The determination of causal genetic variants involves vitamin D genes *CYP27B1* and *VDR*, and the innate antiviral response gene *SP140*, in Multiple Sclerosis.

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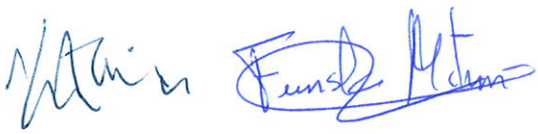

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Abbreviations

1,25(OH)2D	1-alpha,25-dihydroxivytamin D_3 , calcitriol
25(OH)D	25-hydroxyvitamin D_3, calcifediol
ACB	African Caribbeans in Barbados
AFR	African
AI	Autoimmune
AMR	Ad Mixed American
APC	Antigen representative cell
ASW	Americans of African Ancestry in SW USA
BEB	Bengali from Bangladesh
BM	Bone marrow
CD	Crohn's disease
CDX	Chinese Dai in Xishuangbanna, China
CEU	Utah Residents (CEPH) with Northern and Western European Ancestry
CHB	Han Chinese in Bejing, China
CHS	Southern Han Chinese
CLL	Chronic lymphocytic leukemia
CLM	Colombians from Medellin, Colombia
CNS	Central nervous system
CYP24A1	Cytochrome P450 monooxygenase
CYP27B1	Cytochrome P450 enzyme, 1a-hydroxylase
CYP2R1	Cytochrome P450 vitamin D 25 hydroxylase
DC	Dendritic cell
EAE	Experimental autoimmune encephalomyelitis
EAS	East Asian
EBV	Epstein–Barr Virus
eQTL	Expression quantitative trait loci
ESN	Esan in Nigeria
EUR	European
FGF	Fibroblast growth factor
FIN	Finnish in Finland
GBR	British in England and Scotland

GEUVADIS	Genetic European variation in health and disease
GIH	Gujarati Indian from Houston, Texas
GTEx	Genotype-tissue expression
GWAS	Genome wide association studies
GWD	Gambian in Western Divisions in the Gambia
HHV	Human herpesvirus
HLA	Human leukocyte antigen
HTLV	Human T-lymphotropic virus
IBD	Inflammatory bowel disease
IBS	Iberian Population in Spain
IFN	Interferon
IL	Interleukin
Indels	Insertion or the deletion of bases
ITU	Indian Telugu from the UK
JCV	John Cunningham virus
JPT	Japanese in Tokyo, Japan
Kb	Kilo base pairs
KHV	Kinh in Ho Chi Minh City, Vietnam
LCL	Lymphoblastoid cell line
LD	Linkage disequilibrium
LLT	Lectin-like transcript
LPS	Lipopolysaccharide
LWK	Luhya in Webuye, Kenya
MAF	Minor allele frequency
Mb	Mega base pairs
MBP	Myelin basic protein
MHC	Major histocompatibility complex
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSC	Mesenchymal stem cell
MSL	Mende in Sierra Leone

MXL	Mexican Ancestry from Los Angeles USA
PBMC	Peripheral blood mononuclear cells
PEL	Peruvians from Lima, Peru
PJL	Punjabi from Lahore, Pakistan
PLP	Myelin proteolipid protein
PML	Progressive multifocal leukoencephalopathy
PPMS	Primary progressive multiple sclerosis
PRMS	Progressive-relapsing multiple sclerosis
PTH	parathyroid hormone
PUR	Puerto Ricans from Puerto Rico
RA	Rheumatoid arthritis
RRMS	Relapsing–remitting multiple sclerosis
RXR	Retinoid X receptor
SAS	South Asian
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphisms
SPMS	Secondary-progressive multiple sclerosis
SS	Systemic scleroderma
STU	Sri Lankan Tamil from the UK
T1D	Type 1 diabetes
TNF	Tumour necrosis factor
Treg	Regulatory T cell
TSI	Toscani in Italia
TTV	Torque teno virus
VDR	1,25-Dihydroxyvitamin D_3 receptor
VDRE	Vitamin D response element
VZV	Varicella–zoster virus
YRI	Yoruba in Ibadan, Nigeria

Resumen

Antecedentes

La esclerosis múltiple (EM) es una enfermedad autoinmune que afecta al sistema nervioso central (SNC): el cerebro y la médula espinal, y que cursa principalmente con desmielinización neuronal. Como consecuencia, la capacidad de los nervios de transmitir los impulsos eléctricos desde el cerebro y al cerebro, se interrumpe y produce la aparición de los diferentes síntomas que aparecen y remiten, denominados "brotes", o que progresan lentamente a lo largo del tiempo.

La Esclerosis Múltiple, como enfermedad compleja, está caracterizada por una moderada heredabilidad y por la interacción de factores genéticos y ambientales. La variabilidad genética es un determinante importante en la susceptibilidad y progresión de la Esclerosis Múltiple.

Recientemente han surgido nuevas tecnologías de estudios: los Estudios de Asociación del Genoma Completo (GWAS) que se basan en el análisis de variantes a lo largo de todo el genoma en grandes poblaciones y sin una hipótesis establecida. De este modo se buscan *loci* asociados a la enfermedad sin establecer una determinada función en la patología de la misma. Por medio de los GWAS se han determinado alrededor de 100 *loci* asociados a la susceptibilidad a padecer EM aunque en su mayoría se desconoce el gen causal de esta asociación y el efecto de la variante génica sobre la función de este gen que en último término es la causa de la asociación genética.

Objetivo

El principal objetivo de este trabajo es la determinación de las variantes causales de los *loci* asociados con la EM por estudios GWAS y revelar, por el análisis del efecto funcional de la variante causal, los genes implicados y su efecto en la enfermedad.

Metodología

En este trabajo hemos estudiado 4 *loci* que se han visto asociados a EM. Hemos analizado la correlación de los niveles de expresión de los distintos genes de los loci con las variantes de la región asociadas con EM. Para ello hemos empleado datos de expresión procedente de RNA-Seq de líneas linfoblastoides de población europea del proyecto GEUVADIS y GTEx y análisis de expresión por sistemas de cuantificación por qPCR a tiempo real con muestras de RNA de monocitos, con distintos tratamientos, de 109 individuos recogidos en el Biobanco de Andalucía. Hemos hechos estudios funcionales para identificar las variantes que afectan el *splacing* de genes y que se asocian con la enfermedad y hemos hecho estudios caso-control en una cohorte española de 4000 enfermos y 4000 controles para validar la asociación con la enfermedad de las variantes funcionales identificadas aquí frente a las descritas por el GWAS.

Resultados

La expresión del gen *CYP27B1,* enzima activadora de la vitamina D, en monocitos activados con LPS y IFNγ correlaciona con los genotipos del SNP rs10877013 que es la variante causal de la asociación con MS en el locus 12q13-14. Esta correlación no se observa en otro tipo celular como es las células LCLs en las que no se produce activación de la expresión del gen con LPS e IFNγ o vitamina D. La expresión del gen VDR, receptor de la vitamina D, que se localiza a 10Mb del gen *CYP27B1* SNP también correlaciona con la variante rs10877013 en células LCL tratadas con vitamina D. En ambos casos el alelo de riesgo a EM se asocia con una baja expresión de los genes.

La variante rs2248359 que se ha visto asociada con EM en el locus 20q13.2 se localiza a 1 Kb del gen *CYP24A1,* enzima que degrada la vitamina D. Hemos determinado que este gen se expresa de forma inducible tras la activación con la forma activa de la vitamina D ($1,25(OH)_2D_3$), sin embargo no detectamos una correlación entre los niveles de expresión de este gen y los genotipos de la variante asociada a EM ni con otra localizada en la región.

Nuestros resultados indican que la desregulación en el splicing del gen *SP140* es la causa de la asociación genética del locus 2q37.1 con EM. El alelo T del SNP rs28445040 que se asocia con aumento de riesgo de EM correlaciona con la aparición de un transcrito que no lleva el exón 7 y con una disminución del transcrito completo. La variante rs28445040 se localiza en el exón 7 y por análisis de minigén hemos demostrado que es la causa del splicing alternativo del exón 7. Con estudios caso-control en una cohorte española de 4000 pacientes de EM y 4000 controles hemos confirmado que este SNP se asocia a la enfermedad y apunta a que es la variante causal de la asociación.

Mediante estudios de colocalización de las variantes que se asocian a cambios de expresión de genes (expression quantitative trait loci: eQTLs) obtenidas del proyecto GEUVADIS y las variantes que se asocian a EM en el estudio de mapeo fino ImmunoChip observamos que ambos efectos confluyen en un grupo de variantes en total LD en el locus 12p13.31. Estos resultados apuntan a que la variante rs3764022*,* que produce un splicing alternativo del gen *CLEC2D* es la causa de la asociación con MS. Sin embargo, estudios de colocalización de las eQTLs de GEUVADIS con los datos de asociación del GWAS con 10000 pacientes y 30000 controles, indican que la variante causal correlaciona con cambios del gen *CLECL1*. El estudio caso-control entre las variantes mejor asociadas en el ImmunoChip y en el GWAS en nuestra cohorte española mostró que ninguna de las dos variantes se asocia con EM en la población española.

Conclusiones

El estudio de la correlación entre las variantes que se asocian con EM y aquellas que se asocian con cambios de expresión de genes en las mismas regiones nos ha permitido determinar que los cambios en la expresión de los genes *CYP27B1*, *VDR* y *SP140* son la causa de la asociación de estos loci con la EM y hemos determinado sus respectivos mecanismos genético-moleculares asociados a la patotología.

Por otra parte, hemos observado que el sistema tiene limitaciones a la hora de determinar los genes causales como son la expresión específica de tejido o tipo de enfermedad, que nos ha llevado a buscar sistemas en los que se exprese el efecto de la variante como es el caso del gen *CYP27B1* o *VDR,* que se manifiesta únicamente en condiciones de activación concretas. Esta metodología se está aplicando en otras enfermedades complejas lo que está permitiendo determinar los genes que están detrás de las asociaciones genéticas.

Una de las consecuencias que se derivan de esos resultados es la confluencia de factor genético y factor ambiental en el mismo locus. Por ejemplo, en el caso del CYP27B1, que activa la vitamina D, el alelo de riesgo para EM y otras enfermedades asociadas a este locus, es la variante T, que conlleva baja expresión del gen CYP27B1 en monocitos y, a su vez, baja expressión del receptor de vitamina D, VDR, en células B. Si estas personas, ademas de portar el genotipo de riesgo, no toman el sol ni suficientes alimentos o suplementos de vitamina D, conllevaría una deficiencia several de esta hormona.

Introduction

Multiple sclerosis definition and history

Multiple sclerosis (MS) is primarily an inflammatory disorder of the brain and spinal cord in which leads to damage of myelin and axons *(Compston A, 2008).* The first illustration of MS (Fig.1) was in 1838 by a young Scottish physician and artist, Dr Robert Carswell (1793–1857). He spent years in the hospitals and mortuaries of Paris and Lyon painting watercolours and pen and ink drawings of patients and post mortem preparations, he was looking for creating an anatomy and pathology atlas. So, he drew 1034 paintings, 99 are of the brain and spinal cord *(Murray TJ, 2009).* However, the first description of MS as a distinct disease was in 1868 by Jean-Martin Charcot (1825 –1893), a French neurologist and professor of anatomical pathology, he called it "*sclérose en plaques"(Charcot J, 1868).*

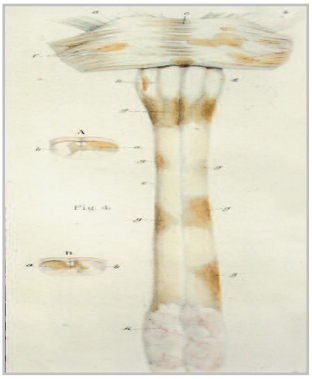

Figure 1.

The spinal cord and pons illustration by Robert Carswell (1838) showing lesions of MS. Scattered hard, brown discoloured and atrophied patches were mentioned in the pons, medulla and cord. The lesions were in the white matter of the cord extended into the grey matter (Courtesy of the Wellcome Library, London).

Types of MS

Neurologists identified 4 types of MS based on the course of the disease *(Hauser and Goodwin, 2008)*:

1- Relapsing–remitting MS (RRMS): is the most common form, affecting about 85% of MS patients. It is marked by attacks of neurologic function "relapse" followed by partial or complete recovery periods "remission", when symptoms improve or disappear.

2- Primary progressive MS (PPMS): affects approximately 10% of MS patients. Symptoms continue to worsen gradually from the beginning. There are no distinct relapses or remissions. This form of MS is more resistant to the drugs typically used to treat the disease.

3- Secondary progressive MS (SPMS): this type may develop in some patients with relapsing–remitting disease. Most patients who are initially diagnosed with RRMS will eventually transition to SPMS, which means that the disease course continues to worsen (not necessarily more quickly) with or without relapses.

4- Progressive-relapsing MS (PRMS): is a rare form, affecting fewer than 5% of patients. It is progressive from the beginning, and occasional relapses along the way. There are no periods of remission.

Symptoms and Diagnostic

About 2,5 million people worldwide were registered as diagnosed MS patients, with a sex ratio 3:1 (female:male). Most people are diagnosed between the ages of 20 and 40 *(Otron et al., 2006)*. The global median prevalence has been increased from 30 per 100 000 in 2008 to 33 per 100 000 in 2013 according to the Multiple Sclerosis International Federation (MSIF).

MS diagnostic can be very difficult as many as 10 % of people diagnosed with MS actually have some other condition that mimics MS such as inflammation in the blood vessels, multiple strokes, vitamin deficiency, and brain infection.

The most common symptoms include disturbances in the visual system (nystagmus, optic neuritis, diplopia), musculoskeletal system (muscle weakness, spasms, ataxia), sensory-tactile system (pain, hypoesthesias, paraesthesias), bladder and bowel (incontinence, frequency or retention), and neuropsychological functioning. Other symptoms include fatigue, speech problems (dysarthria), sexual dysfunction, and sleep disorders (Raine, McFarland, & Hohlfeld, 2008) (Fig.2).

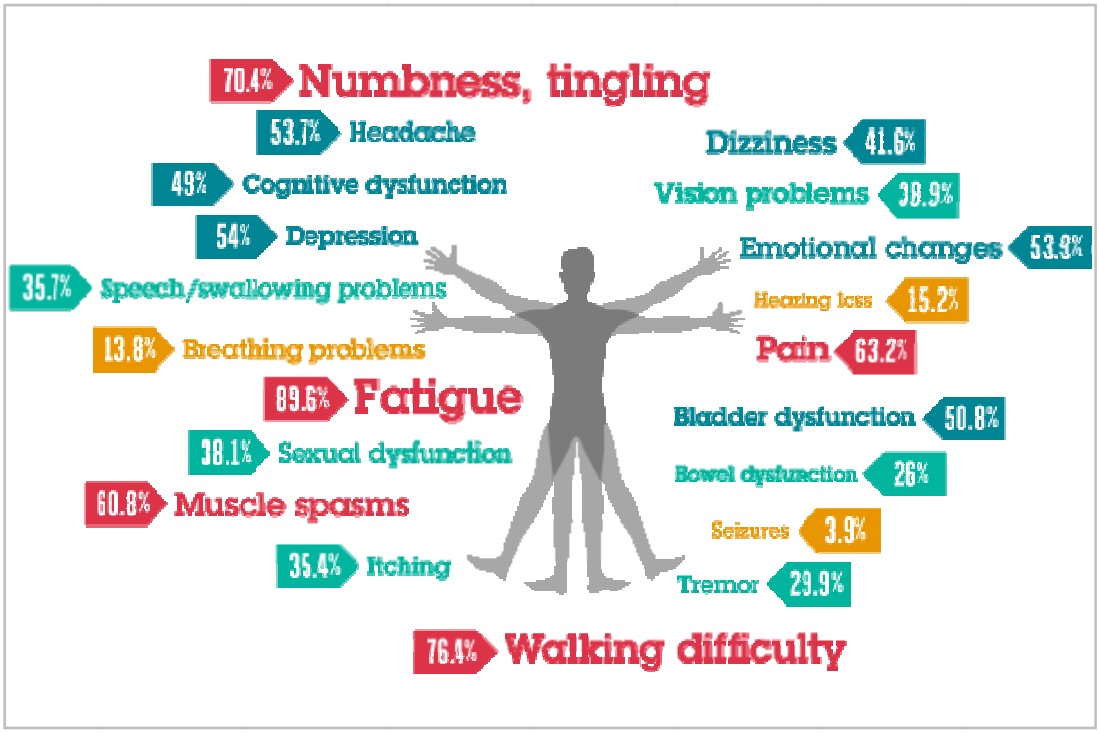

Figure 2.
MS symptoms frequency reported in the survey done by MS Health Union Community in America in 2013 with 3135 patients. (http://multiplesclerosis.net/).

There is no single diagnostic test to MS. So the criteria for MS diagnosis are:

-Onset usually between 10 and 60 years of age
-Symptoms and signs indicating lesions of central nervous system (CNS) white matter
-Evidence of two or more lesions upon examination by Magnetic resonance imaging (MRI) scan (Fig.3)
-Objective evidence of CNS disease on neurological examination
-A course following one of two patterns: two or more episodes lasting at least 24 hours and occurring at least one month apart, or a progressive course of signs and symptoms over at least six months
-No other explanation for the symptoms

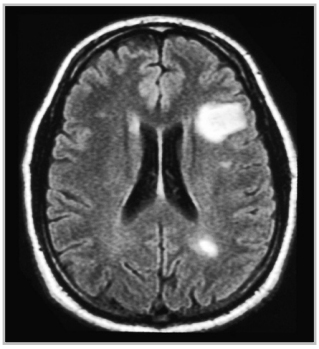

Figure 3.
MRI of 35-year-old man with RRMS, reveals the demyelinating lesions in the white matter.

Pathology and treatment of MS

The primary cause of neuron damage in MS is an inflammation of CNS. However, the specific elements that start this inflammation are still unknown. Studies have suggested that genetic, epigenetic, environmental and infectious agents as factors influencing the development of MS.

The immune system and MS

Numerous immunological studies have been done in human MS and in the animal model for the disease, known as the experimental autoimmune encephalomyelitis (EAE). They elucidated how the innate and the adaptive immune system are involved in MS pathology *(*Fig.4). ***(Loma and Heyman, 2011)***

The role of innate immune system in MS

The innate system plays opposite roles in MS; it is involved in MS pathology by promoting Th1 and Th17 differentiation, generating inflammatory reactions. On the contrary the same system can prevent the autoimmunity reaction by activation Treg cells and manage to repair the CNS by secretion of neurotrophic factors *(Gandhi et al., 2009).*

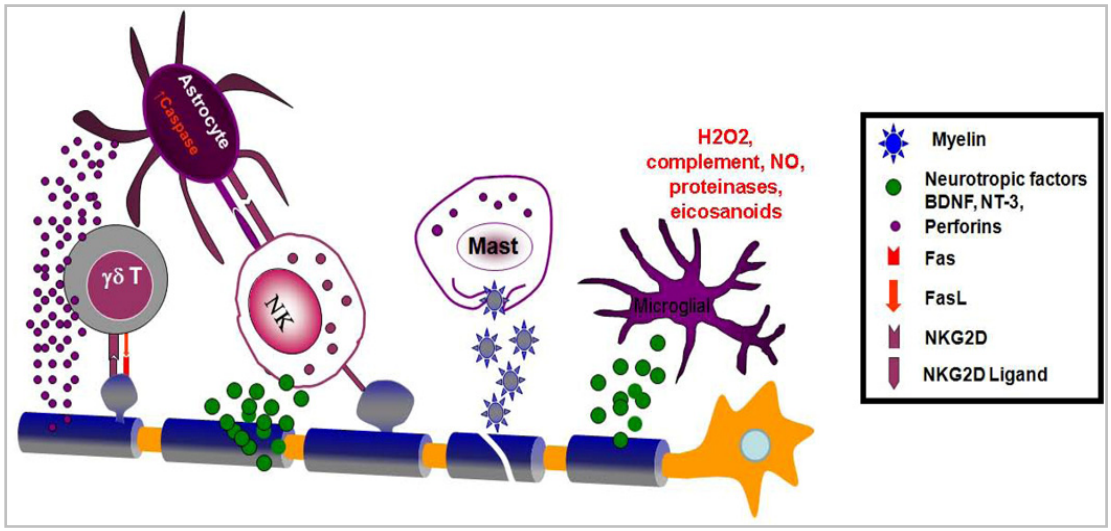

Figure 4.
Neuro-protective and neuro-destructive effects of innate immune system. Innate immune cells attack against the myeline via cytoxicity (oxygen species and perforins secretion by γ-δ T cells) or via direct interaction (Fas-FasL γ-δ T cells, phagocytose by mast cells and microglia). Innate immune cells also play a neuro-protective role by secretion of various neurotropic factors that help in promoting neurogenesis by NK cells *(Gandhi et al., 2010)*.

Dendritic Cells (DCs)

In MS patients, DCs are in the active phenotype with high expression of the activation markers CD40 and CD80, and with an increase in secretion of the proinflammatory cytokines. This activated phenotype of DCs is accompanied by an enhanced pro-inflammatory T cell response as defined by increased secretion of tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ). Additionally, it has been demonstrated that monocyte-derived DCs differentiated from MS patients, secrete more pro-inflammatory cytokines such as IFNγ, TNFα (Th-1 bias cytokine), IL6 *(Huang et al., 1999),* and IL23 (Th-17 bias cytokine) *(Vaknin- Dembinsky et al., 2006) (Vaknin- Dembinsky et al., 2008).*

Microglial/macrophage

Microglial cells constitue 10–20% of glial cells found in the CNS, where they are considered as resident macrophages contributing to MS by their involvement in phagocytosis, antigen presentation and production of cytokines. There are no markers distinguishing microglial cells from blood-derived macrophages in the CNS. Microglial cells are rapidly activated in response to injury, neuro-degeneration, infection, tumors and inflammation. In addition, microglial cells express all known toll-like receptors TLRs (TLR 1–9), the importance of these receptors in MS pathology is revealed in the increasing of their expression in brain lesions in EAE and in MS *(Andersson et al., 2008).* Morever, microglial and macrophages cells are involved in demyelination and phagocytosis of the degraded myelin which results in

augmentation of the expression of myeloperoxidases enzyme, causing neuronal damage *(Benveniste, 1997)*

Natural Killer cells (NK cells)

It has been shown that NK cells have cytotoxic activity *in vitro* towards oligodendrocytes, astrocytes and microglial cells during inflammation *(Saikali et al., 2007).* In addition, the presence of NK cells in demyelinating lesions has been detected in MS patients *(Traugott, 1985).* However, NK cells from mice with EAE are able to produce nuerotrophic factors such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), thus lead to repair and protect the CNS (*Hammarberg et al., 2000).* Consequently, all these data show that NK cells may play a regulatory role in MS (Gandhi et al., 2010)

Mast cells

Because of their presence in the brain, mast cells can interact with the myelin *(Medic et al., 2008),* furthermore these cells can phagocyte myelin vesicles. An *in vitro study* by *Johnson et al.* (*1988)* demonstrated that myelin proteins can stimulate mast cells which in turn liberate the protease contained in the granules that at the end leads to myelin basic protein (MBP) degradation.

NK-T cells

The number of total NK-T cells has shown to be decreased in MS. Nevertheless, these cells produce more IL10 which induces the Treg cells *(Sonoda et al., 2001)* and more IL4 (Th2 bias). Accordingly, it has been suggested that NK-T cells play an "immunoregulatory" role and might be involved in mediating the remission phase of MS *(Araki et al., 2003).*

Gamma-delta T cells (γ-δ T cells)

In MS patients, γ-δ T cells have been detected in the MS lesions *(Selmaj et al., 1991)*, their number has also shown to be increased in the cerebrospinal fluid (CSF) *(Shimonkevitz et al., 1993).* Additionally, the oligodendrocytes affected directly by γ-δ T cells cytotoxicity through perforine secretion and/or Fas- Fas Ligand interaction in MS *(Zeine et al., 1998).*

The adaptive immune system in MS

B cells

Many studies have proved the critical role of B cells in MS immune pathology. The crucial roles of antibody response in the development of CNS demyelination have been demonstrated in EAE studies (*Schluesener et al., 1987*) *(Genain et al., 1995)*. In addition, the antigen presentation by B cells has been shown as an important step for autoimmune attack against the myelin oligodendrocyte glycoprotein in CNS. *(Molnarfi et al., 2013)*. However, not all B cells participate in CNS attack. It has been demonstrated that the central B cells are intact in MS patients. Conversely, the peripheral B cells are defected and this defect potentially resulting from defective Treg function. *(Kinnunen et al., 2013)*. These B cells shown to produce high levels of IL6 (T cells stimulator) in MS patients compared with healthy individuals. *(Barr et al., 2012)*. In addition, myelin-reactive memory B-cells can be found in the peripheral blood of MS patients *(Harp et al., 2010).* These memory B-cells express high levels of CD20 in MS *(Roll et al., 2006).* The Igs secreted by B cells against minor myelin components have also been shown. Anti-MOG (myelin oligodendrocyte glycoprotein) antibodies are able to cause myelin destruction in EAE *(Schluesener et al., 1987; Litzenburger et al.,1998)* , in contrast to anti-MBP (myelin basic protein) or anti-PLP (myelin proteolipid protein) antibodies *(Genain et al., 1995)*. Anti-MOG antibodies have also been found in human MS lesions *(Genain et al., 1999).*

T cells

In the CNS, T-helper cells (Th, CD4+) recognize HLA class II antigens presented by antigen presenting cells (APCs), B cells, DCs, microglia and macrophages, the antigens represented by. However, cytotoxic T cells (CD8+) recognize HLA class I antigens, which are expressed by all nucleated cells. Depend on the cytokines secreted, CD4+ cells polarized to differentiate into effector T cells (Th1, Th2 and Th7). It has been demonstrated that in MS the cells promoting the inflammation are the proinflammatory cells Th1 which produce cytokines such as IFNγ and Th17 cells that secrete IL17, IL21, IL22 and IL26 *(Miyama et al., 2006)*. A migration of these cells has been detected from the periphery to the central nervous system followed by demyelination and axonal loss *(Gandhi et al., 2010)*. Another CD4+ T cells, known as regulatory T cells (Treg), that regulate the effector T cells (Th1, Th2 and Th17), also are involved in MS pathology. No difference has been found in total number of Treg between MS patients and healthy individuals; however the function of these cells has shown to be reduced in MS patients *(Haas et al., 2005)*. Besides the CD4+T cells, CD8+T cells are

involved in MS pathogenesis. CD8+ T cells have been detected in MS lesions, these cytotoxic cells act against CD4+T by perforin secreting leading to their inactivation; they also provoke the death of oligodendrocyte and glial cells *(Weber et al., 2007).*

MS treatments

Currently, there is no cure for MS; the therapies for MS are either immunomodulatory or immunosuppressive *(Duddy, 2015).* Most of these disease-modifying therapies are effective in the relapsing–remitting stage by reducing the frequency of relapses, and decreasing the formation of inflammatory lesions *(Hauser et al., 2013)*; however they do not influence the course of progressive MS and therefore are not sufficient enough to cure chronic neurological disability. In addition, other medications are used to treat the symptoms of MS (symptomatic treatment) improving the quality of patient life.

Disease-modifying therapies are used to reduce the frequency and severity of clinical attacks and the accumulation of lesions within the brain and spinal cord seen on MRI and to slow down the accumulation of disability. The 8 medication belonging to disease-modifying therapies have been approved by the US Food and Drugs Administration (FDA), they are classified as immunomodulators or immunosuppressants. The immunomodulators or receptor modulators are indicated for the treatment of patients with relapsing forms of MS, such as the interferon-beta (Avonex, Rebif, Betaseron and Extavia), glatiramer acetate (Copaxone) and natalizumab (Tysabri) *and* Fingolimod (*FTY720*). These medications help to slow the accumulation of physical disability and decrease the frequency of clinical exacerbations. On the other hand the immunosuppressants are used for their ability to suppress immune reactions such as Mitoxantrone (*Novantrone*), classified also as antineoplastic.

MS symptomatic treatments are aimed at maintaining function and improving quality of life *(Brunton et al., 2005).* It is common practice to treat acute relapses of MS with a short course typically 3 to 5 days. The first drug approved by the FDA was Dalfampridine (Ampyra, Acorda) to improve walking in MS patients *(Zivadinov et al., 2001)*.

Lately, stem cell therapy in axonal demyelination and neurological disability has had promising results in animal models as well as in patient clinical treatment *(Ben-Hur et al., 2013)*. Stem cell therapies may serve as potential therapy for neurodegenerative disease.

Mesenchymal stem cells (MSCs) have the capacity to modulate the intensity of an immune attack in MS by inhibiting antigen-specific T-cell proliferation and cytotoxicity and promoting the generation of Tregs and by promoting self-tolerance by inhibiting the DC

ability to become antigen presenting cells. Recently, many clinical trials have been done on MS patients treated with MSCs: In 2012, 10 SPMS patients received IV injection of autologous BM-MSCs, and six months after treatment, the results have been shown to improve in visual acuity and visual evoked response latency (Connick et al., 2012). In 2014, 9 RRMS patients have been treated with MSCs for 6 months, the results shown that this treatment reduced the lesions visualized by MRI; however non-significant decrease of in Th1 cells in peripheral blood was observed (Llufriu et al., 2014). Therefore, these results proved the neuroprotective effect of MSCs by promotion of endogenous oligodendrogenesis and remyelination *(Rivera et al., 2006).*

Environmental Factors in MS etiology

MS is a multifactorial disease. Its etiology likely to be an interplay of a variety of exogenous and genetic factors. In addition, the increase in incidence rates in short time intervals and in subgroups of patients suggests the strong action of environmental factors in developing and modulating MS (Pugliatti M. et al., 2012)

Infectious causes of MS

Specific transmissible agents have been proposed as possible causes of MS such as human herpesvirus (EBV and HHV-6), coronaviruses, JC virus, Varicella–Zoster virus, Torque Teno virus and *Chlamydia pneumonia*.

EBV

Many studies have been suggested the relation between EBV infection and MS. Numerous studies have shown that MS patients are almost universally seropositive for EBV, but not for other viruses *(Bray PF et al., 1983; Wandinger K-P et al., 2000)*. A meta-analysis of 13 case–control studies has found out that 99.5% of MS patients were EBV seropositive compared with 94.0% of controls, with EBV seronegativity (p<1E-9) *(Ascherio A et al., 2007)*. It has also demonstrated that among subjects not infected with EBV the risk of developing MS is extremely low, but after EBV infection there is an important increase in risk *(Levin LI et al., 2010).* Whereas most of these studies have suggested that EBV infection is a prerequisite for developing MS, this infection is not sufficient, by itself, to cause MS because the great majority of people infected with EBV do not develop the disease (Pakpoor J et al., 2013).

Recently the proposed role of EBV infection in the development of MS has been summarized by Pender and Burrows (2014). During primary infection, EBV infects autoreactive naïve B

cells, and then these cells proliferate intensely and differentiate into latently infected autoreactive memory B cells circulating in the blood. Proliferating and lytically EBV-infected B cells attacked by EBV-specific cytotoxic CD8+ T cells. However EBV-infected autoreactive memory B cells survived, so they enter the CNS where they take up residence and produce oligoclonal IgG and pathogenic autoantibodies, which attack myelin and other components of the CNS. In addition, in CNS, the autoreactive T cells activated by EBV-infected autoreactive B cells presenting CNS peptides, they produce cytokines such as interleukin-2 (IL2), IFNγ and TNFβ and orchestrate an autoimmune attack on the CNS with resultant oligodendrocyte and myelin destruction.

HHV-6

HHV-6 DNA and antibody to the virus were detected in blood samples from patients with MS but were not associated with clinical disease. (Liedtke et al.,1995) In addition, increased concentrations of IgG to HHV-6 were found in blood samples from patients with relapsing-remitting MS than in those with chronic-progressive MS, other neurological diseases, and healthy controls. (Gutierrez et al., 2002) HHV-6 antigen was also found in oligodendrocytes in 12 (80%) of 15 brain specimens from patients with MS. Other cells (neurons, astrocytes, macrophages, ependymal cells, choroid plexus, and endothelial cells) were also positive in brains from patients and controls; Overall, HHV-6 DNA and increased concentrations of antibody to HHV-6 in blood and CSF have been found in only a minority of patients with MS. Detection of HHV-6 DNA and antigen in brain might reflect HHV-6 reactivation from latency in blood T cells trafficking through the brains of patients with inflammatory CNS disease.

Coronaviruses

By use of in-situ hybridisation, Murray and colleagues (1992) detected coronavirus RNA in brains of 12 of 22 patients with MS. Human coronavirus 229E RNA was detected by in four of 11 patients with MS Stewart and colleagues (1992), but not in brains of six patients with neurological disease or in the brains of five healthy people. (Dessau et al., 2001)

JC virus

Polyoma JC virus is the cause of progressive multifocal leukoencephalopathy (PML), the only human demyelinating disease with a proven viral cause. The kidney is the only known site of latent infection. JC virus was not found in the urine of 53 patients with clinically definite MS or 53 controls matched for age and sex *(Boerman et al., 1993).* In a study of 37

patients with MS who were taking ciclosporin *(Stoner et al., 1996),* it was shown by PCR the DNA of JC virus in the urine of 30 (81%). JC virus DNA was detected in the CSF of 9% of patients with MS but not in any patients with other neurological diseases or in other controls *(Ferrante et al., 1998).*

Varicella–zoster virus

VZV is the causative agent of chickenpox. Recent studies conducted by *(Sotelo et al., 2007)* indicated the presence of VZV DNA in CSF and mononuclear blood cells of MS patients in relapse, while VZV viral particles were observed by electron microscopy in patients' CSF *(Sotelo et al., 2008).* Conversely, another study failed to show the presence of VZV virions or DNA in the CSF or in the acute plaques of MS patients *(Burgoon et al., 2009).* Therefore, the role of VZV in MS remains controversial.

Torque Teno virus

Not only pathogenic but also nonpathogenic infectious agents have been suggested to be involved in exacerbation and/or induction of MS. A study by Sospedra et al., (2005) determined the specificity of clonally expanded T cells from CSF of MS patients during disease exacerbation. These T cells were shown to recognize poly-arginine regions of Torque Teno virus (TTV) as well as evolutionary conserved motifs of other common viruses and prokaryotes, suggesting a mechanism of misdirected autoantigen response as a result of molecular mimicry. However, due to the paucity of data, the relation of TTV infection and MS remains ill defined.

Chlamydia pneumonia

C. pneumoniae is a gram-negative bacterium recently implicated in MS, as *C. pneumoniae* DNA and specific antibody has been detected in CSF of some patients with MS *(Sriram et al., 1999*). In an analysis of the humoral immune responses to *C. pneumoniae* in paired serum and CSF samples of patients with definite MS and other inflammatory and non-inflammatory neurological disorders, no difference in seropositivity was found between the groups, although titres of IgG specific for *C. pneumoniae* were substantially higher in the CSF of patients with MS than in controls. 16 (31%) of 52 patients with MS who were seropositive showed intrathecal synthesis of IgG specific for *C. pneumoniae* compared with only one (2%) of 43 seropositive controls (Krametter, et al., 2001). Overall, many studies have assessed a possible relation between *C. pneumoniae* and MS, however it is still not confirmed *(Tsai and Gilden, 2001).*

Vitamin D and MS risk

Many studies have demonstrated a strong association between vitamin D levels and risk of MS.

Vitamin D is a steroid vitamin *(Margherita et al. 2015)*. Vitamin D3 is the primary form of vitamin D, can be taken through the diet such as fish oils, or synthesized in the skin from 7-dehydroxycholesterol upon exposure to ultraviolet B radiation (UVB, wavelength 290–315 nm). Thus, vitamin D produced by UVB depends on seasons and latitude *(Webb et al., 1988)*. Also, many studies have demonstrated that sunscreen and clothes affected the production of Vitamin D from 7-dehydroxycholesterol. *(Matsuoka et al., 1987) (Matsuoka et al., 1992)*.

In addition, it was confirmed that higher childhood and early adolescence sunlight exposure associated with lower MS risk *(Van der Mei et al., 2003; Islam et al., 2007)*. Month of birth also was considered as a factor that influence MS susceptibility, fewer MS patients were born in late spring in compare to who were born in fall.*(Sadovnick et al., 2007)*. Geographic distribution also influence MS prevalence, in the areas further away from the equator where there is less sunshine MS is more common , which show a relationship between vitamin D and the risk of developing MS *(Rahnavard et al., 2010; Allison, 1960)*. It has been also reported that the highest prevalence was in North America (140 per 100 000) and Europe (108 per 100 000), however the lowest was in sub-Saharan Africa (2,1 per 100 000) and east Asia (2,2 per 100 000) *(Lazaros et al., 2015)* (Fig.5).

It has been proved that MS patients have lower serum vitamin D levels than healthy individuals and the intake of vitamin D from supplements had a protective effect against MS *(Munger et al., 2004; Munger et al., 2006; Ozgocmen et al., 2005)*. Additionally, in women an increasing of 10 nmol/L of serum 1,25(OH)2D concentration was associated with a 20% reduction in MS development possibility. The 25(OH)D serum level was considered as a significant predictor of MS risk developing. *(Kragt et al., 2009)*.

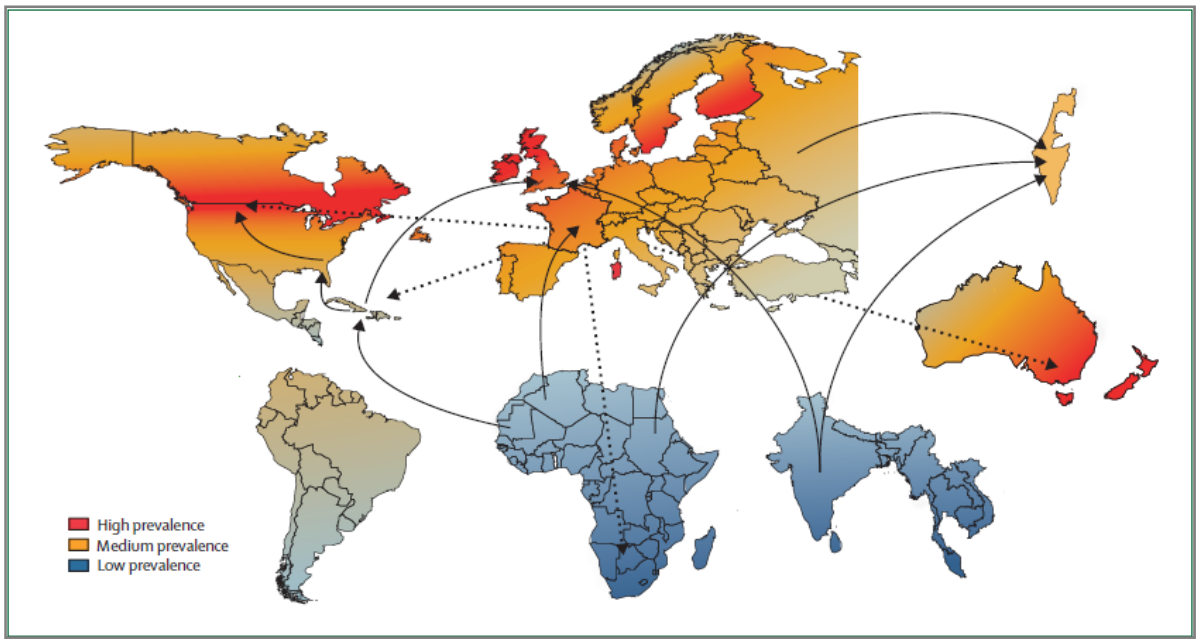

Figure 5.
Prevalence of multiple sclerosis. Map represents the geography distribution of MS. The medium prevalence of multiple sclerosis (orange), areas of exceptionally high frequency (red), and those with low rates (grey-blue) *(Compston and Coles, 2008)*.

Vitamin D pathway

Taken through the diet or synthesized in the skin vitamin D is transported in the blood by the vitamin D binding protein (DBP known as GC) to the liver where is transformed to 25-hydroxyvitamid D_3 (25(OH)D, calcifediol) via an enzymatic hydroxylation reaction by the cytochrome P450 vitamin D 25 hydroxylase (CYP2R1) (*Jones, 2008*) (Fig.6). The 25(OH)D, the major circulating form of vitamin D_3, is carried by the GC to the kidney. *GC* gene has reported to be associated with vitamin D insufficiency *(Wang et al., 2010*) and with serum vitamin D-binding protein level (*Moy at al., 2014).* In the kidney another cytochrome P450 enzyme, 1α-hydroxylase (CYP27B1), converts 25(OH)D to the biologically active form of vitamin D_3, 1,25-dihydroxyvitamin D_3 (1,25(OH)2D) also called calcitriol. *(Omdahl et al., 2002; Holick, 2007*). In addition, *CYP27B1* can be controlled by serum parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) in response to serum calcium and phosphate. The half-life of 25(OH)D is approximately 10–15 days (*Jones, 2008*), the highest amount of this vitamin D_3 form noted in the plasma (usually its concentration in the serum is 20–150 nmol/L or 8–60 ng/mL), however the largest amount is stored in adipose tissue and muscle (Mawer et al., 1972) with half-life of 2-3 months (Vieth, 2005). In addition, the affinity of DBP for 25(OH)D is approximately ten times higher than that for 1,25(OH)2D *(Kawakami et al., 1979),* that is explain the shorter plasma half-life of 1,25(OH)2D (4–20 h) (*Jones, 2008*) . Accordingly, the measurement of the level of 25(OH)D in the serum is

considered as an indicator of vitamin D status. The monooxygenase cytochrome P450 protein CYP24A1 can convert the 25(OH)D to an inactive component 24,25(OH)2D and able to catalyze the active form of vitamin D_3, 1,25(OH)2D to the inactive form 1,24,25(OH)2D *(Zimmerman et al., 2001; Plum and DeLuca, 2010).*

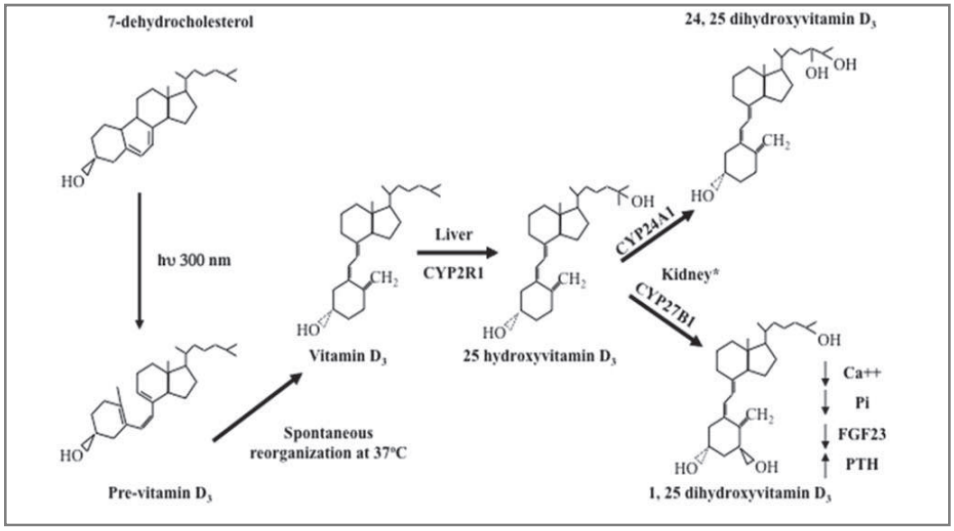

Figure 6.
Scheme of the vitamin D metabolism pathway (web: www.hcare.com).

1,25(OH)2D binds to the 1,25-dihydroxyvitamin D_3 receptor (VDR) which is a nuclear hormone receptor for vitamin D_3, ligand-VDR forms heterodimer with retinoid X receptor (RXR). This complex acts as an active transcription factor on the vitamin D_3 response element (VDRE) regulating the expression of genes that maintain mineral homeostasis and skeletal health, as well as immune, renal, and cardio-vascular function.*(Dusso, 2011)* (Fig.7).

Furthermore, VDR can regulate between 500 to 1000 coding genes, that means it can bind to up to 8000 loci in the human genome *(Haussler et al., 2013).*

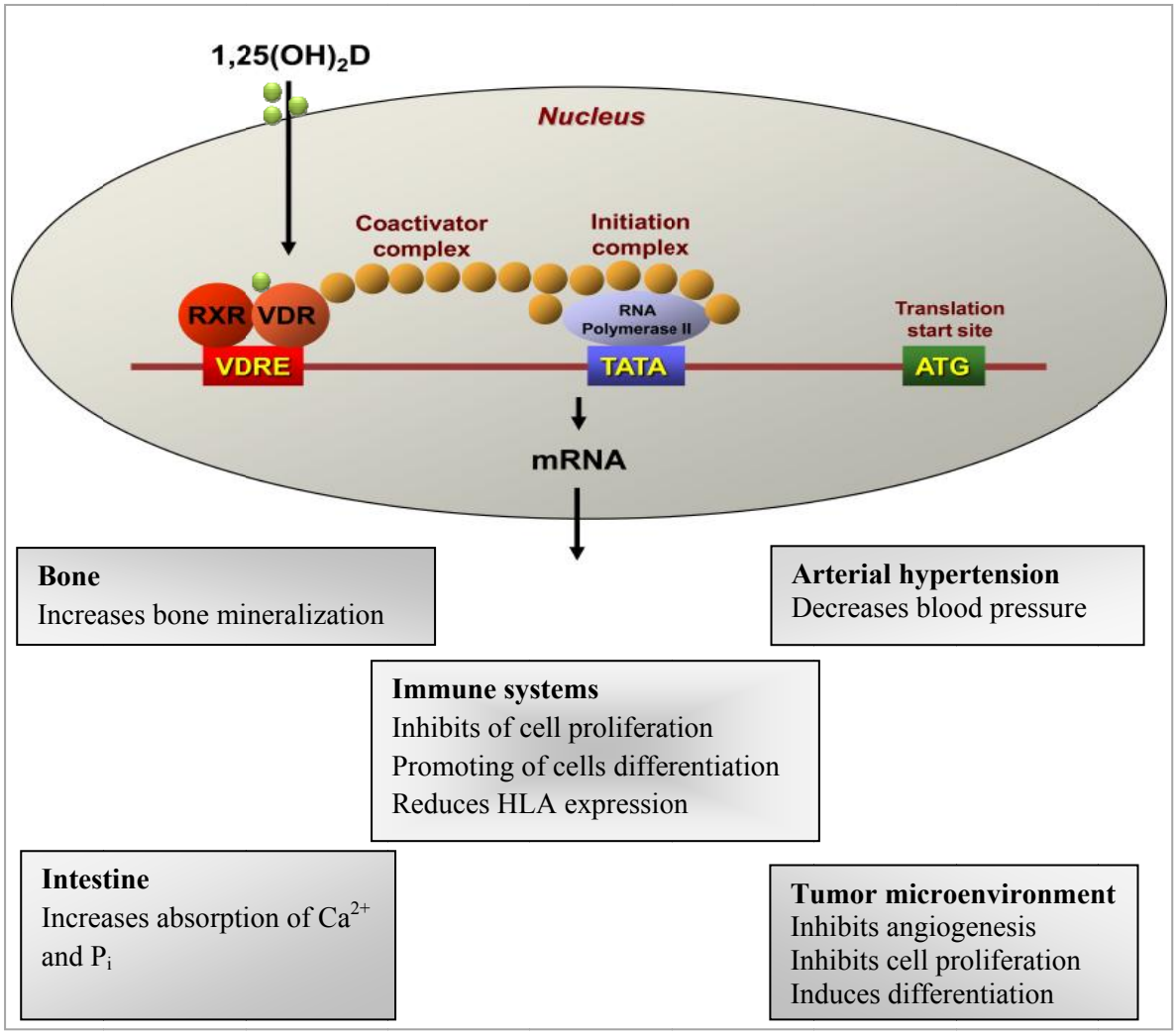

Figure 7.
The mechanism of action of 1,25(OH)2D. Calcitriol binds to VDR forming a heterodimer with RXR. These heterodimers bind to VDREs and start regulating the expression of different genes that may have activating or suppressing activity *(modified from Bikle, 2010*).

Vitamin D and immune system

Vitamin D_3 plays a crucial role in immune system (Fig.8), transforming immune cells into tolerogenic cells in the innate system and controlling cell proliferation and differentiation in the adaptive system that reflects how vitamin D_3 can be benefit in autoimmune reactions.

Vitamin D and the Innate Immune System

1,25(OH)2D can alter function and morphology of monocyte-derived DCs transforming them to tolerogenic cells. These cells have been shown to decrease the MHC class II and co-stimulatory molecule expression (CD40, CD80, CD86) that reduces antigen presentation and IL12 secretion and increases IL10, the tolerogenic inteleukin. Additionally, 1,25(OH)2D able to inhibit T cells cytokines such as IL12 and IL17 *(Banchereau and Steinman, 1998; Rigby et*

al., 1990; Baeke et al., 2010). Another study has been demonstrated that a high dose of 1,25(OH)2D supplementation in healthy humans reduces significantly proinflammatory cytokine IL6 produced by peripheral blood mononuclear cells (PBMC) *(Müller et al., 1991).* Accordingly, all these effect favour induction of Treg, which have a critical role in the immune responses control and autoreactivity development. *(Steinman et al., 2003).* However, the effect of Vitamin D on NKT and NK cells still unclear. *(Peelen et al., 2011).* On the other hand, 1,25(OH)2D able to induce the expression of CAMP gene which encode the cathelicidin, antimicrobial peptide, that plays a critical role in mammalian innate immune defence against invasive bacterial infection by binding to bacterial lipopolysaccharides LPS. It also has an antifungal and antiviral activity *(Zanetti , 2004).* Furthermore, cathelicidin play a role in cell chemotaxis, immune mediator induction, and inflammatory response regulation. (Niyonsaba et al.,2002).

Vitamin D and the adaptive Immune System

1,25(OH)2D has antiproliferative effects on B cells inhibiting their differentiation, proliferation, inducing apoptosis leading to a decrease in the immunoglobulin production. In addition, 1,25(OH)2D prevents the generation of B memory and plasma cells *(Lemire et al., 1984; MChen et al., 2007; Baeke et al., 2010)*. As for T cells, 1,25(OH)2D suppresses Th cells proliferation, differentiation and modulates their cytokines production, inhibiting the secretion of proinflammatory Th1, such as IL2, IFNγ and TNFα. On the other hand induce Th2 to produce more anti-inflammatory cytokines (IL3, IL4, IL5, and IL10). Th17 also are affected by 1,25(OH)2D to produce less IL17 *(Prietl et al. 2013)*. A combination of 1,25(OH)2D and IL2 effects has been shown to change T cells into tolerogenic cells via increasing the expression of Tregs genes *(Jeffery et al., 2009).*

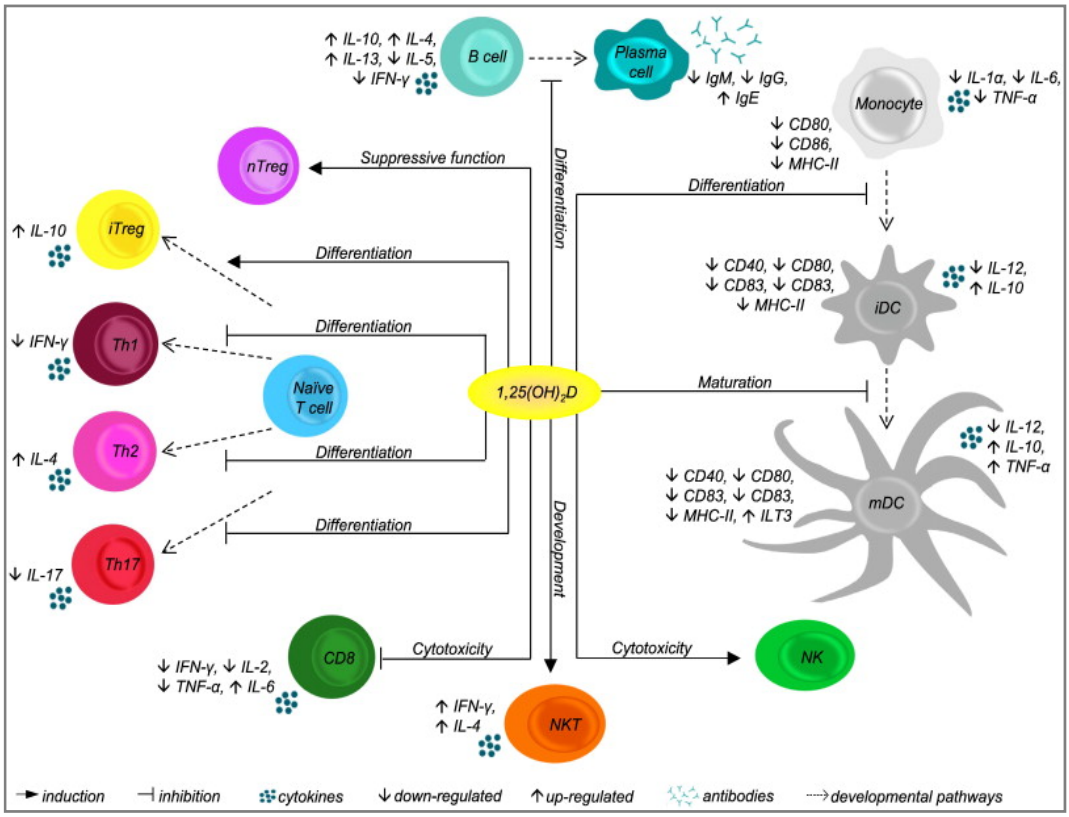

Figure 8.
An overview of the overall effects of $1,25(OH)_2D_3$ on monocytes, dendritic, T , NKT, NK and B cells in both immune systems. *(Peelen et al., 2011).*

Vitamin D with pro-inflammatory transcription factors and signaling pathways

1,25(OH)2D and its receptor complex VDR/RXR can interact with transcription factors such as NF-κB, nuclear factor of activated T-cells (NFAT), or TGF-β receptor which leads to anti-inflammatory effects (Fig.9)

NFκB

Active VDR inhibits NF-κB activation and signaling. NFκB is a ubiquitously expressed transcription factor which represents a heterodimer. When NFκB is inactive it interacts with IκB which keeps it in the cytosol (*Karin and Lin, 2002*). Upon cell activation by proinflammatory stimuli, IκB is phosphorylated and subsequently ubiquitinylated, which leads to proteasomal degradation of the IκB protein. Free NFκB translocates to the nucleus where it activates transcription of proinflammatory cytokines, antiapoptotic factors as well as of enzymes involved in the generation of proinflammatory mediators such as COX-2 (*Karin and Lin, 2002; Tsatsanis et al., 2006*).

It has been shown in lymphocytes that 1,25(OH)2D down-regulates NF-κB levels (*Yu et al., 1995*) and that the vitamin D analog TX 527 prevents NF-κB activation in monocytes (*Stio et*

al., 2007). NFκB activation by 1,25(OH)2D-mediated up-regulation of IκB expression was reported in human peritoneal macrophages (*Cohen-Lahav et al., 2006*) (Fig. A). Additionally, interference of vitamin D signaling with DNA binding of NFκB was found (*Harant et al., 1998*).

In addition, it was shown that 1,25(OH)2D inhibits NF-κB activity in human MRC-5 fibroblasts but not translocation of its subunits p50 and p65. The partial inhibition of NFκB DNA binding by 1,25(OH)2D was dependent on de novo protein synthesis, suggesting that 1,25(OH)2D may regulate expression of cellular factors which contribute to reduced DNA binding of NFκB (*Harant et al., 1998*). Thus, it seems that vitamin D is able to inhibit NFκB activation as well DNA binding (Fig9).

NFAT

Another interesting target for the anti-inflammatory signaling of vitamin D is transcription factor NFAT (Fig. A). NFAT activated by dephosphorylation by calcineurin which leads to translocation of this protein and transcriptional activation of proinflammatory genes such as IL2 and cyclooxygenase-2 (Duque et al., 2005; Muller and Rao, 2010). In T-lymphocytes, it was shown for the IL2 promoter that VDR-RXR heterodimers bind to an NFAT binding site and thus inhibit NFAT activity *(Takeuchi et al., 1998).* Similar results were obtained for IL17 where 1,25(OH)2D blocked NFAT activity which contributed to repression of IL17A expression in inflammatory CD4+ T cells by the hormone (*Joshi et al., 2011*).

TGF-β

TGF-β is a pleiotropic cytokine with a broad range of biologic effects, which is involved in the regulation of inflammatory processes on several levels. A main mechanism in this respect is the maintenance of T cell tolerance to self or innocuous antigens (*Li and Flavell, 2008*). In cancer-associated inflammation, TGF-β suppresses the anti-tumor activity of diverse immune cells, including T-cells, natural killer (NK) cells, neutrophils, monocytes and macrophages (*Bierie and Moses, 2010*). A great number of studies focused on the role of TGF-β in fibrosis and associated inflammation. In these diseases, TGF-β regulates influx and activation of immune cells, as well as the actual fibrotic process, and thus the delicate balance between an appropriate inflammatory response and the development of pathologic fibrosis (*Flanders, 2004; Sheppard, 2006; Lan, 2011*). TGF-β signaling has been attributed both to canonical TGF-β signaling via the Smad proteins (signal-dependent transcription factors).

The influence of vitamin D on inflammation-related signaling via TGF-β and Smad has mainly been investigated in models of fibrosis, and distinct mechanisms have been

elucidated. Activation of 1,25(OH)2D signaling by the natural ligand itself or its synthetic analogs reduces TGF-β expression (*Kim et al., 2013*)

More than 104 genomic sites were found to be co-occupied by both VDR and SMAD3 in hepatic stellate cells, and an analysis of the spatial relationships between the two transcription factors revealed that the respective response elements were located within a range of 200 base pairs (one nucleosomal window). Mechanistically, TGF-β signaling seems to deplete nucleosomes from the co-occupied sites and thus allow access of VDR to these sites. Vitamin D signaling on the other hand seems to limit TGF-β activation by inhibited coactivator recruitment. Spatiotemporal analysis revealed that 1,25(OH)2D / TGF-β-induced VDR and SMAD3 binding to the co-occupied sites were inversely correlated. The maximum of SMAD3 binding occurred 1 h after treatment and was reduced by 70% after 4 h, when VDR binding was maximal. Therefore, TGF-β signaling seems to change the chromatin architecture in a way in which liganded VDR can reverse Smad activation.

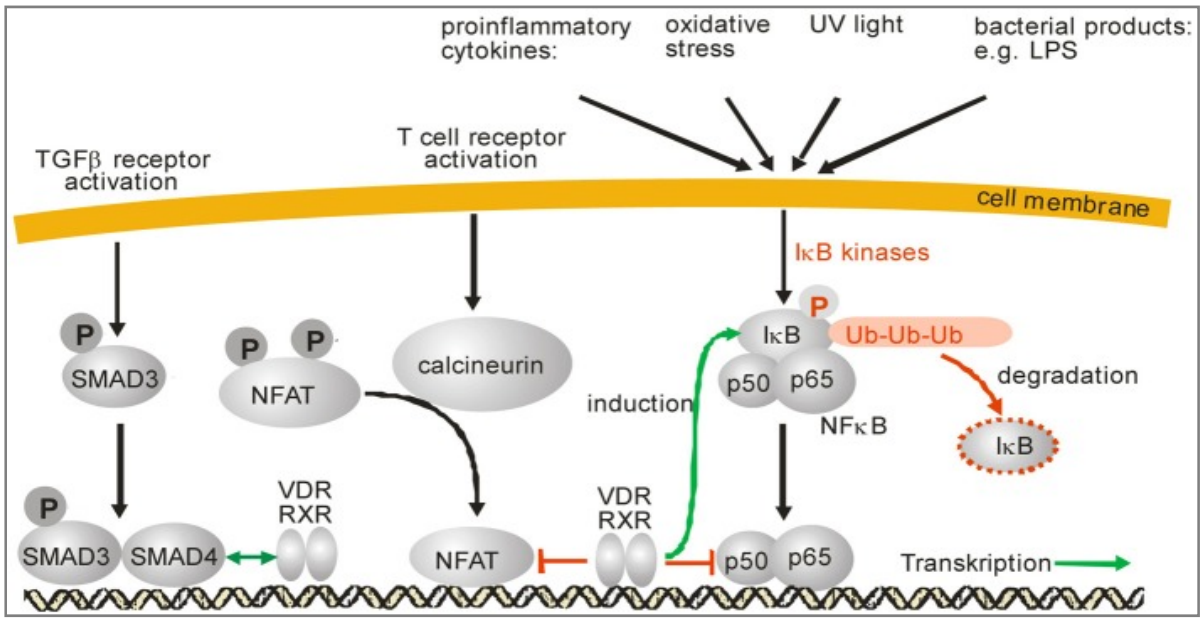

Figure 9.
SMAD, NFAT and NFκB signaling and modulation of these signaling pathways by 1α,25$(OH)_2D_3$-VDR/RXR**.** IκB phosphorylation after various cell stress signals leads to its ubiquitinylation and subsequent proteosomal degradation. After IκB degradation, NFκB is released and translocates into the nucleus where it binds to DNA and modulates gene expression. Activation of NFAT is mediated by the protein phosphatase calcineurin which dephosphorylates NFAT. After dephosphorylation, NFAT translocates into the nucleus, interacts with a variety of other transcription factors and modulates gene expression. Activation of TGFβ receptors leads to phosphorylation of SMAD2 and SMAD3 as well as subsequent translocation into the nucleus. SMAD3 forms a complex with SMAD4 and modulates gene expression of its target genes. After activation by 1α,25(OH)2D the VDR/RXR heterodimer can inhibit NFκB signaling either by induction of IκB or by interference with NFκB DNA binding. Also, inhibition of NFAT signaling was reported by prevention of NFAT binding to its response elements.

Tobacco smoke

Tobacco smoking is a well-established environmental risk factor for MS since many case-control and meta-analysis studies had been done and demonstrated the association between MS incidence and smoking in different population such as Canadian (*Ghadirian et al. 2001*) , European (*Hedstrom et al. 2009*) and Swedish (*Carlens et al. 2010*). Several other smaller case–control or cohort studies have been published. Overall, most of them (*Rodriguez Regal et al. 2009; Pekmezovic et al. 2006; Hernan et al. 2005; Riise et al. 2003*), but not all (*Simon et al. 2010; Silva et al. 2009; Russo et al. 2008*) studies showed that smoking is associated with increased MS susceptibility. Moreover, it has been showed that MS risk increased with increasing duration of exposure (*Hedstrom et al. 2011*) as well as increasing with nicotinine levels (*Sundstrom et al. 2008)*. However, the mechanisms by which smoking might influence the risk of MS and/or its clinical course are unclear (*Wingerchuket al., 2012*). It might increase MS susceptibility through epigenetic modifications *(Koch et al. 2013b).*

Epigenetic consequences

Environmental exposures such as malnutrition, tobacco smoke, air pollutants, metals, organic chemicals, sun exposure, sources of oxidative stress, and the microbiome may induce changes in epigenetic state (*Cortessis et al., 2012*). Epigenetics represents all heritable or non-heritable modification (DNA methylation, histone modifications and RNA interference) that can alter the expression or translation of the gene with no modification in DNA sequences. The epigenetics changes can be generated by the external and environmental factors that turn genes on or off, such as vitamin D deficiency, sun exposure, smoking, chemicals poducts and Ebstein–Barr virus. In addition, these changes are specific to tissues.

Many studies have been also proved that the risk of MS have increased in smoking individuals (*Herna´n et al. 2001*). In addition, smoking has been shown to alter histone modification, pattern of DNA methylation and miRNA expression and therefore might potentially increase MS susceptibility through epigenetic modifications *(Koch et al. 2013b).* Additionally, EBV causes chronic latent viral infection in lymphocytes and upregulates DNMTs that play a mean role in cell proliferation and genome stability. Vitamin D also can change the expression of genes that modify histones and thus might be a potential epigenetic regulator in MS *(Koch et al. 2013a).*

MS Genetics

It has been demonstrated that the family history reveals predisposition to MS. The family recurrence in monozygotic twins represents 35%. The age-adjusted risk is higher for siblings (3%), parents (2%), and children (2%) than for second-degree and third-degree relatives. In addition, recurrence is higher in the children of conjugal pairs with MS (20%) than in the offspring of single affected (2%) (Fig.10) (*Compston and Coles, 2002*).

The first genetic factor related with MS was the human leukocyte antigen (HLA) locus in the 1970s *(Jersild and Fog, 1972)*. This locus is located in the short arm of chromosome 6, in a region called major histocompatibility complex (MHC). MHC genes encode highly polymorphic cell-surface glycoproteins that are key components of the immune system. However, HLA by itself cannot explain the whole genetic component of MS. On the other hand, many non-MHC genes have been found to be associated with MS. The majority of the genes studied are related to immune response *(McElroy and Oksenberg, 2011).*

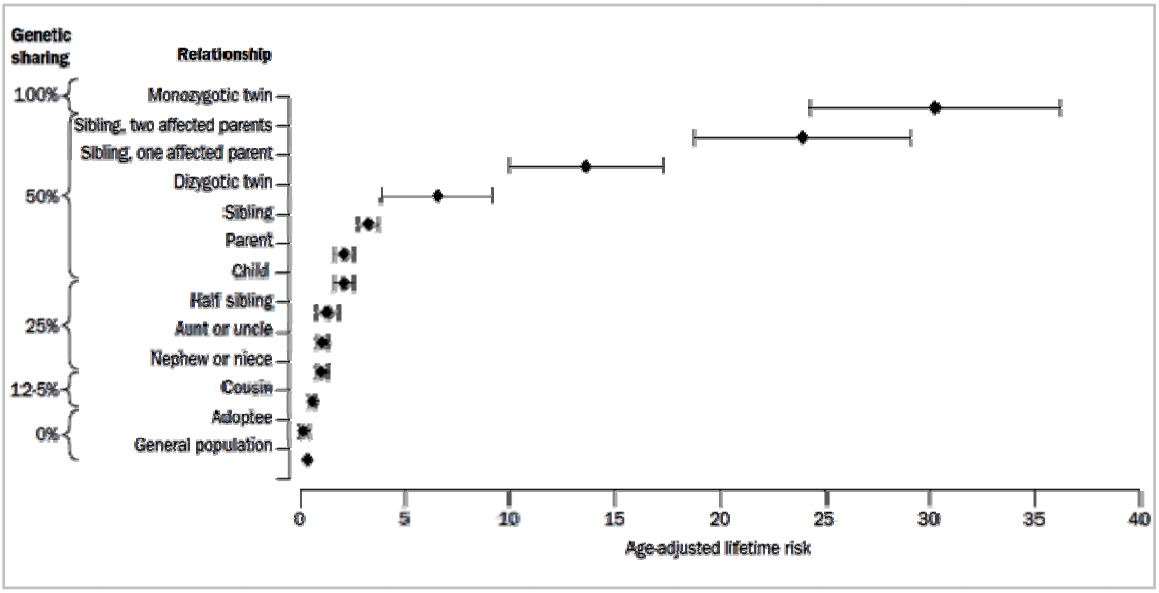

Figure 10.
Recurrence risks for multiple sclerosis in families. Age adjusted recurrence risks for different relatives of probands with multiple sclerosis. Pooled data from population based surveys. Estimated 95% Cl are shown (kindly prepared by Simon Broadley) (Compston and Coles, 2002).

Genome investigation tools and methods

New powerful tools for investigating the genetic architecture of human disease have been developed recently such as Genome Wide Association Studies (GWAS), ImmuChip studies and their meta-analysis. They have quickly become a fundamental part of modern genetic studies playing a central role in the human genetics revolution. These studies are based on data of genetic projects dedicated to provide detailed catalogue of human genetic variation: HapMap and 1000 Genomes projects.

International HapMap Project

The International HapMap Project is a collaboration among researchers at academic centers, non-profit biomedical research groups and private companies in Canada, China, Japan, Nigeria, the United Kingdom, and the United States. The International HapMap Consortium launched the International HapMap Project in 2001, to develop a haplotype map ("HapMap") of the human genome and to describe the common patterns of human genetic variation. It comprised three phases.

In 2005, the International HapMap Consortium released the Phase I HapMap, a resource consisting of over a million accurate and complete single nucleotide polymorphism (SNP) genotypes generated in 269 individuals from four geographically diverse populations: the Yoruba in Ibadan, Nigeria; Japanese in Tokyo, Japan; Han Chinese in Beijing, China; and the CEPH (U.S. Utah residents with ancestry from northern and western Europe). The Phase I HapMap includes data from ten 500-kb regions (the "HapMap ENCODE I regions") that were sequenced to assess the genotyping.

Phase II HapMap was released in 2007, which added over 2.1 million SNPs to the original map in the same 269 individuals. The Phase II HapMap enables an improved choice of tag SNPs. Phase III was finished in 2009; 1.6 million SNPs were genotyped in 1,184 reference individuals from 11 global populations, and sequenced ten 100-kilobase regions in 692 of these individuals. This integrated data set of common and rare alleles, called 'HapMap 3', includes both SNPs and copy number polymorphisms (CNPs). Thus, the HapMap has become an important tool for researchers to use to find genes that affect health / disease, and response to drugs and environmental factors. All HapMap data are freely available to the public through the database dbSNP. A graphical browser for HapMap genotypes is also available at http://www.hapmap.org/cgi-perl/gbrowse/gbrowse.

Moreover, the B lymphocytes from all blood samples of project have been converted by the non-profit Coriell Institute for Medical Research into lymphoblastoid cell lines (LCLs). Therefore, Coriell provides purified DNA and different type of cell lines most of them are LCLs, fibroblast and somatic cell hybrid for research projects that have been approved by the appropriate ethics committees.

1000 Genomes Project

The 1000 Genomes Project, launched in January 2008, it was the first project to sequence the genomes of a large number of people from 26 different ethnic populations to provide a comprehensive resource on human genetic variation, using newly developed technologies faster and less expensive. The goal of the 1000 Genomes Project was to find most genetic variants that have frequencies of at least 1% in the populations studied. (Durbin et al., 2010).

1000 genomes project combined data from 2504 unrelated samples; in addition it released 84 millions variants, including SNPs, indels (insertion or the deletion of bases) and structural variations such as deletions, duplications, copy-number variants, insertions, inversions and translocations. These data not only include several new populations, but also include the populations in the HapMap. It started with three Pilot projects, to provide data that help to design the full-scale project: Pilot 1 sequenced lightly 179 samples from the HapMap CEU, YRI, CHB, and JPT populations. Pilot 2 sequenced deeply two trios. First trios: CEU, NA12878 (daughter) and mother NA12892 and father NA12891. Second trios: YRI, NA19240 (daughter) and mother NA19238 and father NA19239. Pilot 3, sequenced deeply in the exons of 906 genes, in 697 samples from the CEU, TSI, YRI, LWK, CHB, JPT, and CHD HapMap III populations.

Genome Wide Association Studies (GWASs) of MS

GWAS is an approach that involves scanning of multiple markers across the entire genomes of many people belonging to two big populations: cases (patients) and controls (healthy), to find genetic variations associated with a particular disease. So the GWAS is a test for statistical associations between common gene variants (SNPs) and a phenotype.

Besides the GWASs, there is a strong trend for studies to combine data from multiple GWAS studies into a meta-analysis to validate previous findings, expand findings from single populations to universal effects, and identify novel gene effects. Collectively, these analysis have increased the power of the GWAS studies, reduced the numbers of false positives, and enabled the detection of small genetic effects that are associated with a number of diseases.

MS GWASs have detected hundreds of variants at genomic loci that are associated with this disease in many human populations. Despite the fact that the determination of loci facilitates the basic research in MS human genetics, the challenge is to identify causal genes in these loci and to exploit subtle association signals. From 2007 to the present, 24 GWAS and meta-analysis of MS have been published, these GWASs uncovered around 200 SNPs associated with MS disease, and they also reported more than 100 loci as associated with MS (Table 1).

In general, the loci found to reach genome-wide significance have weak additive predictive power for specific phenotypes, which for several traits limits their clinical relevance at present. Most of the loci are noncoding, and many are far from discovered genes and, because of linkage disequilibrium (LD), encompass many variants; therefore, they are not immediately informative or biochemically tractable for experimental work. GWASs results sometimes are not replicated across studies or populations *(Nebert et al., 2008)*, revealing the reports of false positives, that introduce the suspicion of the validity of novel associations, especially when they involve non-coding sequence *(Ward and Kellis, 2012)*.

MS genetic studies based on ImmunoChip

Deep replication of meta- GWASs and fine mapping of GWAS loci were done without the filtering of SNPs on spacing or LD, as had been used in earlier GWAS.

The Immunochip is a consortium based custom Illumina Infinium SNP genotyping array, specific to 12 immunologically related human diseases. The array design integrates relevant 1000Genomes data (CEU population), disease-specific resequencing data and known immune-mediated disease loci identified by common variant GWAS. The probes on this array interrogate 195,806 SNPs and 718 small insertion–deletions.

The final design incorporates 186 distinct loci containing markers meeting genome wide significance criteria ($P<5\times10\text{-}8$) from twelve such diseases (autoimmune thyroid disease, ankylosing spondylitis, Crohn's disease (CD), celiac disease (CCD), IgA deficiency, multiple sclerosis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) , type 1 diabetes (T1D) and ulcerative colitis) (*Parkes et al., 2013*).

Study (Reference)	SNPs	SNPs nb	Report gene(s)	Genes nb	Initial Sample Size		Replication Sample Size		Ance-stry
					Case	Control	Case	Control	
Fortune et al., 2015	rs1858037.		*SPRED2.*		5112	12370			GBR
Beechman et al., 2013	rs1335532, rs11810217, rs4648356, rs11581062, rs7522462, rs1323292, rs12048904, rs233100, rs3761959, rs7595037, rs10201872, rs12466022, rs17174870, rs281783, rs669607, rs9282641, rs11129295, rs2293370, rs771767, rs4285028, rs4308217, rs10936599, rs9821630, rs2243123, rs1500710, rs228614, rs6821894, rs4613763, rs2546890, rs6897932, rs4075958, rs756699, rs1062158, rs350058, rs2302103, rs1738074, rs11154801, rs17066096, rs802734, rs13192841, rs12212193, rs9321490, rs11755724, rs854917, rs354033, rs6952809, rs1843938, rs11984075, rs2066992, rs2214543, rs2019960, rs4410871, rs1520333, rs6986386, rs290986, rs7090512, rs3118470, rs7923837, rs1250550, rs793108, rs7912269, rs650258, rs630923, rs4409785, rs7941030, rs694739, rs491111, rs1800693, rs12368653, rs10466829, rs806321, rs17594362, rs4902647, rs2119704, rs2300603, rs7200786, rs13333054, rs2744148, rs386965, rs11864333, rs9891119, rs180515, rs4792814, rs8081176, rs1373089, rs7238078, rs12456021, rs1077667, rs2303759, rs874628, rs307896, rs8112449, rs7255066, rs281380, rs2278442, rs2248359, rs2425752, rs6062314, rs2762932, rs2283792, rs140522, rs2072711.	102	*CD58, EVI5, MMEL1, SLC30A7, C1orf106, RGS1, EXTL2, DDAH1, FCRL3, PLEK, **SP140**, MERTK, C2orf69, CD86, EOMES, TIMMDC1, NFKBIZ, SLC15A2, CD86, MYNN, PLCL2, IL12A, ARHGEF3, MANBA, SORBS2, PTGER4, IL12B, IL7R, RGS14, TCF7, NDFIP1, DIAPH1, TAGAP, AHI1, IL22RA2, PTPRK, BACH2, MYB, RREB1, RRAGD, ZNF767P, CHST12, CARD11, ELMO1, IL6, NDUFA4, PVT1, MYC, PKIA, TNKS, SYK, IL2RA, IL2RA, HHEX, ZMIZ1, ZNF438, KCNMA1, CD5, CXCR5, UBASH3B, PRDX5, TNFRSF1A, AGAP2, **CLECL1**, DLEU1, Vwa8, ZFP36L1, GPR65, BATF, CLEC16A, IRF8, SOX8, MAF, RMI2, STAT3, RPS6KB1, MAP3K14, RNF213, WNT9B, MALT1, ALPK2, TNFSF14, DKKL1, MPV17L2, SAE1, CDC37, PVR, MAMSTR, ICAM3, **CYP24A1**, NCOA5, ZBTB46, , MAPK1, ODF3B, NCF4.*	95	9772	17376	4218	7296	CEU
Goris et al., 2013	rs2935183.	1	*NPEPPS.*	1	4088	12030			CEU
Leone et al., 2013.	rs9320598.	1	*C6orf167, MMS22L, KLHL32, KIAA1900.*	4	513	49	1684	200	CEU
Lill et al., 2013	rs228614, rs630923, rs2744148, rs180515, rs6062314.	5	*MANBA, CXCR5, SOX8, RPS6KB1, ZBTB46.*	5					CEU
Mero et al., 2013.	rs17411949, rs6659742, rs9283487, rs3817963, rs3129871, rs3828840, rs9271640, rs3129720, rs9275563.	9	*CLSTN2, C10ORF204, PRKRA, BTNL2, HLA-DRA, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DQA.*	9	1367	161	3029	374	CEU
Gourraud et al., 2013.	rs13279485, rs4669226, rs9568281, rs7924357, rs733724, rs17267338, rs17749211, rs1821625, rs12513380, rs4916321, rs533259, rs17090640, rs4271113, rs2920001, rs11026091, rs5978649.	16	*ARHGEF10, LOC339788, ARL11, MMP10, HACE1, VTI1A, SAMD12, NAP5, TLL1, AK127238, RNASEL, LOC285501, TMEM47, DSC1, MRGPRE, EGFL6.*	16	284				CEU

Table 1a.
GWAS and meta-analysis studies of MS from 2013 to 2015.

Study (Reference)	SNPs	SNPs nb	Report gene(s)	Genes nb	Initial Sample Size		Replication Sample Size		Ance-stry
					Case	Control	Case	Control	
Matesanz F, 2012.	rs9292777.	1	*PTGER4.*	1	2127	4558	2785	2940	CEU
Martinelli-Boneschi F, 2012.	rs3129934.	1	*C6orf10.*	1	197	234	379	398	CEU
Patsopoulos NA, 2011.	rs170934, rs2150702, rs6718520, rs2300747, rs9260489, rs3129889, rs12722489, rs2293152, rs6074022, rs2546890, rs8070463, rs10411936, rs2681424, rs7592330, rs1738074, rs1250542, rs7191700, rs9596270, rs10866713.	19	*EOMES, MLANA, THADA, CD58, HLA-B, HLA-DRB1, IL2RA, STAT3, CD40, KPNB1, TBKBP1, TBX21, EPS15L1, ILDR1, CD86, PLEK, FBXO48, C2orf13, TAGAP, ZMIZ1, TNP2, PRM3, PRM2, PRM1, C16orf75, IL12B.*	25	5545	12153	NA	NA	CEU
Sawcer S, 2011.	rs12368653, rs11154801, rs12456021, rs12212193, rs2300603, rs7522462, rs2293370, rs650258, rs1335532, rs9282641, rs8112449, rs6952809, rs7200786, rs10466829, rs630923, rs2248359, rs233100, rs2303759, rs806321, rs11129295, rs11810217, rs3761959, rs2119704, rs7923837, rs2243123, rs2546890, rs17066096, rs7090512, rs6897932, rs13333054, rs17594362, rs386965, rs7238078, rs281380, rs228614, rs4792814, rs2283792, rs17174870, rs4648356, rs874628, rs9321490, rs4410871, rs10936599, rs12466022, rs669607, rs13192841, rs4409785, rs2425752, rs1062158, rs771767, rs140522, rs1520333, rs9821630, rs7595037, rs4613763, rs802734, rs7255066, rs2019960, rs1323292, rs4075958, rs180515, rs11755724, rs307896, rs11581062, rs2744148, rs10201872, rs9891119, rs290986, rs1738074, rs756699, rs1077667, rs6062314, rs4902647, rs1250550, rs793108, rs354033, rs12048904, rs4285028, rs4308217, rs3118470.	80	*AGAP2, **CYP27B1**, AHI1, ALPK2, BACH2, BATF, C1orf106, KIF21B, C3orf1, TMEM39A, CD5, CD6, CDC37, TYK2, CHST12, CLEC16A, **CLECL1**, CXCR5, **CYP24A1**, DDAH1, DKKL1, DLEU1, EOMES, EVI5, EXTL2, FCRL3, GPR65, HHEX, IL12A, IL12B, IL22RA2, IRF8, KIAA0564, MAF, MALT1, MAMSTR, MANBA, NFKB1, MAP3K14, MAPK1, MERTK, MMEL1, MPV17L2, MYB, MYC, MYNN, OLIG3, NCOA5, CD40, NDFIP1, NFKBIZ, ODF3B, SCO2, PKIA, IL7, PLCL2, PLEK, , PTPRK, THEMIS, PVR, PVT1, RGS1, RGS14, RPS6KB1, RREB1, SAE1, SLC30A7, VCAM1, SOX8, **SP140**, SYK, TAGAP, TCF7, TNFSF14, ZBTB46, ZFP36L1, ZNF438, ZNF767, ZNF746, EXTL2, VCAM1, SLC15A2, CD86, CD86, DRB1.*	85	9772	16849	4218	7296	CEU
Briggs FB, 2011.	rs12644284, rs17149161, rs7789940, rs758944, rs7779014, rs11962089.	6	*TRIM2, YWHAG, POPDC3.*	3	1470	NA	NA	NA	CEU
Wang JH, 2011.	rs4939490, rs12025416, rs931555.	3	*CD6, CD58, IL7R.*	3	2124	6720	1618	1988	CEU

Table 1b.
GWAS and meta-analysis studies of MS in 2011 and 2012.

Study (Reference)	SNPs	SNPs number	Report gene(s)	Genes number	Initial Sample Size		Replication Sample Size		Ancestry
					Case	Control	Case	Control	
Baranzini et al., 2010.	rs794185, rs8074980, rs2038256, rs13117816, rs8007846.	5	*SUMF1, CUEDC1, FOXG1B, FLJ11017, FUT8.*	5	382		NA	NA	
Nischwitz S et al., 2010.	rs9271366, rs3780792, rs2503875, rs3745672.	4	*DQA1, VAV2, ZNF433.*	3	590	825	NA	NA	CEU
Sanna et al., 2010.	rs9657904, rs2040406.	2	*CBLB, HLA-DRB,HLA-DQB1.*	2	882	872	1775	2005	SARD
Jakkula et al., 2010.	rs744166, rs3135338.	2	*STAT3, HLA.*	2	68	136	1202	3399	FLN
Brynedal et al., 2010	rs4953911.	1	*MGAT5.*	1	1040		873		CEU
Bahlo et al.,, 2009.	rs703842, rs6074022, rs9271366, rs1335532, rs6604026, rs2104286, rs6984045, rs8049603.	8	*METTL1,* ***CYP27B1,*** *CD40, HLA-DRB1, CD58, EVI5, RPL5, IL2RA, ASAP1, DDEF.*	10	1618	3413	2256	2310	CEU
De Jager et al., 2009.	rs3135388, rs2523393, rs2300747, rs2104286, rs11865121, rs6897932, rs4149584, rs17445836, rs17824933, rs882300, rs6896969, rs1790100, rs1250540, rs4680534, rs1800693.	15	*HLA-DRB1, HLA-B, CD58, IL2RA, CLEC16A, IL7R, , IRF8, CD6, CXCR4, PTGER4, MPHOSPH9, ZMIZ1, IL12A, TNFRSF1A.*	14	2624	7220	2215	2116	CEU
Baranzini et al., 2008.	rs397020, rs1458175, rs1529316, rs908821, rs1755289, rs651477, rs7672826, rs1841770, rs1109670, rs9523762, rs1386330, rs17157903, rs2116078, rs1557351, rs2842483, rs12047808, rs4704970, rs1437898, rs12638253, rs1478091, rs299175, rs10259085, rs10518025, rs6941421, rs7191888, rs180358, rs10243024, rs337718, rs7253363.	29	*C20orf46, PDZRN4, CSMD1, SLC25A36, SH3GL2, EN1, MGC45800, ZIC1, DDEF2, GPC5, RAB38, RELN, KCNB2, WDR7, RFK, C1orf125, SGCD, FLJ34870, FLJ16641, LOC132321, NLRP11, C1GALT1, CENPC1, JARID2, C16orf47, MGC13125, MET, CBLN2, ACP5.*	29	978	883	NA	NA	CEU
Aulchenko et al., 2008.	rs10492972.	1	*KIF1B.*	1	45	195	1316	1423	CEU
Comabella et al., 2008.	rs3129934.	1	*HLA-DRB1.*	1	242	242	553	1033	CEU
Hafler et al., 2007.	rs12722489, rs6897932, rs6498169, rs6604026, rs10984447, rs3135388.	6	*IL2RA, IL7RA, KIAA0350, RPL5, DBC1, HLA-DRA.*	6	931	2431	609 trios, 2322	2987	CEU

Table 1c.

GWAS and meta-analysis studies of MS from 2007 to 2010.

MS associated *loci*

HLA-locus

Many association studies based first on serological typing and newly on GWAS have been associated MS and other autoimmune diseases with HLA-DR/DQ genes. However, it was very difficult to detect the allele risk because of the strong LD across the HLA region. This Class II association has been mapped to the *DRB5*0101- DRB1*1501-DQA1*0102-DQB1*0602* haplotype in the North European population *(Fogdell et al., 1995).* These alleles are almost always present together in this population, making it impossible to distinguish the primary association. The mechanism for the strong LD in these HLA haplotypes has been shown to be consistent with a functional epistatic interaction between *DRB1*1501* and *DRB5*0101* alleles. Also, this functional epistasis has been demonstrated to be associated the EAE in mice *(Gregersen et al., 2006).*

Further, association studies in African-American populations have suggested that the *DRB1*1501* allele itself determines MS-associated susceptibility *(McElroy et al., 2010)*. However, in other populations, the risk allele or haplotype is different or does not contain *DRB1*1501* as in Sardinians where MS is associated with the *DRB1*0301–DQA1*0501–DQB1*0201* and *DRB1*0405–DQA1*0501–DQB1*0301* haplotypes (*Marrosu et al 1998*) , or in African–Brazilian MS patients where the strongest association was observed with *DQB1*0602* rather than *DRB1*1501* (*Caballero et al., 1999*). In Caucasians, heterogeneity at the *DRB1* locus has also been found with respect to MS risk (*Barcellos et al., 2006*). In Canadian MS families it has been observed that some *DRB1*1501*haplotypes determine susceptibility while others do not (*Chao et al., 2008*) and that *DRB1*, *DQA1* and *DQB1* alleles contribute to MS susceptibility through epistatic interactions suggesting haplotypic rather than allelic HLA association (*Lincoln et al., 2009*).

As *DRB1* alleles have different structural properties for antigen presentation according to their amino acid sequence, MS-HLA association has been used to support the concept that disease pathogenesis is the result of an autoimmune reaction, perhaps against myelin-related antigens in the restricting context of *DRB1*1501* (*Smith et al., 1998*). However, this structural theory alone does not fully explain the association study results in MS. The description of polymorphisms that alter HLA gene expression (*Vincent et al., 1996*) , identification of several cis-acting genetic variants on expression of HLA class II genes (*Schadt et al., 2008*) (*Dixon et al., 2007*) as well as the recent observation that vitamin D may

influence *DRB1*1501* expression via a vitamin D response element (*Ramagopalan et al., 2009*), makes it possible that association of HLA class II polymorphisms with MS may be related to the levels of gene expression to the same or a greater extent than restriction of antigen response.

The HLA represents the largest genetic association to MS susceptibility, however it is not yet completely understood how can alert the risk developing of the disease. (*Ramagopalan et al., 2009*; Handunnetthi et al., 2010)

Non-HLA loci

In the last few years, a large number of non-HLA loci have been associated with MS *(Baranzini et al., 2010)*. In 30% of the associated regions, the nearest gene to the associated SNP is an immune system gene. Such as genes coding for cytokine pathway (*CXCR5*, *IL2RA*, *IL7R*,*IL7*, *IL12RB1*, *IL22RA2*, *IL12A*, *IL12B*, *IRF8*, *TNFRSF1A*, *TNFRSF14* , *TNFSF14*), co-stimulatory molecules (*CD37*,*CD40*, *CD58*, *CD80*, *CD86*, *CLECL1*) , signal transduction molecules of immunological relevance (*CBLB*, *GPR65*, *MALT1*, *RGS1*, *STAT3*,*TAGAP*, *TYK2*), genes encode protein related with environmental risk factors of MS and implicated in the immune system response such as vitamin D (*CYP27B1*, *CYP24A1*), genes encode immunological molecules implicated in the MS therapies (*VCAM1*, *IL2RA*), genes involved in neurodegeneration independent of inflammation (*GALC*, *KIF21B*) (*Sawcer et al., 2011)*, and genes that may be involved in the pathogenesis of acute promyelocytic leukemia and viral infection such as (SP140) *(ANZgene, 2009; Pierrot-Deseilligny , Souberbielle, 2010).*

eQTLs in GWAS analysis

GWASs have shown a large number of genetic variants associated with complex diseases. The identification of the causal variant within an associated locus is complex task because most of the GWAS loci contain multiple genes, or no genes at all, or they are often in a high linkage disequilibrium (LD) . In addition, some trait associated SNPs have no effect on the protein structure, or they are located in non-coding regions, such variants could have a regulatory function. For these reasons it is difficult to identify the causal variant for each locus through traditional fine-mapping methods *(Westra and Franke, 2014).*

So, in order to identify which genes are regulated by genetic variation, in 2001 it has been introduced the concept of 'genetical genomics' (*Jansen and Nap, 2001*). This concept is

based on the correlation between the genetic variants and intermediate molecular quantitative traits, such as gene expression levels, protein levels or methylation levels. It is possible to identify quantitative trait loci (QTLs). The easiest to quantify is the mRNA levels by microarrays and more recently by RNA sequencing (RNA-Seq), reflecting the profile of genes expression. The genetic variants that affect gene expression levels are expression quantitative trait loci "eQTLs"*(*Farrall*, 2004)*

In consequence, the new strategy to identify the candidate gene(s) is combining the QTL information from all genes and gene products. It will indicate the portion of the genes expression affected by the genes themselves (*cis*-eQTLs), or to other genomic location (*trans*-eQTLs) (Jansen and Nap, 2001).

Many studies have recently analyzed such variants in the context of gene expression measured in cells or tissues to understand inherited susceptibility to disease. These studies have spawned a big field in human genetics studying expression quantitative trait loci (eQTLs). The majority of human eQTL studies have been performed on blood-derived cells or cell lines. The two biggest and most important projects in this field are GUEVADIS and GTEx projects (Table 2).

GEUVADIS project

GEUVADIS (Genetic European Variation in Health and Disease) is an RNA-sequencing project (*Lappalainen et al. Nature 2013*) which has combined transcriptome and genome sequencing data by performing mRNA and small RNA sequencing on 465 lymphoblastoid cell line samples from 5 populations of the 1000 Genomes Project: the CEPH (CEU), Finns (FIN), British (GBR), Toscani (TSI) and Yoruba (YRI). Of these samples, 423 were part of the 1000 Genomes Phase 1 dataset (*Abecasis et al. Nature 2012*) with low-coverage whole genome and high-coverage exome sequencing data, and the remaining 42 are part of the later phases of 1000 Genomes with Omni 2.5M SNP array data available at the time of this study; these genotypes were imputed from the array data using Phase 1 as the reference.

Additionally, GEUVADIS project developed a browser (http://www.ebi.ac.uk/Tools/geuvadis-das/) for visualization and download of exon and transcript quantifications of protein-coding genes and miRNAs, and quantitative trait loci (QTL) for exon expression levels (eQTL), transcript ratios (trQTL), and miRNA expression levels (mirQLTs)

The GTEx Project

GTEx (Genotype-Tissue Expression) project studies the correlations between genotype and tissue-specific gene expression. This project collected and analysed multiple human tissues from donors (1421 RNAseq samples) who were also densely genotyped, to assess genetic variation within their genomes. By analyzing global RNA expression within individual tissues and treating the expression levels of genes as quantitative traits, 24339 significant eQTLs were determined, between them 8930 unique genes. GTEx project used genome-wide association studies (http://www.ebi.ac.uk/gwas) to identify genetic changes associated with common human diseases.

The comprehensive identification of human eQTLs helped greatly to identify genes whose expression was affected by genetic variation, and provided a valuable basis on which to study the mechanism of gene regulation. This project provided a browser of its database (http://www.gtexportal.org/) which allows users to view and download computed eQTL.

Study reference	Cell/Tissue	Sample size	Ancestry	Method
GTEx Consortium, 2015	Adipose	113	Any racial and ethnic group	RNA-seq
	Adrenal Gland	12		
	Artery	145		
	Brain	313		
	Breast Mammary tissue	27		
	LCLs	39		
	Fibroblasts	14		
	Colon Transverse	12		
	Esophagus	38		
	Fallopian tube	1		
	Heart	108		
	Kidney Cortex	3		
	Liver	5		
	Lung	119		
	Muscle Skeletal	138		
	Nerve Tibial	88		
	Ovary	6		
	Pancreas	19		
	Pituitary	13		
	Prostate	9		
	Skin Not sun exposed	23		
	Skin Sun exposed	96		
	Stomach	12		
	Testis	14		
	Thyroid	105		
	Uterus	7		
	Vagina	6		
	Whole blood	156		
Fairfax et al., 2014	Monocytes (LPS and/or IFN-Y)	432	CEU	RNA -Chip
Lappalainen et al, 2013 (GEUVADIS)	LCLs	465	CEU, FIN, GBR, TSI and YRI.	RNA-seq
Pique-Regi et., al, 2012	LCLs	70	YRI	DNase sensivity QTLs
Pickrell et al., 2010	LCLs	69	YRI	RNA-seq
Gaffney et al., 2012	LCLs	210	CEU, CHB, JPT and YRI.	RNA-Chip
Innocenti et al., 2010	Liver	266	AFR, CEU	RNA-Chip
Montgomery et al., 2010	LCLs	60	CEU	RNA seq for transcript QTL
Zeller et al., 2010	Monocytes	1490	German	RNA-Chip
Dimas et al., 2009	Fibroblasts	75	CEU	
Veyrieras et al., 2008	LCLs	210	CEU, CHB, JPT, YRI and ASN.	
Schast et al., 2007	Liver	427	CEU	
Myres et al., 2007	Brain cortex	279	CEU	
Stranger et al., 2007	LCLs	210	CEU, CHB, JPT and YRI.	

Table 2
The eQTL studies from 2007 to 2015.

Objectives and justification

GWASs have shown a large number of genetic variants significantly associated with MS in several loci. Though some of the variants identified may actually represent the genetic alteration responsible for the disease association, most of these SNPs merely 'tag' an allele of a nearby gene (or a haplotype of a genetic region), which is actually the base of the observed disease relationship "the causal variant". The identification of the causal variant within an associated locus is complex task because most of the GWAS loci contain multiple genes, or no genes at all, or they are often in high linkage disequilibrium (LD). Thus, **the main objective** of this work was to determine the causal variants of the loci associated with MS by GWAS studies and reveals, by the analysis of the functional effect of the causal variant, the genes implicated and their effect in the disease.

Specific objectives:

1- To determine the functional effect of the MS causal variant in the 12q13-14 locus on the transcription of the gene *CYP27B1*.

2- To determine the effect of MS associated variants on the transcription of genes implicated in vitamin D function as vitamin D receptor (*VDR*) and vitamin D degradation (*CYP24A1*).

3- To determine the potential relation in expression of the different genes of the vitamin D pathway under different conditions.

4- To determine which MS associated variants are eQTLs.

5- To determine the causative variant of the 2q37.1 locus.

6- To validate the effect of the MS causal variant at the 2q37.1 locus on the splicing of *SP140* gene.

7- To validate this association in a Spanish cohort through case-control study.

8- To determine of the possible common causal variant at the 12p13.31 locus.

Materials and Methods

Samples collection

25 ml of Buffy coat (50ml of whole blood) were collected from 119 healthy individuals subjects (voluntary blood donors: 67 females and 52 males) between the ages of 20–65. Samples were collected from Spanish subjects.

109 LCLs (lymphoblastoid cell lines) from 94 CEU (Utah residents with Northern and Western European ancestry from the CEPH collection, International Hapmap Project) and 15 TSI (Tuscan in Italy, 1000 Genomes and International Hapmap projects) were purchased from Coriell cell repositories (Coriell Institute for Medical Research, NJ, USA).

Monocyte separation

Peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation, using Histopaque (Sigma), cells were washed two times in phosphate-buffered saline (PBS) solution without Ca^{2+} and $Mg^{2+.}$ Then, monocytes were isolated from PBMC by positive selection using anti-CD14 (MicroBeads, anti-human CD14 MACS, Miltenyi). This method provides a sample of ~99% purity. 2.10^6 of purified $CD14^+$ were rested overnight (12 hours) in $25cm^2$ flask (Cell Culture Treated Flasks, Thermo Scientific) at 37°C, 5% CO2 in 5ml RPMI 1640 complete medium (Supplemented with 10% fetal calf serum, penicillin/streptomycin, and L-glutamine).

LCLs culture

1.10^6 cells per well were incubated in plates (Nunclon 12-Well PS MultiDish, Thermo Scientific) at 37°C, 5% CO2 in 2ml RPMI 1640 complete medium, supplemented with 10% fetal calf serum, penicillin/streptomycin, and L-glutamine.

Cell stimulation

After overnight resting, $CD14^+$ cells were stimulated with 2 ng/ml Interferon-gamma (IFNγ, Roche) and 20 ng/ml Lipopolysaccharide (LPS, Sigma) for 24 hrs.

LCLs were treated with 200 nM 1α,25-dihydroxyvitamin D_3 (1,25(OH)2D, Sigma) or with 500nM 25-hydroxyvitamin D_3 monohydrate (25(OH)D, Sigma) for 24 hours.

RNA extraction

Total RNA was extracted using the RNeasy Mini Kit from cells collected in the RLT buffer following the manufacturer's instruction (Qiagen). Then RNA was quantified and qualified

by the Experion automated electrophoresis system (Bio-Rad), the RNA quality indicator (RQI) was 10 (intact RNA) for all RNA samples.

Genotyping

Genomic DNA was isolated from 300 µL buffy coat samples of healhy controls used for monocyte purificaion with FlexiGene DNA Kit (Qiagen). DNA was diluted to 10ng/ul with RNase-/DNase-free water and analyzed by real-time PCR using the TaqMan Genotyping Master Mix (Applied Biosystems) and primer sets (Life Technologies) for rs10877013 (*CYP27B1*), rs10466829 (*CLECL1*) and rs3764022 (*CLEC2D*). (Table 3)

The genotype data of 109 LCLs (CEU and TSI) were obtained from the 1000 GenomeS and Hapmap projects database.

SNP	Gene	Assay ID	Context Sequence [VIC/FAM]
rs10877013	CYP27B1	C_25985808_10	AGAAGCATGAGAAGGTTGGTCACAC**[C/T]** ATTGCAGAAGTGGTACTGTGAGAGT
rs10466829	CLECL1	C_30127235_10	ACATGACCTGAAGCCATTGAAGAGT**[A/G]** AACAAAGCCAGACAGATTCTAGAGG
rs3764022	CLEC2D	C_25992569_10	TTTTCAATAATTTTTTCCAGGTTGT**[C/G]** TGCATTCAAAAGAGCATTCTATTAA

Table 3.
TaqMan SNPs Genotyping Assay Data from Life Technology.

PCR

cDNA was generated by reverse transcriptase PCR using Superscript III First-Strand Synthesis SuperMix (Invitrogen) from 150 ng of total RNA. Then, *CYP27B1, CYP24A1, VDR, SP140 and CLE2D* mRNA levels were quantified by real-time PCR using Go Taq qPCR Master Mix (Promega) and normalized to *UBE2D2* mRNA levels using $2^{-\Delta CT}$ method. Reactions were run in triplicate. The primers sequences of studied genes were designed using Primer3 browser (Table 4).

Gene primers	Forward (5'-3')	Reverse (5'-3')
CYP27B1	CCAGACAGCACTCCACTCAG	ACAGAGTGACCAGCGTATTT
CYP24A1	CATCATGGCCATCAAAACAAT	GCAGCTCGACTGGAGTGAC
VDR	TCCTCCTGCTCAGATCACTG	AGGGTCACAGAAGGGTCATC
SP140 E6:E8	CAGTTAGCTCTCCCAAAGGC	CTGTGCTGTATGTCCTTGCC
SP140 E6:E7_8	TGGTGGAGGAGATGCTGAAG	CCGTTGCTTTCTAGAACTTC
SP140 E6_8:E8	CTACCAGGTGGGGGAGTTCT	TTCCCTCTGGACTCTCTTGG
CLEC2D E1:E3	CATCTGAATTGCCTGCAAAC	CAACCTGAGCAAGATCAGCA
CLEC2D E1_3:E3	TTGCCTGCAAACCCAGCAAT	CAACCTGAGCAAGATCAGCA
UBE2D2	CAATTCCGAAGAGAATCCACAAGGAATTG	GTGTTCCAACAGGACCTGCTGAACAC

Table 4
The primers sequences of studied genes.

Exon-Trapping analysis

Exon-Trapping analysis was performed as described by Desviat et al. (33). Briefly: PCR amplification of a DNA fragment containing the exon 7 of *SP140* gene (78bp) and the flanking intronic sequences of 103bp at the 5' and 75bp at the 3' of the exon was done. The primers for this amplification were: forward 5'- *CCCGAATATTAGAGCTCAGCA*-3' and reverse 5'- *TGGGAAGGGAGATGAAAGAG*-3'. The amplification of the DNA fragment was performed from the LCL NA12383 which is heterozygous for the rs28445040 variant. The PCR product was cloned in TOPO-TA vector and sequenced to identify the clones carrying each allele and to discard potential PCR errors. An EcoRI fragment from each TOPO vectors was subcloned in the EcoRI site of the pSPL3 minigene plasmid and orientation checked by sequencing. 1ug allele *T* and allele *C* pSPL3 (pC, pT) plasmids and plasmid without insert (p) were transfected in HEK cells using jetPRIME (Polyplus) and harvested 24 h after transfection. 10 ug from the same plasmids were transfected in $1{,}5.10^6$ LCL cells by electroporation using the Mirus Ingenio kit , Amaxa® Nucleofector® II Device with the program M-013, 4 hours after, cells were activated with 200nM 1,25(OH)2Dfor 24 h. RNA was extracted and amplified by RT-PCR using SD6 and SA2 primers. The PCR products were visualized in 2% agarose gel electrophoresis and sequenced to confirm the DNA origin.

Statistical analysis

LD patterns between SNPs were analyzed with Haploview 4.2 (Barrett, J.C., 2005).

All real-time PCR mRNA expression was measured relative to *UBED2D* and, error margins were calculated using the standard error.

Mean mRNA values from different experimental conditions were compared by using the Student's *t* test.

The association between gene expression and SNP was tested using Spearman's rank correlation test Sig. (2-tailed) .This method has been previously shown to produce robust results and avoids the effect of outliers in gene expression values (*Dimas AS , 2009*).

The association between genes expression and genome loci was tested using Genetranassoc software (http://bios.ugr.es/Genetranassoc/), the software to compute association (Spearman correlation coefficient, including permutation test) using 1000 Genomes and Hapmap SNp genotype data.

A direct link between the studied genes expressions was tested using Pearson's linear correlation.

Results

Determination of the causal gene in the 12q13-14 locus responsible for the association with MS

The MS-GWAS associated variants correlate with the expression of several genes in the locus

For many risk loci, the association signals do not directly implicate a single gene and the causative role for candidate genes in the region can only be speculated. One of these loci is 12q13–14 which has been associated in GWAS with rheumatoid arthritis (RA) (Okada Y *et al., 2014;* Orozco G *et al.2014*; Zhernakova et al., 2011; Raychaudhuri *et al.* 2014), celiac disease (CD) (Zhernakova et al., 2011) and MS (Sawcer et al., 2011) (Bahlo et al., 2009)). However, different genes have been suggested in each study based on the main associated signal. A meta-analysis of two published GWAS totalling 3393 RA cases and 12 462 healthy controls identified an association at rs1678542 localised in the *KIF5A* intronic region (Raychaudhuri et al., 2008). Another meta-analysis of two published GWAS on CD (4533 cases and 10 750 controls) and RA (5539 cases and 17 231 controls) described the association of both diseases at rs10876993 localized in the intergenic region between *B4GALNT1* and *OS9* genes (Zhernakova et al., 2011). Association at this locus was also described in a MS GWAS performed by the Australian and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene) in 1618 MS-cases. In this case, an associated SNP (rs703842) was located at the 3' untranslated region (3' UTR) of the *METTL1* gene (Bahlo et al., 2009). The last GWAS performed by the International Multiple Sclerosis Genetics Consortium (IMSGC) with 10 000 MS patients also reported the association with this region at rs12368653 in the*AGAP2* gene (Sawcer et al., 2011). In candidate gene studies, the *KIF5A* variant was demonstrated to be associated to MS (Raychaudhuri et al., 2008) and type 1 diabetes (Fung et al., 2009). Also rare variants in the *CYP27B1* gene have been associated with MS (Ramagopalan et al., 2011). Other candidate-gene studies had demonstrated association of variants located at the *CYP27B1* gene with type 1 diabetes (Bailey et al. 2007) and MS (Sundqvist et al., 2010). In previous work we performed a fine mapping of the 12q13.3–12q14.1 region by a Tag-SNP approach determining a functional variant which alters the enhancer activity of a regulatory element in the locus affecting the expression of several genes and explains the association of the 12q13.3–12q14.1 region with MS (Fig.11). This SNP, rs10877013, was in total LD with other polymorphisms that associated with the expression levels of *FAM119B, AVIL, TSFM, TSPAN31* and *CYP27B1* genes in different eQTL studies.

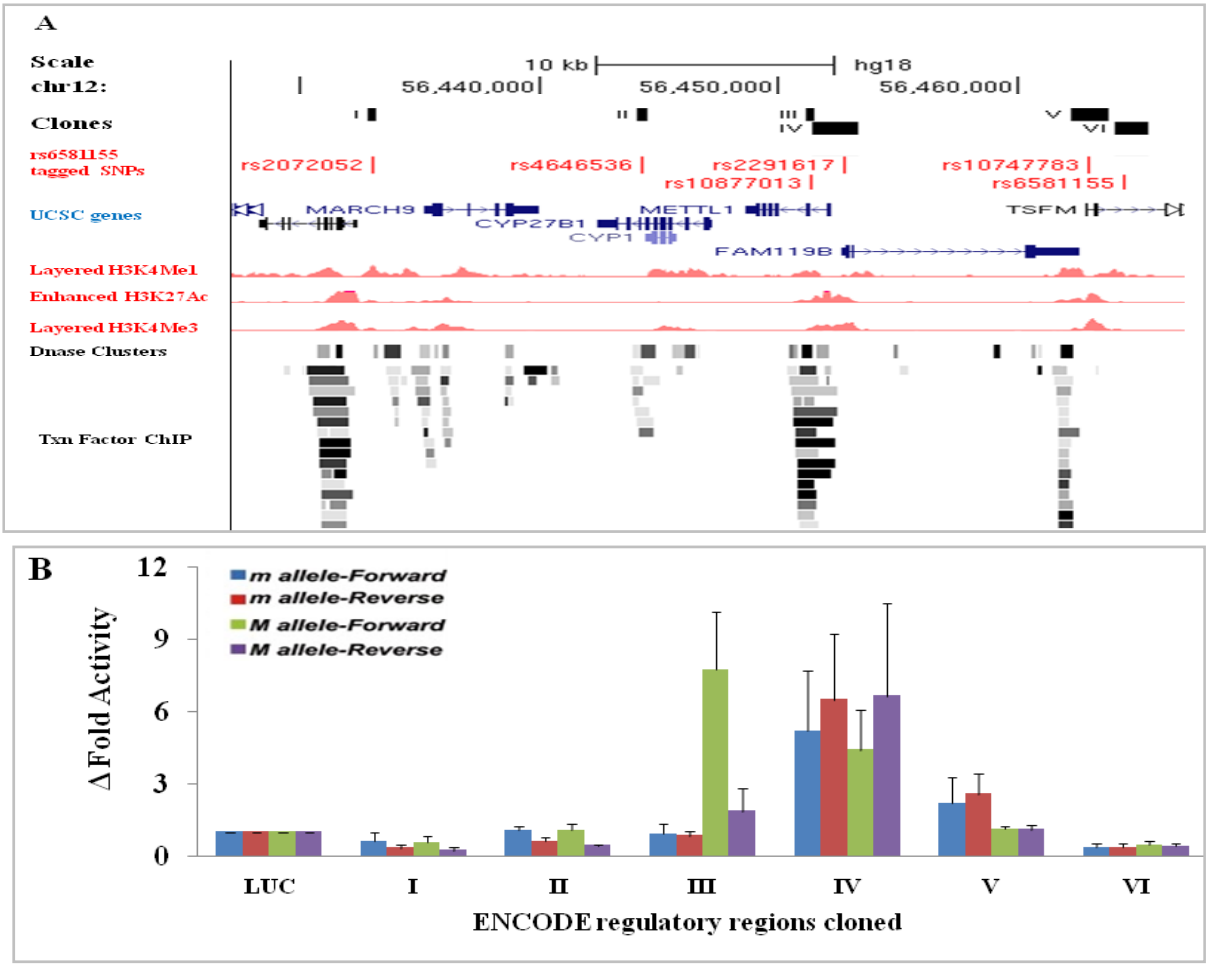

Figure 11.
Enhancer-suppressor activity of the rs10877013 variant. (A) Schematic illustration to show the localization of the six tagged SNPs (in red) in potential regulatory regions (in black, I to VI) as indicated by ENCODE for the GM12878 lymphoblastoid cell line and the genes present in the region: *Enhancer H3K4Me1* track shows where modification of histone proteins is suggestive of enhancer; *Promoter H3K4Me3* track shows a histone mark associated with promoters; *Layered H3K4Me3* track shows histone mark associated with promoters that are active or poised to be activated; *DNase Clusters* track shows regions where chromatin is hypersensitive to DNase I enzyme; *Txn Factor ChIP* track shows DNA regions where transcription factors bind to DNA as assayed by chromatin immunoprecipitation (ChIP) with antibodies specific to the transcription factor followed by sequencing of the precipitated DNA (ChIP-Seq). (B) Luciferase activity of the different constructs corresponding to the six regions (I to VI) with potential regulatory activity, containing tagged polymorphisms transfected into Raji B cells. Four clones for each region bearing the different alleles (allele m, minor and M, major) and in both orientations (Forward and Reverse) from three independent transfection experiments are represented. Luciferase activity levels are referred to the level of the control plasmid containing only the basic promoter and the Renilla activity. (C) Expression of different genes in the 12q13.3-12q14.1 region correlate with rs10877013 SNPs .In the plot are represented the media and standard deviation respect to genotypes of rs10877013, for *FAM119B,TSFM, AVIL* and *TSPAN31* genes obtained by Zeller et al. from monocytes obtained from 1490 German individuals.

Determination of the effect of the MS-associated variant on the *CYP27B1* expression

Given that the variant associated with MS in the region is correlated with the expression of various genes in the locus, it is difficult to determine which of those genes is implicated in the pathology. However, given the critical role of Vitamin D in MS pathology, the candidate gene with high potency in the region would be *CYP27B1*. This enzyme catalyses the conversion of 25(OH)D to 1,25(OH)2D.

The *CYP27B1* gene is expressed in proximal tubule cells of the kidney and the disease-activated macrophages, which are the major source of *CYP27B1* (Adams and Hewison, 2012). In order to determine the potential connection between the variant genotypes, gene expression and MS disease, we studied the relationship between the rs10877013 genotypes and the expression of *CYP27B1* when in immune cells were challenged by inflammatory stimuli as IFNγ and LPS, or active form of vitamin D (1,25(OH)2D).

For this study we used CD14+ monocytes, genotyped for the rs10877013, purified from blood of 119 individuals and 109 LCLs of the HapMap-1000 Genomes collection with known genotypes.

CD14 monocytes did not express *CYP27B1* gene after extraction. However when CD14 stimulated with LPS (2 ng/ml) and IFNγ (20 ng/ml), *CYP27B1* was highly up-regulated ($P<0.0005$) (Fig.12A) reaching a peak after 24-36 h of stimulation (Fig.11B). *CYP27B1* mRNA expression levels was associated with the rs10877013 genotypes in 119 samples of stimulated CD14+ monocytes. High expression of *CYP27B1* was clearly associated with the MS protective genotype (rs10877013-T allele), consequently the low level was associated with the MS risk allele rs10877013-C (Spearman correlation rho= 0.4, P=5.0E-6) as shown in (Fig.12C).

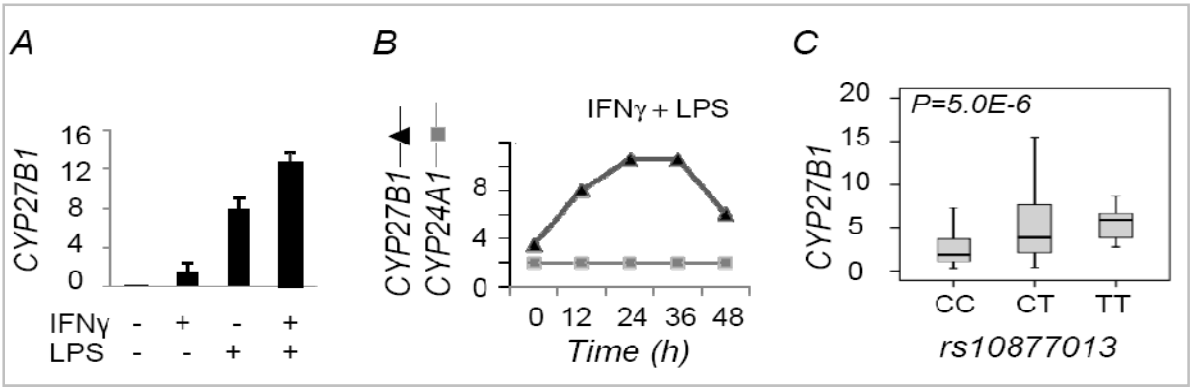

Figure 12.

CYP27B1 mRNA expression levels in CD14+ monocytes incubated with LPS+IFNγ. (A) *CYP27B1* mRNA expression under different inflammatory stimuli (each bar is the mean of 4 samples ±SE). (B) Time course of *CYP27B1* and *CYP24A1* mRNA expression under LPS+IFNγ stimulation (each point represents the mean of 4 samples in which SE was less than 5%). (C) *CYP27B1* mRNA expression levels with respect to the rs10877013 genotypes in 119 samples of CD14+ monocytes represented by boxplot distributions with medians and quartiles. P-value *(P)* stands for the significance of the statistical comparisons. Transcript levels were normalized according to the *UBE2D2* transcript levels**.**

To determine if the expression of *CYP27B1* could be affected by vitamin D we analyzed the expression of the gene in presence of 25(OH)D or 1,25(OH)2D in LCLs. LCLs expressed *CYP27B1* mRNA at low levels independently of vitamin D stimulation (Fig.13A). Also no association has been observed between *CYP27B1* mRNA expression of 109 LCLs samples and rs10877013 genotypes (Fig.13B)

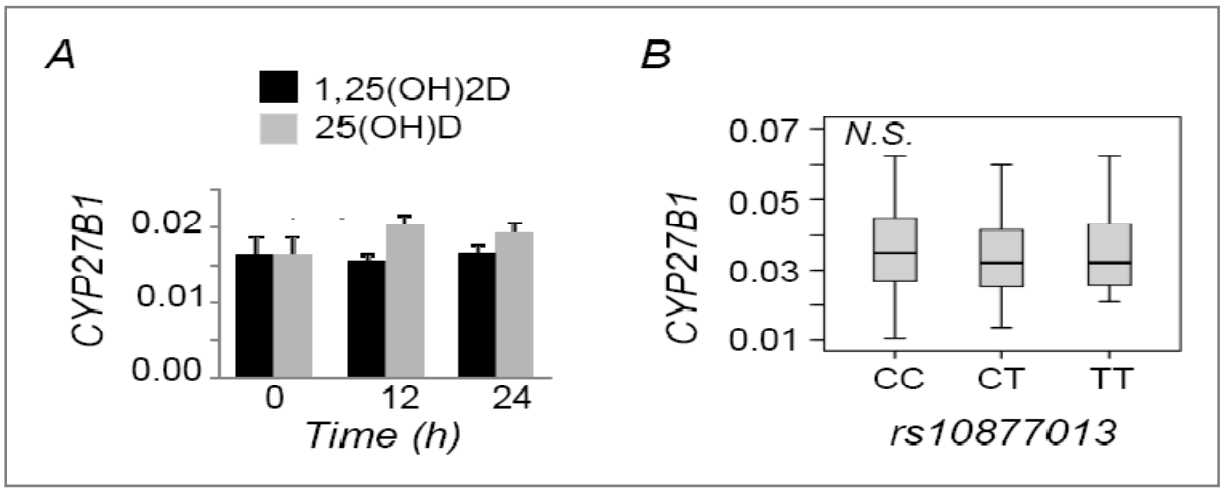

Figure 13.

CYP27B1 mRNA expression levels in LCLs incubated with vitamin D. (A) *CYP27B1* expression with respect to the incubation time (h) and vitamin D form stimulation (each bar is the mean of 4 samples ±SE). (B) *CYP27B1* mRNA expression levels with respect to the rs10877013 genotypes in 109 LCLs represented by boxplot distributions with medians and quartiles. *N.S.* stands for non significant. Relative quantification of the indicated transcript was performed by RT-qPCR using *UBE2D2* as reference gene.

Effect of the genetic variants on the *VDR* expression

VDR gene is located on the chr12 approximately 10 Mb from CYP27B1 (Fig.14).

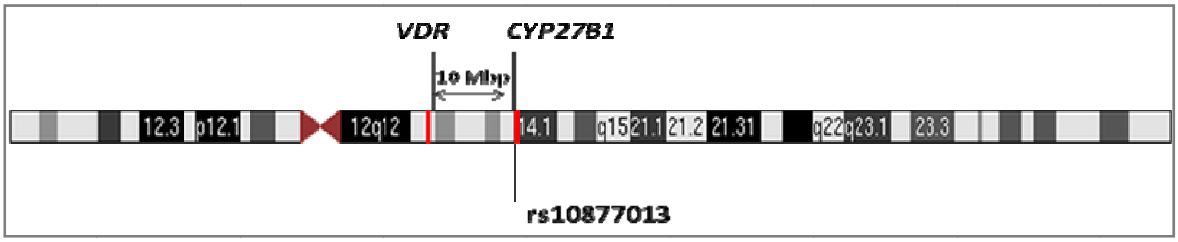

Figure 14.
Localisation of *VDR* and *CYP27B1* genes respect to the functional variant rs10877013 on chr 12.

VDR mRNA expressed at low level in unstimulated CD14+ monocytes (Fig.15A) when they stimulated with IFNγ+LPS, *VDR* expression was highly up-regulated after 24 h of stimulation (P<0.0005). However, *VDR* mRNA was not associated with rs10877013 genotypes (P=0.34) in 119 samples of CD14+ monocytes, as shown in (Fig.15B).

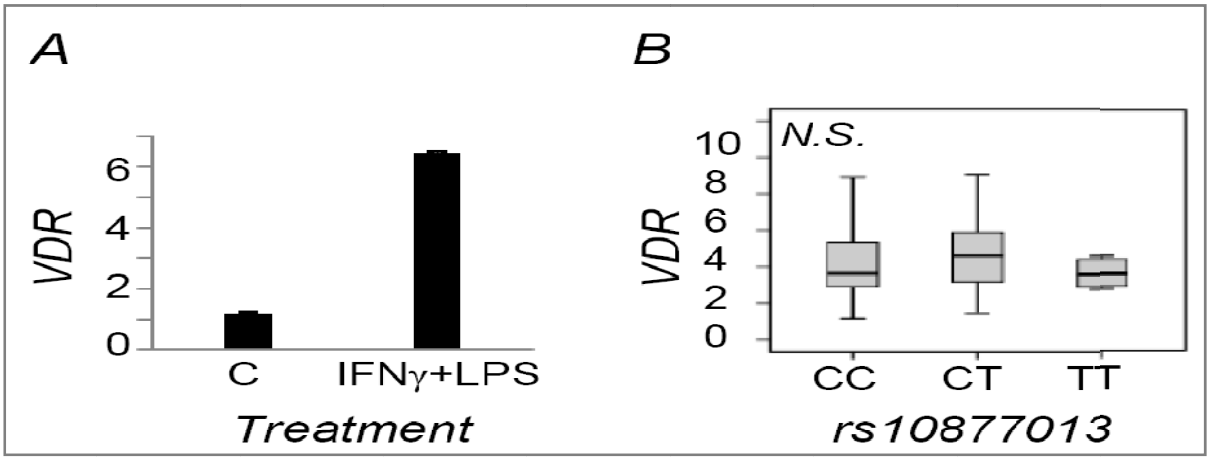

Figure 15.
VDR mRNA expression levels in CD14+ monocytes incubated with LPS+ IFNγ for 24h. (A) Induction of *VDR* mRNA expression (each bar is the mean of 4 samples ±SE). (B) *VDR* mRNA expression levels with respect to the rs10877013 genotypes in 119 activated monocytes represented by boxplot distributions with medians and quartiles. *N.S.* stands for non significant. Relative quantification was performed by RT-qPCR using *UBE2D2* as a reference gene.

VDR mRNA was transcribed at low level in untreated LCLs. A significant increasing observed in LCLs treated with 200 nM of 1,25(OH)2Dafter 12h (P<0.005) and 24 h (P<0.05) of incubation (Fig.16A). In addition, *VDR* mRNA expression showed a significant association with rs10877013 genotypes in 109 LCLs (Spearman's rho=0.328, P=6.03E-4) (Fig.16B).

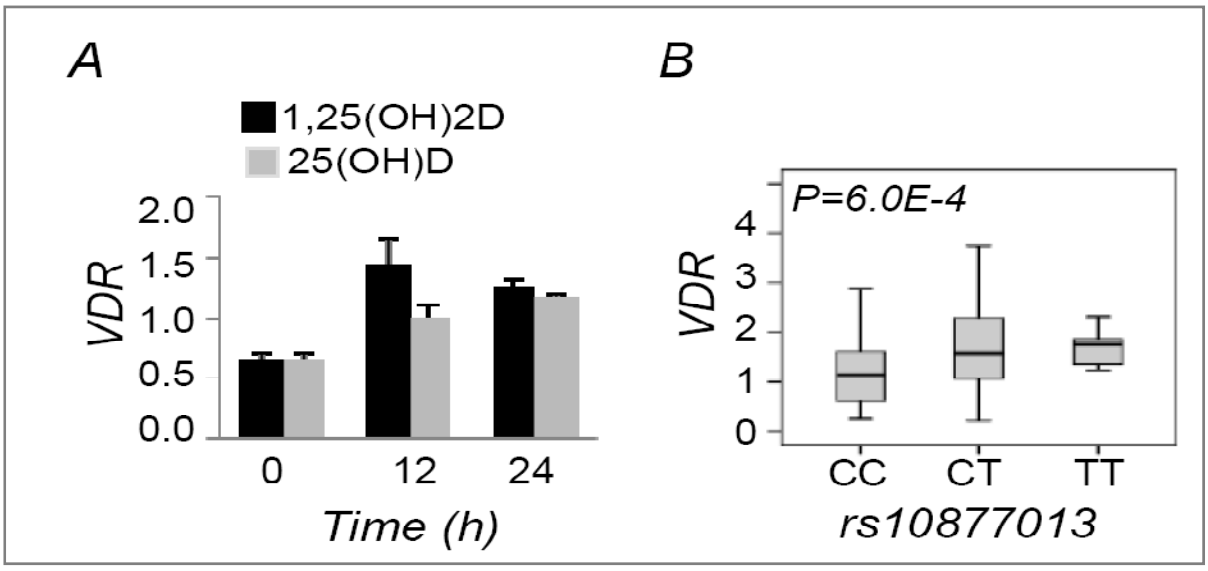

Figure 16.
VDR mRNA expression in LCLs incubated with both forms of vitamin D. (A) *VDR* expression with respect to the incubation time (h) and vitamin D form stimulation (each bar is the mean of 4 samples ±SE). (B) *VDR* expression levels with respect to the rs10877013 genotypes in 109 LCLs treated with 200nM $1,25(OH)_2D$ represented by boxplot distributions with medians and quartiles. *P* stands for significance. Relative quantification was performed by RT-qPCR using *UBE2D2* as a reference gene.

The effect of the genetic variants on the *CYP24A1* expression

CYP24A1 gene is located on the locus 20q13.2 between *PFDN4* gene and *BCAS1* gene (Homo sapiens breast carcinoma amplified sequence 1). rs2248359 has been associated to MS (risk allele, rs2248359-C) *(Sawcer S, 2011)*, it is located 1Kbp upstream of CYP24A1 (Fig.17) In addition this locus was associated to bipolar disorder (BD) and schizophrenia (SCZ) with rs2276498 (Wang et al., 2010), IgG glycosylation with *rs6064045 (Lauc et al., 2013)*, calcium level (CaL) with rs1570669 *(O'Seaghdha et al., 2013),* atopic dermatitis (AD) with rs16999165 *(Hirota et al., 2012)* and Obesity related trait with rs2585417 (*Comuzzie et al., 2012).*

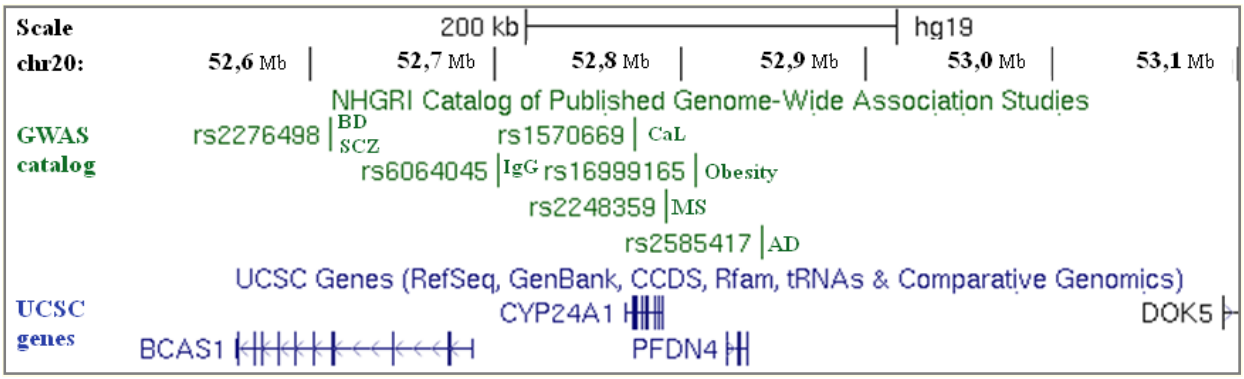

Figure 17.
Schematic representation of the 20q1.2 locus (chr20:52469742-53162587), from the UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly. Trait GWAS from GWAS catalog. rs2248359 MS was associated with MS in GWAS. UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics).

CYP24A1 was characterized as a highly cell-specific and stimulus-specific inducible gene. CD14+ monocytes did not express *CYP24A1* in unstimulated CD14+ or in incubated CD14+ with several inflammatory stimuli, including IFNγ+LPS (Fig.12B). On the other hand, LCLs expressed *CYP24A1* only after stimulation with the active form of vitamin D (1,25(OH)2D). In order to determine the most optimal vitamin D concentration to induce *CYP24A1*, LCLs were incubated with different concentrations of 1,25(OH)2D or 25(OH)D. (Fig.18A). 1,25(OH)2D(200nM) stimulation led to a significant induction of *CYP24A1* expression at 12 h ($p<0.005$) and 24h ($P<0.0005$). However, 25(OH)D (500nM) didn't modify the transcription level of *CYP24A1* (Fig. 18B). Additionally, adding both of vitamins D forms had the same effect as 1,25(OH)2D (no significant difference) (Fig.18C).

Furthermore, *CYP24A1* mRNA levels were analyzed depend on the SNPs genotypes in the region chr20:52561127-53253972. However, no association was observed between the expression levels and the SNPs genotypes in this region including the GWAS MS-associated rs2248359 (p=0.81) (Fig.18D)

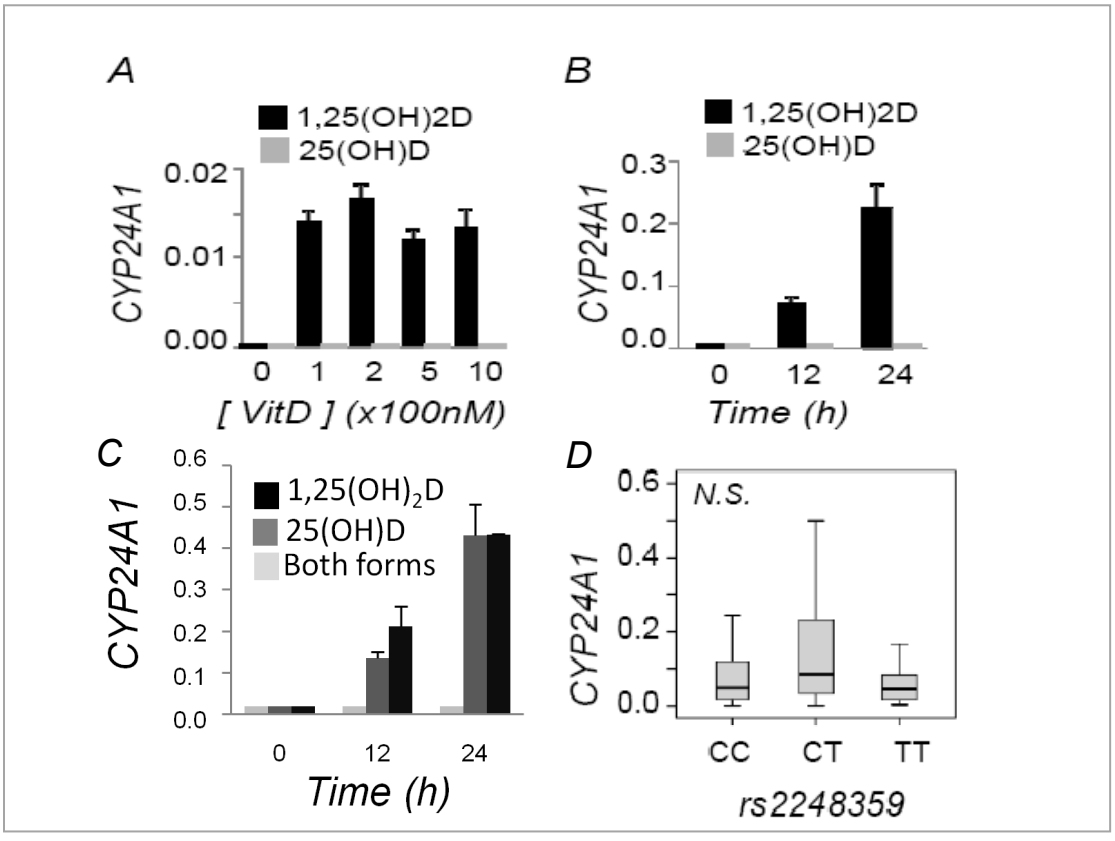

Figure 18.

CYP24A1 mRNA expression in LCLs incubated with both forms of vitamin D. (A) mRNA expression with respect to different vitamin D concentration [VitD] and (B) incubation time (h) (each bar is the mean of 4 samples ±SE). (C) The effect of both vitamin D forms on CYP24A1 expression. (D) mRNA expression levels with respect to the rs2248359 genotypes in 109 LCLs treated with $1,25(OH)_2D$ represented by boxplot distributions with medians and quartiles. *N.S.,* stands for non significant. Relative quantification was performed by RT-qPCR using *UBE2D2* as a reference gene.

Genes correlation

A direct relationship between the studied genes expressions was tested in LCLs (Table 5). *CYP24A1* and *VDR* expression levels were directly correlated (Pearson's r= 0.56, P=1.1x10E-10) (Fig. 19)

Genes expression		***CYP24A1***	***VDR***
CYP27B1	r	-0.02	0.14
	p-value	0.85	0.14
VDR	r	**0.40**	-
	p-value	**1.43E-5*** **	-
CYP24A1	r	-	-
	p-value	-	-

Table 5.
Expression-correlation analysis between the genes: *CYP27B1, CYP24A1, VDR* and *SP140* in 109 LCLs treated with 200nM of the active form of vitamin D for 24h. Correlations analyzed using Pearson's linear correlation (2-tailed). r, stands for Pearson correlation index; P, significance.

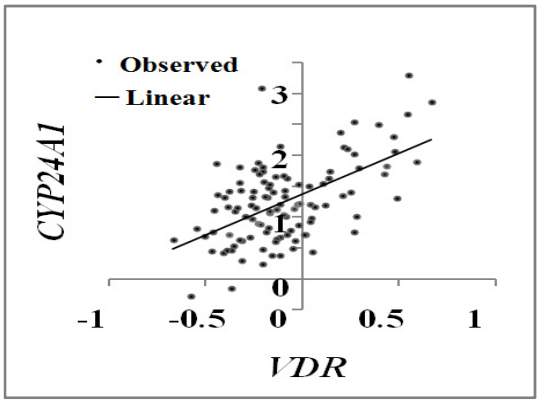

Figure 19.
Scatter plot represents the correlation between *VDR* and *CYP24A1* transcript levels from table 5 (-Log expression data).

Determination of MS/GWAS-associated variants that are eQTLs from LCLs of European origin

To determine the relationship between the MS associated variants from GWAS (MS/GWAS) and the best eQTLs obtained from the GEUVADIS Project, we calculated the LD (r^2) between the best-eQTLs and the 202 MS/GWAS-associated variants of the GWAS catalogue (http://www.genome.gov/gwastudies/). Thirty six best-eQTLs were in LD between r^2 0.05 to 1 with MS/GWAS-associated SNPs (Table 6). We selected the 2q37.1 and 12p13.31 loci to analyze with more detail the colocalization of eQTL and association signals.

Study (Reference)	chr	MS-SNP	position	eQTL	position	LD (r2)	Transcript	Gene	rho
(Patsopoulos et al., 2011)	chr16	rs7191700	11406802	rs918737	11439638	0,12	ENST00000381820	RMI2	0,39
(Sawcer et al., 2011)	chr2	rs10201872	231106723	rs6705808	231091340	0,97	ENST00000392045	SP140	0,48
	chr2	rs10201872	231106723	rs35540610	231121828	0,68	ENST00000343805	SP140	0,50
	chr2	rs10201872	231106723	rs10498245	230246707	0,45	ENST00000350136	SP140	0,40
	chr3	rs4285028	121660663	rs9808981	121429560	0,66	ENST00000273668	EAF2	0,35
	chr3	rs4285028	121660663	rs6780316	121544090	0,34	ENST00000349820	IQCB1	0,34
	chr4	rs228614	103578636	rs223473	103693620	0,41	ENST00000273986	CISD2	0,34
	chr4	rs228614	103578636	rs223413	103732865	0,25	ENST00000226578	MANBA	0,33
	chr4	rs228614	103578636	rs223363	103767338	0,46	ENST00000394801	UBE2D3	0,47
	chr6	rs9321490	135494874	rs9471935	42840317	0,06	ENST00000493763	RPL7L1	0,44
	chr8	rs1520333	79401037	rs2953477	79624604	0,25	ENST00000263849	ZC2HC1A	0,50
	chr8	rs1520333	79401037	rs10093113	79585247	0,26	ENST00000520351	RP11-578O24.2	0,63
	chr12	rs10466829	9876090	rs2080210	9838051	0,13	ENST00000444971	CLEC2D	0,33
	chr12	rs10466829	9876090	rs11052604	9862547	0,47	ENST00000261339	CLEC2D	0,41
	chr12	rs10466829	9876090	rs7977720	9866348	0,86	ENST00000542530	CLECL1	0,54
	chr12	rs10466829	9876090	rs2080206	9867965	0,69	ENST00000540988	CLECL1	0,45
	chr12	rs10466829	9876090	rs9634110	9543483	0,19	ENST00000229402	KLRB1	0,37
	chr12	rs10466829	9876090	rs11051176	9626489	0,20	ENST00000518709	RP11-726G1.1	0,54
	chr12	rs10466829	9876090	rs10843723	9566301	0,19	ENST00000540982	RP11-599J14.2	0,32
	chr12	rs12368653	58133255	rs2291617	58166402	0,40	ENST00000300209	METTL21B	0,39
	chr12	rs12368653	58133255	rs701008	58117644	0,29	ENST00000323833	TSFM	0,34
	chr12	rs12368653	58133255	rs238517	58117736	0,72	ENST00000389146	OS9	0,39
	chr12	rs12368653	58133255	rs10431506	58311523	0,26	ENST00000300145	XRCC6BP1	0,42
	chr12	rs12368653	58133255	rs12368653	58133256	0,50	ENST00000552423	OS9	0,41
	chr12	rs703842	58162738	rs2291617	58166402	0,97	ENST00000300209	METTL21B	0,39
	chr12	rs703842	58162738	rs701008	58117644	0,75	ENST00000323833	TSFM	0,34
	chr12	rs703842	58162738	rs238517	58117736	0,31	ENST00000389146	OS9	0,39
	chr12	rs703842	58162738	rs10431506	58311523	0,17	ENST00000300145	XRCC6BP1	0,42
	chr12	rs703842	58162738	rs701006	58106836	0,62	ENST00000552423	OS9	0,41
	chr14	rs2119704	88487688	rs1372362	88467872	0,12	ENST00000261304	GALC	0,39
	chr19	rs2303759	49869050	rs1864142	49854221	0,09	ENST00000426897	CD37	0,33
	chr22	rs140522	50971265	rs131752	51023423	0,14	ENST00000423069	CPT1B	0,34
(Briggs et al., 2011)	chr6	rs11962089	105612219	rs6905641	105795405	0,09	ENST00000448705	PREP	0,41
(De Jager et al., 2009)	chr12	rs1790100	123656724	rs4275659	123447927	0,66	ENST00000315580	ARL6IP4	0,50
	chr12	rs1790100	123656724	rs4759416	123746356	0,92	ENST00000538446	CDK2AP1	0,31
	chr12	rs1790100	123656724	rs28855757	123885936	0,89	ENST00000544658	CDK2AP1	0,33
	chr12	rs1790100	123656724	rs28659953	123912212	0,16	ENST00000280571	RILPL2	0,32
(Baranzini et al., 2008)	chr1	rs12047808	179469313	rs479094	179842106	0,05	ENST00000271583	TOR1AIP1	0,48
	chr2	rs1109670	9250037	rs35132160	9239634	0,06	ENST00000471753	MBOAT2	0,40
(Aulchenko et al., 2008)	chr1	rs10492972	10353111	rs2506892	10598215	0,21	ENST00000492696	PEX14	0,31

Table 6.

LD between the GWAS associated variants and the best-eQTLs for the EUR LCLs. rho: for Spearman correlation between the transcript expression level and the eQTL allele.

Determination of the causal gene responsible for the association with MS in the 2q37.1 locus

The 2q37.1 locus locus has been reported to be associated to MS (rs10201872, risk allele A) *(Sawcer et al., 2011)* and to two other diseases after different GWAS. rs13397985 (G risk allele) has been showed to be associated with Chronic lymphocytic leukemia (CLL) in four GWAS. *(Di Bernardo et al., 2008) (Slager et al., 2012) (Berndt et al., 2013) (Speedy et al., 2014)*. rs7423615, rs6716753 with T and C as risk alleles respectively, have been associated to Chorn's disease *(Franke et al., 2010) (Jostins et al., 2012)* (Fig.20).

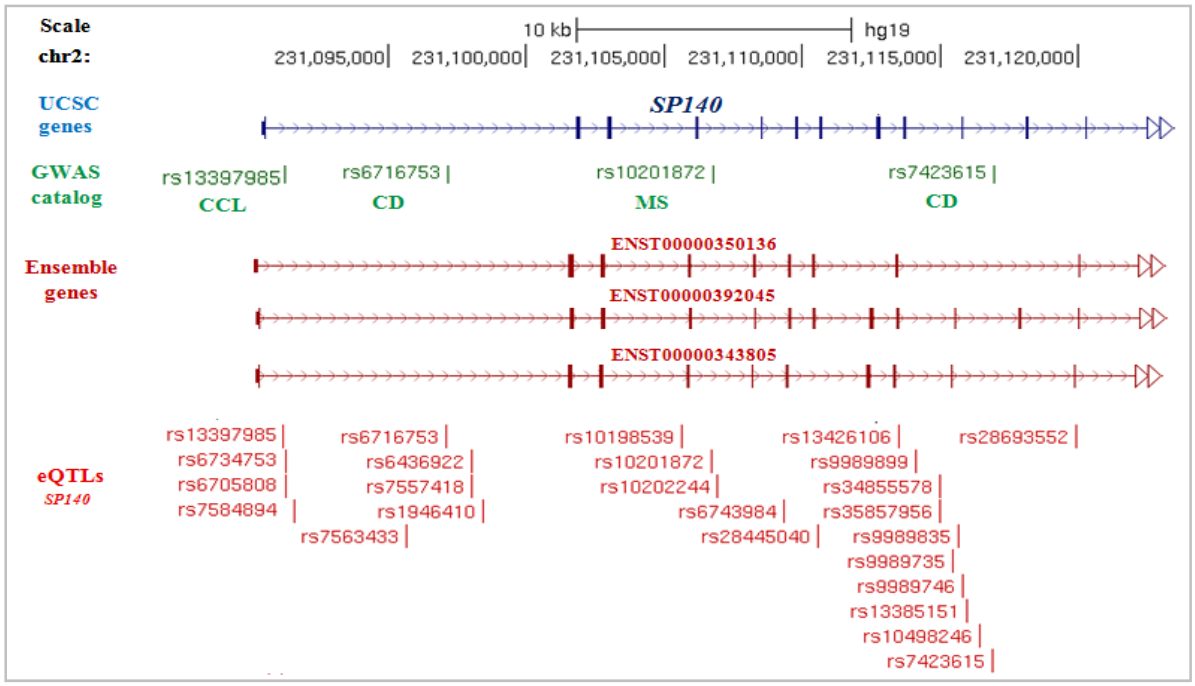

Figure 20.
Schematic representation of the the 2q37.1 locus (chr2:231090445-231123336) 33Mb, from the UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly. UCSC indicate the position of *SP140 gene* .GWAS catalog shows the 2 SNPs rs7423615 and rs6716753 associated with seven CD (Chorn's disease), the SNP associated with CLL rs13397985 and the MS SNP rs1022001872. Ensembl genes represent three trascripts of SP140: ENST00000350136, ENST00000392045 and ENST00000343805. Best-eQTLs for ENST00000392045 and ENST00000343805 transcripts of the *SP140* gene, which correlated with the best MS-associated variants in the Immunochip.

Determination of eQTLs in the 2q37.1 locus

In order to determine the causal polymorphisms of the association with the different disease we determined the variants that correlate with expression of different genes in the locus that colocalize with the disease associated variants. We first identified at this locus the eQTLs in LCLs from European origin from the GEUVADIS Project *(Lappalainen et al., 2013)*. The selection of the eQTLs at coordinates chr2:230856224-231357223, 250 Mb flanking each side of the SNP associated with MS, unrevealed 4 eQTLs, one associated with a transcript of

the *SP100* gene and three with transcripts of the *SP140* gene (Table 7). The SNPs that best correlated with each of the four transcripts (best-eQTLs) were different ones, but those for the transcripts ENST00000343805 and ENST00000392045 of the *SP140* gene were in strong LD (r^2 =0.99) in European population.

						LD between eQTLs and associated SNP (r^2) *(2)*			
						CLL	MS	CD	CD
Gene	**Transcript**	**rho** *(1)*	**P value**	**FDR_P value**	**eQTL**	**rs13397985**	**rs10201872**	**rs6716753**	**rs7423615**
SP100	ENST00000452345	0.32	1.37E-07	5.974E-05	rs1649884	0.009	0.005	0.013	0.009
SP140	ENST00000350136	-0.35	2.87E-09	1.54E-06	rs10498245	0.492	0.449	0.509	0.449
SP140	ENST00000343805	-0.49	3.26E-17	6.18E-14	rs13426106	0.991	0.899	0.959	1
SP140	ENST00000392045	0.44	5.81E-14	7.603E-11	rs13397985	1	0.891	0.967	0.991

Table 7.
Linkage disequilibrium (LD) between the eQTLs at the chr2:230856224-231357223 locus and the GWAS associated variants for different diseases in the region. (1) Spearman's rho correlations between RNA expression levels and genotypes; (2) the LD has been calculated with the EUR population of 1000 Genomes Project; MS, multiple sclerosis; CD Crohn's disease; CLL, chronic lymphocytic leukemia.

Colocalization of best-GWAS variants and best-eQTLs

To determine whether the GWAS-variants in the 2q37.1 locus colocalized with the eQTLs, we calculated the LD between the best-eQTLs and the best-associated SNP for each disease (Table 7). The best-eQTLs for the *SP140* transcripts ENST00000343805 and ENST00000392045 were in almost total LD with the best GWAS-variants of MS, CD, and CLL (Fig. 20A and Table 6). These variants belong to a LD block including 18 variants with r^2 ranging between 0.94 and 1, as calculated from EUR populations of the 1000 Genomes Project. To verify the colocalization between eQTLs and association signals, we examined data of the Immunochip project for MS *(Beechman et al. 20013).* This dataset provides high genotyping density for this region in a large cohort of 14277 cases and 23605 controls. Given that both eQTLs and Immunochip have the 1000 Genomes as base of design, the data of MS association and the eQTLs were available for the same SNPs. Thus, we integrated both signals to determine whether they shared the causative variant (Fig. 21). Complete colocalization was observed between the best MS-associated SNPs and the best-eQTLs for the transcripts ENST00000343805 and ENST00000392045 of the *SP140* gene (Fig. 21A). However, there was no colocalization with the best-eQTLs for the other *SP140* transcript ENST00000350136 and the *SP100* transcripts. In the *locuszoom* graphs (Fig. 21B), we observed that the best associated variant in the Immunochip data for MS, rs9989735, is in total LD with a group of SNPs that are those top correlated with the transcription levels of ENST00000392045 and ENST00000343805, but not with transcript ENST00000350136.

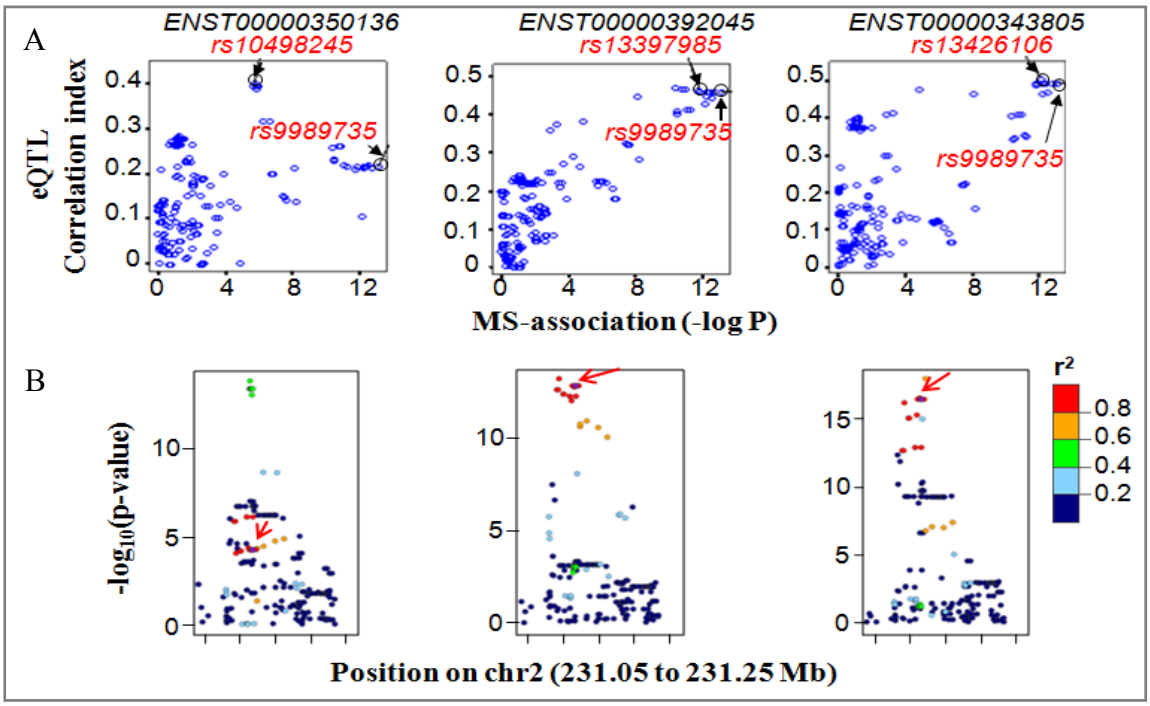

Figure 21.
(A) Scatter plots representing the expression correlation coefficient (absolute value of Spearman's rho coefficient) for each indicated transcript versus the MS-association values (-log *P*). Determination of eQTLs in the region was performed in this work using the RNA sequencing data from GEUVADIS Project, together with the genotype information from the 1000 Genomes Project. In each plot, the best MS-associated SNP and the best-eQTL are indicated. (B) LocusZoom plots showing the expression-correlation levels of variants in the region. The best MS-associated SNP in the locus from the Immunochip dataset is in purple and indicated with an arrow. Colours scale represents the linkage disequilibrium (r^2 values) respect to this variant obtained from the 1000 Genomes EUR population.

Changes in the RNA isoform profile associated with disease

The expression levels of the two *SP140* RNA isoforms showed opposite correlations with the genotypes of the best MS-associated variant (Fig. 22A). The main difference between these two RNA isoforms was the alternative splicing of exon 7. In the LD block, one of the best associated variants rs28445040 was located in exon 7, at 5 bases downstream of the splicing acceptor site. To explore whether the skipping of exon 7 is the functional cause of the association, we turned to eQTL exon-level analysis of the *SP140* for the European (EUR, n= 373) and African-Yoruba (YRI, n= 89) populations of the GEUVADIS Project *(Lappalainen et al., 2013)*. For YRI population, we observed that the best eQTL for *SP140* exon 7 was rs28445040 with a PICS score of 0.6429, much higher than the next one, rs13426106, with a PICS score of 0.0715. For the EUR population the results indicated that rs28445040 was an eQTL for all *SP140* exons, except for exons 24 and 25, albeit a significantly higher correlation coefficient was observed for exon 7 (Fig. 22B). It seemed that the reduction of the expression levels of the ENST00000392045 transcript was compensated by the increase in the ENST00000343805 isoform except for exon 7, not present in the latter.

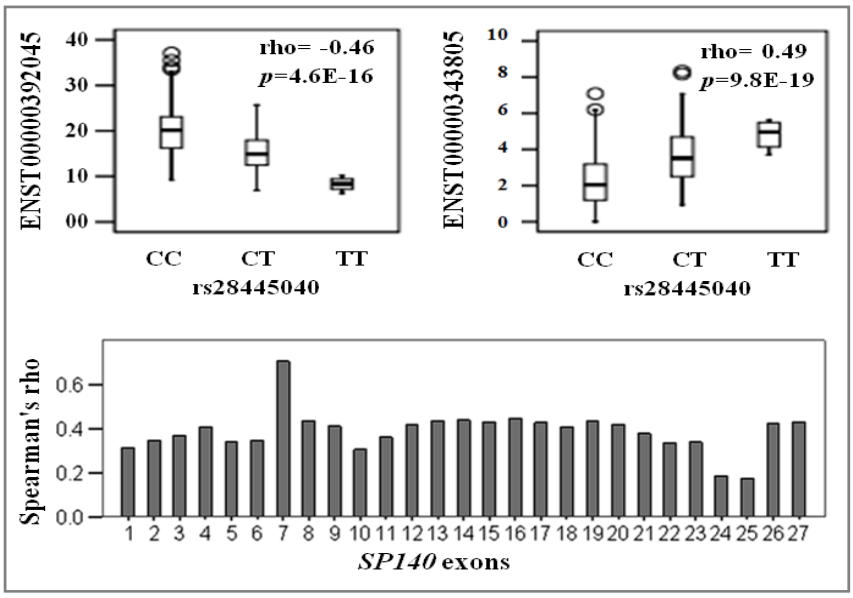

Figure 22.
(A) Box plots represent the mRNA levels from RNA-Seq (GEUVADIS Project) of both *SP140* transcripts ENST00000392045 and ENST00000343805 in 344 LCLs versus the rs28445040 genotype. Spearman's correlation index (rho) and p-value are indicated inside the plots. (B) Spearman's correlation index (*rho*-values) between each *SP140* exon and the rs28445040 genotypes.

To confirm these data experimentally, we analyzed *SP140* RNA levels by a reverse transcriptase (RT)-PCR in LCLs from individuals carrying the different genotypes of rs28445040 (NA12004: CC; NA20766: CT; NA20518: TT) (Fig. 23A) using primers that hybridized in the flanking exon 6 and exon 8. The PCR products were visualized by polyacrylamide-gel electrophoresis. The samples from TT and TC carries showed a band corresponding in size to a fragment lacking exon 7, and with a T-allele dose effect that was absent in the CC carriers.

In order to quantify the expression levels of the two spliced variants and to validate the data obtained from the RNA-Seq from GEUVADIS, we studied SP140 expression levels in 59 LCLs (32 CC, 22 CT and 5 TT for rs28445040) using a bridge primer that hybridized between exons 6 and 8 for the transcript with skipped-exon 7 and another qPCR with a bridge primer between exon 7 and 8 for the full-length transcript, as shown in (Fig. 23B). After Sperman's rho correlation test of expression levels respect to the rs28445040 genotypes, we observed that the expression of the exon 7-skipped transcript was highly correlated with the T-allele dose while the full-length transcript, containing exon 7, was inversely correlated (Fig. 23C).

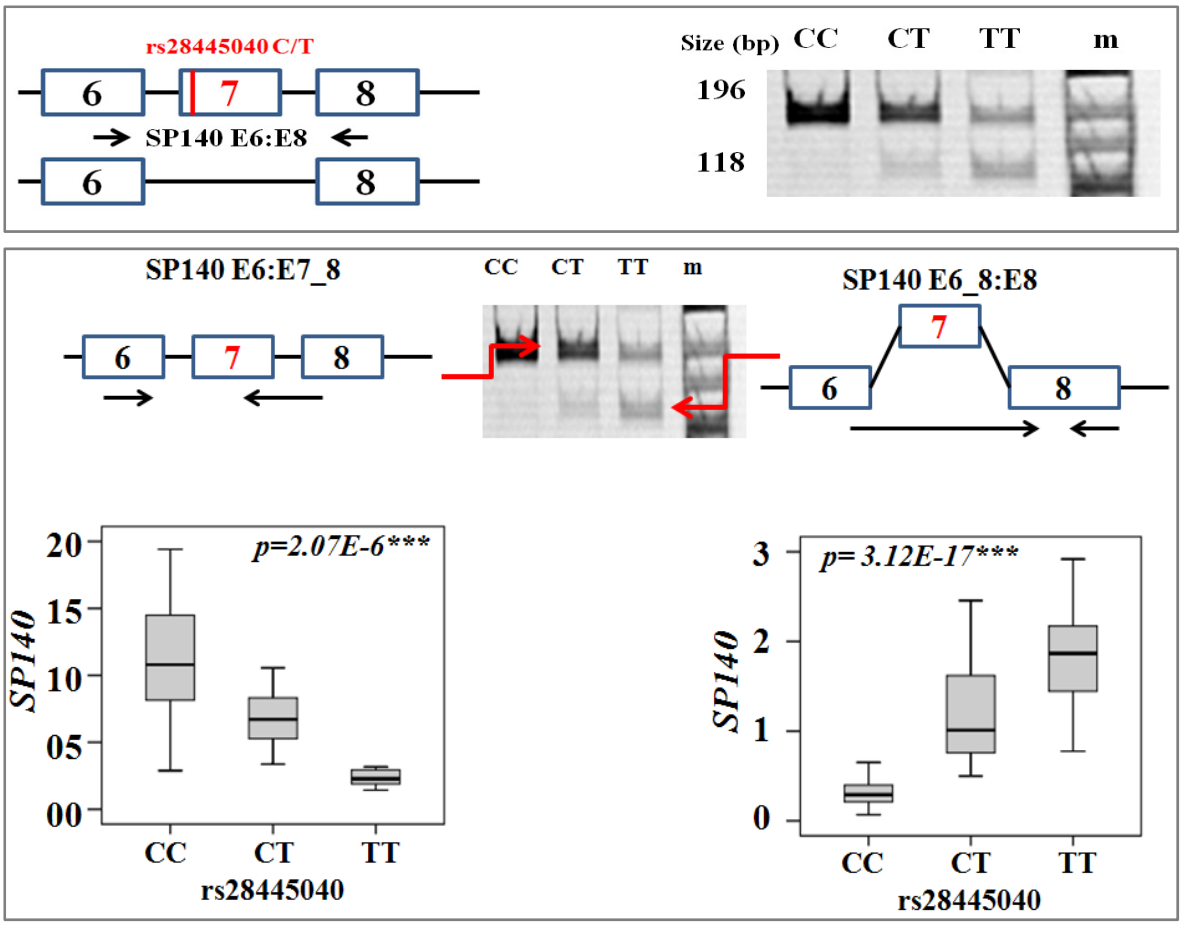

Figure 23.
(A) Schema of the positions of 5' forward and 3' reverse primers in exon 6 and 8 respectively (SP140 E6:E8) and the position of rs28445040 in exon 7 in *SP140* gene. The Polyacrylamide gel electrophoresis (PAGE) showing the results of the RT-PCR amplification of RNA from LCLs with different rs28445040 genotypes (cell lines: NA12004: CC, NA20766: CT, NA20518: TT). The Lane m is the molecular weight marker. (B) Schema of the specific primers SP140 E6:E7_8 used to amplify the full length transcript (exon 7 included) and SP140 E6_8:E8 to amplify the exon7-skipped transcript of the gene *SP140*. (C) Box plots represent the SP140 mRNA levels of the full length and exon7-skipped transcripts respect to rs28445040 genotypes measured by real time qPCR from 59 LCLs . Spearman's correlation index (rho) and p-value are indicated inside the plots stand for the significance of the statistical comparisons by Spearman's rank correlation test Sig. (2-tailed). Relative quantification was performed by RT-qPCR using UBE2D2 as a reference gene using $2^{-\Delta CT}$ method.

rs28445040 as a functional variant affecting the exon 7-skipped RNA isoform

To confirm that rs28445040 is the causal variant of the splicing alteration observed in the *SP140* transcript profile, we used an alternative splicing strategy by cloning the exon 7 and its flanking sequences carrying the two alleles into the pSPL3 plasmid (Fig. 24A). After transfection in HEK cells, RNA purification, RT-PCR amplification, analysis of the RNA products by agarose-gel

electrophoresis and sequencing, we determined that the exon 7- T allele was spliced in about 60% of the molecules and the C allele was spliced in < 10% of the molecules. These data were in agreement with the results shown in (Fig. 24B) confirming that the rs28445040-T allele produced splicing alterations by exon 7-skipping.

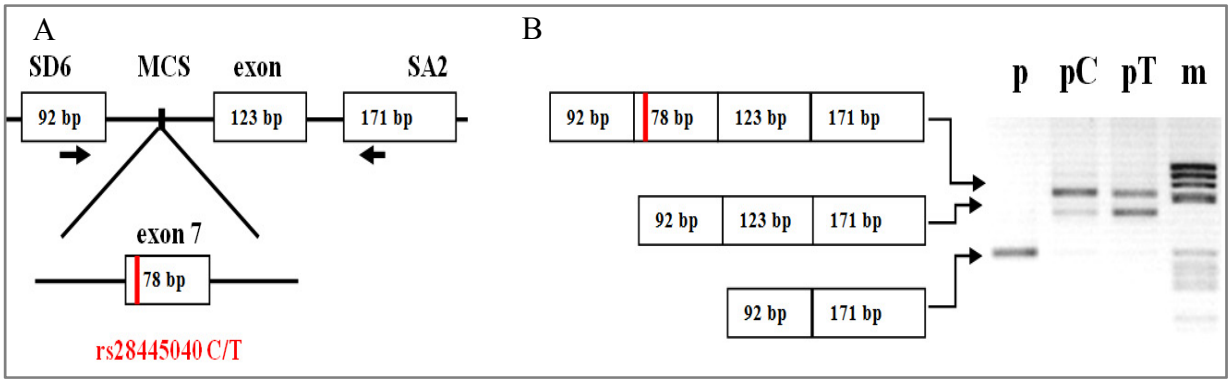

Figure 24.
Alternative splicing of SP140 exon 7. (A)) Genomic DNA construct showing the cloned sequence within the pSPL3 vector in the multi-cloning site position (MCS). The scheme represents the size in bp of the different exons corresponding to the vector, containing a cryptic exon, and the recombinant fragment of the SP140 exon 7 with intron flanking sequences. The position of the rs28445040 variant (C/T) and the primers for RT-PCR amplification are also indicated. **(B)** Agarose gel electrophoresis of RT-PCR from HEK cells RNA transfected with pSPL3- rs28445040 C allele insert (pC), T allele insert (pT) or with no insert as control plasmid (p). Lane m is the molecular weight marker.

Confirmation of the genetic association by a case-control study

We focused our attention on rs28445040 as the causal variant due to its location in the SP140 exon 7 and its high LD with the variant best associated with MS risk by GWAS rs10201872. Initially we studied the LD using the Haploview program version 4.2. with SNPs data of CUE and TSI populations from 1000 genome project, a very high correlation between the causal SNP rs28445040 and the MS variant rs10201872 has been reported (D': 0.951 r2=0.9). In addition, we observed that all risk alleles of GWAS SNPs associated with MS, CLL and CD traits, form one LD block (haplotype) with the T allele of the causal variant rs28445040 which induces the splicing of SP140 gene. The frequency of the haplotype is 0.187 (Fig. 25). Then, the LD between these two SNPs was calculated from the CEU and TSI populations of the 1000 Genomes Project (r2=0.96); however, when the LD was analyzed in other populations we observed that holds great variability. In African populations the LD between these two variants is lower than in the CEU population, ranging from 0.6 to 0.64, and in Asiatic populations both variants are missing. The highest LD, though with differences, was observed in the Amerindian and European populations (Table 8).

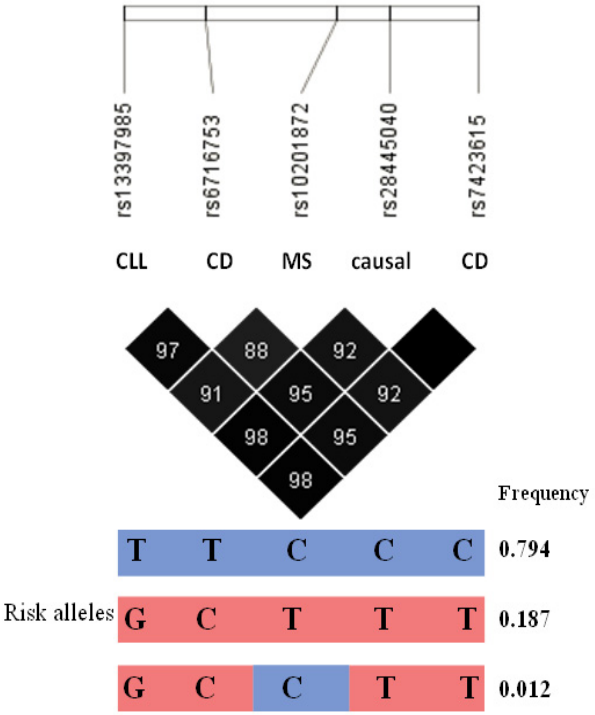

Figure 25.
LD plot represented by r-square in a gray-scale and haplotypes for the CEU and TSI populations for the GWAS SNPs and the functional SNP. Visualized using default settings (MAF 0.1 %) in Haploview version 4.2

		rs28445040 causal variant	**rs10201872** MS-GWAS
Populations	**LD** (r^2)	**MAF** (T)	**MAF** (T)
ASW	0.64	0.156	0.107
LWK	0.63	0.16	0.108
YRI	0.6	0.108	0.068
CLM	1	0.192	0.192
MXL	0.92	0.106	0.098
PUR	1	0.127	0.127
TSI	0.89	0.179	0.163
GBR	0.83	0.208	0.18
FIN	0.82	0.14	0.118
CEU	0.96	0.206	0.2
CHB		0	0
CHS		0	0
JPT		0	0

Table 8.
Linkage disequilibrium between the functional variant (rs28445040) and the GWAS variant (rs10201872) in different human populations and the minor allele frequency (MAF) for each variant.

Therefore, we considered important to estimate the LD and to discern the primary association signal in our Caucasian Spanish population. Thus, we performed a case-control study with 4384 patients and 3197 controls to confirm the association of this functional variant (rs28445040) with MS and to compare it with the best associated variant from GWAS (rs10201872). Results shown in Table 9 indicated that both variants were in strong LD (r^2=0.93) and evidenced similar MS risk association p-values (MAF (T allele) p-values, odds ratios: 1.9 E-9, OR=1.35 [1.22-1.49] and 4.9 E-10, OR=1.37 [1.24-1.51], respectively). After logistic regression analyses, we found that the dominant model was the one that best fitted the data for both SNPs.

	MS patients			Control			Dominant model	
Genotypes / SNPs	TT	CT	CC	TT	CT	CC	p-value	OR (CI 0.95)
rs10201872	158 (3.6)	1390 (31.7)	2836 (64.7)	88 (2.7)	824 (25.8)	2285 (71.5)	4.9 E-10	1.37 (1.24-1.51)
rs28445040	167 (3.8)	1434 (32.7)	2783 (63.5)	99 (3.1)	857 (26.8)	2241 (70.1)	1.9 E-9	1.35 (1.22-1.49)

Table 9.
MS-association of the best GWAS-MS variant (rs10201872) and the functional variant described in this work (rs28445040) by logistic regression analysis. Genotype distributions are shown as the number (%); odds ratio (OR), 95% confidence interval (CI), and p-values were determined by logistic regression analysis with dominant model.

SP140 in CD14 and LCLs

In CD14 monocytes, *SP140* mRNA expression was increased significantly (P<0.005) when cells were stimulated with LPS (20 ng/ml) and IFNγ (2 ng/ml) for 24h (Fig. 26A). However, any significant change in *SP140* expression level was observed in LCLs when they were treated with 1,25(OH)2D200nM for 24h (Fig. 26B). While comparing between the *SP140* expression levels in CD14 and LCL, low level of *SP140* mRNA expressed by CD14 monocytes was observed. However, in LCLs *SP140* is expressed at high level. Furthermore, *SP140* mRNA expression level in LCLs showed a significant association with the MS-GWAS rs10201872 (Spearman's rho=0.50, p=3.2E-05) (Fig. 26C). Low expression level of *SP140* was clearly associated with the MS risk allele rs10201872-T. Thus, the high level was associated with the MS protective allele rs10201872- C.

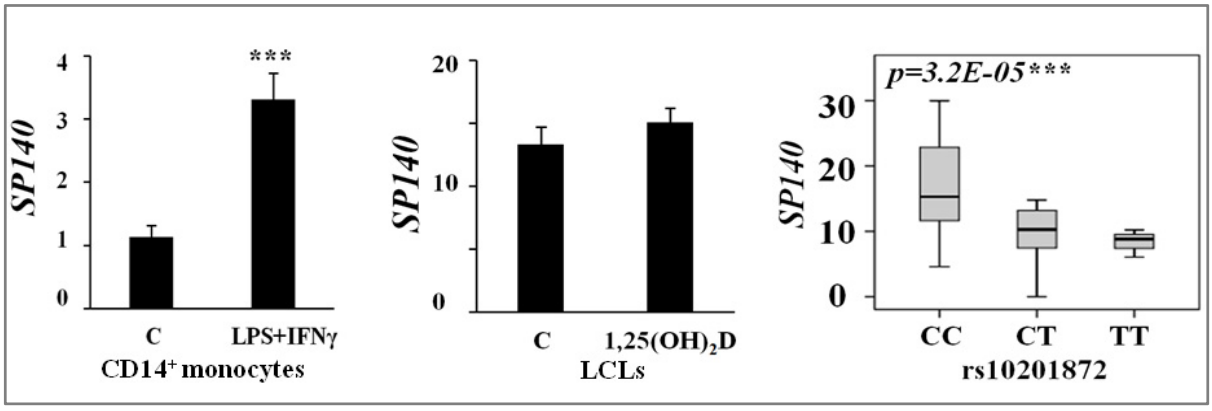

Figure 26.
SP140 mRNA expression in CD14+ monocytes and LCLs. (A) Induction of SP140 expression in CD14+ stimulated with LPS (20 ng/ml) and IFNγ (2 ng/ml) for 24h. ***P<0.0005 compared with the positive control (cells not stimulated). (B) SP140 mRNA expression in LCLs control and LCLs incubated with 200nM of 1,25(OH)2Dfor 24h. Each bar is the mean of eight samples ±SE. (C) *SP140* mRNA expression levels respect to the rs10201872 genotypes in 59 LCLs represented by boxplot distributions with medians and quartiles. p-value= 3.2E-05 stands for the significance of the statistical comparisons by Spearman's rank correlation test Sig. (2-tailed). Relative quantification was performed by RT-qPCR using UBE2D2 as a reference gene using $2^{-\Delta CT}$ method.

SP140 and Vitamin D

In order to study the effect of vitamin D on SP140 in LCL we tranfected LCL cells with pSPL3-rs28445040 (p, pC and pT). Then the cells were incubated with 200nM of 1,25(OH)2Dfor 24 h. The PCR products amplified the plasmid inserts using the specific primers (SD6 and SA2) run in agarose gel. No difference was observed between control and vitamin D treated cells (Fig. 27).

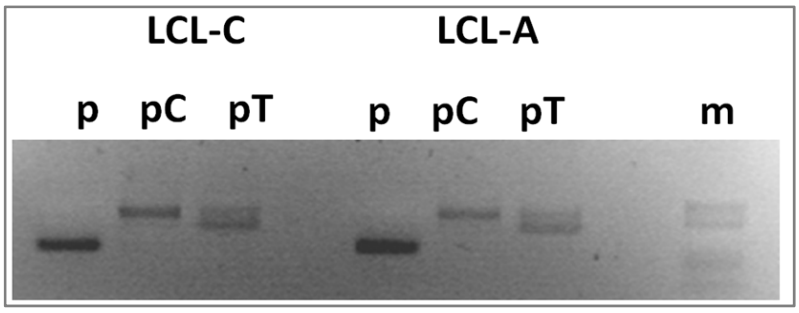

Figure 27.
Agarose gel electrophoresis of RT-PCR from LCL-C as control and LCL-A cells incubated with 1,25(OH)2D200nM for 24h transfected with pSPL3-rs28445040 C allele insert (pC), T allele insert (pT) or with no insert as control plasmid (p). Lane m is the molecular weight marker.

Determination of the causal gene responsible for the association with MS in the 12 p13.31 locus

The 12p13.31 locus (Fig. 28) has been associated to MS in GWAS by the variant rs10466829 (risk allele A) *(Sawcer S et al., 2011)* which is located in the first intron of *CLECL1* gene. In addition, this region has been associated with the autoimmune disease type 1 diabetes by 2 different GWAS by the variants rs3764021, rs11052552 *(WTCCC et al., 2007)* and rs4763879 *(Barrett et al., 2009).* Then the p13.31 locus was associated with N-glycosylation of human immunoglobulin G, interestedly, most of the loci associated with this trait have been strongly associated with autoimmune and inflammatory conditions *(Lauc et al., 2013).* On the other hand, this locus has been also associated with Obesity-related traits (Comuzzie et al., 2012).

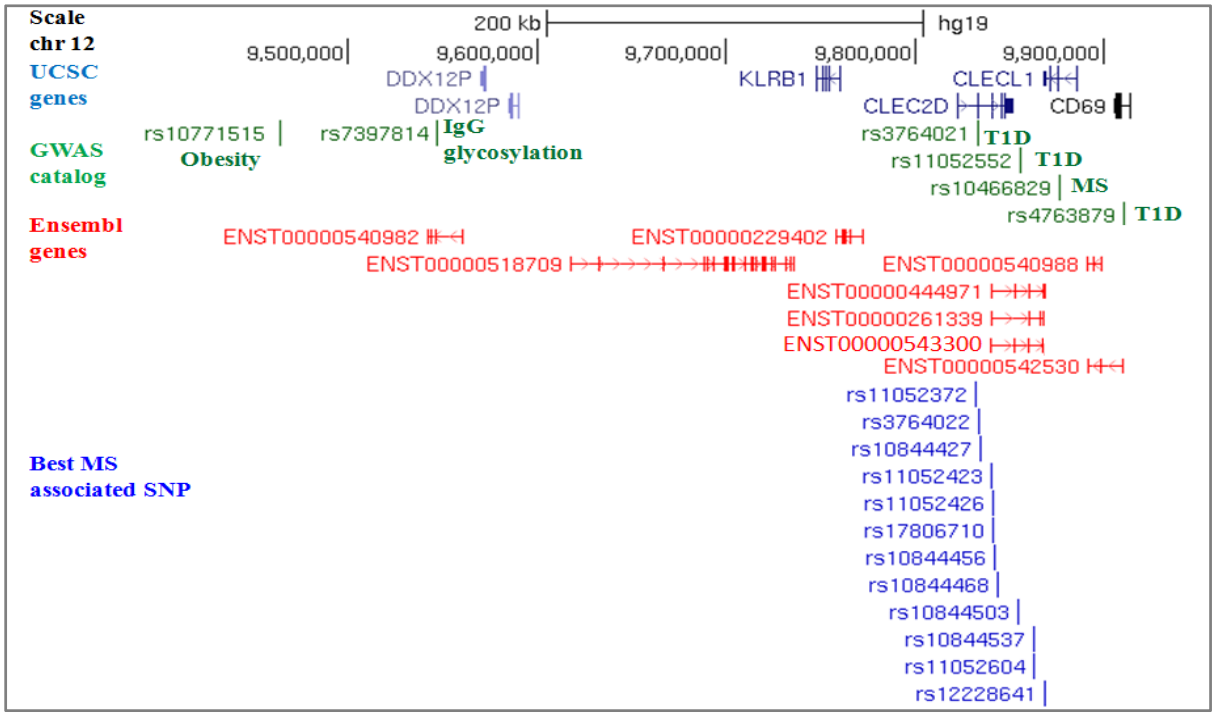

Figure 28.
Schematic representation of the locus 13.31 on chr12 (chr12:9436282-9973552) 537Mb, from the UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly. UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics) indicate the position of the genes in this region.GWAS catalog shows the MS associated SNP rs10466829, the SNPs associated to T1D, IgG glycosylation and to the obesity relatred trait. Ensembl genes represents the trascripts studied. Best MS associated SNPs from ImmunoChip.

Determination of eQTLs in the chr12 p13.31 locus

In order to determine the gene associated with MS in the region of chr12 p13.31, we determined the eQTLs that can affect the expression of the locus genes in LCLs using the GUEVADIS project *(Lappalainen et al., 2013).* The selection of the eQTLs at coordinates chr12:9436282-9973552, 250 Mb flanking each side of the SNP associated with MS, unrevealed 8 eQTLs. One for the *KLRB1* gene, two for *ncRNAs,* three for *CLEC2D* and two for *CLECL1* (Table 10).

Gene	Transcript	Best eQTL	eQTL correlation(1)	MS association(2)	Best MS SNP(3)	eQTL correlation(1)	MS association(2)
KLRB1	ENST00000229402	*rs7137213*	0.30	1.85	*rs10844503*	0.14	8.22
RP11599J14.2	ENST00000540982	*rs7137213*	0.23	1.85		0.11	8.22
RP11726G1.1	ENST00000518709	*rs10771920*	0.52	3.30		0.23	8.22
CLEC2D	ENST00000444971	*rs2401388*	0.33	0.62		0.10	8.22
CLEC2D	ENST00000261339	*rs2401394*	0.41	7.91		0.40	8.22
CLEC2D	ENST00000543300	*rs4763283*	0.38	6.67		0.31	8.22
CLECL1	ENST00000542530	*rs7977720*	0.52	6.92		0.43	8.22
CLECL1	ENST00000540988	*rs2080206*	0.45	6.62		0.38	8.22

Table 10.
Comparison of transcript level correlations and MS-association *P*-values between best eQTLs and best MS-associated SNPs at the chr12 p13.31 locus. (1) Spearman's rho correlations between RNA expression levels and genotypes. (2) Data obtained from ImmunoChip as -Log *P*-values. (3) Best MS associated SNP from ImmunoChip.

Colocalization of best-GWAS variants and best-eQTLs

To verify the colocalization between eQTLs and association signals in the chr12 p13.31 locus, we examined data of the Immunochip project for MS *(Beecham et al., 2013)*. This dataset provides high genotyping density for this region in a large cohort of 14277 cases and 23605 controls. Given that both eQTLs and Immunochip have the 1000 Genomes as base of design, the data of MS association and the eQTLs were available for the same SNPs. Thus, we integrated both signals to determine whether they shared the causative variant (Fig. 29).

Complete colocalization was observed between the best MS-associated SNPs and the best eQTLs for the transcript ENST00000261339, and ENST00000543300 corresponding to the *CLEC2D* gene (C-type lectin domain family 2, member D). However, as observed in the Figure 29, there was no colocalization between the best eQTLs for other RNA isoforms in the same locus, corresponding to *CLECL1*, *KLRB1*, *CLEC2D* and two long non-coding RNA (*lncRNA*), and the best MS-associated variants.

The best eQTLs and the best MS associated SNPs were not unique but rather they were a group of several SNPs in almost total LD (Fig. 29). Although, both datasets were obtained from Caucasian populations, there were some differences between the SNPs that integrated the groups of best MS variants and the ones obtained from the eQTL analysis. These divergences were reflected in differences of LD between cohorts, as can be observed in the *locuszoom* graphs (Fig. 30).

Since the best eQTL for CLECL1 and CLEC2D were in high LD, we wanted to discarded possible error using a second set of data from the GWAS of the IMSGC. In this case when we represented the data by LocusZoom, taking a reference the best associated variant of the GWAS (rs10466829), we observed that these data colocalize better with the CLECL1 eQTLsENST00000542530 (Fig. 30A).

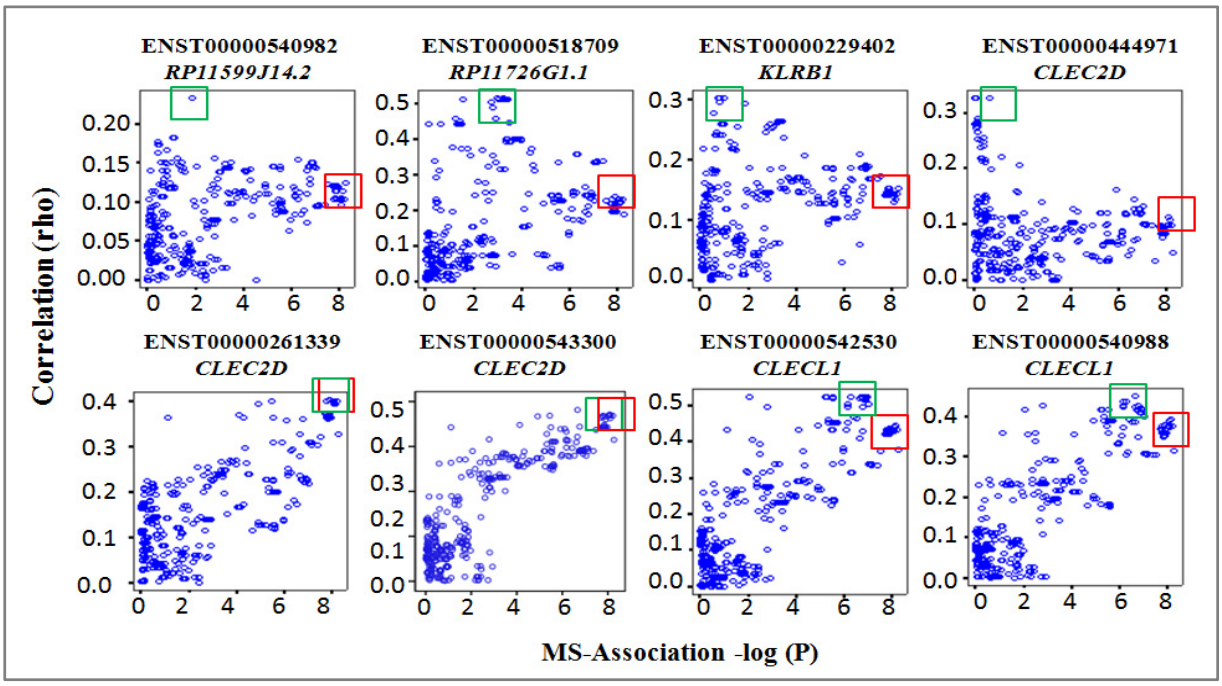

Figure 29.
Scatter plots representing the expression correlation coefficient (absolute value of Spearman's rho coefficient) versus the MS-association values (-log *P*) of the SNPs for each indicated transcript. Data for MS-association was obtained from the ImmunoChip Project at http://www.immunobase.org/. Determination of eQTLs in the region was performed in this work using the RNA sequencing data from GEUVADIS Project together with the genotype information from the 1000 Genomes Project. In each plot, the green square frames the best eQTLs for the indicated transcripts and the red square frames the best MS-associated SNPs.

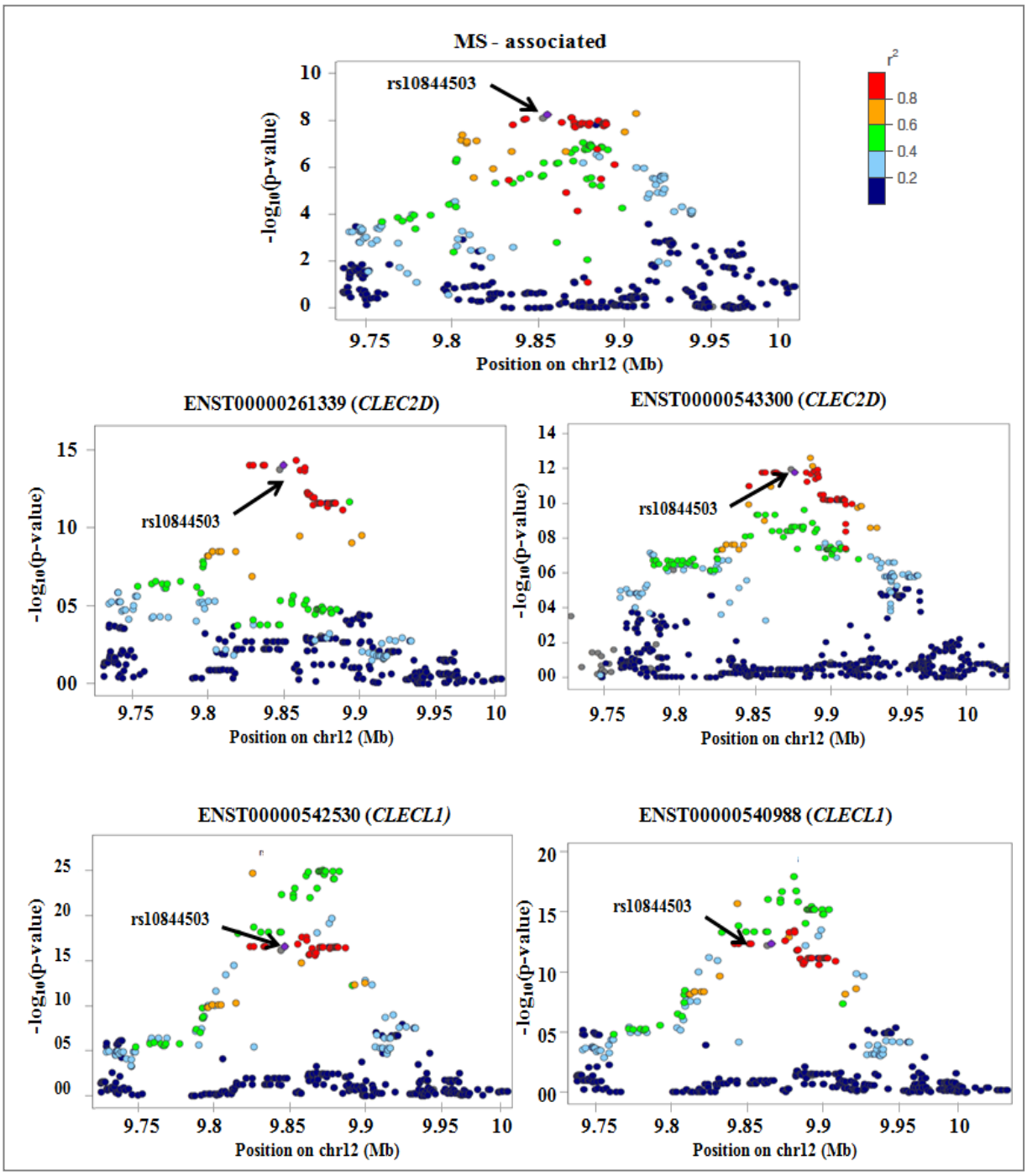

Figure 30 A.
LocusZoom plots showing the expression-correlation levels of variants in the region chr12: 9700000-1000000. The best MS-associated SNP from the Immunochip dataset rs10844503 in the locus is in purple and indicated with arrow. Colours scale represents the linkage disequilibrium (r^2 values) respect to this variant obtained from the 1000 Genomes EUR population.

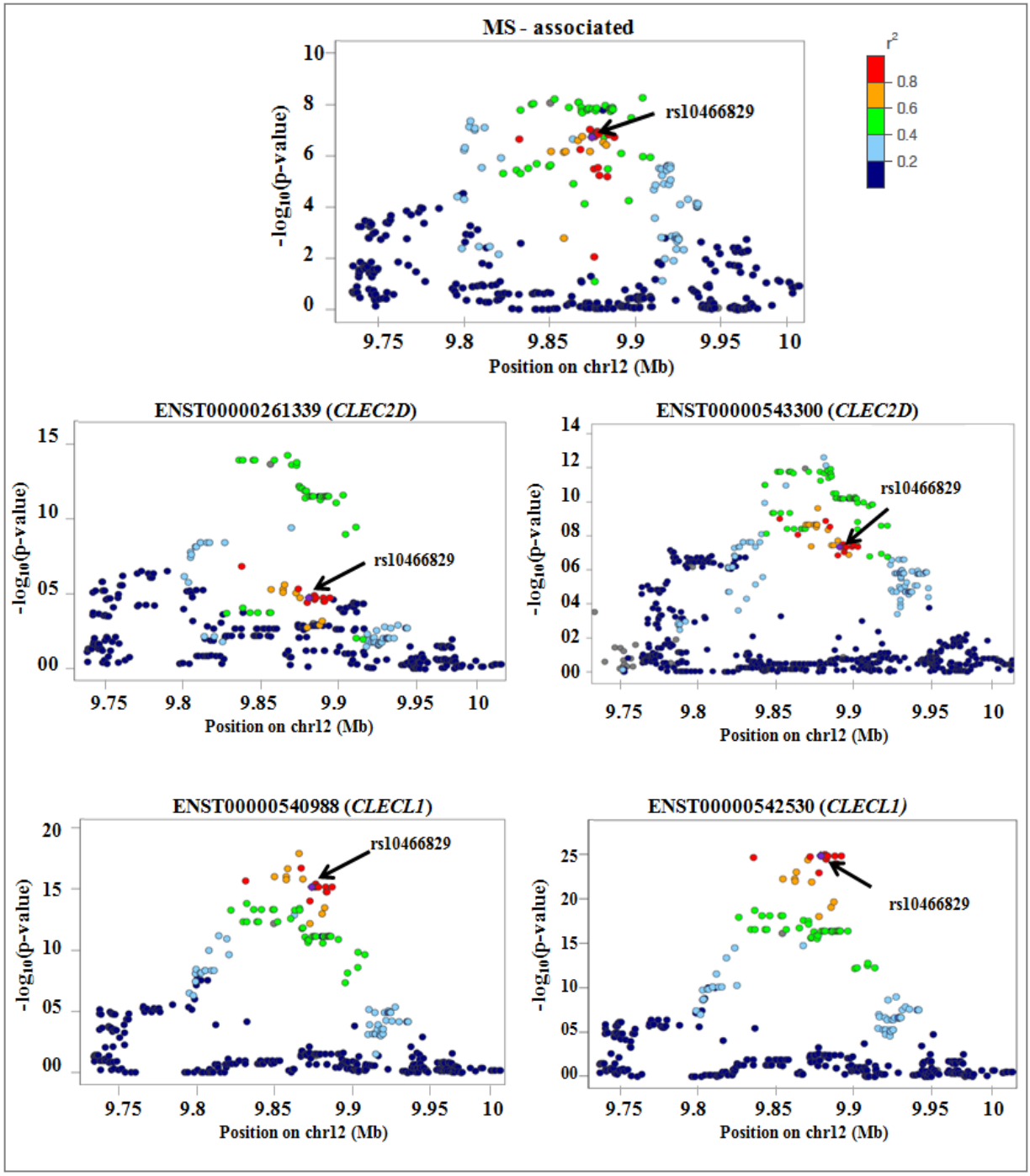

Figure 30 B.
LocusZoom plots showing the expression-correlation levels of variants in the region chr12: 9700000-1000000. The best MS-associated SNP rs10466829 from GWAS is in purple and indicated with arrow. Colours scale represents the linkage disequilibrium (r^2 values) respect to this variant obtained from the 1000 Genomes EUR population.

Changes in the RNA isoform profile associated with disease

Using the GEUVADIS data, we got that the expression levels of the two *CLEC2D* RNA isoforms (ENST00000261339 and ENST00000543300) showed opposite correlations with the genotypes of the best MS-associated variant (Fig. 31). The main difference between these two RNA isoforms was the alternative splicing of exon 2. In the LD block, one of the best associated variants rs3764022 was located to 6 bases of the splicing acceptor site of exon 2. It seemed that the reduction of the expression levels of the ENST00000543300 transcript was compensated by the increase in the ENST00000261339 isoform.

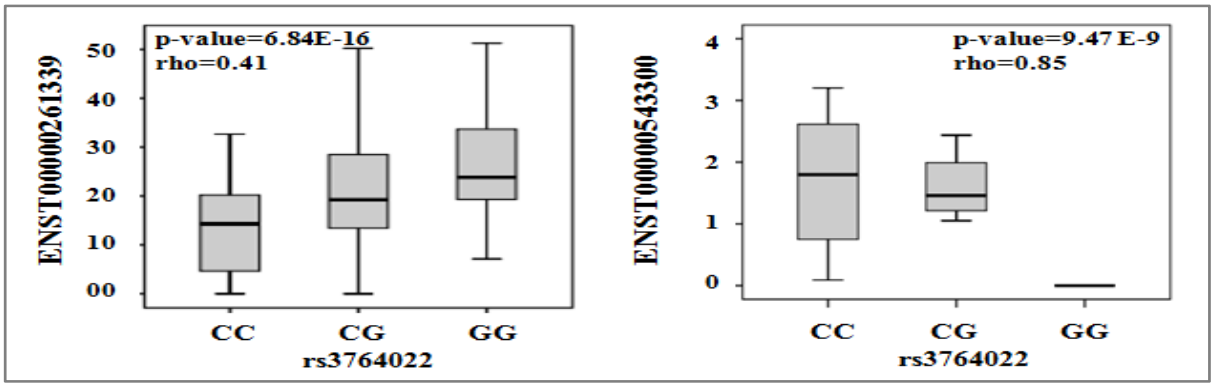

Figure 31.
Box plots represent the mRNA levels from RNA-Seq (GEUVADIS Project) of both *CLEC2D* transcripts ENST00000261339 and ENST00000543300 in 344 LCLs versus the rs3764022 genotype. Spearman's correlation index (rho) and p-value are indicated inside the plots.

To have an experimental confirmation, we performed RT-PCR using primers that hybridized in the flanking exon 1 and exon 3 (*CLEC2D* E1:E3) with LCLs carrying the different genotypes of the rs3764022 (Fig. 32). The cell lines bearing the CG and GG genotypes showed a band corresponding in size to a fragment lacking exon 2 and with a G allele doses effect.

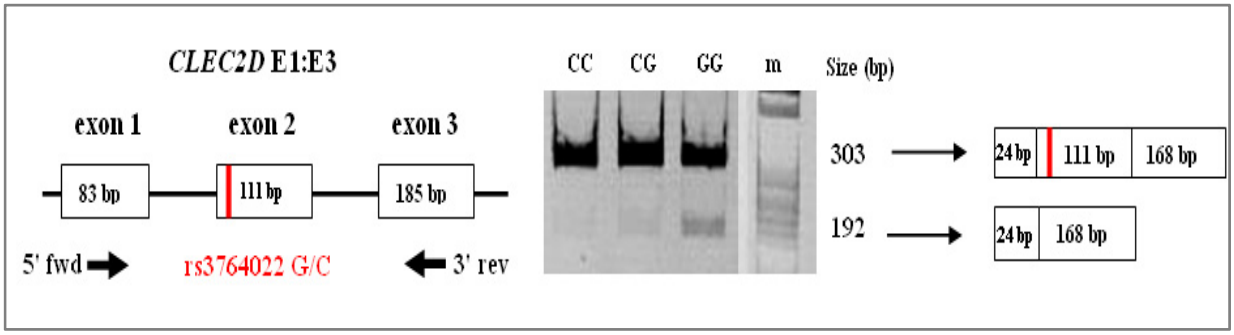

Figure 32.
Schema of the positions of 5' forward and 3' reverse primers in exon 1 and 3 respectively (CLEC2D E1:E3) and the position of rs3764022 in exon 2 in *CLEC2D* gene. The Polyacrylamide gel electrophoresis (PAGE) showing the results of the RT-PCR amplification of mRNA from LCLs with different rs3764022 genotypes CC, CG and GG. the Lane m is the molecular weight marker.

Then we proved this association using 24 LCLs samples (8 sample for each genotype of rs3764022) with relative quantification by real time PCR using a 1st-3th exon bridge primer (CLEC2D E1_3:E3). Results showed a strong association between rs3764022-G allele and high expression level of *CLEC2D*, therefore rs3764022-C allele showed to be associated with low gene expression (Fig. 33).

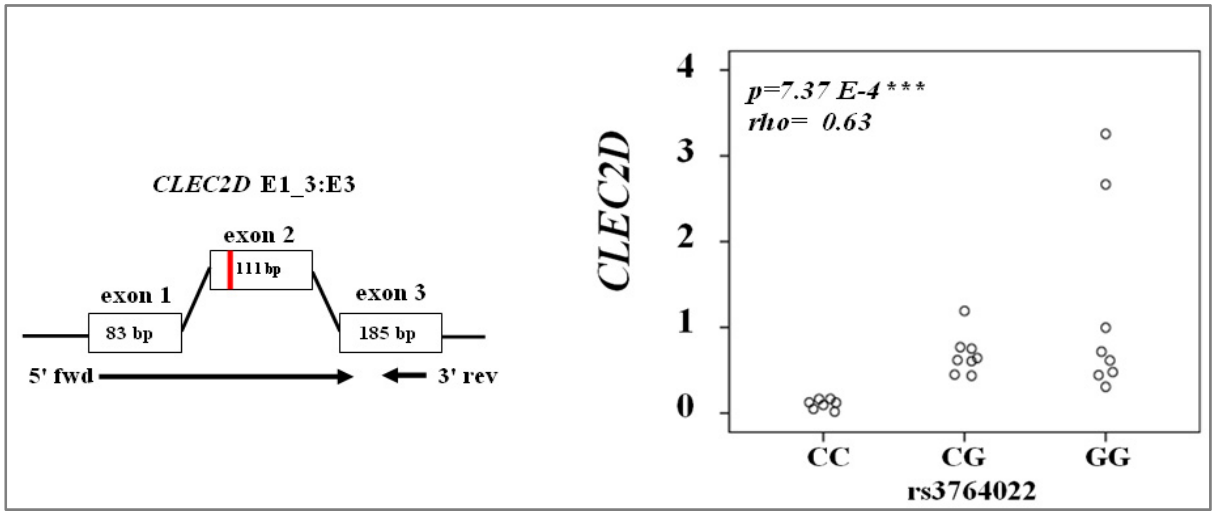

Figure 33.
The mRNA expression levels depend on rs3764022 genotypes in 24 LCLs samples (8 sample for each genotype) represented by simple scatter graph p= 5.89E-05 stands for significance. Also the scheme of the position of the primers used to amplify the *CLEC2D* gene are indicated. Relative quantification was performed by RT-qPCR using UBE2D2 as a reference gene using $2^{-\Delta CT}$ method.

Analysis of the genetic association by a case-control study

We focused our attention on rs3764022 as the causal variant due to its location in the *CLEC2D* exon 2 and its high LD with the variant best associated with MS risk by ImmunoChip rs10844503.

Initially we studied the LD using the Haploview program version 4.2 with SNPs data of EUR populations (CUE, TSI, GBR, FIN and IBS) from 1000 genome project, a very high correlation between the causal SNP rs3764022 and the MS variant by ImmunoChip rs10844503 has been reported. Also, important correlations have been detected between the functional SNP and rs12227655 and rs10844609 which are the best eQTLs for ENST00000543300 (*CLEC2D*) and ENST00000261339 (*CLEC2D*) respectively (Fig. 34).

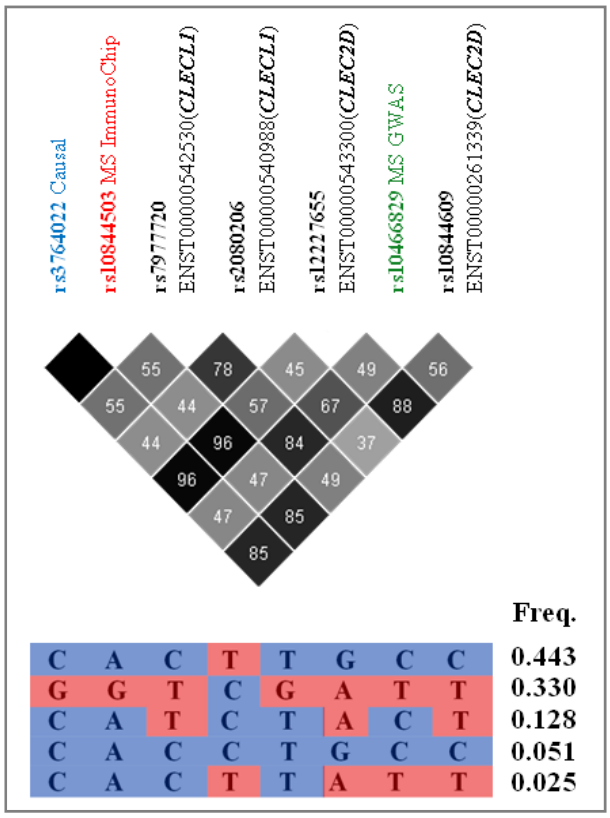

Figure 34.
LD plot represented by r-square in a gray-scale and the haplotypes for the EUR populations for the GWAS and Immunochip SNPs, the functional SNP and for the best eQTLS for *CLECL1* and *CLEC2D* transcripts mentioned above. Visualized using default settings (MAF 0.1 %) in Haploview version 4.2

To try to determine which of the eQTLs, the one for *CLECL1* or for *CLEC2D*, is the actual causal variant of the MS association we performed a case-control study with 4046 patients and 3120 controls with the functional variant (rs3764022) that affect *CLEC2D* slicing or the best associated variant from GWAS (rs10466829). Results shown in Table 11A indicate that both variants were not associated with the disease in our cohort. Also, a meta-analysis study has been done with Caucasian Spanish population and German populations (Table 11B).

SNP	A1/A2	TEST	MS patients	Control	P-value
rs3764022	G/C	GENO	497/1881/1620	358/1461/1269	0.5542
		TREND	2875/5121	2177/3999	0.3774
		ALLELIC	2875/5121	2177/3999	0.3841
		DOM	2378/1620	1819/1269	0.6257
		REC	497/3501	358/2730	0.2829
rs10466829	G/A	GENO	833/2001/1212	682/1507/931	0.3998
		TREND	3667/4425	2871/3369	0.4118
		ALLELIC	3667/4425	2871/3369	0.4087
		DOM	2834/1212	2189/931	0.9155
		REC	833/3213	682/2438	0.1915

SNP	A1/A2	p-value	OR	Q
rs3764022	G/C	0.3831	1.0262	0.8098
rs10466829	G/A	0.08139	0.9568	0.4693

Table11.
(A). MS-association of the functional variant (rs3764022) and the best GWAS-MS variant (rs10466829) by logistic regression analysis.(B) Results of the meta-analysis study. A: minor allele. A2: major allele. TEST: type of test, GENO (genotypes), TREND (Cochran-Armitage test), ALLELIC, DOM (dominant), REC (recessive). OR: Odds ratio. Q: p-value for Cochrane's Q statistic.

Discussion

MS and Vitamin D genes expression: *CYP27B1*, *VDR* and *CYP24A1*

MS is an inflammatory demyelinating and neurodegenerative disease of the CNS causing lesion in its white matter where usually the infiltration of monocytes, T and B lymphocytes, and plasma cells is seen.

In this study we examined the relationship between the MS-associated regulatory variant (rs10877013) (Alcina et al., 2012) and the expression of genes involved in vitamin D activation (*CYP27B1*), vitamin D receptor (*VDR*) and vitamin D degradation (*CYP24A1*), in 119 $CD14^+$ monocytes samples under inflammatory conditions challenged by IFNγ+LPS, and in 109 LCLs under autocrine-like stimulation with vitamin D. This polymorphism may be considered causal for MS and, most likely, a common genetic determinant for several autoimmune diseases associated with the same LD block, increasing disease susceptibility by down-regulating *CYP27B1* expression.

We found that, in non stimulated $CD14^+$ monocytes *CYP27B1* and *VDR* expressed at low levels (Fig.11A.B and 14A); *CYP24A1* was not expressed in $CD14^+$ neither in the nonstimulated nor in stimulated cells with IFNγ and LPS. However, the transcription of *CYP27B1* and *VDR* in monociytes was upregulated after IFNγ and LPS treatment. Considering that there was no vitamin D in the culture medium to induce *VDR* expression and no correlation was found in the expression of these two genes, they seem to be independently regulated. In addition, the MS-risk allele rs10877013-C was associated with low expression of *CYP27B1* (Fig. 12C), but not with *VDR*. Thus, a strong pro-inflammatory stimulus upregulated the expression of *CYP27B1* in monociytes not in LCLs (B cells), and the expression levels were affected by rs10877013 genotypes. The differential effect of this variant in monocytes and B lymphocytes (LCLs), regarding *CYP27B1* expression, indicates that it exerts its action in cell type-specific manner, affecting regulatory sequences of the enhancer where it is localized, and only functioning in monocytes after their activation with LPS-IFNγ.

The association of *CYP27B1* expression with rs10877013 genotypes may have a total effect in gene expression and may alter the anti-inflammatory effect of vitamin D in monocytes, *(Wöbke et al., 2014)* DCs *(Shahijanian et al., 2014) and* NK cells (*Morán-Auth et al., 2013).*

As it is known,during inflammatory stimuli in $CD14^+$ monocytes , the p38 MAPK pathway is induced through TLR activation to promote the production of the proinflammatory cytokines and T cells differentiating cytokines: IFNγ and IL2, polarizing Th1 and Th17 respectively. The production of active vitamin D upregulates *(Zhang et al., 2012)* the expression of

mitogen-activated protein kinase phosphatases-1 MKP-1, which in turn inhibits p38 MAPK, thus preventing the proinflammatory cytokine production in monocytes / macrophages and enhancing the apoptotic death of inflammatory $CD4^+$ T cells in experimental EAE *(Pedersen et al., 2007)* (Fig. 34). In addition, it has been demonstrated that he ***p38 MAPK*** expression was 5 fold elevated in MS lesion *(Lock et al., 2002)*. As we have proved in our study that the MS risk allele rs10877013-C is associated with low expression of *CYP27B1* in monocytes so it means to be associated with reduction in vitamin D active form ($1,25(OH)_2D_3$). Consequently, minimal inhibition of the proinflammmatory pathway p38 MAPK by MKP-1could lead to CNS damage directly or indirectly (Fig. 35). So Vitamin D plays the role of immunomodulator in inflammmatory responses.

Consistent with previous studies, these data support the hypothesis that low vitamin D level is more a cause than a consequence of illness *(Pakpoor and Ramagopalan, 2014; Autier et al., 2014; Gillie et al., 2014)*.

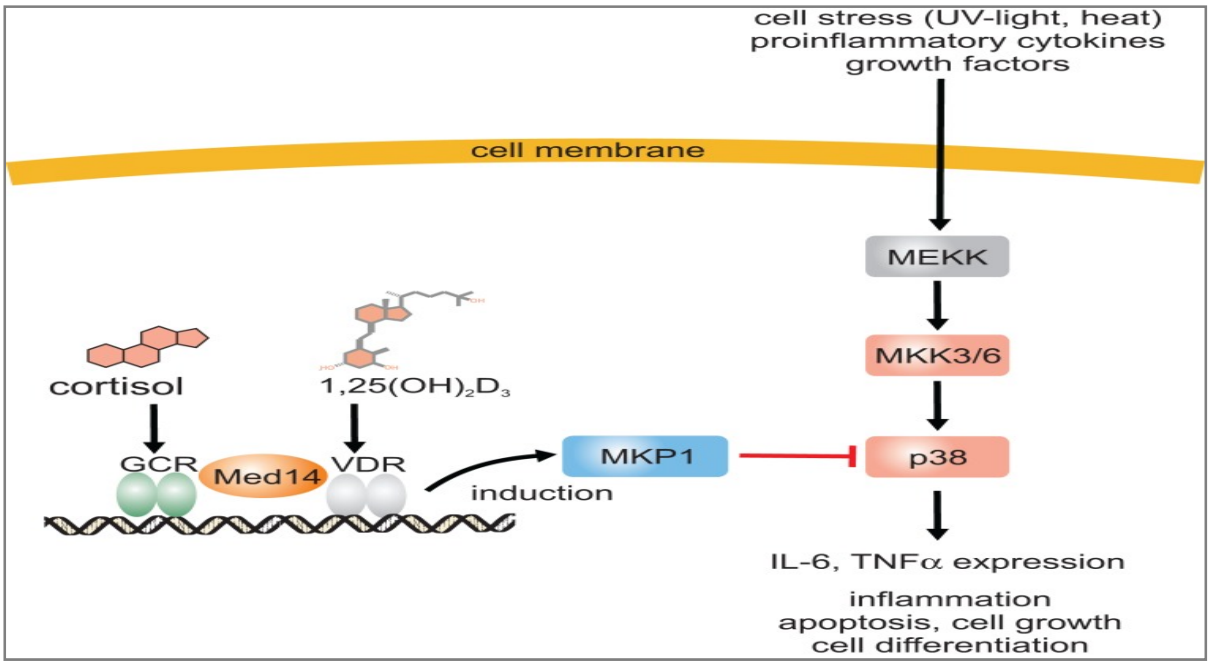

Figure 35.
Inhibition of the p38 MAP kinase pathway by 1,25(OH)2Dand a mechanism for the synergistic anti-inflammatory effects of 1,25(OH)2Dand glucocorticoids. Proinflammatory stimuli lead to p38 MAP kinase phosphorylation and activation which subsequently induces expression of many proinflammatory proteins such as IL-6 and TNFα. $1\alpha,25(OH)_2D_3$ induces MKP1 expression which dephosphorylates and inactivates p38 MAP kinase. 1,25(OH)2Dstimulates glucocorticoid-induced MKP1 expression via enhanced expression of Med14.

CYP27B1 and *VDR* are expressed constitutively in LCLs, however *CYP24A1* is not expressed. We observed that 1,25(OH)2Dhas no effect on *CYP27B1* expression in LCL, however $25(OH)D_3$ induces slightly the *CYP27B1* (not significantly). On the other hand, *VDR*

being upregulated by both vitamin D forms, even it is thought to be induced more significantly when cells are treated with $1,25(OH)_2D_3$. We also found that the *VDR* transcription, but not of the *CYP27B1*, was associated with the MS-risk allele in 109 LCLs stimulated with $1,25(OH)_2D_3$. The cause of this association is unknown. VDR is located at approximately 10 Mb from the variant site (Fig.14) , close to *CYP27B1*, with a high potential of regulating over long distances in the same chromosome, since chromatin interaction between promoters and promoter-enhancer of many genes have been described. (Chepelev et al., 2012) (Li et al., 2012).

Referring to our data, CYP24A1 was not expressed in $CD14^+$ monocytes even after treatment with several stimuli, but it was specifically upregulated by $1,25(OH)_2D_3$ in LCLs, and its expression correlated with the expression of *VDR*. The MS-GWAS associated variant in the region *(Sawcer et al., 2011)* has been associated with *CYP24A1* expression in the brain tissue of frontal cortex and temporal cortex, rs2248359-G risk allele is associated with high expression level of *CYP24A1 (Ramasam et al., 2014*). *CYP24A1* encodes the enzyme responsible for initiating of the $1,25(OH)_2D_3$ degradation (the physiologically active form of vitamin D_3) proving the possible pathogenic role for low levels of $1,25(OH)_2D_3$ in MS. However, in our study, neither the MS-GWAS associated variant in the *CYP24A1* region (rs2248359) nor any other variant located in 1 Mb around the *CYP24A1* from the 1000 Genome Project database, seemed to associate with its expression levels after vitamin D activation.

Additionally, studying the gene expression in LCLs stimulated with 1,25(OH)2D , we found that the expression levels of *VDR* and *CYP24A1* were directly correlated. The VDR activated by vitamin D can induce the transcription of *CYP24A1* which in turn inactivates $1,25(OH)_2D_3$ by hydroxylation. The important role of VDR in MS can be explained through the results concerning that VDR directly interacts with enhancer and promoter elements and likely modifies their action. In addition, VDR binding is also more likely to occur within MS-related regions when compared with the rest of the genome and more than 60% of MS-related regions are bound by the VDR. These include the genomic regions containing the MS-GWAS reported genes *EVI5, VCAM1, CXCR4, SP140, TME M39A, CD86, NFKB1, CXCR5, TNFRSF1A, CLECL1, CYP27B1, CLEC16A, IRF8, STAT3, TNFSF14, TYK2, CD40* and *TNFRSF6B.*. (*Disanto G et al., 2012)*

Many studies suggested that childhood or early adolescence is the crucial time period for vitamin D-dependent MS risk. The interactions of genetic and other environmental risk factors, contributing to the onset of disease, appear to occur later in life *(Goodin, 2012).* In

such a context, the relevance of our findings is based on the regulatory role that the polymorphism rs10877013 exert on two vitamin D metabolism genes (*CYP27B1* and *VDR*) in two different immune cell types (monocytes and B cells).

Thus, the rs10877013 C allele is a "low producer" of *CYP27B1* in activated monocytes (CC genotype carriers express about a third of TT) as well as a "low producer" of *VDR* in B cells (LCLs) (CC genotype carriers express about a half of TT). This genetic constitution, in combination with a potential vitamin D deficit in the early years of life, caused by low sunlight exposure or low vitamin D intake, could produce a synergistic effect, leaving these individuals unable of efficiently process different infectious agents or inflammatory situation that could occur in adolescence or later in life. The inflammatory stimulus could be alone or in a combination of infectious agents: Epstein Barr virus (EBV), Human Herpes virus type 6 (HHV-6), endogenous retroviruses, etc, all of them MS risk candidates. Monocytes could carry out a special role in innate immunity, by inducing the expression of *CYP27B1*, and with the encoding enzyme, converting the serum vitamin D ($25(OH)D_3$) into active vitamin D ($1,25(OH)_2D_3$) intracellularly and mounting an efficient immune response against pathogens.

In clinically definitive MS, active vitamin D ($1,25(OH)_2D_3$) may reduce differentiation of monocytes to DCs and proliferation, thus decreasing T-cell stimulation; controling T-cell activation and inhibiting T-cell proliferation. At the same time, it promotes activity of the Th2 phenotype cells (with a protective role), resulting in an anti-inflammatory effect and enhances the production of the anti-inflammatory cytokine IL10 *(Munger et al., 2011 and 2014; Schwalfenberg et al., 2011; Wöbke et al., 2014).* The regulatory effect of the rs10877013, depending on the genotype, can alter the expression of *CYP27B1* in monocytes and *VDR* in B cells (LCLs), and consequently interfering with these anti-inflammatory processes (for instance, an infectious agent). So the CC genotype carriers, "low producers", have less capability of mount an anti-inflammatory response.

In conclusion, these findings confirm associations of vitamin D activity with diverse inflammatory pathways in monocytes, *(Shahijanian et al., 2014)* although the magnitude of this connection is largely and differentially is influenced by the rs10877013 genotypes, suggesting the importance of the genetic component in the final result of vitamin D system network *(Hossein-nezhad et al., 2013a; Barry et al., 2014 ; Ahn et al., 2010).*

Additional elucidation of the regulatory mechanisms of this causal variant in *CYP27B1* and *VDR*, including potential epigenetic regulation in different cellular types and tissues, may be relevant for many diseases associated with vitamin D deficit or with sunlight exposure deficit during childhood *(Schwalfenberg et al., 2011).* We consider also important to determine

regulatory allelic variants that may be in the DNA regions interacting with vitamin D receptor in target genes since they could influence the efficacy of vitamin D treatment in diverse clinical trials *(Hossein-nezhad et al., 2013b; Kuhle et al., 2015).*

MS and innate antiviral response: the SP140 gene in CLL, CD and MS

Referring to our results, we identified that rs28445040 is the causal SNP for the association of the *SP140* locus with MS susceptibility. To identify this SNP causality we followed strategy of integration of the high density map of SNPs associated with MS-risk available from the ImmunoChip Project (*Parkes et al., 2013)* and the high density map of eQTLs generated in our study using RNA sequences from the GEUVADIS Project *(Lappalainen, et al., 2013)*, which have been obtained from the LCLs of the 1000 Genomes Project *(Abecasis et al., 2012).*

We have demonstrated that this variant responsible of the splicing of the exon 7 of the *SP140* gene is leading to a decrease of the full length transcript, and, as a consequence, the reduction of the produced protein in blood cells.

Moreover, this causal SNP of the *SP140* association with MS rs28445040 is in strong LD with the variants associated with CLL and CD obtained by different GWAS (Fig.24). Both autoimmune diseases CD and MS have been repetitively associated with the same susceptibility loci in many studies *(Voight and Cotsapas, 2012).* Even though CLL is not an autoimmune disease, it has been associated with autoimmune susceptibility loci such as: *IRF4* locus associated with rheumatoid arthritis (RA) (Okada et al., 2014) and the *IRF8* locus associated with MS *(Sawcer et al., 2011*), RA *(Okada et al., 2012),* systemic lupus erythematosus (SLE) (*Martin et al., 2013)*), inflammatory bowel disease (IBD) *(Jostins et al., 2012)* and systemic scleroderma (SS) (*Martin et al., 2013)*. So, the common characteristic between the pathologies of AI diseases and CLL could be the immunological tolerance failure *(Garcia-Munoz et al., 2015).*

Because of different density of markers used in GWAS and eQTLs studies, the colocalization of both signals in a locus does not always indicate a common origin of effects *(Battle and Montgomery, 2014)***.** In addition, the high LD between variants prevents the identification of a unique SNP as the causal variant of eQTL and risk association. This is the case for the *SP140* locus in which 18 SNPs, with r^2 ranging between 0.965 and 1, are potential causal variants, and therefore, the identification of the causal one had required functional studies.

The opposite effects of the eQTLs on the levels of expression of two *SP140* transcripts, differing in the presence or absence of exon 7, and the localization of the variant rs28445040 one of the 18 associated SNPs, were suggestive of rs28445040 as the causal SNP. We have demonstrated by alternative splicing construct experiments that rs28445040 is the responsible of the alternative splicing of exon7. Morevere, referring to our collaborators results, they found by western blot that there was a T-allele dependent reduction in full-length protein expression. Therefore, the ultimate effect of the exon-skipping seems to be the reduction of the SP140 protein.

The association assay performed in an Spanish cohort with the best MS variant from the GWAS *(Lappalainen, et al., 2013)* and the rs28445040 did not allow distinguishing which one was the primary signal of the association due to the strong LD between them *(Malo et al., 2008)*. Nevertheless, our collaboratores have confirmed the association of the locus in the Spanish cohort, showing that the T carriers, producing lower expression of the protein, had a higher MS risk. The use of eQTL data from an African population, having different LD pattern in the *SP140* locus respect to the EUR population, resulted in an important help to narrow down the causal variant. Thus, data of YRI eQTLs, obtained from the GEUVADIS Project *(Lappalainen, et al., 2013)*, pointed to rs28445040 as the most likely functional variant affecting the splicing of *SP140* exon 7.

It is so difficult to envisage the pathogenic relevance of the reduction of *SP140* protein expression in any of the associated diseases because of the limited knowledge of the functional activity of *SP140*. To explain this we considered two plausible, non exclusive, hypotheses. First, due to its strong sequence homology with the autoimmune regulator AIRE, a transcriptional activator which plays an important role in immunity by regulating the expression of autoantigens and negative selection of autoreactive T-cells in the thymus, we see that the implication of SP140 in MS and other immune-mediated diseases could be related with the process of immune self-tolerance acquisition, potentially contributing to the autoimmune component of MS, CLL and CD. The second hypothesis is based on the potential role of SP140 as an antiviral component of nuclear bodies induced by interferons as showed our results in monocytes (Fig.25A). SP140 protein is implicated in innate immune response to HIV-1 by its interaction with the virus Vif protein. As we know that one of the putative risk factors for MS is infection with Epstein-Barr virus (EBV) *(Tzartos et al., 2012)* . The “low producer” of SP140, rs28445040-T carriers, could have lower effective antiviral response against viruses potentially implicated in MS and in the other SP140-associated diseases.

Moreover, $1,25(OH)_2D_3$ seems to activate slightly the expression of *SP140* in LCLs with no effect on the alternative allele-depending splicing (Fig.25A and Fig.26). These results can be explained by the role of VDR activated by vitamin D that can bind the promoter element of *SP140* gene (*Disanto G et al., 2012).*

CLEC2D in MS

The same strategy as *SP140* has been followed with the locus of CLEC2D to determine the causal SNP of the association in the region. So, we determined the best eQTLs for the transcripts of each gene in the locus, we integrated the high density map of SNPs associated with MS-risk available from the Immunochip Project *(Parkes et al., 2013),* and the high density map of eQTLs generated in our study using RNA sequences from the GEUVADIS Project *(Lappalainen, et al., 2013)*, which in turn have been obtained from the LCLs of the 1000 Genomes Project *(Abecasis et al., 2012).* This strategy has pointed to rs3764022 as the causative variant of the association.

Then we have demonstrated that this variant responsible of the exon 2 splicing of the *CLEC2D* gene, leading to a decrease of the full length transcript. The variant rs3764022 , suggested as the causal SNP, has the opposite effects on the expression levels of two *CLEC2D* transcripts, differing in the presence or absence of exon 2 and the localization of this eQTLs. Therefore, the ultimate effect of the exon-skipping seems to be the reduction of the *CLEC2D* protein.

Our results indicate the association between the risk MS variant and the increase of the expression of one specific RNA of *CLEC2D* isoform, which lacked the exon2. *CLEC2D* encodes a member of the natural killer cell receptor C-type lecitin, it's also called lectin-like NK cell receptor or lectin-like transcript 1 (LLT1). This receptor protects target cells against natural killer cell-mediated lysis. Also, it is able to discriminate between the self and non-self and modulate the IFNγ production. *(Zelensky and Gready, 2005)*

LLT1 contains a transmembrane domain near the N-terminus as well as the C-type lectin-like extracellular domain. The protein translated from the RNA exon2-skipped lacks the entire transmembrane region *(Germain et al., 2011)* and so it accumulates in the endoplasmic reticulum where it forms homodimers or heterodimers with the full length protein. Thus, we would expect a lower expression of LLT1 protein on the cell surface of the individuals carrying the *G* allele of the eQTL rs3764022. At the same time, *G* variant is the risk allele for

MS; therefore, the increased expression of the short protein seems to be the cause of the association with MS.

However, when we followed the same strategy using the GWAS SNP instead the Immunibase, *CLEC1L* would be the gene associated with MS (Fig. 28B). This difference may be due to the number of markers used by GWAS and Immunochip.

Moreover, no association has been detected between best MS variant from the GWAS rs10466829 and the rs3764022 as in the association assay study performed in Spanish cohort as in the meta-analysis that has been done with Spanish and German populations. These unexpected results can be owing to the difference ancestry between controls and cases in the populations studied, that affected the genotypes distribution.

Conclusions

1. The risk variant of the association of the 12q13-14 locus with MS (rs10877013) correlates with lower expression of *CYP27B1* and *VDR* genes in cell and activation dependent manner, pointing to the rs10877013 variant as a genetic determinant that affects the function of vitamin D system linking environmental and genetic factors.

2. The SNP associated with MS in the 20q13.2 locus is close to the *CYP24A1*gene but it does not seem to correlate with the expression of the gene even after vitamin D expression induction.

3- *VDR* and *CYP24A1* expression levels are directly correlated.

4-Low vitamin D level is more a cause than a consequence of MS disease.

5- rs28445040 explain the association in the *SP140* locus as the functional variant in this region. Risk allele is the responsible for exon-7 splicing of *SP140* genes. Thus, the reduction in the full length transcript gene expression.

6- rs3764022 is the causal variant in the locus 12p13.31. The risk allele rs3764022-G is the responsible for exon-2 splicing of *CLEC2D* gene, so the decrease of the full length transcript gene expression level.

References

Abecasis, G.R., Auton, A., Brooks, L.D., DePristo, M.A., Durbin, R.M., Handsaker, R.E., Kang, H.M., Marth, G.T. and McVean, G.A. (2012) An integrated map of genetic variation from 1,092 human genomes. Nature, 491, 56-65.

Adams JS, Hewison M. Extrarenal expression of the 25-hydroxyvitamin D-1-hydroxylase. Arch Biochem Biophys 2012;523:95–102

Aggarwal, S.; Pittenger, M.F. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005, 105, 1815–1822.

Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010; 19: 2739-45.

Allison RS. Geographic distribution of multiple sclerosis. Preliminary notes on a comparative study of prevalence in Charleston, S.C. and Halifax, N.S. Acta Psychiatr Scand Suppl. 1960; 35(147):18-22.

Andersson A, Covacu R, Sunnemark D, Danilov AI, Dal Bianco A, Khademi M, Wallstrom E, Lobell A, Brundin L, Lassmann H, Harris RA. Pivotal advance: HMGB1 expression in active lesions of human and experimental multiple sclerosis. J Leukoc Biol 2008;84:1248–1255.

Angelini DF, Borsellino G, Poupot M, Diamantini A, Poupot R, Bernardi G, Poccia F, Fournie JJ, Battistini L. FcgammaRIII discriminates between 2 subsets of Vgamma9Vdelta2 effector cells with different responses and activation pathways. Blood 2004;104:1801–1807.

Araki M, Kondo T, Gumperz JE, Brenner MB, Miyake S, Yamamura T. Th2 bias of CD4+ NKT cells derived from multiple sclerosis in remission. Int Immunol 2003;15:279–288.

Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: The role of infection. Ann Neurol. 2007;61:288–299.

Aulchenko YS et al. Genetic variation in the KIF1B locus influences susceptibility to multiple sclerosis. Nat Genet. 2008-11-09.

Aulchenko YS, Hoppenbrouwers IA, Ramagopalan SV, Broer L, Jafari N, Hillert J, Link J, Lundström W, Greiner E, Dessa Sadovnick A, Goossens D, Van Broeckhoven C, Del-Favero J, Ebers GC, Oostra BA, van Duijn CM, Hintzen RQ. Genetic variation in the KIF1B locus influences susceptibility to multiple sclerosis. Nat Genet. 2008 Dec;40(12):1402-3.

Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene). Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. Nat Genet. 2009 Jul;41(7):824-8.

Autier P, Boniol M, Pizot C and Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol 2014; 2: 76-89.

Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C Vitamin D: modulator of the immune system. Curr Opin Pharmacol. 2010 Aug; 10(4):482-96.

Baeke F, Takiishi T, Korf H, Gysemans C. Mathieu C Vitamin D: modulator of the immune system.Curr Opin Pharmacol. 2010 Aug; 10(4):482-96.

Bahlo M, Booth DR, Broadley SA, Brown MA, Foote SJ, Griffiths LR, et al. Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. Nat Genet. 2009;41:824–828.

Bailey R, Cooper JD, Zeitels L, Smyth DJ, Yang JH, Walker NM, Hyppönen E, Dunger DB, Ramos-Lopez E, Badenhoop K, Nejentsev S, Todd JA. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. Diabetes 2007;56:2616–21

Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998 Mar 19; 392(6673):245-52.

Baranzini SE et al. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. Hum Mol Genet. 2008-11-14.

Baranzini SE, Srinivasan R, Khankhanian P, Okuda DT, Nelson SJ, Matthews PM, Hauser SL, Oksenberg JR, Pelletier D. Genetic variation influences glutamate concentrations in brains of patients with multiple sclerosis. Brain. 2010 Sep;133(9):2603-11.

Baranzini SE, Wang J, Gibson RA, Galwey N, Naegelin Y, Barkhof F, Radue EW, Lindberg RL, Uitdehaag BM, Johnson MR, Angelakopoulou A, Hall L, Richardson JC, Prinjha RK, Gass A, Geurts JJ, Kragt J, Sombekke M, Vrenken H, Qualley P, Lincoln RR, Gomez R, Caillier SJ, George MF, Mousavi H, Guerrero R, Okuda DT, Cree BA, Green AJ, Waubant E, Goodin DS, Pelletier D, Matthews PM, Hauser SL, Kappos L, Polman CH, Oksenberg JR. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. Hum Mol Genet. 2009 Feb 15;18(4):767-78.

Barbara Prietl, Gerlies Treiber, Thomas R. Pieber, and Karin Amrein. Vitamin D and Immune Function Nutrients. 2013 Jul; 5(7): 2502–2521.

Barcellos LF, Sawcer S, Ramsay PP, Baranzini SE, Thomson G, et al. Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. Hum Mol Genet. 2006;15:2813–2824.

Barr TA, Shen P, Brown S, et al: B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. J Exp Med 2012;209:1001-1010.

Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, Rich SS; Type 1 Diabetes Genetics Consortium. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009 Jun;41(6):703-7. doi: 10.1038/ng.381. Epub 2009 May 10.

Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, Rich SS; Type 1 Diabetes Genetics Consortium. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009 Jun;41(6):703-7.

Barry EL, Rees JR, Peacock JL, et al. Genetic variants in CYP2R1, CYP24A1, and VDR modify the efficacy of vitamin D_3 supplementation for increasing serum 25-hydroxyvitamin D levels in a randomized controlled trial. J Clin Endocrinol Metab 2014; 99: E2133-7.

Bartel, D. P. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 2004; 116, 281–297.

Battle, A. and Montgomery, S.B. (2014) Determining causality and consequence of expression quantitative trait loci. Hum Genet, 133, 727-35.

Beecham, A.H. and Patsopoulos, N.A. and Xifara, D.K. and Davis, M.F. and Kemppinen, A. and Cotsapas, C. and Shah, T.S. and Spencer, C. and Booth, D. and Goris, A. et al. (2013) Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet, 45, 1353-60.

Ben-Hur, T.; Fainstein, N.; Nishri, Y. Cell-based reparative therapies for multiple sclerosis. Curr. Neurol. Neurosci. Rep. 2013, 13, 397.

Benveniste EN. Cytokines: influence on glial cell gene expression and function. Chem Immunol 1997;69:31–75.

Berndt SI, Skibola CF, Joseph V, Camp NJ, Nieters A, Wang Z et al. Genome-wide association study identifies multiple risk loci for chronic lymphocytic leukemia. Nat Genet. 2013 Aug;45(8):868-76.

Bielekova B, et al. Effect of anti-CD25 antibody daclizumab in the inhibition of inflammation and stabilization of disease progression in multiple sclerosis. Arch Neurol. 2009;66:483–489.

Bikle DD. Vitamin D: newly discovered actions require reconsideration of physiologic requirements. Trends Endocrinol Metab 2010, 21:375-384.

Bloch DB, de la Monte SM, Guigaouri P, Filippov A, Bloch KD. Identification and characterization of a leukocyte-specific component of the nuclear body. J Biol Chem. 1996 Nov 15;271(46):29198-204.

Boerman RH, Bax JJ, Beekhuis-Brussee JA, et al. JC virus and multiple sclerosis: a refutation?. Acta Neurol Scand, 87 (1993), pp. 353–355

Bray PF, Bloomer LC, Salmon VC, Bagley MH, Larsen PD. Epstein-Barr virus infection and antibody synthesis in patients with multiple sclerosis. Arch Neurol. 1983;40:406–408.

Briggs FB et al. Genome-wide association study of severity in multiple sclerosis. Genes Immun. 2011-06-09.

Brunton LL. Immunomodulators. In: Lazo JS, Parker KL, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill Medical; 2005. pp. 1424–1427.

Brynedal B1, Wojcik J, Esposito F, Debailleul V, Yaouanq J, Martinelli-Boneschi F, Edan G, Comi G, Hillert J, Abderrahim H. MGAT5 alters the severity of multiple sclerosis. J Neuroimmunol. 2010 Mar 30;220(1-2):120-4.

Burgoon M.P., Cohrs R.J, Bennett J.L.,. Anderson S.W, Ritchie A.M., Cepok S., Hemmer B., Gilden D., Owens G.P.. Varicella zoster virus is not a disease-relevant antigen in multiple sclerosis. Annals of Neurology, 65 (2009), pp. 474–479

Caballero A, Alvés-León S, Papais-Alvarenga R, Fernández O, Navarro G, et al. DQB1*0602 confers genetic susceptibility to multiple sclerosis in Afro-Brazilians. Tissue Antigens. 1999;54:524–526.

Caplan, A.I. Mesenchymal stem cells. J. Orthop. Res. 1991, 9, 641–650.

Carlens C, Hergens MP, Grunewald J, Ekbom A, Eklund A, Höglund CO, Askling J. Smoking, use of moist snuff, and risk of chronic inflammatory diseases. Am J Respir Crit Care Med. 2010 Jun 1; 181(11):1217-22.

Chang, T. C., & Mendell, J. T. (2007). microRNAs in vertebrate physiology and human disease. Annual Review of Genomics and Human Genetics 2007; 8, 215–239.

Chao MJ, Barnardo MC, Lincoln MR, Ramagopalan SV, Herrera BM, et al. HLA class I alleles tag HLA-DRB1*1501 haplotypes for differential risk in multiple sclerosis susceptibility. Proc Natl Acad Sci U S A. 2008;105:13069–13074.

Charcot, J. Histologiede la sclerose en plaues. Gazette des hopitaux, Paris 1868, 41, 554-5.

Chepelev I, Wei G, Wangsa D, Tang Q, Zhao K.Characterization of genome-wide enhancer-promoter interactions reveals co-expression of interacting genes and modes of higher order chromatin organization. Cell Res 2012; 22: 490-503.

Christy AL, Brown MA. The multitasking mast cell: positive and negative roles in the progression of autoimmunity. J Immunol 2007;179:2673–2679.

Cohen J, Barkhof F, Comi G, Hartung HP, Khatri BO, Montalban X, Pelletier J, Capra R, Gallo P, Izquierdo G, Tiel-Wilck K, de Vera A, Jin J, Stites T, Wu S, Aradhye S, Kappos L. and TRANSFORMS Study Group. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. N. Engl. J. Med.2010;362:402–415.

Comabella M, Craig DW, Camiña-Tato M, Morcillo C, Lopez C, Navarro A, Rio J; BiomarkerMS Study Group, Montalban X, Martin R. Identification of a novel risk locus for multiple sclerosis at 13q31.3 by a pooled genome-wide scan of 500,000 single nucleotide polymorphisms. PLoS One. 2008;3(10):e3490.

Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372:1502–1517.

Compston A, Coles A. Multiple sclerosis. The Lancet 2002 , Volume 360, Issue 9333, Page 648.

Comuzzie AG, Cole SA, Laston SL, Voruganti VS, Haack K, Gibbs RA, Butte NF. Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. PLoS One. 2012;7(12):e51954.

Connick, P.; Kolappan, M.; Crawley, C.; Webber, D.J.; Patani, R.; Michell, A.W.; Du, M.Q.; Luan, S.L.; Altmann, D.R.; Thompson, A.J.; et al. Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: An open-label phase 2a proof-of-concept study. Lancet Neurol. 2012, 11, 150–156.

Cortessis VK, Thomas DC, Levine AJ, et al. Environmental epigenetics: prospects for studying epigenetic mediation of exposure–response relationships. Human Genetics. 2012;131(10):1565-1589.

D Krametter, G Niederwieser, A Berghold, *et al.* *Chlamydia pneumoniae* in multiple sclerosis: humoral immune responses in serum and cerebrospinal fluid and correlation with disease activity marker. Mult Scler, 7 (2001), pp. 13–18

De Jager PL et al. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. Nat Genet. 2009-06-14.

De Jager PL1, Jia X, Wang J, de Bakker PI, Ottoboni L, Aggarwal NT, Piccio L, Raychaudhuri S, Tran D, Aubin C, Briskin R, Romano S; International MS Genetics Consortium, Baranzini SE, McCauley JL, Pericak-Vance MA, Haines JL, Gibson RA, Naeglin Y, Uitdehaag B, Matthews PM, Kappos L, Polman C, McArdle WL, Strachan DP, Evans D, Cross AH, Daly MJ, Compston A, Sawcer SJ, Weiner HL, Hauser SL, Hafler DA, Oksenberg JR. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. Nat Genet. 2009 Jul;41(7):776-82.

Degner JF, Pai AA, Pique-Regi R, et al. DNaseI sensitivity QTLs are a major determinant of human expression variation. Nature. 2012;482(7385):390-394.

Dessau RB, Lisby G, Frederiksen JL. Coronaviruses in brain tissue from patients with multiple sclerosis. Acta Neuropathol (Berl), 101 (2001), pp. 601–604

Dhib-Jalbut S, Marks S. Interferon-beta mechanisms of action in multiple sclerosis. Neurology. 2010; 74:S17–S24.

Di Bernardo MC1, Crowther-Swanepoel D, Broderick P, Webb E, Sellick G et al. A genome-wide association study identifies six susceptibility loci for chronic lymphocytic leukemia. Nat Genet. 2008 Oct;40(10):1204-10

Dimas AS, Deutsch S, Stranger BE, et al. Common regulatory variation impacts gene expression in a cell type dependent manner. Science (New York, NY). 2009;325(5945):1246-1250.

Dixon AL, Liang L, Moffatt MF, Chen W, Heath S, et al. A genome-wide association study of global gene expression. Nat Genet. 2007;39:1202–1207.

Duddy, M. NICE approval of dimethyl fumarate could benefit thousands living with

Durbin, R. M.; Abecasis, G. R.; Altshuler, R. M.; Auton, G. A. R.; Brooks, D. R.; Durbin, A et al. A map of human genome variation from population-scale sequencing. Nature 2010; 467 (7319): 1061–1073

Dusso AS. Kidney disease and vitamin D levels: 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and VDR activation. Kidney inter., Suppl. 2011; 1: 136–141.

Fairfax BP, Humburg P, Makino S, et al. Innate Immune Activity Conditions the Effect of Regulatory Variants upon Monocyte Gene Expression. Science (New York, NY). 2014;343(6175):1246949.

Farh, K.K., Marson, A., Zhu, J., Kleinewietfeld, M., Housley, W.J., Beik, S., Shoresh, N., Whitton, H., Ryan, R.J., Shishkin, A.A. et al. (2015) Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature, 518, 337-43.

Farrall M. Quantitative genetic variation: a post-modern view. Hum Mol Genet. 2004 Apr 1;13 Spec No 1:R1-7

Fatemi, M., Pao, M. M., Jeong, S., Gal-Yam, E. N., Egger, G., Weisenberger, D. J., et al. Footprinting of mammalian promoters: Use of a CpG DNA methyltransferase revealing nucleosome positions at a single molecule level. Nucleic Acids Research 005; 33, e176.

Ferrante P, Omodeo-Zorini E, Caldarelli-Stefano R, et al. Detection of JC virus DNA in cerebrospinal fluid from multiple sclerosis patients. Mult Scler, 4 (1998), pp. 49–54

Fogdell A, Hillert J, Sachs C, Olerup O. The multiple sclerosis- and narcolepsy-associated HLA class II haplotype includes the DRB5*0101 allele. Tissue Antigens. 1995;46:333–336.

Fortune MD, Guo H, Burren O, Schofield E, Walker NM, Ban M, Sawcer SJ, Bowes J, Worthington J, Barton A, Eyre S, Todd JA, Wallace C. Statistical colocalization of genetic risk variants for related autoimmune diseases in the context of common controls. Nat Genet. 2015 Jul;47(7):839-46.

Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet. 2010 Dec;42(12):1118-25.

Fraser KB, Millar JHD, Haire M, McCrea S. Increased tendency to spontaneous in-vitro lymphocyte transformation in clinically active multiple sclerosis. Lancet. 1979;314:715–717.

Fung EY, Smyth DJ, Howson JM, Cooper JD, Walker NM, Stevens H, Wicker LS, Todd JA. Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. Genes Immun 2009;10:188–91

Gaffney DJ, Veyrieras J-B, Degner JF, et al. Dissecting the regulatory architecture of gene expression QTLs. Genome Biology. 2012;13(1):R7.

Gandhi R, Laroni A, Weiner HL. Role of the innate immune system in the pathogenesis of multiple sclerosis. J Neuroimmunol2010; 221:7–14.

Gandhi, R., Laroni, A., Weiner, H.L. Role of the innate immune system in the pathogenesis of multiple sclerosis. J. Neuroimmunol., 2009, 221, 7-14.

Garcia-Munoz, R., Feliu, J. and Llorente, L. (2015) The top ten clues to understand the origin of chronic lymphocytic leukemia (CLL). J Autoimmun, 56, 81-6.

Genain CP, Cannella B, Hauser SL, Raine CS. 1999. Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat. Med. 5:170–75

Genain CP, Nguyen MH, Letvin NL, et al: Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. J Clin Invest 1995;96:2966-2974.

Genain CP, NguyenMH,Letvin NL, Pearl R, Davis RL, et al. 1995. Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. J. Clin. Invest. 96: 2966–74

Germain, C., Meier, A., Jensen, T., Knapnougel, P., Poupon, G., Lazzari, A., Neisig, A., Håkansson, K., Dong, T., Wagtmann, N., et al. (2011). Induction of lectin-like transcript 1

(LLT1) protein cell surface expression by pathogens and interferon-γ contributes to modulate immune responses. J. Biol. Chem. 286, 37964-37975.

Ghadirian P, Dadgostar B, Azani R, Maisonneuve P. A case-control study of the association between socio-demographic, lifestyle and medical history factors and multiple sclerosis.Can J Public Health. 2001 Jul-Aug; 92(4):281-5.

Gillie O. Controlled trials of vitamin D, causality and type 2 statistical error. Public Health Nutr 2014.

Giovannoni G, Comi G, Cook S, Rammohan K, Rieckmann P, Soelberg SP, Vermesch P, Greenberg S. and CLARITY Study Group. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis.N. Engl. J. Med. 2010;362:416–426.

Goodin DS. The genetic and environmental bases of complex human-disease: extending the utility of twin-studies. PLoS One 2012; 7:e47875.

Goris A, van Setten J, Diekstra F, Ripke S, Patsopoulos NA, Sawcer SJ; International Multiple Sclerosis Genetics Consortium, van Es M; Australia and New Zealand MS Genetics Consortium, Andersen PM, Melki J, Meininger V, Hardiman O, Landers JE, Brown RH Jr, Shatunov A, Leigh N, Al-Chalabi A, Shaw CE, Traynor BJ, Chiò A, Restagno G, Mora G, Ophoff RA, Oksenberg JR, Van Damme P, Compston A, Robberecht W, Dubois B, van den Berg LH, De Jager PL, Veldink JH, de Bakker PI.No evidence for shared genetic basis of common variants in multiple sclerosis and amyotrophic lateral sclerosis.Hum Mol Genet. 2014 Apr 1;23(7):1916-22.

Gourraud PA, Sdika M, Khankhanian P, Henry RG, Beheshtian A, Matthews PM, Hauser SL, Oksenberg JR, Pelletier D, Baranzini SE. A genome-wide association study of brain lesion distribution in multiple sclerosis. Brain. 2013 Apr;136(Pt 4):1012-24.

Gregersen JW, Kranc KR, Ke X, Svendsen P, Madsen LS, et al. Functional epistasis on a common MHC haplotype associated with multiple sclerosis. Nature. 2006;443:574–577.

GTEx Consortium. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. Science. 2015 May 8;348(6235):648-60.

Gutierrez J, Vergara MJ, Guerrero M, et al. Multiple sclerosis and human herpesvirus 6. Infection, 30 (2002), pp. 145–149

Haas, J., Hug, A., Veihover, A., Fritzching, B., Falk, C.S, Filser, A., Vetter, T., Milkova L, Korporal, M., Fritz, B., Storch- Hagenlocher, B., Krammer, P.H., Suri-Payer, E., Wildeman, B. Reduced suppressive effect of CD4+CD25 high regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. Eur. J. Immunol., 2005, 35, 3343-3352.

Hammarberg H, Lidman O, Lundberg C, Eltayeb SY, Gielen AW, Muhallab S, Svenningsson A, Linda H, van Der Meide PH, Cullheim S, Olsson T, Piehl F. Neuroprotection by encephalomyelitis: rescue of mechanically injured neurons and neurotrophin production by CNS infiltrating T and natural killer cells. J Neurosci 2000;20:5283–5291.

Handunnetthi L, Ramagopalan SV, Ebers GC, Knight JC. Regulation of major histocompatibility complex class II gene expression, genetic variation and disease. Genes Immun. 2010;11:99–112.

Hannah SS, Norman AW. 1 alpha,25(OH)2 vitamin D3-regulated expression of the eukaryotic genome. Nutr Rev. 1994 Nov;52(11):376-82.

Harp CT, Ireland S, Davis LS, et al: Memory B cells from a subset of treatment-naïve relapsing-remitting multiple sclerosis patients elicit CD4(+) T-cell proliferation and IFN-γ production in response to myelin basic protein and myelin oligodendrocyte glycoprotein. Eur J Immunol 2010;40:2942-2956.

Hauser S, Waubant E, Arnold D, Vollmer T, Antel J, Fox R, Bar-Or A, Panzara M, Sarkar N, Agarwal S, Langer-Gould M, Smith C. and Hermes Trial Group. B-cell depletion with rituximab in relapsing remitting multiple sclerosis. N. Engl. J. Med. 2008;358:676–688.

Hauser SL, Goodwin DS. Multiple sclerosis and other demyelinating diseases. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, editors. Harrison's Principles of Internal Medicine. 17th ed. II. New York: McGraw-Hill Medical; 2008. pp. 2611–2621.

Hauser, S.L.; Chan, J.R.; Oksenberg, J.R. Multiple sclerosis: Prospects and promise. Ann. Neurol. 2013, 74, 317–327.

Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, Jurutka PW. Molecular mechanisms of vitamin D action. Calcif Tissue Int. 2013 Feb;92(2):77-98.

Hawkes CH. Is multiple sclerosis a sexually transmitted infection?. J Neurol Neurosurg Psychiatry, 73 (2002), pp. 439–443

Hayday AC. [gamma][delta] cells: a right time and a right place for a conserved third way of protection. Annu Rev Immunol 2000;18:975–1026.

Hedström AK, Bäärnhielm M, Olsson T, Alfredsson L. Exposure to environmental tobacco smoke is associated with increased risk for multiple sclerosis. Mult Scler. 2011 Jul; 17(7):788-93.

Hedström AK, Bäärnhielm M, Olsson T, Alfredsson L. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. Neurology. 2009 Sep 1; 73(9):696-701.

Hernán MA, Jick SS, Logroscino G, Olek MJ, Ascherio A, Jick H. Cigarette smoking and the progression of multiple sclerosis. Brain. 2005 Jun; 128(Pt 6):1461-5.

Hohlfeld R. Multiple Sclerosis: A Comprehensive Text. Saunders/Elsevier, 2008.

Holick MF . Vitamin D deficiency. N Engl J Med 2007; 357: 266–81.

Hossein-nezhad A and Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc 2013b; 88: 720-55.

Hossein-nezhad A, Spira A and Holick MF. Influence of vitamin D status and vitamin D3 supplementation on genome wide expression of white blood cells: a randomized double-blind clinical trial. PLoS One 2013a; 8: e58725.

Huang YM, Xiao BG, Ozenci V, Kouwenhoven M, Teleshova N, Fredrikson S, Link H. Multiple sclerosis is associated with high levels of circulating dendritic cells secreting pro-inflammatory cytokines. J Neuroimmunol 1999;99:82–90.

Innocenti F, Cooper GM, Stanaway IB, et al. Identification, Replication, and Functional Fine-Mapping of Expression Quantitative Trait Loci in Primary Human Liver Tissue. Storey JD, ed. PLoS Genetics. 2011;7(5):e1002078.

International Multiple Sclerosis Genetics Consortium. Genome-wide association study of severity in multiple sclerosis. Genes Immun. 2011 Dec;12(8):615-25.

International Multiple Sclerosis Genetics Consortium1, Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, De Jager PL, de Bakker PI, Gabriel SB, Mirel DB, Ivinson AJ, Pericak-Vance MA, Gregory SG, Rioux JD, McCauley JL, Haines JL, Barcellos LF, Cree B, Oksenberg JR, Hauser SL. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med. 2007 Aug 30;357(9):851-62. Epub 2007 Jul 29.

International Multiple Sclerosis Genetics, C., Wellcome Trust Case Control, C., Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C. C., Patsopoulos, N. A., Moutsianas, L., Dilthey, A., Su, et al. (2011) Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011 Aug 10;476(7359):214-9.

Islam T, Gauderman WJ, Cozen W, Mack TM. Neurology. Childhood sun exposure influences risk of multiple sclerosis in monozygotic twins. 2007 Jul 24; 69(4):381-8.

Jakkula E1, Leppä V, Sulonen AM, Varilo T, Kallio S, Kemppinen A, Purcell S, Koivisto K, Tienari P, Sumelahti ML, Elovaara I, Pirttilä T, Reunanen M, Aromaa A, Oturai AB, Søndergaard HB, Harbo HF, Mero IL, Gabriel SB, Mirel DB, Hauser SL, Kappos L, Polman C, De Jager PL, Hafler DA, Daly MJ, Palotie A, Saarela J, Peltonen L. Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. Am J Hum Genet. 2010 Feb 12;86(2):285-91.

Jansen R.C., Nap J.P. Genetical genomics: the added value from segregation. Trends Genet., 17 (2001), pp. 388–391

Jeffery L.E., Burke F., Mura M., Zheng Y., Qureshi O.S., Hewison M., Walker L.S.K., Lammas D.A., Raza K., Sansom D.M. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J. Immunol. 2009;183:5458–5467.

Jersild C, Fog T. Histocompatibility (HL-A) antigens associated with multiple sclerosis. Acta Neurol Scand Suppl. 1972;51:377.

Jocher R, Rethwilm A, Kappos L, Meulen V ter. Search for retroviral sequences in peripheral blood mononuclear cells and brain tissue of multiple sclerosis patients. J Neurol, 237 (1990), pp. 352–355.

Johnson D, Seeldrayers PA, Weiner HL. The role of mast cells in demyelination. 1. Myelin proteins are degraded by mast cell proteases and myelin basic protein and P2 can stimulate mast cell degranulation. Brain Res 1988;444:195–198.

Jones G . Pharmacokinetics of vitamin D toxicity. Am J Clin Nutr 2008; 88: 582S–6S.

Joseph K. Pickrell, John C. Marioni, Athma A. Pai, Jacob F. Degner, Barbara E. Engelhardt, Everlyne Nkadori , Jean-Baptiste Veyrieras, Matthew Stephens , Yoav Gilad & Jonathan K.

Pritchard. Understanding mechanisms underlying human gene expression variation with RNA sequencing. Nature. 2010; 464, 768-772

Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP et al.Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012 Nov 1;491(7422):119-24.

Jostins, L., Ripke, S., Weersma, R.K., Duerr, R.H., McGovern, D.P., Hui, K.Y., Lee, J.C., Schumm, L.P., Sharma, Y., Anderson, C.A. et al. (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature, 491, 119-24.

Kawakami M , Imawari M , Goodman DS . Quantitative studies of the interaction of cholecalciferol (vitamin D3) and its metabolites with different genetic variants of the serum binding protein for these sterols. Biochem J 1979; 179: 413–23.

Kinnunen T, Chamberlain N, Morbach H, et al: Specific peripheral B cell tolerance defects in patients with multiple sclerosis. J Clin Invest 2013;123:2737-2741.

Koch, M. W., Metz, L. M., & Kovalchuk, O. Epigenetic changes in patients with multiple sclerosis. Nature Reviews Neurology 2013b; 9, 35–43.

Koch, M. W., Metz, L. M., & Kovalchuk, O. Epigenetics and miRNAs in the diagnosis and treatment of multiple sclerosis. Trends in Molecular Medicine 2013a; 19, 23–30.

Kouzarides, T. Chromatin modifications and their function. Cell 2007; 128, 693–705.

Kragt J, van Amerongen B, Killestein J, Dijkstra C, Uitdehaag B, Polman Ch, Lips P. Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women. Mult Scler. 2009 Jan; 15(1):9-15.

Krementsov DN, Thornton TM, Teuscher C, Rincon M. The emerging role of p38 mitogen-activated protein kinase in multiple sclerosis and its models. Mol Cell Biol. 2013 Oct;33(19):3728-34.

Kuhle J, Disanto G, Dobson R, et al. Conversion from clinically isolated syndrome to multiple sclerosis: A large multicentre study. Mult Scler 2015.

Lappalainen T, Sammeth M, Friedländer MR, et al. Transcriptome and genome sequencing uncovers functional variation in humans. Nature. 2013;501(7468):506-511.

Lappalainen, T., Sammeth, M., Friedlander, M.R., t Hoen, P.A., Monlong, J., Rivas, M.A., Gonzalez-Porta, M., Kurbatova, N., Griebel, T., Ferreira, P.G. et al. (2013) Transcriptome and genome sequencing uncovers functional variation in humans. Nature, 501, 506-11.

Lappalainen, T., Sammeth, M., Friedlander, M.R., t Hoen, P.A., Monlong, J., Rivas, M.A., Gonzalez-Porta, M., Kurbatova, N., Griebel, T., Ferreira, P.G. et al. (2013) Transcriptome and genome sequencing uncovers functional variation in humans. Nature, 501, 506-11.

Lappalainen, T., Sammeth, M., Friedlander, M.R., t Hoen, P.A., Monlong, J.,

Lappalainen, T., Sammeth, M., Friedlander, M.R., t Hoen, P.A., Monlong, J.,Rivas, M.A., Gonzalez-Porta, M., Kurbatova, N., Griebel, T., Ferreira, P.G. et al. Transcriptome and genome sequencing uncovers functional variation in humans. Nature,2013; 501, 506-11.

Lauc G, Huffman JE, Pučić M, Zgaga L, Adamczyk B, Mužinić A, Novokmet M, Polašek O, Gornik O, Krištić J, Keser T, Vitart V, Scheijen B, Uh HW, Molokhia M, Patrick AL, McKeigue P, Kolčić I, Lukić IK, Swann O, van Leeuwen FN, Ruhaak LR, Houwing-Duistermaat JJ, Slagboom PE, Beekman M, de Craen AJ,Deelder AM, Zeng Q, Wang W, Hastie ND, Gyllensten U, Wilson JF, Wuhrer M, Wright AF, Rudd PM, Hayward C, Aulchenko Y, Campbell H, Rudan I. Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. PLoS Genet. 2013;9(1):e1003225.

Lazaros Belbasis, Vanesa Bellou, Evangelos Evangelou, John P A Ioannidis, Ioanna Tzoulaki. Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses . Lancet Neurol. 2015 Mar;14(3):263-73.

Lemire JM, Adams JS, Sakai R, Jordan SC. 1 alpha,25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. J Clin Invest. 1984 Aug; 74(2):657-61.

Leone MA, Barizzone N, Esposito F, Lucenti A, Harbo HF, Goris A, Kockum I, Oturai AB, Celius EG, Mero IL, Dubois B, Olsson T, Søndergaard HB, Cusi D, Lupoli S, Andreassen BK; International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium 2, Myhr KM, Guerini FR; PROGEMUS Group; PROGRESSO Group, Comi G, Martinelli-Boneschi F, D'Alfonso S. Association of genetic markers with CSF oligoclonal bands in multiple sclerosis patients. PLoS One. 2013 Jun 13;8(6):e64408.

Levin LI, Munger KL, O'Reilly EJ, Falk KI, Ascherio A. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. Ann Neurol. 2010;67:824–830.

Li G, Ruan X, Auerbach RK, et al. Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell 2012; 148: 84-98.

Liedtke W, Malessa R, Faustmann PM, Eis-Hubinger AM. Human herpesvirus 6 polymerase chain reaction findings in human immunodeficiency virus associated neurological disease and multiple sclerosis. J Neurovirol, 1 (1995), pp. 253–258

Lincoln MR, Ramagopalan SV, Chao MJ, Herrera BM, Deluca GC, et al. Epistasis among HLA-DRB1, HLA-DQA1, and HLA-DQB1 loci determines multiple sclerosis susceptibility. Proc Natl Acad Sci U S A. 2009;106:7542–7547.

Litzenburger T, Fassler R, Bauer J, Lassmann H, Linington C, et al. 1998. B lymphocytes producing demyelinating autoantibodies: development and function in gene-targeted transgenic mice. J. Exp. Med. 188:169–80

Liu J, Wang L, Zhan S, Tan J, Xia Y. Daclizumab for relapse remitting multiple sclerosis. Cochrane Database Syst. Rev. 2007;6:CD008127.

Llufriu, S.; Sepulveda, M.; Blanco, Y.; Marin, P.; Moreno, B.; Berenguer, J.; Gabilondo, I.; Martinez-Heras, E.; Sola-Valls, N.; Arnaiz, J.A.; et al. Randomized placebo-controlled phase II trial of autologous mesenchymal stem cells in multiple sclerosis. PLoS ONE 2014, 9, e113936.

Loma I, Heyman R. Multiple sclerosis: Pathogenesis and treatment. Curr Neuropharmacol. 2011;3:409–16.

Lowe KE, Maiyar AC, Norman AW. Vitamin D-mediated gene expression. Crit Rev Eukaryot Gene Expr. 1992;2(1):65-109.

Maccario, R.; Podesta, M.; Moretta, A.; Cometa, A.; Comoli, P.; Montagna, D.; Daudt, L.; Ibatici, A.; Piaggio, G.; Pozzi, S.; et al. Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. Haematologica 2005, 90, 516–525.

Madani N, Millette R, Platt EJ, Marin M, Kozak SL, Bloch DB & Kabat D. Implication of the lymphocyte-specific nuclear body protein Sp140 in an innate response to human immunodeficiency virus type 1. J Virol. 2002 Nov;76(21):11133-8

Malaviya R, Twesten NJ, Ross EA, Abraham SN, Pfeifer JD. Mast cells process bacterial Ags through a phagocytic route for class I MHC presentation to T cells. J Immunol 1996;156:1490–1496.

Malo, N., Libiger, O. and Schork, N.J. (2008) Accommodating linkage disequilibrium in genetic-association analyses via ridge regression. Am J Hum Genet, 82, 375-85.

Margherita T. Cantorna, Lindsay Snyder, Yang-Ding Lin, and Linlin Yang. Vitamin D and 1,25(OH)2D Regulation of T cells. Nutrients. 2015 Apr; 7(4): 3011–3021.

Marrosu MG, Murru MR, Costa G, Murru R, Muntoni F, et al. DRB1-DQA1-DQB1 loci and multiple sclerosis predisposition in the Sardinian population. Hum Mol Genet. 1998;7:1235–1237.

Martin, J.E., Assassi, S., Diaz-Gallo, L.M., Broen, J.C., Simeon, C.P., Castellvi, I., Vicente-Rabaneda, E., Fonollosa, V., Ortego-Centeno, N., Gonzalez-Gay, M.A. et al. (2013) A systemic sclerosis and systemic lupus erythematosus pan-meta-GWAS reveals new shared susceptibility loci. Hum Mol Genet, 22, 4021-9.

Martinelli-Boneschi F1, Esposito F, Brambilla P, Lindström E, Lavorgna G, Stankovich J, Rodegher M, Capra R, Ghezzi A, Coniglio G, Colombo B, Sorosina M, Martinelli V, Booth D, Oturai AB, Stewart G, Harbo HF, Kilpatrick TJ, Hillert J, Rubio JP, Abderrahim H, Wojcik J, Comi G. A genome-wide association study in progressive multiple sclerosis. Mult Scler. 2012 Oct;18(10):1384-94. Epub 2012 Mar 28.

Matesanz F1, González-Pérez A, Lucas M, Sanna S, Gayán J, Urcelay E, Zara I, Pitzalis M, Cavanillas ML, Arroyo R, Zoledziewska M, Marrosu M, Fernández O, Leyva L, Alcina A, Fedetz M, Moreno-Rey C, Velasco J, Real LM, Ruiz-Peña JL, Cucca F, Ruiz A, Izquierdo G. Genome-wide association study of multiple sclerosis confirms a novel locus at 5p13.1. PLoS One. 2012;7(5):e36140.

Matsuoka LY, Ide L, Wortsman J, et al. Sunscreens suppress cutaneous vitamin D3 synthesis. J Clin Endocrinol Metab. 1987;64:1165–8.

Matsuoka LY, Wortsman J, Dannenberg MJ, et al. Clothing prevents ultraviolet-B radiation-dependent photosynthesis of vitamin D3. J Clin Endocrinol Metab. 1992;75:1099–103.

Mawer EB , Backhouse J , Holman CA , Lumb GA , Stanbury SW . The distribution and storage of vitamin D and its metabolites in human tissues. Clin Sci 1972; 43: 413–31.

Mazerski J, Martelli S, Borowski E."The geometry of intercalation complex of antitumor mitoxantrone and ametantrone with DNA: molecular dynamics simulations. Acta Biochim. Pol. 1998; 45 (1): 1–11

McElroy JP, Cree BA, Caillier SJ, Gregersen PK, Herbert J, et al. Refining the association of MHC with multiple sclerosis in African Americans. Hum Mol Genet. 2010;19:3080–3086

McElroy JP, Oksenberg JR. Multiple sclerosis genetics. Neurol Clin. 2011 May;29(2):219-31.

MChen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE odulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol. 2007 Aug 1; 179(3):1634-47.

Medic N, Vita F, Abbate R, Soranzo MR, Pacor S, Fabbretti E, Borelli V, Zabucchi G. Mast cell activation by myelin through scavenger receptor. J Neuroimmunol 2008;200:27–40.

Mero IL, Gustavsen MW, Sæther HS, Flåm ST, Berg-Hansen P, Søndergaard HB, Jensen PE, Berge T, Bjølgerud A, Muggerud A, Aarseth JH; International Multiple Sclerosis Genetics Consortium, Myhr KM, Celius EG, Sellebjerg F, Hillert J, Alfredsson L, Olsson T, Oturai AB, Kockum I, Lie BA, Andreassen BK, Harbo HF. Oligoclonal band status in Scandinavian multiple sclerosis patients is associated with specific genetic risk alleles. PLoS One. 2013;8(3):e58352.

Minagar A, Alexander S, Sharain M, Zivadinov R. Alemtuzumab and multiple sclerosis: therapeutic application. Expert Opin. Biol. Ther. 2010;10:421–429.

Minghetti PP, Norman AW . 1,25(OH)2-vitamin D3 receptors: gene regulation and genetic circuitry. FASEB J. 1988 Dec;2(15):3043-53.

Miyama, K.J., Nakae, S., Matsuki, T., Nambu, A., Ishigame, H, Kakuta, S., Sudo, K., Iwakura, Y. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. J. Immunol., 2006, 177, 566-573.

Molnarfi N, Schulze-Topphoff U, Weber MS, et al: MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. J Exp Med 2013;210:2921-2937.

Montgomery SB, Sammeth M, Gutierrez-Arcelus M, Lach RP, Ingle C, Nisbett J, Guigo R, Dermitzakis ET. Transcriptome genetics using second generation sequencing in a Caucasian population. Nature. 2010 Apr 1;464(7289):773-7.

Morán-Auth Y, Penna-Martinez M, Shoghi F, Ramos-Lopez E, Badenhoop K. Vitamin D status and gene transcription in immune cells. J Steroid Biochem Mol Biol 2013; 136: 83-5.

Moretta A, Marcenaro E, Parolini S, Ferlazzo G, Moretta L. NK cells at the interface between innate and adaptive immunity. Cell Death Differ 2008;15:226–233.

Moy KA, Mondul AM, Zhang H, Weinstein SJ, Wheeler W, Chung CC, Männistö S, Yu K, Chanock SJ, Albanes D. Genome-wide association study of circulating vitamin D-binding protein. Am J Clin Nutr. 2014 Jun;99(6):1424-31.

Müller K, Diamant M, Bendtzen K. Inhibition of production and function of interleukin-6 by 1,25-dihydroxyvitamin D3. Immunol Lett. 1991 May; 28(2):115-20.

Munger KL and Ascherio A. Prevention and treatment of MS: studying the effects of vitamin D. Mult Scler 2011; 17: 1405-11.

Munger KL, Köchert K, Simon KC, et al. Molecular mechanism underlying the impact of vitamin D on disease activity of MS. Ann Clin Transl Neurol 2014; 1: 605-17.

Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296(23):2832-8.

Munger KL, Zhang SM, O'Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis.Neurology. 2004;62(1):60–5

Murray RS, Brown B, Brain D, Cabirac GF. Detection of coronavirus RNA and antigen in multiple sclerosis brain. Ann Neurol, 31 (1992), pp. 525–533

Murray TJ. Robert Carswell: the first illustrator of MS. Int MS J. 2009 Sep;16(3):98–101.

Myers AJ, Gibbs JR, Webster JA, Rohrer K, Zhao A, Marlowe L, Kaleem M, Leung D, Bryden L, Nath P, Zismann VL, Joshipura K, Huentelman MJ, Hu-Lince D,Coon KD, Craig DW, Pearson JV, Holmans P, Heward CB, Reiman EM, Stephan D, Hardy J.A survey of

genetic human cortical gene expression. Nat Genet. 2007 Dec;39(12):1494-9. Epub 2007 Nov 4.

Nebert, D.W., Zhang, G. & Vesell, E.S. From human genetics and genomics to pharmacogenetics and pharmacogenomics: past lessons, future directions. Drug Metab. Rev. 40, 187–224 (2008).

Nebert, D.W., Zhang, G. & Vesell, E.S. From human genetics and genomics to pharmacogenetics and pharmacogenomics: past lessons, future directions. Drug Metab. Rev. 40, 187–224 (2008).

Nischwitz S, Cepok S, Kroner A, Wolf C, Knop M, Müller-Sarnowski F, Pfister H, Roeske D, Rieckmann P, Hemmer B, Ising M, Uhr M, Bettecken T, Holsboer F, Müller-Myhsok B, Weber F. Evidence for VAV2 and ZNF433 as susceptibility genes for multiple sclerosis. J Neuroimmunol. 2010 Oct 8;227(1-2):162-6.

Niyonsaba F., Iwabuchi K., Someya A., Hirata M., Matsuda H., Ogawa H., and Nagaoka I. A cathelicidin family of human antibacterial peptide LL-37 induces mast cell chemotaxis.Immunology. 2002 May; 106(1): 20–26.

Okada, Y., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Kawaguchi, T., Stahl, E.A., Kurreeman, F.A., Nishida, N. et al. (2012) Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. Nat Genet, 44, 511-6.

Okada, Y., Wu, D., Trynka, G., Raj, T., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Yoshida, S. et al. (2014) Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature, 506, 376-81.

Omdahl JL , Morris HA , May BK . Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. Annu Rev Nutr 2002; 22: 139–66.

Orozco G, Viatte S, Bowes J, Martin P, Wilson AG, Morgan AW, Steer S, Wordsworth P, Hocking LJ; UK Rheumatoid Arthritis Genetics Consortium; Wellcome Trust Case Control Consortium; Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate Consortium, Barton A, Worthington J, Eyre S. Novel rheumatoid arthritis susceptibility locus at 22q12 identified in an extended UK genome-wide association study. Arthritis Rheumatol. 2014 Jan;66(1):24-30.

Orton SM, Herrera BM, Yee IM, et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol 2006; 5: 932–936.

Ozgocmen S, Bulut S, Ilhan N, Gulkesen A, Ardicoglu O, Özkan Y. Vitamin D deficiency and reduced bone mineral density in multiple sclerosis: effect of ambulatory status and functional capacity. Journal of Bone and Mineral Metabolism. 2005;23(4):309–13

Pakpoor J, Disanto G, Gerber JE, Dobson R, Meier UC, Giovannoni G, et al. The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. Mult Scler.2013;19:162–166.

Pakpoor J, Ramagopalan S. Evidence for an Association Between Vitamin D and Multiple Sclerosis. Curr Top Behav Neurosci 2014.

Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. Nat Rev Genet. 2013 Sep;14(9):661-73.

Parkes, M., Cortes, A., van Heel, D.A. and Brown, M.A. (2013) Genetic insights into common pathways and complex relationships among immune-mediated diseases. Nat Rev Genet, 14, 661-73.

Patsopoulos NA et al. Genome-wide meta-analysis identifies novel multiple sclerosis susceptibility loci. Ann Neurol. 2011-12-01

Patsopoulos NA1; Bayer Pharma MS Genetics Working Group; Steering Committees of Studies Evaluating IFNβ-1b and a CCR1-Antagonist; ANZgene Consortium; GeneMSA; International Multiple Sclerosis Genetics Consortium, Esposito F, Reischl J, Lehr S, Bauer D, Heubach J et al. Genome-wide meta-analysis identifies novel multiple sclerosis susceptibility loci. Ann Neurol. 2011 Dec;70(6):897-912.

Pedersen LB, Nashold FE, Spach KM, Hayes CE. 1,25-dihydroxyvitamin D3 reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking. J Neurosci Res 2007; 85: 2480-90.

Peelen E, Knippenberg S, Muris AH, Thewissen M, Smolders J, Tervaert JW, Hupperts R, Damoiseaux J. Effects of vitamin D on the peripheral adaptive immune system: a review. Autoimmun Rev. 2011 Oct;10(12):733-43.

Pekmezovic T, Drulovic J, Milenkovic M, Jarebinski M, Stojsavljevic N, Mesaros S, Kisic D, Kostic J. Lifestyle factors and multiple sclerosis: A case-control study in Belgrade. Neuroepidemiology. 2006; 27(4):212-6.

Pender MP, Burrows SR. Epstein–Barr virus and multiple sclerosis: potential opportunities for immunotherapy. Clinical & Translational Immunology. 2014;3(10)

Pierrot-Deseilligny C, Souberbielle JC. Is hypovitaminosis D one of the environmental risk factors for multiple sclerosis? Brain. 2010;133:1869–1888

Plum LA , DeLuca HF . Vitamin D, disease and therapeutic opportunities. Nat Rev Drug Discov 2010; 9: 941–55.

Preiningerova J. Oral laquinomod therapy in relapse remitting multiple sclerosis. Expert Opin. Investig. Drugs. 2009;18:985–989.

Pugliatti M, Harbo HF, Holmøy T, Kampman MT, Myhr KM, Riise T, Wolfson C. Environmental risk factors in multiple sclerosis.Acta Neurol Scand Suppl. 2008;188:34-40.

R.M. Steinman, D. Hawiger, M.C. Nussenzweig. Tolerogenic dendritic cells. Annu Rev Immunol, 21 (2003), pp. 685–711

Racke M, Lovett-Racke A, Krandikar N. The mechanism of action of glatiramer acetate treatment in multiple sclerosis. Neurology. 2010;74:S25–S30.

Rahnavard Z, Eybpoosh S, Homami MR, Meybodi HA, Azemati B, Heshmat R, Larijani B. Vitamin d deficiency in healthy male population: results of the Iranian multi- center osteoporosis study. Iran J Public Health. 2010; 39(3):45-52.

Ramagopalan SV, Dyment DA, Cader MZ, Morrison KM, Disanto G, Morahan JM, Berlanga-Taylor AJ, Handel A, De Luca GC, Sadovnick AD, Lepage P, Montpetit A, Ebers GC. Rare variants in the CYP27B1 gene are associated with multiple sclerosis. Ann Neurol 2011;70:881–6

Ramagopalan SV, Knight JC, Ebers GC. Multiple sclerosis and the major histocompatibility complex.Curr Opin Neurol. 2009;22:219–225.

Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton SM, et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. PLoS Genet. 2009;5:e1000369.

Raychaudhuri S, Remmers EF, Lee AT, Hackett R, Guiducci C, Burtt NP, Gianniny L, Korman BD, Padyukov L, Kurreeman FA, Chang M, Catanese JJ, Ding B, Wong S, van der Helm-van Mil AH, Neale BM, Coblyn J, Cui J, Tak PP, Wolbink GJ, Crusius JB, van der Horst-Bruinsma IE, Criswell LA, Amos CI, Seldin MF, Kastner DL, Ardlie KG, Alfredsson L, Costenbader KH, Altshuler D, Huizinga TW, Shadick NA, Weinblatt ME, de Vries N, Worthington J, Seielstad M, Toes RE, Karlson EW, Begovich AB, Klareskog L, Gregersen PK, Daly MJ, Plenge RM. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat Genet 2008;40:1216–23

Razin, A. CpG methylation, chromatin structure and gene silencing-a three-way connection. EMBO Journal 1998; 17, 4905–4908.

Reik, W., Dean, W., & Walter, J. Epigenetic reprogramming in mammalian development. Science 2001; 293, 1089–1093.

relapsing–remitting multiple sclerosis. Neurodegener. Dis. Manag. 2015, 5, 7–10.

Rice GP, Oger J, Duquette P, et al. Treatment with interferon beta-1b improves quality of life in multiple sclerosis. Can J Neurol Sci 1999;26:276–282.

Riise T, Nortvedt MW, Ascherio A. Smoking is a risk factor for multiple sclerosis. Neurology. 2003 Oct 28; 61(8):1122-4

Rivas, M.A., Gonzalez-Porta, M., Kurbatova, N., Griebel, T., Ferreira, P.G. et al. (2013) Transcriptome and genome sequencing uncovers functional variation in humans. Nature, 501, 506-11.

Rivera, F.J.; Couillard-Despres, S.; Pedre, X.; Ploetz, S.; Caioni, M.; Lois, C.; Bogdahn, U.; Aigner, L. Mesenchymal stem cells instruct oligodendrogenic fate decision on adult neural stem cells. Stem Cells 2006, 24, 2209–2219.

Rodríguez Regal A, del Campo Amigo M, Paz-Esquete J, Martínez Feijoo A, Cebrián E, Suárez Gil P, Mouriño MA. A case-control study of the influence of the smoking behaviour in multiple sclerosis. Neurologia. 2009 Apr; 24(3):177-80.

Roll P, Palanichamy A, Kneitz C, Dorner T, Tony HP: Regeneration of B cell subsets after transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. Arthritis Rheum 2006;54:2377-2386.

Rose JW, Burns JB, Bjorklund J, Klein J, Watt HE, Carlson NG. Daclizumab phase II trial in relapsing and remitting multiple sclerosis: MRI and clinical results". Neurology. 2007; 69 (8): 785–789.

Rozniecki JJ, Hauser SL, Stein M, Lincoln R, Theoharides TC. Elevated mast cell tryptase in cerebrospinal fluid of multiple sclerosis patients. Ann Neurol 1995;37:63–66.

Russo C, Morabito F, Luise F, Piromalli A, Battaglia L, Vinci A, Trapani Lombardo V, de Marco V, Morabito P, Condino F, Quattrone A, Aguglia U. Hyperhomocysteinemia is associated with cognitive impairment in multiple sclerosis. J Neurol. 2008 Jan; 255(1):64-9.

S Sriram, CW Stratton, S Yao, *et al.* *Chlamydia pneumoniae* infection of the central nervous system in multiple sclerosis. Ann Neurol, 46 (1999), pp. 6–14

Sadovnick AD, Duquette P, Herrera B, Yee IM, Ebers GC. A timing-of-birth effect on multiple sclerosis clinical phenotype. Neurology. 2007 Jul 3; 69(1):60-2.

Saikali P, Antel JP, Newcombe J, Chen Z, Freedman M, Blain M, Cayrol R, Prat A, Hall JA, Arbour N. NKG2D-mediated cytotoxicity toward oligodendrocytes suggests a mechanism for tissue injury in multiple sclerosis. J Neurosci 2007;27:1220–1228.

Sanna S1, Pitzalis M, Zoledziewska M, Zara I, Sidore C, Murru R, Whalen MB, Busonero F, Maschio A, Costa G, Melis MC, Deidda F, Poddie F, Morelli L, Farina G, Li Y, Dei M, Lai S, Mulas A, Cuccuru G, Porcu E, Liang L, Zavattari P, Moi L, Deriu E, Urru MF, Bajorek M, Satta MA, Cocco E, Ferrigno P, Sotgiu S, Pugliatti M, Traccis S, Angius A, Melis M, Rosati G, Abecasis GR, Uda M, Marrosu MG, Schlessinger D, Cucca F. Variants within the immunoregulatory CBLB gene are associated with multiple sclerosis. Nat Genet. 2010 Jun;42(6):495-7.

Sawcer S et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011-08-11.

Sawcer S, Franklin RJ, Ban M. Multiple sclerosis genetics. Lancet Neurol. 2014;13:700–709.

Schadt EE, Molony C, Chudin E, et al. Mapping the Genetic Architecture of Gene Expression in Human Liver. Abecassis G, ed. PLoS Biology. 2008;6(5):e107.

Schadt EE, Molony C, Chudin E, Hao K, Yang X, et al. Mapping the genetic architecture of gene expression in human liver. PLoS Biol. 2008;6:e107.

Schluesener HJ, Sobel RA, Linington C, Weiner HL. 1987.Amonoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. J. Immunol. 139:4016–21

Schluesener HJ, Sobel RA, Linington C, Weiner HL: A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. J Immunol 1987;139:4016-4021.

Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. Mol Nutr Food Res 2011; 55: 96-108.

Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. Mol Nutr Food Res. 2011 Jan;55(1):96-108.

Selmaj K, Brosnan CF, Raine CS. Colocalization of lymphocytes bearing gamma delta T-cell receptor and heat shock protein hsp65+ oligodendrocytes in multiple sclerosis. Proc Natl Acad Sci U S A 1991;88:6452–6456.

Shahijanian F, Parnell GP, McKay FC, et al. The CYP27B1 variant associated with an increased risk of autoimmune disease is underexpressed in tolerizing dendritic cells. Hum Mol Genet 2014; 23: 1425-34.

Shimonkevitz R, Colburn C, Burnham JA, Murray RS, Kotzin BL. Clonal expansions of activated gamma/delta T cells in recent-onset multiple sclerosis. Proc Natl Acad Sci U S A 1993;90:923– 927.

Silva KR, Alvarenga RM, Fernandez Y Fernandez O, Alvarenga H, Thuler LC. Potential risk factors for multiple sclerosis in Rio de Janeiro: a case-control study. Arq Neuropsiquiatr. 2009 Jun; 67(2A):229-34.

Simon JH, Jacobs LD, Campion M, et al. Magnetic resonance studies of intramuscular interferon beta-1a for relapsing multiple sclerosis. Ann Neurol 1998;43:79–87.

Simon KC, van der Mei IA, Munger KL, Ponsonby A, Dickinson J, Dwyer T, Sundström P, Ascherio A. Combined effects of smoking, anti-EBNA antibodies, and HLA-DRB1*1501 on multiple sclerosis risk. Neurology. 2010 Apr 27; 74(17):1365-71.

Slager SL1, Skibola CF, Di Bernardo MC, Conde L, Broderick P et al. Common variation at 6p21.31 (BAK1) influences the risk of chronic lymphocytic leukemia. Blood. 2012 Jul 26;120(4):843-6.

Smith KJ, Pyrdol J, Gauthier L, Wiley DC, Wucherpfennig KW. Crystal structure of HLA-DR2 (DRA*0101, DRB1*1501) complexed with a peptide from human myelin basic protein. J Exp Med.1998;188:1511–1520.

Sonoda KH, Faunce DE, Taniguchi M, Exley M, Balk S, Stein-Streilein J. NK T cell-derived IL-10 is essential for the differentiation of antigen-specific T regulatory cells in systemic tolerance. J Immunol 2001;166:42–50

Sospedra M., Zhao Y., zur Hausen H., Muraro P.A., Hamashin C., de Villiers E.M., Pinilla C., Martin R. Recognition of conserved amino acid motifs of common viruses and its role in autoimmunity. PLoS Pathogens, 1 (2005)

Sotelo J., Ordoñez G., Pineda B.. Varicella–zoster virus at relapses of multiple sclerosis. Journal of Neurology, 254 (2007), pp. 493–500

Sotelo J.,. Martínez-Palomo A, Ordoñez G., Pineda B.. Varicella–zoster virus in cerebrospinal fluid at relapses of multiple sclerosis. Annals of Neurology, 63 (2008), pp. 303–311

Speedy HE, Di Bernardo MC, Sava GP, Dyer MJ, Holroyd A et al. A genome-wide association study identifies multiple susceptibility loci for chronic lymphocytic leukemia. Nat Genet. 2014 Jan;46(1):56-60.

Steinman L. A molecular trio in relapse and remission in multiple sclerosis. Nat Rev Immunol.2009;9:440–447.

Stelekati E, Orinska Z, Bulfone-Paus S. Mast cells in allergy: innate instructors of adaptive responses. Immunobiology 2007;212:505–519.

Stewart JN, Mounir S, Talbot PJ.Human coronavirus gene expression in the brains of multiple sclerosis patients. Virology, 191 (1992), pp. 502–505

Stoner GL, Agostini HT, Ryschkewitsch CF, et al. Characterization of JC virus DNA amplified from urine of chronic progressive multiple sclerosis patients. Mult Scler, 1 (1996), pp. 193–199

Stranger BE, Nica AC, Forrest MS, et al. Population genomics of human gene expression. Nature genetics. 2007;39(10):1217-1224.

Sumaya CV, Myers LW, Ellison GW. Epstein-Barr virus antibodies in multiple sclerosis. Arch Neurol. 1980;37:94–96.

Sundqvist E, Bäärnhielm M, Alfredsson L, Hillert J, Olsson T, Kockum I. Confirmation of association between multiple sclerosis and CYP27B1. Eur J Hum Genet 2010;18:1349–52

Sundström P, Nyström L, Hallmans G. Smoke exposure increases the risk for multiple sclerosis. Eur J Neurol. 2008 Jun; 15(6):579-83.

Tennakoon DK, Mehta RS, Ortega SB, Bhoj V, Racke MK, Krandikar NJ. Therapeutic induction of regulatory cytotoxic, CD8 T cells in multiple sclerosis. J. Immunol. 2006;176:7119–7129.

The International Multiple Sclerosis Genetics A high-density screen for linkage in multiple sclerosis. Am J Hum Genet. 2005; 77, 454–467.

Toms R, Weiner HL, Johnson D. Identification of IgE-positive cells and mast cells in frozen sections of multiple sclerosis brains. J Neuroimmunol 1990;30:169–177.

Traugott U. Characterization and distribution of lymphocyte subpopulations in multiple sclerosis plaques versus autoimmune demyelinating lesions. Springer Semin Immunopathol 1985;8:71–95.

Tupin E, Kronenberg M. Activation of natural killer T cells by glycolipids. Methods Enzymol 2006;417:185–201.

Tzartos, J.S., Khan, G., Vossenkamper, A., Cruz-Sadaba, M., Lonardi, S., Sefia, E., Meager, A., Elia, A., Middeldorp, J.M., Clemens, M. et al. (2012) Association of innate immune activation with latent Epstein-Barr virus in active MS lesions. Neurology, 78, 15-23.

Urdinguio, R. G., Sanchez-Mut, J. V., & Esteller, M. Epigenetic mechanisms in neurological diseases: Genes, syndromes, and therapies. Lancet Neurology 2009; 8, 1056–1072.

Vaknin-Dembinsky A, Balashov K, Weiner HL. IL-23 is increased in dendritic cells in multiple sclerosis and down-regulation of IL-23 by antisense oligos increases dendritic cell IL-10 production. J Immunol 2006;176:7768–7774.

Vaknin-Dembinsky A, Murugaiyan G, Hafler DA, Astier AL, Weiner HL. Increased IL-23 secretion and altered chemokine production by dendritic cells upon CD46 activation in patients with multiple sclerosis. J Neuroimmunol 2008;195:140–145.

Van der Mei IA, Ponsonby AL, Dwyer T, Blizzard L, Simmons R, Taylor BV, Butzkueven H, Kilpatrick T. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study.BMJ. 2003 Aug 9; 327(7410):316.

Veyrieras J-B, Kudaravalli S, Kim SY, et al. High-Resolution Mapping of Expression-QTLs Yields Insight into Human Gene Regulation. Gibson G, ed.PLoS Genetics. 2008;4(10):e1000214.

Vieth R , ed. The Pharmacology of Vitamin D, Including Fortification Strategies. 2nd edn. London: Elsevier, 2005.

Vincent R, Louis P, Gongora C, Papa I, Clot J, et al. Quantitative analysis of the expression of the HLA-DRB genes at the transcriptional level by competitive polymerase chain reaction. J Immunol.1996;156:603–610.

Voight, B.F. and Cotsapas, C. (2012) Human genetics offers an emerging picture of common pathways and mechanisms in autoimmunity. Curr Opin Immunol, 24, 552-7.

Vollmer T, Stewart T, Baxter N. Mitoxantrone and cytotoxic drugs' mechanisms of action. Neurology. 2010;74:S41–S46

Vollmer T, Stewart T, Baxter N. Mitoxantrone and cytotoxic drugs' mechanisms of action. Neurology. 2010;74:S41–S46

W.F. Rigby, M. Waugh, R.F. Graziano. Regulation of human monocyte HLA-DR and CD4 antigen expression, and antigen presentation by 1,25-dihydroxyvitamin D3. Blood, 76 (1990), pp. 189–197

Wandinger K-P, Jabs W, Siekhaus A, Bubel S, Trillenberg P, Wagner H-J, et al. Association between clinical disease activity and Epstein-Barr virus reactivation in MS. Neurology.2000;55:178–184.

Wang JH1, Pappas D, De Jager PL, Pelletier D, de Bakker PI, Kappos L, Polman CH; Australian and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene), Chibnik LB, Hafler DA, Matthews PM, Hauser SL, Baranzini SE, Oksenberg JR. Modeling the

cumulative genetic risk for multiple sclerosis from genome-wide association data. Genome Med. 2011 Jan 18;3(1):3.

Wang S. Epidemiology of vitamin D in health and disease. Nutr Res Rev 2009; 22(2): 188-203.

Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010 Jul 17;376(9736):180-8.

Ward LD and Kellis M. Interpreting noncoding genetic variation in complex traits and human disease. Nature Biotechnology, 2012; 30, 1095–1106.

Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab. 1988;67:373–8.

Weber, M.S., Prod'homme, T., Youssef, S., Dunn, S.E, Rundle, C., Lee, L., Patarroyo, J.C., Stuve, O., Sobel, R.A., Steinman, L., Zamvil, S.S. Type II monocytes modulate T cell mediated central nervous system autoimmune disease. Nat. Med., 2007, 13, 935-943.

Wellcome Trust Case Control Consortium et) al Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007 Jun 7; 447(7145): 661–678.

Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007 Jun 7;447(7145):661-78.

Westra H.-J., Franke L. From genome to function by studying eQTLs. Biochim. Biophys. Acta (2014), pp. 1896–1902.

Wingerchuk DM. Smoking: effects on multiple sclerosis susceptibility and disease progression. Therapeutic Advances in Neurological Disorders. 2012;5(1):13-22.

Wöbke TK, Sorg BL and Steinhilber D.Vitamin D in inflammatory diseases. Front Physiol 2014; 5: 244.

Xiao J, Yang R, Biswas S, Qin X, Zhang M, Deng W. Mesenchymal Stem Cells and Induced Pluripotent Stem Cells as Therapies for Multiple Sclerosis. Int J Mol Sci. 2015 Apr 24;16(5):9283-9302.

Zanetti M . Cathelicidins, multifunctional peptides of the innate immunity. Journal of Leukocyte Biology 2004; 75 (1): 39–48.

Zeine R, Pon R, Ladiwala U, Antel JP, Filion LG, Freedman MS. Mechanism of gammadelta T cellinduced human oligodendrocyte cytotoxicity: relevance to multiple sclerosis. J Neuroimmunol 1998;87:49–61.

Zeller T, Wild P, Szymczak S, et al. Genetics and Beyond – The Transcriptome of Human Monocytes and Disease Susceptibility. Bochdanovits Z, ed. PLoS ONE. 2010;5(5):e10693.

Zhang H, Shih DQ, Zhang X. Mechanisms underlying effects of 1,25-Dihydroxyvitamin D3 on the Th17 cells. Eur J Microbiol Immunol (Bp) 2013 Dec; 3(4): 237–240.

Zhang Y, Leung DY, Richers BN, et al. Vitamin D inhibits monocyte/ macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. J Immunol 2012; 188: 2127-35.

Zhernakova A, Stahl EA, Trynka G, Raychaudhuri S, Festen EA, Franke L, Westra HJ, Fehrmann RS, Kurreeman FA, Thomson B, Gupta N, Romanos J, McManus R, Ryan AW, Turner G, Brouwer E, Posthumus MD, Remmers EF, Tucci F, Toes R, Grandone E, Mazzilli MC, Rybak A, Cukrowska B, Coenen MJ, Radstake TR, van Riel PL, Li Y, de Bakker PI, Gregersen PK, Worthington J, Siminovitch KA, Klareskog L, Huizinga TW, Wijmenga C, Plenge RM. Meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis identifies fourteen non-HLA shared loci. PLoS Genet 2011;7:e1002

Zimmerman DR , Reinhardt TA , Kremer R , Beitz DC , Reddy GS , Horst RL . Calcitroic acid is a major catabolic metabolite in the metabolism of 1 alpha-dihydroxyvitamin D(2). Arch Biochem Biophys 2001; 392: 14–22.

Zivadinov R, Rudick R, De Masi R, Nasuelli D, Ukmar M, Pozzi–Mucelli R, Grop A, Cazzato G, Zorzon M. Effects of IV methylprednisolone on brain atrophy in relapsing-remitting multiple sclerosis.Neurology. 2001;573:1239–1247.