) and environmental (the practice of physical activity) variables, using multivariate logistic regression models. We estimated the probabilities for depression by combining the Val66Met genotype (Val/Val homozygous vs Met allele carrier) and physical activity (binomial, number of hours or intensity). All the association and interaction analyses were performed both crudely and including sex, age and BMI as covariates.

3. Results

Description of the sample

From the 4507 PISMA-ep total sample, 4286 (95.1%) participants accepted to provide a saliva sample for the genetic studies. From those, 3194 (74.52 %) were genotyped for the *BDNF* Val66Met polymorphism. A total of 71 (2.22%) participants with BMI under 18.5 were excluded from the analyses. The final sample consisted of 3123 community-based adults, of which 209 were cases with depression (6.69%) (Table 11).

The characteristics of the sample including the frequencies of the independent variables analysed, both genotypic (Val66Met polymorphism) and phenotypic (practice of physical activity, number of hours and intensity) have been detailed in Table 11.

Table 11. Summary of frequencies of independent variables.
* Mean (s.d.) of reported hours of physical activity was calculated excluding participants reporting no physical activity.

	Total sample (3123)	Women 1554 (49.76%)	Men 1569 (50.24%)
Mean age (s.d.)	43.18 (15.18)	43.76 (14.94)	42.61 (15.39)
Mean BMI (s.d.)	26.16 (4.50)	25.73 (4.97)	26.59 (3.93)
Diagnosis of depression	No 2914 (93.31%) Yes 209 (6.69%)	No 1406 (90.48%) Yes 148 (9.52%)	No 1508 (96.11%) Yes 61 (3.89%)
Val66Met genotype	ValVal 1947 (62.34%) ValMet 1044 (33.43%) MetMet 132 (4.23%)	ValVal 969 (62.36.%) ValMet 512 (32.95%) MetMet 73 (4.69%)	ValVal 978 (62.33%) ValMet 532 (33.91%) MetMet 59 (3.76%)
Met allele carrying	1176 (37.66%)	585 (37.64%)	591 (37.67%)
Physical activity	No 1260 (40.35%) Yes 1863 (59.65%)	No 676 (43.5%) Yes 878 (56.5%)	No 584 (37.22%) Yes 985 (62.78%)
Mean hours of physical activity (s.d.)*	9.73 (9.79)	9.62 (9.90)	9.83 (9.70)
Intensity of physical activity	No 1260 (40.35%) Light 624 (19.98%) Moderate 988 (31.64%) Vigorous 251 (8.04%)	No 676 (43.5%) Light 350 (22.52%) Moderate 452 (29.09%) Vigorous 76 (4.89%)	No 584 (37.22%) Light 274 (17.46%) Moderate 536 (34.16%) Vigorous 175 (11.15%)

The BDNF Val66Met polymorphism and Depression

There was no significant association between carrying the Met allele of the Val66Met polymorphism and depression, in the total sample in crude analyses nor after adjusting for sex, BMI and age (OR = 1.04, 95%CI = 0.77-1.39, P = 0.81). Besides, these results were not significant neither in women (OR = 1.05, 95%CI = 0.73-1.50, P = 0.78) nor in men (OR = 1.01, 95%CI = 0.59-1.70, P = 0.974) after adjusting for age and BMI. The frequencies of the Val66Met genotypes are detailed in Table 12.

Table 12. Associations between depression and genetic factors or physical activity variables. Statistically significant results are highlighted in bold.

Abbreviations: CI, confidence interval; OR, odds ratio; *P*, p-value; $\bar{x}$, mean; s.d., standard deviation.

	Total sample (3123)			***Women (1554)***			***Men (1569)***		
	Cases (209)	***Controls (2914)***	***Adjusted* OR (95% CI), P***	***Cases (148)***	***Controls (1406)***	***Adjusted** OR (95% CI), P***	***Cases (61)***	***Controls (1508)***	***Adjusted** OR (95% CI), P***
					Genotypes				
Val/Val	130 (62%)	1817 (62%)		92 (62%)	877 (62%)		38 (62%)	940 (62%)	
Val/Met	66 (32%)	978 (34%)	0.98 (0.71-1.33), 0.891	45 (31%)	467 (33%)	0.96 (0.65-1.40), 0.842	21 (35%)	511 (34%)	1.02 (0.58-1.75), 0.936
Met/Met	13 (6%)	119 (4%)	1.50 (0.78-2.69), 0.195	11 (7%)	62 (5%)	1.71 (0.81-3.32) 0.131	2 (3%)	57 (4%)	0.89 (0.14-3.02), 0.876
					Met allele carrying				
Val/Val	130 (62%)	1817 (62%)		92 (62%)	877 (62%)		38 (62%)	940 (62%)	
Met allele carriers	79 (38%)	1097 (38%)	1.04 (0.77-1.39), 0.81	56 (38%)	529 (38%)	1.05 (0.73-1.50) 0.780	23 (38%)	568 (38%)	1.01 (0.59-1.70), 0.974
					Physical activity				
No	111 (53%)	1149 (39%)		83 (56%)	593 (42%)		28 (46%)	556 (37%)	
Yes	98 (47%)	1765 (61%)	**0.69 (0.51-0.92), 0.011**	65 (44%)	813 (58%)	**0.64 (0.45-0.91), 0.013**	33 (54%)	952 (63%)	0.74 (0.44-1.26), 0.26

	Total sample (3123)			*Women (1554)*			*Men (1569)*		
	Cases (209)	*Controls (2914)*	*Adjusted* OR (95% CI), P*	*Cases (148)*	*Controls (1406)*	*Adjusted** OR (95% CI), P*	*Cases (61)*	*Controls (1508)*	*Adjusted* OR (95% CI), P*
Intensity of physical activity									
No	111 (53%)	1149 (39%)		83 (56%)	593 (42%)		28 (46%)	556 (37%)	
Light	35 (17%)	589 (21%)	**0.59 (0.39-0.87), $9.68x10^{-3}$**	26 (17%)	324 (23%)	**0.57 (0.35-0.89), 0.018**	9 (15%)	265 (18%)	0.64 (0.28-1.32) 0.253
Moderate	52 (25%)	936 (32%)	0.73 (0.51-1.04), 0.083	32 (22%)	420 (30%)	0.65 (0.41-1.00), 0.053	20 (33%)	516 (34%)	0.85 (0.46-1.55), 0.607
Vigorous	11 (5%)	240 (8%)	0.89 (0.44-1.65), 0.734	7 (5%)	69 (5%)	1.23 (0.49-2.66), 0.630	4 (6%)	171 (11%)	0.57 (0.16-1.52), 0.307
Hours of physical activity									
Means expressed in hours	$\bar{x}$ = 4.72, s.d. = 8.16	$\bar{x}$ = 5.88, s.d. = 9.00	0.99 (0.97-1.01), 0.240	$\bar{x}$ = 4.60, s.d. = 8.88	$\bar{x}$ = 5.52, s.d. = 8.84	0.99 (0.97-1.01), 0.378	$\bar{x}$ = 5.00, s.d. = 6.12	$\bar{x}$ = 6.22, s.d. = 9.13	0.98 (0.95-1.01), 0.367

* Adjusted by age, BMI and sex.

** Adjusted by age and BMI.

Depression and physical activity

We found a statistically significant protective effect against depression in those participants reporting any physical activity, which remained significant after adjusting for covariates (age and BMI), both in the total sample (OR = 0.69, 95%CI = 0.51-0.92, P = 0.011) and in women (OR = 0.64, 95%CI = 0.45-0.91, P = 0.013), but not in men (OR = 0.74, 95%CI = 0.44-1.26, P = 0.26).

Regarding the intensity of physical activity, we found a protective effect for depression in those individuals who practiced light intensity (crude and after adjusting for sex, age and BMI) and a trend association for those showing moderate intensity. However, no association with vigorous physical activity was found (light intensity: OR = 0.59, 95%CI = 0.39-0.87, P = 9.68x10-3; moderate intensity: OR = 0.73, 95%CI = 0.51-1.04, P = 0.083; vigorous intensity: OR = 0.89, 95%CI = 0.44-1.65, P = 0.734). We observed similar results in women (light intensity: OR = 0.57, 95%CI = 0.35-0.89, P = 0.018; moderate intensity: OR =

0.65, 95%CI = 0.41-1.00, P = 0.053; vigorous intensity: OR = 1.23, 95%CI = 0.49-2.66, P = 0.630), but not in men (data shown in Table 12).

Finally, when we explored the effect of hours of physical activity on depression in the total sample, we did not find any association neither in crude analyses nor after adjusting for age, sex and BMI (OR = 0.99, 95%CI = 0.97-1.01, P = 0.240). These results remained non-significant when women and men were analysed separately (data available in Table 12).

The BDNF Val66Met polymorphism and physical activity

When exploring the association between Val66Met genotype and physical activity, we found a statistically significant association between practicing physical activity and carrying the Met allele. This association was found in the total sample (OR = 1.26, 95%CI = 1.08-1.47, P = 0.002) and in men (OR = 1.48, 95%CI = 1.19-1.84, P = 5.28x10-4), but not in women. The association between carrying the Met allele and the intensity of physical activity was also assessed, but we did not find any significant effect, except for moderate physical activity only in males (OR = 1.41, 95%CI = 1.14-1.76, P = 1.74x10-3). Additionally, we did not find a significant association between hours of physical activity and carrying the Met allele in the total sample, nor in women or men (data of associations between *BDNF* Val66Met polymorphism and physical activity is available under request).

Interaction between the BDNF Val66Met polymorphism, depression and hours of exercise

We found a significant interaction effect between the number of hours of physical activity and the risk for depression conferred by the Val66Met polymorphism (see Table 13 and Figure 16). Thereby, as the number of hours of exercise increases, the Met alleles carriers have a lower risk of depression compared to Val/Val homozygous. Those results remained statistically significant after adjusting for age, sex and BMI (OR = 0.95, 95%CI = 0.90-0.99, P = 0.027) and was strengthened in women, also after adjusting for age and BMI (OR = 0.93, 95%CI = 0.87-0.98, P = 0.019). However, this interaction effect was not found in men.

In contrast, when we assessed the binomial physical activity variable, or the intensity of physical activity, no significant interactions were found (see Table 13).

Table 13. Interaction between Met allele carriers and physical activity variables on risk of depression. Statistically significant results are highlighted in bold.
Abbreviations: CI, confidence interval; OR, odds ratio; *P*, p-value
* Adjusted by age, BMI and sex.
** Adjusted by age and BMI.

		OR (95%CI), *P*		
		Total sample*	**Women****	**Men****
Physical activity (yes/no) * carrying Met allele		0.86 (0.48-1.55), 0.616	0.91 (0.44-1.86), 0.786	0.78 (0.27-2.29), 0.641
Intensity of physical activity * carrying Met allele	None	Ref. 1	Ref. 1	Ref. 1
	Light	0.54 (0.22-1.26), 0.167	0.63 (0.22-1.67), 0.363	0.35 (0.05-1.89), 0.257
	Moderate	0.94 (0.46-1.90), 0.858	1.07 (0.43-2.60), 0.881	0.73 (0.21-2.46), 0.607
	Vigorous	2.00 (0.54-8.31), 0.308	1.15 (0.22-6.64), 0.870	4.34 (0.47-96.14), 0.234
Hours of physical activity * carrying Met allele		**0.95 (0.90-0.99), 0.027**	**0.93 (0.87-0.98), 0.019**	0.98 (0.91-1.05), 0.648

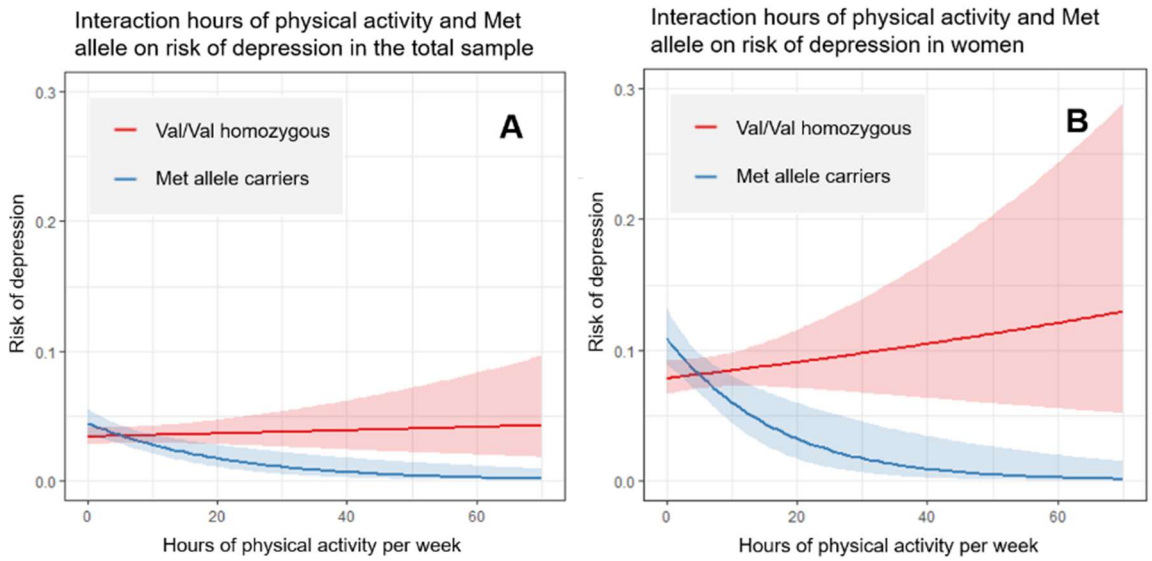

Figure 16. Graphic representation of the interaction between physical activity and the Met allele of the *BDNF* Val66Met polymorphism on the risk of depression, in (A) the total sample and (B) only in women. Results only in men were not statistically significant.

4. Discussion

The main aim of this study was to determine the potential role of the *BDNF* Val66Met polymorphism in the relationship between depression and physical activity in a large sample of community-based adults.

The BDNF Val66Met polymorphism and depression

We did not find a significant association between the *BDNF* Val66Met polymorphism and depression. This result is in line with the ones reported from previous studies, including extensive meta-analyses [31,33]. However, it has been suggested that more accurate assessments of the samples considering different parameters, such as, sex, age, ethnicity and gene-gene interactions would be required in order to unveil the peculiarities of this relationship [52].

Depression and physical activity

In relation to physical activity, we found an association with a lower prevalence of depression. These results remained significant even after considering classical parameters involved in depression, i.e., sex and age, and also BMI, a variable linked to depression and physical activity [53]. Interestingly, in the PISMA-ep cohort the prevalence of depression was significantly higher in those participants that reported no practice of physical activity versus those who practiced exercise. This effect was shown in the total sample and in women, but not in men. In this sense, multiple cross-sectional studies have shown significant associations between practicing physical activity and less prevalence of depression [54–56]. Besides, prospective studies have described a significant effect of physical activity preventing the onset of depression. A systematic review [9] and a recent meta-analysis of prospective studies [10] have led to the conclusion that the practice of physical activity is associated with lower odds of incident depression. In their systematic review of prospective studies performed in the general population, Mammen and Faulkner observed in 25 out of 30 studies that reporting physical activity at the beginning of the studies was inversely associated with incident depression at follow-up. Similarly, another recent meta-analysis reported an increased risk of developing depression when the sedentary behavior was higher [11]. These results support the effect observed in our cohort, which suggests a potential differential role of physical activity depending on the gender. Interestingly, four of these studies found this inverse association between practice of physical activity and incident depression at follow-up in women, but not in men [15,57–59]. Similarly, regarding intensity of physical activity, we found significant differences in the prevalence of depression between participants who practiced light-intensity physical activity and the sedentary ones, suggesting a protective effect of physical activity, in the total sample and in women. This result is highly interesting, since the implication of light-intensity physical activity in depression has been less studied than moderate- and vigorous-intensity physical activity [60]. Our findings have important implications for public health, since they empathise that practicing light-intensity physical activity seems to have an important effect on depression. Light-intensity physical activity is well-accepted in general and clinical populations and it has properties that make it more transferable for certain population groups like the elderly. In this sense, further research in this field should be encouraged.

Regarding high intensity physical activity, we have previously reported a significant association with a lower prevalence of depression in an extended sample form the PISMA-

ep cohort [61]. However, we could not find the same association here, possibly due to the smaller sample used in this study.

The BDNF Val66Met polymorphism and physical activity

When we assessed the association between the Val66Met polymorphism and the practice of physical activity, we found a higher proportion of physically active participants among Met carriers, compared to homozygous Val/Val. Although this polymorphism might be one among many genetic variants influencing a complex behavioral outcome like physical activity, it is worth highlighting this finding. There are few studies investigating this relationship, the majority reporting no association [62–65], whereas one study reported that Val/Val individuals experienced higher exertion than Met carriers, arguing that this could influence adherence to exercise [66]. Another study reported similar results regarding differences in intrinsic motivation during exercise in regular exercisers, finding greater intrinsic motivation in Met carriers compared to Val/Val participants [67]. This evidence makes us hypothesize that, if there is an effect, it could be mediated by intermediate factors.

Interaction between the BDNF Val66Met polymorphism, depression and hours of exercise

Interestingly, we found an interaction effect between the number of hours of physical activity and a decreased risk of depression, only in Met allele carriers. This interaction suggests that the practice of physical activity exerts a dose-dependent protective effect on the risk of depression, moderated by the *BDNF* Val66Met genotype. The practice of physical activity was significantly associated with less risk of depression in Met allele carriers but not in Val/Val homozygous. This effect was found in the total sample and was strengthened in women. These findings are similar to the results from previous research in healthy adolescent women, which reported an association between physical activity and the level of depressive symptoms, also moderated by the Val66Met genotype [42]. Similarly, they also found that women Met allele carriers who practiced physical activity were associated with lower depressive symptoms, compared to the Val/Val homozygous. This interaction could be explained under the hypothesis of the differential susceptibility [68]. According to this, individuals with genetic susceptibility for a certain condition would be more malleable, i.e., would benefit more from a favorable environment. In this sense, risk allele carriers (Met allele), under beneficial conditions (practicing physical activity), would have less risk of depression than those without a certain genetic risk.

The reasons underlying sex differences in this interaction effect remain unclear, although it has been suggested that the social aspects of physical activity (e.g., being encouraged by others, practicing physical activity with family members) have a more beneficial effect on women than in men, and, consequently, this could possibly lead to the observation of less prevalence of depression in physically active women [55,69]. Another potential hypothesis to explain gender differences in the effect of physical activity in depressive symptoms in adults would be the role of estrogen, which has a key role in physical activity and in mood. In this regard, a recent meta-analysis has reported that physical activity, even in light

intensities, is associated with a reduction of depressive symptomatology in adult women of ages around the menopausal transition, repeatedly associated with increased risk of depressive symptoms [70].

In contrast, there is high heterogeneity in studies assessing the potential role of the *BDNF* Val66Met polymorphism on the effect of physical activity on depression. For instance, one cross-sectional study evaluated the role of this polymorphism on the effect of self-reported physical activity on depressive symptoms (using the Center for Epidemiology Depression Scale) in a population-based cohort, not finding a statistically significant moderation effect, possibly due to the sample size [16]. Similar results were found in another study including a sample of 1196 adolescents [71]. Other studies have shown opposite results when comparing between endurance athletes (n=55) and a control group (n=58). The findings showed worse depressive symptoms in the control group participants that were Val/Val homozygous, whereas there were no differences when comparing genotypes in athletes [38]. In a cohort of US veterans, Pitts et al. suggested that the initially observed reduction in cognitive functioning associated with depression would be moderated both by the Val66Met polymorphism and by the practice of physical activity. Thus, in participants with depression, those who practiced physical activity outperformed their not physically active counterparts in different domains of cognition [39]. Interestingly, among Met allele carriers with depression, physically active participants scored better results in subjective cognition, visual learning and work memory tasks in comparison with physically inactive participants.

In experimental studies that analyse interventions with physical activity considering *BDNF* Val66Met genotype, heterogeneous results have also been described. In a 12-week intervention in patients with depression, Rahman et al. observed that the highest proportion of responders to the intervention with physical activity (those who experimented a reduction greater than 50% on Montgomery Asberg Depression rating scale) were Met allele carriers who were not exposed to childhood adversity, compared to Val/Val homozygous [41]. Besides, in a year-lasting intervention with physical activity (aerobic, strength, flexibility and balance training) performed in sedentary community-dwelling participants, Dotson et al. found a decrease in somatic symptoms of depression (one of the four factors of the Center for Epidemiologic Studies Depression Scale) which was more evident in Met allele carriers, but exclusively in men [40].

One strength of this study is the extensive and detailed characterization of our community sample. However, due to the cross-sectional design of the study we are not able to establish causality, thus further longitudinal studies including larger sample sizes would be required. Furthermore, we are also aware that considering the assessment of physical activity compared to accelerometer and objective measurements of physical activity, questionnaires may be influenced by the participant mood, recall bias and memory inaccuracy, and also social desirability bias [72]. Therefore, future studies should include additional objective measures for the assessment of physical activity.

Finally, further research would be required to assess whether the effect of physical activity on depression risk in longitudinal studies is moderated by the *BDNF* Val66Met genotype. In this way, we could consider using this polymorphism as a biological marker to predict the effect that physical activity would exert on depression risk. Furthermore, functional studies are necessary to investigate how the effect of physical activity on depression risk could be mediated by this polymorphism. In this regard, it has been hypothesized that the increase of BDNF caused by the practice of physical activity could partially be implied in a decrease of the hippocampal atrophy, therefore protecting against depression [73].

Conclusions

In conclusion, our findings provide further evidence of a protective effect of physical activity on the risk of depression. We report a gene-environment interaction effect in which Met allele carriers of the *BDNF* Val66Met polymorphism who are more physically active showed a decreased prevalence of depression. Interestingly, this effect is strengthened in women, which has sex differences implications that should be addressed in future studies. Finally, these findings point to physical activity as a potential approach for the prevention of mental disorders in the general population and can also be considered as a non-invasive adjunct treatment for mental diseases.

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RESULTS

CHAPTER IV
Study 6

CHAPTER IV: Genetics of depression and its relationship with obesity and physical activity

Study 6: Relationship between a polygenic risk score (PRS) for depression, obesity and physical activity

1. Introduction

Depression is a disabling disorder with global impact[1], which is aggravated under different conditions. It is common for depression to co-occur with other physical and mental disorders, worsening their prognosis[2,3].

During the past years, physical activity –understood as any motion or movement generated by our skeletal muscles that leads to the use of energy[4]– has been proposed as a target for both prevention and treatment of depression[5–7]. In this regard, results in randomised control trials with resistance exercise training have reported an improvement in depressive symptoms[8], and in observational studies, practising physical activity was observed to be related with decreased risk of depression[9,10]. At a time when the efficacy and acceptability of antidepressants is being questioned[11], the importance of alternative therapies is rising and they are being considered as a primary or adjunct treatment[5]. At the same time, the benefits of physical activity on physical health have been consistently reported, therefore being an option to be considered when physical disorders are comorbid with depression[12,13].

Depression has been largely associated with a genetic component, despite a harsh shortage of GWAS results in its early days. The recent major efforts in order to achieve genome-wide statistically significant associations for depression, through the optimisation of sample size –using a wide definition of depression— resulted in the identification of 44 and 102 independent loci in consecutive years[14,15]. The development of these studies has allowed us to study how the combination of genetic variants is significantly associated with predicting depression[14,15]. As shown in Chapter I, scores of genetic risk generated in a larger sample, even with a different, more heterogeneous phenotype in this case[15], can be useful for association with depression, which is feasible for exploring different subtypes of depression or the relationship of a certain genetic risk with potential risk factors[16–18].

In the present study, we examine whether physical activity reduces the genetic risk for depression, determined with a PRS generated with the base data of the largest up-to-date mega-analysis of GWAS in depression[15]. Our initial hypothesis would be that, in the contextual framework of a population-based cohort, the practice of physical activity might be associated with a lower prevalence of depression, even in those individuals who have a higher polygenic risk.

2. Methods

Study population

The included population consists of a subsample of the PISMA-ep study. This cross-sectional study was conducted on a community-dwelling sample, representative of the Andalusian adult population, with the main objective of establishing the prevalence of the most common mental disorders in this region, as well as to identify their potential risk factors[19]. The *Methodological overview of the studies included* section describes in a deeper level of detail the methodology of the sample collection.

Phenotypic characterisation

Depression: The PISMA-ep study aimed to assess the prevalence of the most common mental disorders. Therefore, a diagnosis of depression –among other mental disorders– was ascertained using the Spanish version of the Mini-International Neuropsychiatric Interview (MINI)[20,21]. This instrument allows the diagnosis of 16 Axis I psychiatric disorders, following DSM-IV and CIE-10 criteria. These interviews were conducted by trained interviewers trained for interviewing techniques, protocol scales and inventories.

Anthropometric measures: Self-reported measures of height and weight were taken for each participant, then their body mass index (BMI) was calculated using the formula: weight (in kilograms) divided by height (in square meters): weight [kg] / height [m^2].

Physical activity: Information concerning the physical activity of the participants was obtained from a questionnaire which included 4 items: a) a dichotomous question to report whether the participant performed physical activity or not; b) in case of a previous affirmative answer, the nature of the physical activity, having to choose between 1) leisure, 2) work, 3) housework; c) the number of hours of physical activity performed per week, and the intensity that the participant considered this activity was, to be chosen between 1) light, 2) moderate and 3) vigorous.

Genotyping

A total of 197 individuals with depression and 1359 controls were genotyped using the Illumina Infinium PsychArray-24 BeadChip (Illumina, San Diego, CA, USA), which was developed in collaboration with the Psychiatric Genomics Consortium and is commonly employed for large-scale genetic studies researching for psychiatric predisposition and risk. This array includes ~593,260 fixed markers, including SNPs from arrays developed for identifying putative functional exonic variants (Exome-24 BeadChip) and informative genome-wide tag SNPs (Infinium Core-24 BeadChip), and 50,000 additional markers associated with common psychiatric disorders. The genotyping of the controls was performed at the Stanley Center for Psychiatric Research at Broad Institute, whereas cases were genotyped in the Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO).

Polygenic risk score

Summary statistics from the largest up-to-date GWAS in depression, which included more than 800,000 individuals and obtained 102 statistically significant loci[15], were the base data for a PRS performed on a subsample from the PISMA-ep study. Following guidelines published by Choi et al. (2020)[23], both base and target data underwent different filters for QC, using PLINK[24,25] and R[26]. Initially, base data had an 'info' score (which provided information of each SNP imputation quality) > 0.8 and a MAF > 1%, duplicated, ambiguous or mismatching SNPs were removed. From 8,483,301 variants included from the base file, 424,528 SNPs were excluded for having a MAF < 1%, and 1,244,172 were ambiguous variants, therefore remaining 6,814,601 total SNPs from the base data.

QC filter was performed on target data in order to keep only SNPs with a MAF > 1%, satisfying Hardy-Weinberg equilibrium ($p < 1 \times 10^{-6}$), with a missingness of < 1% of subjects, and individuals with an acceptable genotyping rate (removing subjects with > 1% missing). Subsequently, heterozygosity of the samples ($p > 1 \times 10^{-6}$) was assessed. Finally, first or second-degree relatives (pi-hat > 0.125) in the sample were excluded.

The PRSice-2 software, a PRS program that implements the standard C+T PRS method, was employed to run the PRS analysis[27]. A first clumping step (LD r^2 threshold < 0.1; 500 kb sliding window) was applied to create independent SNPs adjusting LD. PRS was further calculated assuming an additive model. As recommended when analysing limited sample sizes, we used the standardised calculation of PRS[16,23]. PRS for the following p-value thresholds were estimated: 0.001, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and a full model including every SNP (p = 1). We used Nagelkerke's pseudo R^2 to assess the proportion of phenotypic variance explained. PRS were divided into quintiles, and the ORs for depression in each quintile were calculated considering the first quintile as the reference group. Using PRSice-2, no covariates were included for the PRS calculation.

Statistical analysis

First, the logistic regression model including the generated standardised PRS (the 'genetic model') was further completed in R to include age, sex and the two first principal components (PCs) as covariates to assess whether the effect observed was consistent after adjusting for typical covariates. Then, multivariate logistic regressions were employed to evaluate the association of the depression PRS and different variables with the depression phenotype. The null model only contained the covariates while the genetic model added the depression PRS. The additive models included BMI data and physical activity data (both the dichotomous variable and the standardised number of hours, according to the analysis performed in Choi et al., 2020[18]). The interaction model included BMI as a covariate, and physical activity variables and PRS forming the interaction term.

Analysis of variance (ANOVA) was used for model comparisons in order to assess whether each model increased explanatory value over its immediately reduced model (i.e., the null model was compared with the genetic model; the genetic model was compared with the

additive models, and the additive model was compared with the interaction model). Akaike information criterion (AIC), an estimator of predictive quality for regression models, which is used to compare candidate models by balancing goodness of fit with model complexity, were reported for the different logistic regression models. The preferred model would be the one with the minimum AIC value, taking into account that this parameter considers the model fitness and penalyses the number of estimated parameters. Receiver Operating Characteristic (ROC) curves were generated, and areas under the curve (AUC) were calculated to evaluate the performance of the different models, using the 'pROC' R package[28]. Plotting the AUC represents the true positive rate (sensitivity) versus the false-positive rate (specificity), and in this study indicates the probability of an individual with a diagnosis of depression having a higher predicted risk than an individual without a diagnosis of depression[29].

3. Results

Demographic characteristics of the sample

The sample included in the current study was extracted from the sample in which the GWAS of Study 1 was conducted. Due to the requirements of the sample characterisation, only a subset of the PISMA-ep sample that had complete phenotypic data (BMI and physical activity data, apart from sex, age and PCs) was included. This sample consisted of 1,288 participants (176 cases with depression and 1,112 controls). Then, underweight participants (BMI < 18.5) were filtered out (removing 8 cases and 0 controls). Therefore, the sample prior to the QC steps consisted of 168 cases and 1,112 controls (n = 1,280). Starting from a total of 6,338,411 SNPs and 1,280 individuals, 1,767,514 variants were removed due to missing genotype data, 1,010,143 variants due to not reaching MAF threshold, and no variants were excluded due to Hardy-Weinberg disequilibrium (3,560,754 variants remained). No individuals were removed due to missing genotype data. The following heterozygosity analysis resulted in the exclusion of 1 case and 11 controls, remaining 167 cases and 1,101 controls (n = 1,268). The analysis of duplicates and relatives led to the exclusion of 35 participants with pi-hat > 0.125 (3 cases and 32 controls). Following these QCs and filters described in the *Methods* section, the final sample consisted of 1,233 individuals (164 depression cases and 1,069 controls).

Among the participants finally included in the PRS analysis (n = 1,233), the average age was 42.48 years (SD = 15.15), and 49.64% were female (Table 14). Whereas 39.42% of the participants declared no regular practice of physical activity, among the remaining proportion we observe a variety in the number of hours reported (Table 14 and Figure 17). The proportion of participants with a diagnosis of depression at the moment of the study was 13.3%. We observed that depression was statistically associated with the absence of physical activity (OR = 1.52; 95% CI = [1.08 - 2.15]; p = 1.65 x 10^{-2}, data not shown). In a descriptive manner, it can be observed in Figure 18 that, when stratifying hours of physical activity into quintiles and then comparing "low physical activity" against "high physical activity" (being "low" Q1 and Q2, and "high" Q3, Q4 and Q5, as in Choi et al., 2020[18]), prevalence of depression is below the average in the "low physical activity" group, although when dividing the "high

physical activity" into quintiles, those quintiles reporting more hours of physical activity do not correlate with a lower prevalence.

Table 14. Descriptive summary table of variables measured

	n (%)	mean (SD)
Met criteria for depression	164 (13.3%)	
Reported practice of physical activity	486 (39.42%)	
Physical activity (hours/week)[a]		3.00 (7.00)
Female	612 (49.64%)	
Age		42.48 (15.15)
BMI		26.19 (4.62)

[a] Given the zero-enriched distribution of the variable, the median and the interquartile range were employed as measures of central tendency and dispersion, respectively.

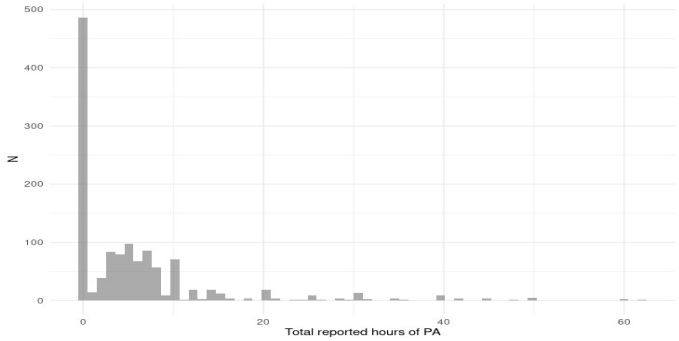

Figure 17. Distribution of the reported hours of physical activity among the participants.

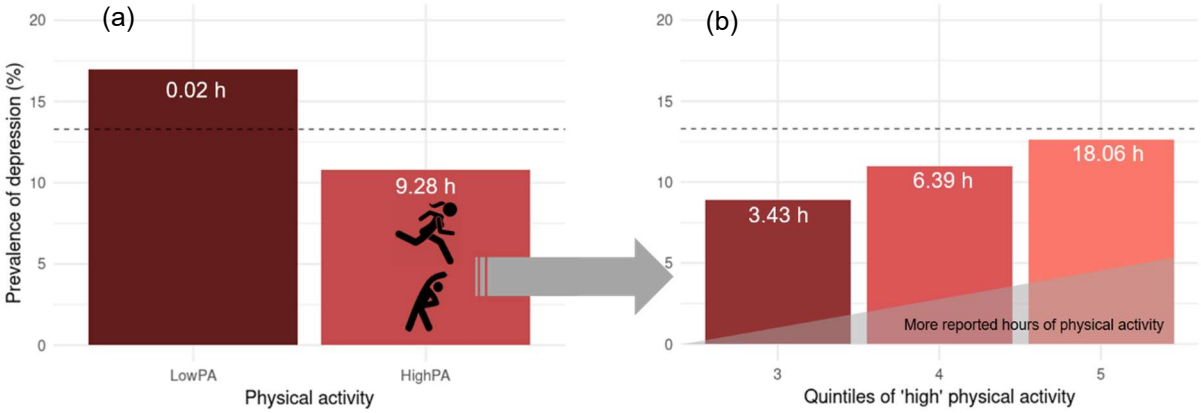

Figure 18. Prevalence of depression (a) per category of physical activity and (b) per quintiles of physical activity in the "high physical activity" group. Numbers within each bar indicate average hours of physical activity reported by participants in each group. The dashed line indicates depression prevalence in this sample (13.3%)

Polygenic risk score

A polygenic risk score for depression was calculated over a PISMA-ep subsample, using summary statistics from Howard, et al (2019)[15]. Among all PRS generated, the most explanatory PRS was obtained with a p-value threshold of ~0.052, which included 19,281 SNPs and explained approximately a 1.37% of the variability of depression (R^2 = 0.014; p = 2.59 x 10^{-3}). A bar-plot comparing the PRS results obtained using different p-value thresholds is represented in Figure 19.

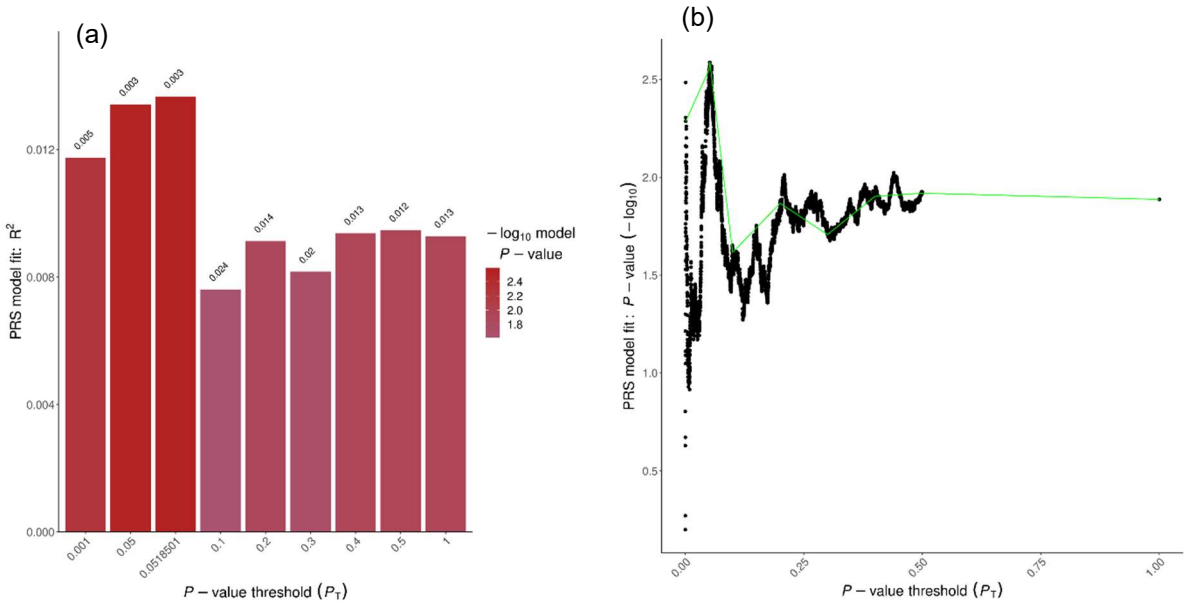

Figure 19. Representation of the model fit of the resulting PRS using different p-value thresholds, as a a) bar plot and b) high-resolution plot.

Figure 20a and 20b represent how cases and controls are distributed across the spectrum of PRS values. It can be observed that the highest PRS values are more frequent in depression cases than in controls. Accordingly, when the prevalence of depression was assessed using a genetic model, which only considered the PRS and the typical covariates –sex, age, and the two first PCs– the resulting OR was 1.26 (SE = 0.088; p = 7.43 x 10^{-3}), suggestive of a certain genetic risk. As depicted in Table 15, the inclusion of PRS as genetic risk in the predictive model showed a more parsimonious fit, according to the results of the ANOVA comparison between null (only classic covariates) and genetic (adding PRS to the classic covariates) models (deviance = 7.26; p = 7.04 x 10^{-3}), and the Nagelkerke's pseudo R^2 values of each model ($R^2_{null} \approx 0.106$; $R^2_{genetic} \approx 0.116$). The model comparison revealed that the following inclusion of BMI and practice of physical activity also improved the model fit (Table 16). In this respect, both each additive model (additive—BMI and additive–physical activity) means an improvement of the model fit, although the best result was obtained with the full-additive model –which included both BMI and physical activity ($R^2_{full\text{-}additive} \approx 0.144$). Moreover, BMI was significantly associated with depression prevalence in the full additive model (OR = 1.07, 95%CI = [1.03 - 1.11], p = 1.09 x 10^{-4}, adjusted for practising/not practising physical activity and the classical covariates). Since the addition of the hours of physical activity did not improve the regression models, the full additive model and the further interaction only considered

physical activity with its dichotomous variable. The interaction terms of GxE (PRS x the different variables of physical activity) did not improve the model fit, as can be observed in the Nagelkerke's pseudo R^2 comparison (Table 16), as well as with the observed AUC, which was lower than the full additive model (Figure 21).

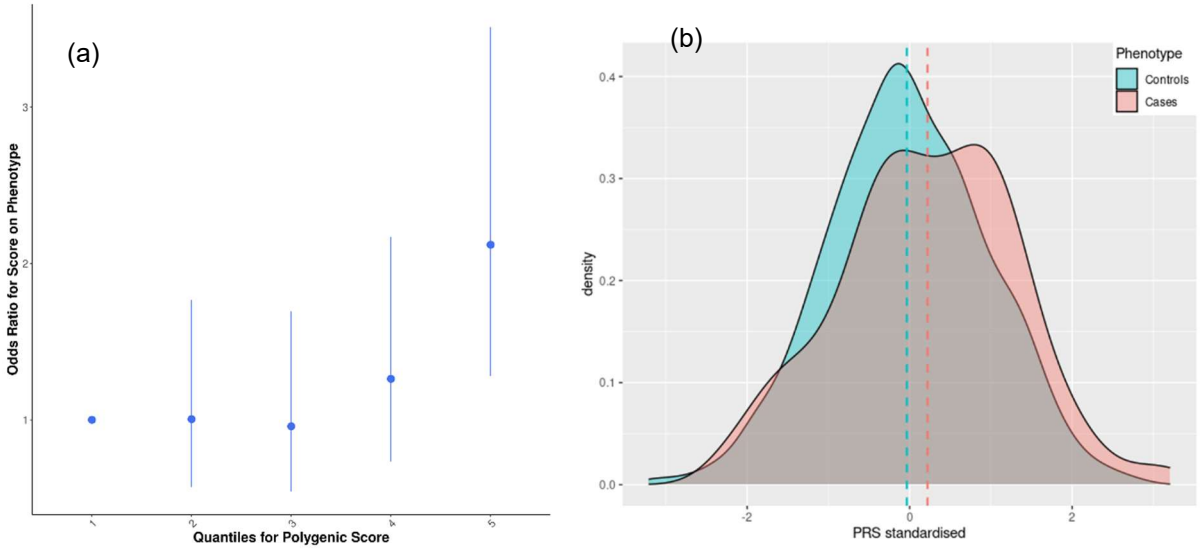

Figure 20. Association of the PRS with the depressive phenotype: a) Quantile plot illustrating the predicted risk of depression as PRS increases, using the most explanatory PRS; b) density plot representing the distribution of PRS values in cases and controls.

Table 15. Summary of findings from the effects of the null, genetic, additive and interaction models of a depression PRS and physical health on depression in a subsample from the PISMA-ep study.

Model	Variable	OR	SE	95% CI	p-value	Nagelkerke's pseudo R2	ANOVA model comparison (p)
Null	-	-	-	-	-	0.106	-
Genetic	PRS	1.26	0.088	1.07 - 1.50	7.43×10^{-3}	0.116	7.04 x 10-3
Additive (G+E)	PRS	1.25	0.089	1.05 - 1.50	0.011	0.144	3.95 x 10-5
	BMI	1.07	0.018	1.03 - 1.11	1.09×10^{-4}		
	PA-no	1.41	0.178	1.00 - 2.00	5.16×10^{-2}		
Interaction (GxE)	PRS	1.17	0.128	0.92 - 1.49	0.206	0.145	0.42
	BMI	1.07	0.018	1.03 - 1.11	1.16×10^{-4}		
	PA-no	1.38	0.181	0.97 - 1.97	0.074		
	PRS * PA-no	1.16	0.179	0.81 - 1.65	0.421		

All the models were adjusted for age, sex, and the two first principal components. The null model only contained these covariates. The PRS variable refers to the PRS constructed using the p-value threshold of ~0.052. G+E=gene plus environment model; GxE=interaction model (effect of G+E plus GxE). PA = physical activity.

Table 16. Comparison of the logistic regression models.

Model	ANOVA model comparison						Nagelkerke's pseudo R^2	AIC
	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)	Signif. code		
Null	1228	893.85	—	—	—	—	0.106	903.85
Genetic	1227	886.59	1	7.26	7.04 x 10-3	**	0.116	898.59
Additive – BMI	1226	870.08	1	16.51	4.84 x 10-5	***	0.139	884.08
Additive –PA-yes/no	1226	880.87	1	5.73	1.67 x 10-2	**	0.124	894.87
Additive – full	1225	866.31	2	20.28	3.95 x 10-5	***	0.144	882.31
Interaction (PA-yes/no)	1224	865.66	1	0.65	0.42		0.145	883.66
Interaction (PA-std. hours)	1224	868.77	1	-2.46	—		0.141	886.77

ANOVAs were performed between models and their immediate more complex model, i.e., the null model was compared with the genetic model; the genetic model was compared with the three additive models; and the additive (full: BMI + PA-yes/no) model was compared with the interaction models. Nagelkerke's pseudo R^2 indicates how accurately different models act as predictors to the model. PA = physical activity. AIC = Akaike information criterion.

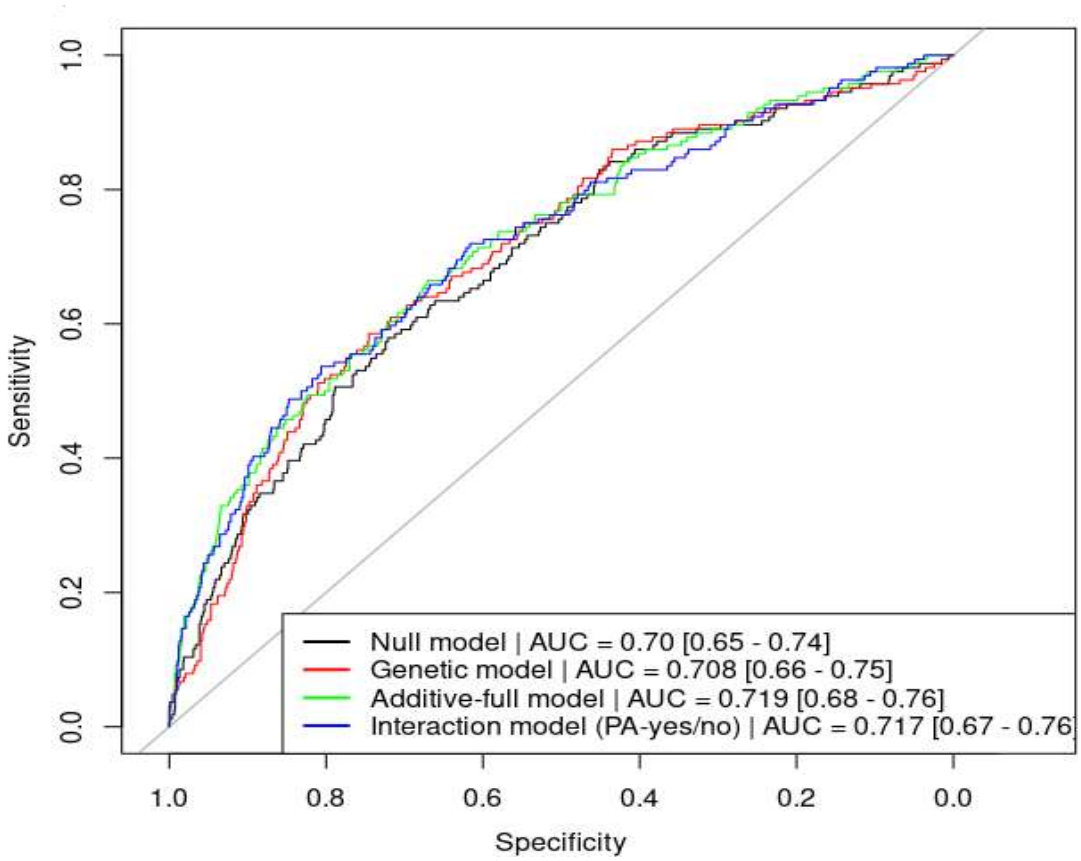

Figure 21. Assessment of the predictive ability of the different constructed predictive models using AUC.

In an exploratory manner, we divided the PRS scores into three categories of genetic risk, as performed in Choi et al., 2020[18], as represented in Figure 22a. When we attempted to compare the prevalence of depression in the different genetic risk groups according to whether or not they reported practising physical activity, we observed that physical activity shows a tendency towards a lower prevalence of depression, which is more pronounced the higher the risk conferred by the genetic background (Figure 22b).

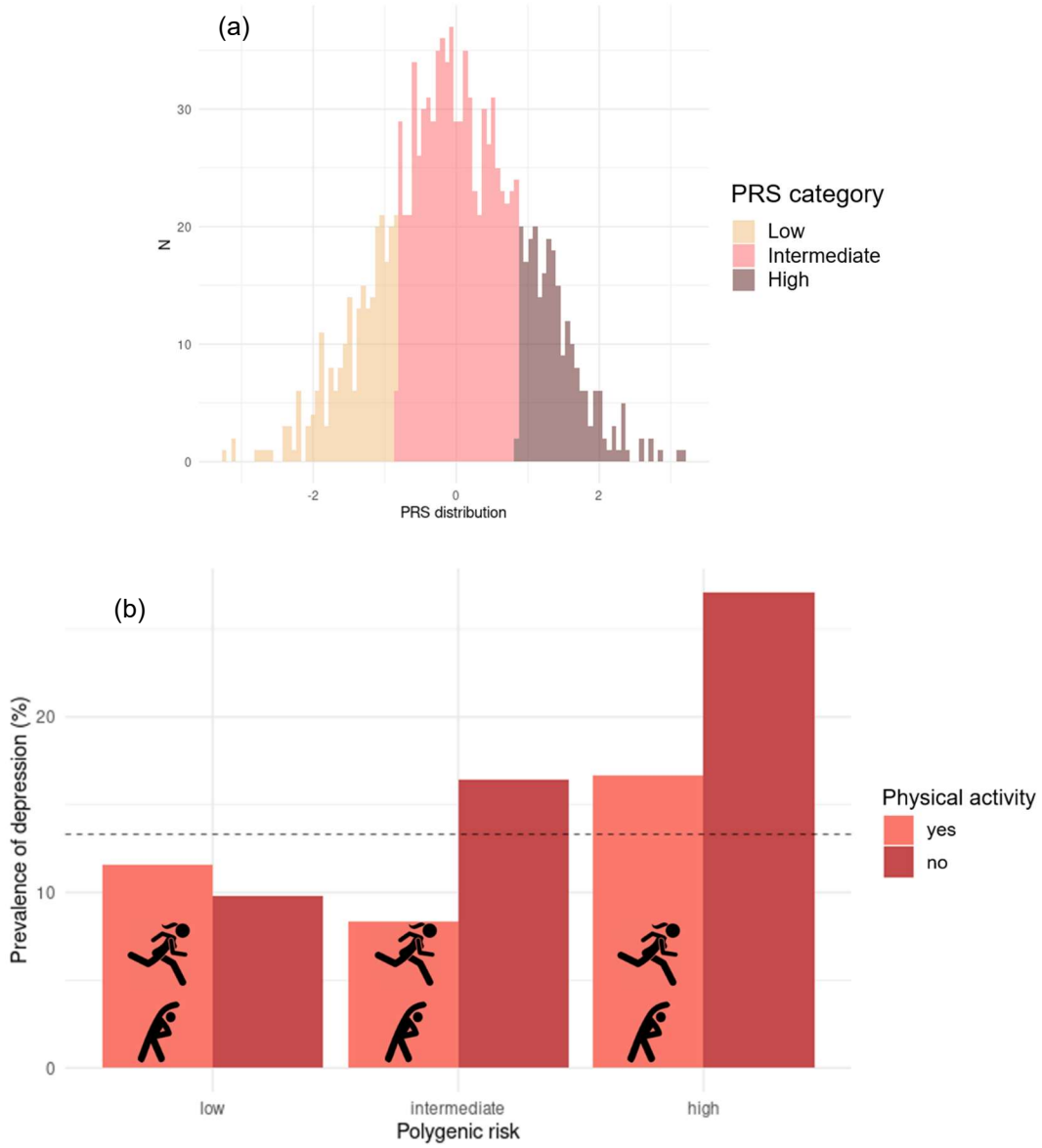

Figure 22. a) Distribution of PRS values divided into categories: quintile 1 corresponds to low genetic risk; quintiles 2, 3 and 4 correspond to intermediate genetic risk, and quintile 5 corresponds to high genetic risk. In b) the prevalence of depression is assessed in the different genetic risk categories previously defined, comparing between participants that reported practice of physical activity and those who did not.

4. Discussion

In this study, we constructed a PRS of depression in a thoroughly characterised sample, representative of the Andalusian adult general population, using the summary statistics from the largest GWAS mega-analysis ever conducted at the moment of the study[15]. Among the different scores evaluated, the best PRS was generated using a p-value threshold of approximately 0.052. The PRS generated with this setting was composed of 19,281 SNPs and explained ~1.37% of the variability of depression. This PRS was then included in different logistic regression models, using phenotypic information indicative of the physical health of the participants, i.e., BMI and physical activity, generating GxE models.

Similarly to what was observed in a recent longitudinal study by Choi and colleagues (2020)[18], we reported a statistically significant association between not practising physical activity and having been diagnosed with depression. This association was found only when BMI was not included as a covariate, whereas the additive model that included BMI and practising/not practising physical activity did not reach statistical significance. Nonetheless, contrary to the above results, we did not find an association between an increase in the number of hours of physical activity reported and decrease in the risk of depression. Our results, suggesting that individuals that did not report any practice of physical activity would be more likely to develop depression, again provides evidence of a protective and modifiable target. Our preliminary data stratifying for genetic risk also pointed towards an association of physical activity and lower prevalences of depression, especially in individuals with a higher genetic risk. Although reverse causality might be a limitation of our cross-sectional study, previous data from meta-analysis of randomised controlled trials with resistance exercise training[8], and prospective studies[9,10], have reported evidence towards the protective effect of physical activity against risk of depression. A Mendelian randomisation study also supported that the direction of the effect was that physical activity exerted a protective effect for depression risk, although this was only observed considering accelerometer data[7].

Among the wide phenotypic range of depression, the effect of physical activity could be key in certain subtypes and clusters. In particular, the atypical depression subtype is characterised by the DSM-5 criteria with hypersomnia and hyperphagia/weight gain[30], and is more prevalent in women, often has an earlier onset age, and has an approximate frequency of 15-29% of patients with depression[31,32]. In a randomised controlled trial designed to evaluate the effect of an aerobic exercise intervention on the reduction of depressive symptoms, a small-to-moderate effect was observed in cases with atypical depression, rather than other subtypes[33]. Also, clusters of patients with increased proinflammatory cytokines and immunometabolic dysregulations have been suggested to report greater improvement in depressive symptoms following interventions with physical activity[34]. It has been suggested that, in these participants, the anti-inflammatory effect indirectly caused by physical activity is probably mediating the antidepressant effects[35]. For a deeper understanding of the relationship between depression prevalence and physical activity, and whether this protective and modifiable factor is able to compensate a pre-established genetic risk, further

studies should be conducted in order to compare the association between physical activity and depression in clusters and subtypes that have been previously studies to react more efficiently to physical activity, as the ones previously mentioned.

Interestingly, we observed a statistically significant association of BMI with the prevalence of depression. Whereas it has been described that atypical depression often results in weight gain[32], this association with BMI appears in a representative sample of the general adult population, thus including all depressive subtypes that may be represented in this cohort. A stratification of the disease could lead to stronger associations, that may reveal the involvement of behavioural patterns –e.g., physical activity– or modifiable factors in the risk of depression for the different subtypes, pointing towards more personalised approaches and indications. In this preliminary study, we observed how the comparison of logistic regression models and AUCs of the different models support that the more comprehensive a model is, complementing genetic information with phenotypic and environmental information, the closer it is to be associated with the phenotype of interest.

In regards to the limitations of the study, first, the ROC and AUC were calculated based on the entire cohort without using cross-validation techniques. While this approach provided an informative assessment of the models' performance, results should be interpreted with caution as performance estimates may be overoptimistic, since the models were not evaluated on independent test sets. Therefore, following steps will consist in applying a cross-validation of the ROC analysis, or using a test sample to assess its predictive ability. Secondly, this study assessed the predictive effect of a PRS which was constructed for a broad phenotype of depression –including a considerable sample of self-reported depressive symptoms[15]–for a clinical diagnosis of depression. Even though there is a significant correlation between these self-reported depressive symptoms and future major depression[36], it would have been more accurate to employ a PRS constructed by using a cohort of clinically diagnosed cases with depression. Finally, our cohort is representative from the Andalusian general population, thus our findings may not be transferable to other cohorts or ethnicities, apart from showing an unbalanced design between depression cases and controls.

In conclusion, a PRS which was constructed from summary statistics of a broad phenotype of major depression was associated with risk of clinically ascertained depression in an Andalusian cohort representative of the general adult population. Although the PRS itself explained a ~1.37% of the phenotypic variance, different phenotype variables were considered, increasing the variance explained in more complex models. Future studies should consider stratification of the cohort in order to assess whether these phenotypic variables, indicative of physical health, explain a higher phenotypic variance in certain subtypes and clusters of depression. Nonetheless, the additive model, combining genetic and phenotypic information related to physical health suggest that, in the analysed sample, promoting different healthy life behaviours are modifiable environmental factors which can exert a protective effect over one's genetic risk, as has been previously reported in different cohorts[18].

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GENERAL DISCUSSION AND FUTURE PERSPECTIVES

General discussion and future perspectives

The overall objective of the Doctoral Thesis was to investigate the genetic differences between cases with depression and controls in a epidemiological sample representative of the Andalusian general population, and to identify the potential interaction between the genetic background and the environment, in terms of physical health. Over the course of four chapters, the focus was placed on different approaches through specific objectives.

A few decades have passed since genetic studies started in depression, starting with familiar and twin studies followed by candidate genes studies, and, since 2007, GWAS studies. Genetic studies are of a pivotal relevance since they provide signals that are, beyond the biological significance of the particular variant, constant markers throughout the lifetime of the individual and detectable from birth. After initial years without significant results[1], during the last years it has been possible to detect a significant number of genetic variants associated with depression[2,3].

Chapter I (Study 1) was designed with the objective of contributing to the existing knowledge about genetic variants associated with depression. Here, the analysed cohort was the PISMA-ep study, representative of the Andalusian general adult population. As expected given the trajectory of GWAS in depression through the past decade, due to the sample size of our study, we did not find any genetic variant reaching genome-wide significance. However, 9 top-hits SNPs were located in the 'grey zone' and were studied, together with their surroundings. Subsequently, we performed a PRS in this cohort –that included clinically ascertained cases with depression– using summary statistics from the largest up-to-date GWAS mega-analysis in depression –which employed a broad definition of depression, including self-reported depressive symptoms in a large proportion of the sample[3]. Interestingly, the PRS was associated with depression prevalence in the PISMA-ep sample, although the proportion of the variance explained with the PRS was of ~0.8%, lower than the same parameter in the different cohorts used in this mega-analysis, which ranged from 1.5% and 3.2% depending on the cohort[3]. It has been suggested that this broad definition of depression might not provide an optimal genetic architecture for depression, since they identify a proportion of variants which are common to diverse psychiatric disorders[4,5]. Therefore, future approaches should consider performing GWAS with clinically ascertained depression, despite the difficulty of achieving a sample size comparable to the last successful GWAS mega-analyses.

The risk posed by depression, beyond the impairment in quality of life and the disability caused by this disorder, is reflected in a decrease in the life expectancy of patients with depression, as has been reported in longitudinal studies, reported to be of 15.3 years less (95% CI = 13.8 - 16.8 years) in males and of 12.5 years less (95% CI = 10.8 - 14.2 years) in women[6]. Although there are multiple causes associated with this lower life expectancy –for instance, the elevated risk of suicide–, there is a higher proportion of deaths caused by physical health conditions, especially cardiovascular disease, metabolic syndrome or type 2 diabetes[6,7], which are often comorbid with depressive disorders. In Chapters II and III we analyse the

relationship between depression and physical health in the PISMA-ep sample, focusing on BMI and physical activity, respectively, since both are closely related to physical health conditions and are suggested to have bidirectional interplays with depression[8–11]. Moreover, genetics plays a relevant role in both BMI and physical activity. Therefore, in these chapters we investigated the participation of genetic factors through different approaches.

In **Chapter II**, we initially present a systematic review (**Study 2**), studying the role of a genetic variant of the *FTO* gene (rs9939609), robustly associated with BMI increase, in the relationship between BMI and depression. However, the results were inconclusive and suggested better outcomes with a more thorough characterisation of depression. Chapter II continued with the **Study 3**, in which we generated an unweighted genetic risk score from alleles of 30 risk loci for depression that was significantly associated with prevalence of depression in the PredictD-CCRT cohort. Here we observed that fitness of the model was improved after including non-genetic risk factors. Remarkably, the highest predictive ability for depression was achieved in a model including an interaction term between GRS and BMI, that could be interpreted as individuals with a high genetic risk, who had a high BMI, had increased risk for depression than those with high genetic risk but lower BMI. The same results, but with the variables permuted, was recently reported, finding that a GRS for BMI improved its predictive ability for BMI by including depressive status in the model[12]. These results would point towards a bidirectional relationship, although it is noteworthy that among depression subtypes, atypical depression is characterised with weight gain/increased appetite and sleepiness/excessive sleeping, among other features[13]. This would suggest that stratification of depressive disorders may favour the identification of more robust associations and the identification of a clustered genetic architecture.

In **Chapter III** the focus is on the relationship between physical activity and depression. In a context of a certain lack of efficiency of antidepressants[14,15], alternative approaches take a pivotal role for depression treatment. In this respect, physical activity has been largely associated with a potential role for prevention and treatment of depressive disorders[16–18]. In this Chapter we present a systematic review (**Study 4**) investigating the role of a molecule with a strong involvement in depression and physical activity as is BDNF, in the relationship between both. Firstly, we analysed the role of the most studied polymorphism in the BDNF *BDNF* gene, rs6265 (Val66Met), suggesting a greater antidepressant effect of physical activity in Met allele carriers of the *BDNF* Val66Met polymorphism, as reported in four of the six included studies. Then, we studied the effect of both acute and chronic physical activity on the BDNF protein, finding a consensus on the increase in protein after a single bout of physical activity, but inconclusive results regarding the chronic effect of physical activity interventions. Subsequently, in **Study 5** we assessed whether the Val66Met polymorphism played a role in the relationship between physical activity and prevalence of depression in the PISMA-ep sample. Here we found a surprising interaction effect between carrying the risk (Met) allele for this *BDNF* genetic variant and reported hours of physical activity, decreasing the prevalence of depression in Met allele carriers as more hours of physical activity were reported. Although this result was observed in the total sample, the effect was more

significant in women. These results are consistent with a previous cross-sectional study in healthy adolescent women, reporting the moderating effect of the Val66Met allele in the association between physical activity and decreased depressive symptoms[19], although as previously stated in Study 4, there is a high heterogeneity between studies, being difficult to establish comparisons. In interventions with physical activity, results improving depressive symptoms were more noteworthy in Met allele carriers[20,21]. Regarding these results, it might be interesting to consider hypotheses that estimate that individuals with a certain genetic susceptibility would benefit more from a favourable environment, as practising physical activity[22]. In this respect, future studies should address how the genetic risk level resulting from stratification may result in a differential effect of physical activity –among other environmental factors– on the risk for depression.

Finally, in **Chapter IV** (**Study 6**), we integrated the scopes of study from the previous chapters. Here we constructed a PRS for depression in a subsample from the PISMA-ep study that had phenotypic information for the variables of study from Chapters II and III (i.e., BMI and physical activity), and assessed the fitness of different multivariate logistic regression models. As depicted in a recent review, the use of PRS for psychiatric disorders can be of considerable value at various points, both prior to the illness, for diagnosis, and during the course of the illness[23]. In this respect, PRS can facilitate risk prediction from birth, detect early symptoms, support diagnosis and contribute to treatment decisions, as well as predict disease progression and outcome. However, at this moment the predictive levels of the PRS for depression are not yet clinically useful, and are lower than compared to other psychiatric disorders, such as schizophrenia. It is also to be considered that the genetic influence is relatively low in depression (for instance, heritability is estimated at 37% in depression, while in schizophrenia it is 81%[24]), although the high lifetime prevalence of this disorder (around 13%, against 4% in schizophrenia, following with the example above[24]) provides an interesting basis for the generation of risk prediction models.

According to recent studies, which propose to improve clinical relevance by means of a phenotypic refining[4] –instead of the latest studies in which a self-reported diagnosis of depressive symptoms is considered, under the term "broad depression"[3]–, in our study, cases with depression stem from an epidemiological sample, following a clinically ascertained diagnosis, which should be taken into consideration as a favourable point for the homogeneity of our cohort. Besides, our results are reported in the PISMA-ep sample, which is representative of the general Andalusian adult population, which should be considered as a strength for the present study. Further studies reaching a certain level of homogeneity towards diagnosis criteria may provide results more translatable for clinical and predictive purposes.

Another strength of this study was the inclusion of BMI and physical activity information in the regression models. During the past decades, interactions between genes and environment have been prolific in multiple fields of study. For example, physical activity was reported to attenuate the genetic predisposition –using the sum of risk alleles from 12 SNPs– for BMI in

large samples[25,26]. However, there is scarce evidence of these interactions using data generated from GWAS and for depression, a recent meta-analysis did not find evidence for an interaction between PRS for depression and childhood trauma, a commonly studied environmental factor associated with depression[27]. Our results did not report an association either, although the inclusion of both variables in the additive logistic regression model did improve the model fitness, suggesting that they could be potentially included in future models for prediction of depression. Finally, as stated above, due to the heterogeneity of depression, stratification may provide larger differences regarding BMI and physical activity information, and is perhaps related to a genetic predisposition more closely associated with a more phenotypically homogeneous subtype.

For the near horizon, future research should account for interactions between genetic risk and environmental factors, in order to generate more accurate predictive models. Only together with other "–omics" areas , and their integration with individual characteristics from each subject, it will be possible to generate enough information to provide more precise diagnosis, the classification into endophenotypes of depression, or the prognosis or prediction of response to certain treatments, leading to a more personalised psychiatry[28].

General limitations:

The results presented in this Doctoral Thesis need to be interpreted with caution since there are some limitations:

— Summary statistics employed for PRS were extracted from GWAS using a broad definition of depression, which included self-reported depressive symptoms, in contrast to our clinically ascertained cohort with diagnosed cases with depression.

— The PISMA-ep is a cross-sectional study. Consequently, it is not possible to conclude how implementation of life habits may affect incidence of depression.

— Measurements of physical activity in the PISMA-ep sample are self-reported, with the risks that this may entail (questionnaires may be influenced by the participant mood, recall bias, memory inaccuracy, social desirability bias, among others)[29].

— BMI provides a simple estimation for obesity, but may not be the optimal manner to obtain a complete vision of obesity risk. Examples of this would be normal-weight people who are metabolically obese[30,31], or how BMI does not provide a measure of adiposity, not being able to discriminate between lean and fat mass[32]. Moreover, BMI was self-reported, although the use of self-reported weight and height is commonly accepted and does not differently differ from measured values[33].

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CONCLUSIONS

Conclusions

Specific conclusions

Chapter I: Genetics of depression in the PISMA-ep epidemiological sample

Study 1 — GWAS and PRS of depression in the PISMA-ep sample

- In a GWAS for depression conducted in an epidemiological sample, representative of the Andalusian adult population, no genetic variant achieved genome-wide statistical significance. However, we found 9 suggestively-significant independent SNPs associated with depression.
- A PRS calculated using summary statistics from the largest up-to-date GWAS mega-analysis performed in broad depression was significantly associated with depression prevalence in the PISMA-ep cohort.

Chapter II: Genetics of the relationship between depression and physical health: Depression and obesity

Study 2 — Systematic review of the role of the *FTO* rs9939609 SNP in the relationship between depression and obesity

- There is robust evidence towards the effect of the *FTO* rs9939609 variant on BMI, although the current level of evidence is not enough to conclude a relationship between this SNP, BMI and depression. Further research is required to cover the role of this gene on the depression-obesity comorbidity, emphasising a more precise characterisation of depression.

Study 3 — Genetic risk score (GRS) for depression and its relationship with BMI

- An unweighted GRS constructed using risk alleles of candidate genes for depression was significantly associated with depression prevalence in the CCRT sample. The predictive model was optimised when an interaction term between BMI and the GRS was considered.

Chapter III: Genetics of the relationship between depression and physical health: Depression and physical activity

Study 4 — Systematic review of the relationship between *BDNF*, physical activity and depression

- In four out of six studies, a greater antidepressant effect of physical activity in Met allele carriers of the *BDNF* Val66Met polymorphism was reported.

- The levels of the BDNF protein increased following a single bout of physical activity independently of the depressive status.
- Chronic effect of physical activity regarding BDNF levels did not reveal any conclusive results, with high heterogeneity across studies.

Study 5 — Interaction effect between physical activity and the BDNF Val66Met Polymorphism on depression in women from the PISMA-ep study

- An interaction effect was observed, suggesting that the decrease of depression prevalence associated with self-reported hours of physical activity is more pronounced in Met allele carriers of the *BDNF* Val66Met polymorphism. This effect was found in the total sample, although the effect was strengthened in women after sex stratification.

Chapter IV: Genetics of depression and its relationship with obesity and physical activity

Study 6 — Relationship between a polygenic risk score for depression, obesity and physical activity

- A PRS of broad depression was associated with risk of clinically ascertained depression in a subsample of the PISMA-ep study, explaining approximately a 1.37% of the phenotypic variance. The model was optimised when BMI and physical activity information were included in an additive model.

General conclusion

Since the emergence of genome-wide association studies, the use of genetic risk assessment tools is contributing to the understanding of the genetic architecture of genetically complex diseases as depression. Accounting for variables related to the physical health of individuals may be useful for developing more accurate risk prediction models for depression, aiming towards precision psychiatry approaches.

Conclusiones

Conclusiones específicas

Chapter I: Genetics of depression in the PISMA-ep epidemiological sample

Study 1 — GWAS and PRS of depression in the PISMA-ep sample

- En un GWAS para depresión realizado en una muestra epidemiológica representativa de la población adulta andaluza, ninguna variante genética alcanzó significancia estadística a nivel de genoma completo. No obstante, encontramos 9 SNPs independientes sugestivamente significativos asociados con la depresión.
- Un PRS calculado utilizando los resultados del mayor mega-análisis GWAS actualizado realizado en depresión de amplio espectro se asoció significativamente con la prevalencia de depresión en la cohorte PISMA-ep.

Chapter II: Genetics of the relationship between depression and physical health: Depression and obesity

Study 2 — Systematic review of the role of the *FTO* rs9939609 SNP in the relationship between depression and obesity

- Existen pruebas sólidas del efecto de la variante rs9939609 de *FTO* sobre el IMC, aunque actualmente la evidencia no es suficiente para concluir que exista una relación entre esta variante genética, el IMC y la depresión. Se requieren más investigaciones para cubrir el papel de este gen en la comorbilidad depresión-obesidad, recalcando la necesidad de una caracterización más precisa de la depresión.

Study 3 — Genetic risk score (GRS) for depression and its relationship with BMI

- Un GRS no ponderado construido empleando alelos de riesgo de genes candidatos para la depresión se asoció significativamente con la prevalencia de depresión en la muestra CCRT. El modelo predictivo se optimizó al considerar un término de interacción entre el IMC y el GRS.

Chapter III: Genetics of the relationship between depression and physical health: Depression and physical activity

Study 4 — Systematic review of the relationship between *BDNF*, physical activity and depression

- En cuatro de seis estudios se observó un mayor efecto antidepresivo de la actividad física en los portadores del alelo Met del polimorfismo Val66Met de *BDNF*.
- Los niveles de la proteína BDNF aumentaron tras una única sesión de actividad física independientemente del estado depresivo.

- El efecto crónico de la actividad física sobre los niveles de BDNF no reveló resultados concluyentes, con una elevada heterogeneidad entre los estudios.

Study 5 — Interaction effect between physical activity and the *BDNF* Val66Met Polymorphism on depression in women from the PISMA-ep study

- Se observó un efecto de interacción que sugería que la disminución de la prevalencia de la depresión que se asoció al número de horas de actividad física autoreportadas es más pronunciada en personas portadoras del alelo Met del polimorfismo Val66Met de *BDNF*. Este efecto se encontró en la muestra total, aunque el efecto se vio reforzado en las mujeres tras la estratificación por sexo.

Chapter IV: Genetics of depression and its relationship with obesity and physical activity

Study 6 — Relationship between a polygenic risk score for depression, obesity and physical activity

- Un PRS calculado para depresión de amplio espectro se asoció con el riesgo de depresión clínicamente diagnosticada en una submuestra del estudio PISMA-ep, explicando aproximadamente un 1,37% de la varianza fenotípica. El modelo se optimizó al incluir información sobre el IMC y la actividad física en un modelo aditivo.

Conclusión general

Desde la aparición de los estudios de asociación del genoma completo, el uso de herramientas de evaluación del riesgo genético está contribuyendo a la comprensión de la arquitectura genética de enfermedades genéticamente complejas como la depresión. Considerar variables relacionadas con la salud física de los individuos puede ser de gran utilidad para desarrollar modelos más precisos de predicción del riesgo de depresión, con el objetivo de un abordaje psiquiátrico de precisión.

ANNEXES

Papers derived from the Doctoral Thesis

Published papers

Zarza-Rebollo, J. A., Molina, E., & Rivera, M. (2021). The role of the FTO gene in the relationship between depression and obesity. A systematic review. *Neuroscience and biobehavioral reviews*, *127*, 630–637. https://doi.org/10.1016/j.neubiorev.2021.05.013

Anguita-Ruiz, A., Zarza-Rebollo, J. A., Pérez-Gutiérrez, A. M., Molina, E., Gutiérrez, B., Bellón, J. Á., Moreno-Peral, P., Conejo-Cerón, S., Aiarzagüena, J. M., Ballesta-Rodríguez, M. I., Fernández, A., Fernández-Alonso, C., Martín-Pérez, C., Montón-Franco, C., Rodríguez-Bayón, A., Torres-Martos, Á., López-Isac, E., Cervilla, J., & Rivera, M. (2022). Body mass index interacts with a genetic-risk score for depression increasing the risk of the disease in high-susceptibility individuals. *Translational psychiatry*, *12*(1), 30. https://doi.org/10.1038/s41398-022-01783-7

Zarza-Rebollo, J. A., Molina, E., López-Isac, E., Pérez-Gutiérrez, A. M., Gutiérrez, B., Cervilla, J. A., & Rivera, M. (2022). Interaction Effect between Physical Activity and the *BDNF* Val66Met Polymorphism on Depression in Women from the PISMA-ep Study. *International journal of environmental research and public health*, *19*(4), 2068. https://doi.org/10.3390/ijerph19042068

Papers in preparation

Zarza-Rebollo, J.A., López-Isac, E., Rivera, M., Gómez-Hernández, L, Pérez-Gutiérrez, A.M., Molina, E. (Under review in *International Journal of Clinical and Health Psychology*). The relationship between BDNF and physical activity on depression. A systematic review.