Interrogating the intersection of genetic- and gender-based risk for multiple sclerosis

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Abstract

Multiple sclerosis (MS) has no effective prevention or cure and only limited treatment options. Significant progress has been made in finding genetic susceptibility loci, but it is still mostly unclear how genetic variants associated with disease are implicated in MS pathogenesis. Meanwhile, epidemiologically, it has long been clear that women get MS two or three times more frequently than men do. It is also known that there is substantial sexual dimorphism in immune regulation. The analysis presented here attempts to triangulate genetic and sex-based risk for MS, first, in order to understand quantitatively the relationship between sex and genetic risk and, second, to highlight particular genes implicated by both genetics and gender in MS etiology. First, I use a Bayesian framework to assess the relationship between male and female effect sizes at variants previously shown to be associated with MS. I show that the effect size of genetic variants for MS risk is systematically lower in females than males, with female effect size estimated at 0.42 times male effect size. Second, I use genome-wide RNAseq data to characterize differential transcription between the sexes, finding over 5000 genes to be differentially expressed, but I do not find sexual dimorphism in *cis*-eQTL effect sizes. I highlight a subset of genes whose expression is affected by an MS risk variant and whose expression is sexually dimorphic. Several of these genes have functions consistent with complicity in MS. Together, these analyses demonstrate methodology to integrate genetic and genomic data in the context of sexual dimorphism in order to explore the mechanism of disease.

Abbreviations and terms

BF	Bayes factor
C57BL/6	C57 black 6 (mouse strain)
CNS	central nervous system
EAE	experimental autoimmune encephalomyelitis
eQTL	expression quantitative trait locus
GWAS	genome-wide association study
HLA	human leukocyte antigen
IL	interleukin
MHC	major histocompatibility complex
MS	multiple sclerosis
PCA	principal components analysis
SJL	Swiss Jim Lambert (mouse strain)
SNP	single nucleotide polymorphism
TLR	toll-like receptor

1 Background

1.1 Introduction

Multiple sclerosis (MS), a devastating disease causing progressive neurological disability, has neither prevention nor cure. It is also not currently possible to predict who will get MS and who will not. Approximately 2.5 million people suffer from MS worldwide (Hauser and Goodin, 2012), and the disease course is long and arduous: median disease course is 23 years (Cottrell et al., 1999) and involves a progressive and debilitating loss of motor function and control. The underlying biological mechanism of the disease remains poorly understood, resulting in a lack of specific and effective treatments. A 2008 study of MS patients in the UK found that the disease cost patients about £25,000 per year, only about £1,300 of which was spent on medication (McCrone et al., 2008).

An autoimmune attack on myelin in the central nervous system (CNS) causes destruction of myelin sheaths surrounding neurons. The origin and mechanism of this anti-myelin autoimmune attack are unclear. Thus, diagnosis is based on clinical findings and on imaging showing immune damage to the CNS. Genome-wide association studies (GWAS) have shed light on some formerly mysterious diseases. It is possible to understand what differs between those who get a disease and those who do not by analyzing large human populations of cases and controls. The genetic variants associated with disease can often help uncover the underlying biological mechanism of disease. For example, based on GWAS results, and through multiple strategies of biological follow-up, autophagy pathways have been shown to be important in Crohn's disease, which has led to the development

of autophagy-enhancing drugs for use in Crohn's (McGovern et al., 2015; Levine et al., 2015). As another example, GWAS results have uncovered pathways important in the biological mechanism underlying obesity, particularly highlighting genes relevant in the brain (El-Sayed Moustafa and Froguel, 2013).

In order for the GWAS approach to reach its full impact it is necessary to go further than mapping associations between genetic variants and disease, especially in a disease as complex as MS has proven to be. Though somewhat heritable, MS is not a Mendelian disorder — many variants contribute small effects to disease risk — so the link between genetics and disease is often unclear. Elucidating subtle biological links between genetic variants and MS may still uncover keys to new therapeutic strategies, but it requires more in-depth analysis of these risk variants, including an understanding of the interplay between genetic variants and other risk factors that together inform who will get MS and how the disease process unfolds.

GWAS allows us to understand genetic heterogeneity in disease risk. However, genetic variants are not the only variables that are unevenly distributed between cases and controls. To dissect the biological relevance of GWAS variants, we can look at disease risk along another axis of heterogeneity: sex. Epidemiologically, MS affects women at 2-3 times the rate that it affects men (Pugliatti et al., 2006; World Health Organization, 2008). The basis of this significantly increased MS susceptibility in women is not understood. The intersection between genetic risk of MS, which has been well characterized, and gender-based risk is even less well studied.

1.2 Immune etiology of multiple sclerosis

An MS patient presents clinically with muscle weakness, spasticity (abnormal muscle stiffness) and ataxia (loss of movement control), along with vision problems, including diplopia (double vision) and optic neuritis (inflammation of the optic nerve, resulting in blurred vision). Some patients suffer from cognitive dysfunction, vertigo and mental health issues such as depression, anxiety and fatigue (Beiske et al., 2008; Hauser and Goodin, 2012). The clinical course of MS is not uniform but instead follows one of four courses: relapsing-remitting, secondary progressive, primary progressive or progressive relapsing. Because no genetic variants or biomarkers in the blood or cerebrospinal fluid can reliably differentiate between these subcategories, and because patients sometimes transition between courses, it is unclear whether disease mechanism differs between these subcategories of MS (Lublin et al., 2014). Thus, MS is considered a single disease for the purpose of the analyses presented here.

It is unclear what prompts the immune system to attack myelin sheaths of neurons in the CNS, nor is it clear which components of the immune system play a critical role in promoting and sustaining the autoimmune attack (Hauser and Goodin, 2012). T cell attacks appear largely responsible for myelin destruction (Babbe et al., 2000). Increasingly, other cell types are additionally thought to be involved in disease, either directly through in adaptive immune response or indirectly by maintaining an inflammatory environment and sustaining the immune response

against myelin (Kappos et al., 2011; Palanichamy et al., 2014). The trigger that initiates the anti-myelin immune attack may be immune dysregulation in some arm of the immune system, a malformed or abnormally expressed protein in the CNS, or both.

Current treatment options for MS patients are limited. Treatment relies on broad immune suppression: depleting lymphocytes, blocking immune cell entry into the CNS, or generally suppressing inflammation (Table 1). These treatments work to some extent, reinforcing an overly aggressive immune response in the CNS as the causal mechanism, broadly, for MS. However, they are accompanied by myriad side effects due to their nonspecific mechanism of action in suppressing the immune system.

Understanding the mechanism of autoimmune attack that causes MS in greater depth is required for rational approaches to prevention and treatment. Because we know neither which cells are key players in the aberrant immune attack in MS nor the molecular pathways that account for immune dysregulation, it is difficult to approach MS research using a cell line or gene knockout approach. Instead, human data from cases and controls can be extremely useful in order to generate hypotheses about the key biological pathways in disease.

Drug/treatment	**Mechanism of action**	**Reference**
alemtuzumab	monoclonal antibody against CD52; depletes circulating T and B cells	Hartung et al. (2015)
azathioprine	purine analogue that blocks purine biosynthesis, preventing lymphocyte proliferation and causing apoptosis of stimulated T cells	Winkelmann et al. (2016) Massacesi et al. (2014)
cladribine, mitoxantrone hydrochloride	inhibit DNA repair, suppressing lymphocyte development	Hauser and Goodin (2012) Giovannoni et al. (2010) Edan et al. (1997)
dimethyl fumarate	limits Th1 and Th17 activity; limits expression of genes downstream of NF-κB	Winkelmann et al. (2016) Gold et al. (2012)
fingolimod	blocks sphingosine–1–phosphate; traps lymphocytes in secondary lymphoid organs, periphery	Hauser and Goodin (2012) Cohen et al. (2010) Kappos et al. (2010)
glatiramer acetate	synthetic 4-amino acid peptide that binds to MHC; induces Th2 and regulatory phenotypes; binds MHC, preventing self-peptide presentation	Winkelmann et al. (2016) Johnson et al. (2000)
IFN–β	broad immune modulator in antiviral response; downregulates T cell proliferation and trafficking to CNS	Hauser and Goodin (2012) Paty and Li (1993)
methylprednisolone, dexamethasone	glucocorticoid therapy, suppressing immune function	Hauser and Goodin (2012) Milligan et al. (1987)
natalizumab	monoclonal antibody against $\alpha_4\beta_1$ integrin, blocking lymphocyte adhesion and trafficking to CNS	Steinman (2014) Polman et al. (2006)
ocrelizumab	humanized anti-CD20 monoclonal antibody; depletes B cells	Kappos et al. (2011)
plasma exchange	depletes antibodies, including anti-self	Hauser and Goodin (2012) Weinshenker et al. (1999)
teriflunomide	limits T and B cell proliferation by interfering with pyrimidine biosynthesis	Winkelmann et al. (2016) O'Connor et al. (2011)

Table 1: A list of commonly used MS treatments and their mechanisms of action underscores the lack of highly specific therapies; most treatment approaches rely on broad immunosuppression or on blocking immune cell trafficking.

1.3 Risk for multiple sclerosis

Genetics plays a major role in risk of MS. A monozygotic twin of an MS patient has a 25–30% chance of getting MS, which is 170 times the risk in the general population (Koch-Henriksen and Sorensen, 2010; Cree, 2014). Loci associated with MS in GWAS explain nearly a third of the risk of developing MS (Watson et al., 2012), so interrogating these variants may provide significant insight into new treatment targets and into the basic biology of MS. But how do we account for the non-genetic risk? It is possible that other genetic risk variants remain to be discovered or that stochastic differences in gene expression account for the <100% risk concordance of MS in monozygotic twins. Epidemiological studies, however, indicate the major role of environmental influences in risk of acquiring MS. These environmental factors may act independently of genetics or may interact with genetic risk variants. If environmental variables do interact with genetics, they may act across the genome or at specific variants.

Several environmental factors have been found to correlate with risk of MS. It has long been recognized that populations living at higher latitudes have a higher prevalence of MS (Ebers and Sadovnick, 1993), though it is debated whether this association is causal, for example due to UV light exposure, or related to some underlying genetic structure (Koch-Henriksen and Sorensen, 2010). Another factor important in MS risk and disease course appears to be concomitant or prior infectious disease. Individuals with a history of infectious mononucleosis, particularly later in life, have a higher risk of developing MS (Handel et al., 2010). Patients with both MS and helminth infections have less severe MS-mediated disability, likely because helminths cause some level of immunosuppresion (Correale

and Farez, 2011). In contrast, systemic infections, like upper respiratory tract infections, are associated with increased relapse rate and worse clinical presentation of MS symptoms, likely due to T cell activation (Correale et al., 2006).

Among the multitude of environmental factors highlighted in the MS literature, a strong female bias, found repeatedly, remains of the most intriguing and mysterious associations with MS. The sexual dimorphism in MS is widely acknowledged but poorly understood; female MS patients outnumber male patients at least 2:1 and, in some studies, upwards of 3:1 (Chao et al., 2010). The MS prevalence ratio may also be increasing, at least in some parts of the world (Kingwell et al., 2015; Greer and McCombe, 2011; Koch-Henriksen and Sorensen, 2010; Orton et al., 2006). Many differences have been observed in the immune systems of males and females, some of which could be explained by sex chromosome genetics and others driven by hormone level variation (especially estrogen, progesterone and testosterone, but including others such as prolactin) — either through the action of a single hormone or, more realistically, through a combination of hormones acting together and with gene expression from sex chromosomes.

1.3.1 Gender in differential immune regulation

MS not the only case in which gender has been found to have an impact on disease course and, more broadly, on immune function. In 1965, a group of pediatricians reported, with respect to infectious disease, "That the human male is more susceptible than the female to many diseases is familiar knowledge" (Washburn et al.,

1965). In contrast, autoimmunity is generally female-skewed (Table 2). Some have attempted to summarize the effects of reproductive hormones by concluding that estrogen is pro-inflammatory while progesterone and androgens are anti-inflammatory, but the reality is much more complicated (Oertelt-Prigione, 2012). Sex bias in autoimmunity is the manifestation of many components of biological sex, including sex chromosomes and sex hormones.

An intuitive explanation for differential immune regulation between the sexes can be found in childbearing. To optimize reproductive success, women have evolved to tolerate both sperm and an immunologically "foreign" fetus for the duration of gestation, nine months in humans. Both constitutively and through menstrual cycling, the female immune system must cope with a unique set of challenges in order to ward off pathogens, while also tolerating foreign antigens contributed by the father during reproduction, particularly at certain times in the menstrual cycle and in certain stages of developmental maturity. For example, regulatory T cells ($CD4^+CD25^+FOXP3^+$ lymphocytes) increase in peripheral blood at the end of the follicular phase of the menstrual cycle in fertile women, but postmenopausal women and women with recurrent spontaneous abortions have no such regulatory T cell increase (Arruvito et al., 2007). Concretely, pregnancy has been demonstrated to have a clinical impact on MS disease course, because disease improves during pregnancy and then rebounds post-partum before settling back to a pre-pregnancy state (Confavreux et al., 1998).

The X chromosome may contribute to differential immune regulation between the sexes. The X chromosome harbors several genes with important roles in

immune function, such as *FOXP3*, the transcription factor that determines the development of regulatory T cells; molecules involved in interleukin signaling: IL-13, IL-2Rγ and IL-9R (Khalid, 2014); TLR9, a toll-like receptor; and CD40L, a molecule important in T cell–B cell signaling (Libert et al., 2010). In females, in each cell, one X chromosome is normally inactivated in order to have balanced dosage of gene expression compared to males. It has been hypothesized that either reactivation and overdosing of X-linked genes, or haploinsufficiency of X-linked genes, may contribute to the immune over-activation observed in autoimmunity. It has also been proposed that skewed X chromosome inactivation in females may contribute to autoimmunity. Dendritic cells in the thymus mediate self tolerance by presenting self antigens; if a preponderance of thymic dendritic cells are expressing genes from one X chromosome, but cells in the periphery express genes from the other X chromosome, T cells may mistakenly recognize those antigens as foreign (Libert et al., 2010).

Reproductive hormones also play an important role in immune function and, unlike a relatively stable karyotype, hormones vary over an individual's lifetime and over the course of a day, month or year, thereby modulating immune response dynamically over time. In keeping with the important role the immune system plays in reproduction, reproductive hormones do not act on reproductive organs alone but also on immune cells. Progesterone, estrogen, and testosterone each play multiple and important roles in immune regulation. As an example, progesterone suppresses the antiviral response of plasmacytoid dendritic cells (Hughes et al., 2008). $CD4^+$ T cells also express an intracellular progesterone receptor, which, when activated, suppresses a pro-inflammatory phenotype, including a

T-dependent antibody response (Hughes et al., 2013). In contrast, estrogen treatment of plasmacytoid dendritic cells enhances the antiviral response, as measured by type I interferon production (Seillet et al., 2012). But estrogen also has many other effects (Fish, 2008), including expansion of regulatory T cell populations, a mechanism of suppressing inflammation (Polanczyk et al., 2004). In humans, elevated testosterone level has been shown to correlate with weaker antibody responses to the influenza vaccine, suggesting some role in suppressing the immune response (Furman et al., 2014). Studies of hormonal effects on immune regulation are complicated by the multitude of effects each hormone has, both immunologically and systemically. Many hormonal effects have been found — many more than the examples presented above — so it is clear that reproductive hormones do have biological implications on immune regulation and function, but specific links to disease mostly remain poorly understood.

	Disease	Female:Male ratio
Male-predominant	reactive arthritis	0.2
	ankylosing spondylitis	0.4
	Guillan-Barré syndrome	0.8
	wegener's granulomatosis	0.9
	type 1 diabetes	0.9
Female-predominant	psoriasis	1.1
	pemphigus vulgaris	1.1
	ulcerative colitis	1.2
	rheumatic fever	1.2
	pemphigoid	1.3
	myasthenia gravis	1.4
	Crohn's disease	1.4
	hemolytic anemia	1.4
	Behçet's disease	1.5
	celiac disease	1.6
	vitiligo	1.6
	juvenile rheumatoid arthritis	1.8
	multiple sclerosis	**1.8**
	rheumatoid arthritis	2.4
	erythema nodosum	2.7
	localized scleroderma	3.2
	systemic sclerosis	3.7
	primary biliary cirrhosis	3.9
	localized lupus erythematosus	4.7
	Graves' disease	5.1
	systemic lupus erythematosus	5.2
	Sjögren's syndrome	7.5
	Hashimoto thyroiditis	8.3

Table 2: Gender ratios of a subset of autoimmune diseases. Not all autoimmune diseases show a strong gender skew, but many do not exhibit equal prevalence between males and females; of autoimmune diseases that show gender bias, female bias is more common. Table modified from Jorgensen et al. (2010).

1.3.2 Current understanding of the biology underlying gender-biased MS risk

Beyond the research on sexual dimorphism in general immune function, many analyses have attempted to elucidate the sexual dimorphism particularly relevant to MS. Work in humans and in mouse models has pointed to both sex chromosomes and reproductive hormones as important arbiters of gender-skewed risk for MS. There has also been some work on gender-skewed heritability patterns.

Based on evidence in humans, reproductive hormones appear to play a role in MS risk, some with protective effect. Epidemiologically, women using oral contraceptives or who have given birth have a later age of MS onset (Khalid, 2014). Clinically, women often report worsening of MS symptoms immediately prior to menstruation, which is a period of large hormonal fluctuation; women using oral contraceptives are less likely to experience this cycle-dependent worsening (Zorgdrager and De Keyser, 1997). The ratio of progesterone to 17-β-estradiol during the luteal phase of the menstrual cycle in pre-menopausal MS patients has been found to correlate with number and size of MS lesions found on MRI (Pozzilli et al., 1999).

It has been suggested for over a decade, through small clinical studies, that reproductive hormones may have therapeutic benefit, underscoring their causal role in modulating MS pathology. Treating with estrogens is somewhat complicated because many different types of estrogen are physiologically important, but estriol has generally been accepted as a therapeutic with few side effects (Gold and Voskuhl, 2009). An estrogen produced during pregnancy, estriol has been shown to

have anti-inflammatory effects in female MS patients and improve the number and severity of MS lesions apparent on MRI (Sicotte et al., 2002). Estrogen levels need to be relatively high — for example, an estriol level similar to that in pregnancy — in order to have an effect on MS (Greer and McCombe, 2011). Testosterone has also been shown to alleviate symptoms (Gold et al., 2008); testosterone treatment in male MS patients caused a decrease in the $CD4^+$ T cell population and a less inflammatory cytokine profile, based on decreased IL-2 levels and increased TGF-β levels (Gold et al., 2008).

Mouse models of MS have also shed light on the biology of the disease, especially because they allow for both hormone and sex chromosome manipulation. Immunizing some strains of mice with myelin peptides and adjuvant causes experimental autoimmune encephalomyelitis (EAE). The immunization prompts differentiation and activation of $CD4^+$ Th1 cells, which attack myelin in the CNS, causing an MS-like phenotype (Bebo et al., 1998) (Sospedra and Martin, 2005). An EAE mouse model using the SJL strain exhibits the same sexual dimorphism as humans, but not when EAE is induced in a C57BL/6 genetic background (Bebo et al., 1998), despite no difference in androgen levels between the two strains (Palaszynski et al., 2004b). The difference in gender effect based on EAE mouse strain suggests that genetic background interacts with gender to inform differential EAE risk in SJL mice but not in C57BL/6 mice (Palaszynski et al., 2004b).

Results in EAE mouse models underscore the importance of reproductive hormones on disease risk and clinical course. When SJL EAE males are castrated, they exhibit similar frequency and severity of EAE to their female counterparts,

further suggesting a substantial hormonal impact in influencing the gender difference in disease course (Bebo et al., 1998). Although C57BL/6 mice do not exhibit sexual dimorphism in disease onset, female reproductive hormones appear to alter disease phenotype; clinical scores vary periodically over the course of the menstrual cycle in female mice (Papenfuss et al., 2004).

Studies of hormone therapy in the EAE model further prove the importance of reproduction in influencing the mechanisms underlying MS, and these mouse studies allow for further dissection of the biological pathways important in hormone-mediated alteration of MS pathology. In C57BL/6 mice, treatment with estriol improves clinical scores in both male and female EAE mice (Palaszynski et al., 2004a). Estriol appears to act through dendritic cells, driving a tolerogenic induction of a Th2 phenotype in $CD4^+$ T helper cells. (Papenfuss et al., 2011). 17-β-estradiol treatment of ovariectomized C57BL/6 mice, to the level found in pregnancy, lowers the risk of developing EAE on immunization and decreases the severity of EAE cases, as does true pregnancy at the time of immunization (Haghmorad et al., 2014). Estrogen receptor β ligand (ERβ ligand) is clinically useful to improve symptoms after EAE onset, but only for the duration of ERβ ligand therapy, (Wisdom et al., 2013; Tiwari-Woodruff et al., 2007). Treating EAE-primed mice with dihydrotestosterone (DHT) has been found to reduce the clinical severity of disease but not delay disease in both SJL and C57BL/6 mouse strains, but only in non-castrated male mice (Giatti et al., 2015; Palaszynski et al., 2004b). DHT is a useful tool because unlike physiological testosterone, it is not metabolized to 17-β-estradiol, proving that testosterone modulates EAE independent of estradiol pathway.

Based on further EAE model experiments, sex chromosome differences also appear to play an important role in MS risk independent of their hormonal implications. Another SJL EAE model manipulated the *Sry* gene, the male sex-determining gene on the Y chromosome. "Female" mice were manipulated to have *Sry* or "male" mice had *Sry* removed; this SJL/$Sry^{+/-}$ model should standardize for hormonal differences between the "males" and "females" and only exhibit EAE differences based on sex chromosome presence. The XX chromosomal complement, as opposed to XY, was shown to cause the increased severity of MS, in part due to differences in immune cells with an XX vs XY background, as shown through adoptive transfer (Smith-Bouvier et al., 2008). It is unclear exactly why two X chromosomes might cause higher EAE susceptibility, but the "Kast-Stewart hypothesis" proposes that skewed X-inactivation in the thymus could cause T cells not to become tolerant of some self antigens, allowing for autoreactivity in the periphery when antigens encoded on the other X chromosome are encountered (Knudsen et al., 2007).

Finally, some work on genetic skew in MS has been conducted; sex-skewed heritability and MHC effects with respect to sex have been explored in humans. Parent-of-origin effects have been proposed to explain, in part, gender differences in MS risk, but results have not been entirely consistent. Risk of MS in an individual whose parent had MS appears to be modulated by the gender of the affected parent. In one study, maternal MS was found to be correlated with higher risk, as shown by a higher rate of MS in half-siblings who share an affected mother rather than affected father (Ebers et al., 2004). However, in another study, it was

found that MS-affected men transmit MS more often to their children (Kantarci et al., 2006). This pattern was explained as a manifestation of the Carter effect, in which men require a greater genetic load of disease-risk variants in order to clinically manifest a polygenic disease, so when a father has the disease, he has a particularly large genetic liability load and is therefore likely to transmit many risk variants to his offspring, who, especially if they are female, will then have a high risk of getting the disease (Kantarci et al., 2006).

The present analysis focuses on non-MHC genetic determinants of MS susceptibility and their relationship to sex in modulating disease risk, but it is worth noting that the MHC may be an important mediator of gender dimorphism in MS. The highly diverse MHC region is a major genetic contributor to MS risk, and MS patients with *HLA-DRB1*15*, a HLA class II allele on chromosome 6 well characterized for its association with MS, exhibit a higher female-to-male ratio than MS patients who do not carry *HLA-DRB1*15*. This allele exhibits an inheritance pattern skewed from what is expected or even observed for other HLA-DRB1 alleles, and the allele is particularly transmitted in mother-daughter pairs, in deviation from expected transmission probability (Chao et al., 2011).

In summary, many studies have been previously conducted, both using epidemiological and mouse model tools, to understand the gender dimorphism in MS and, more broadly, autoimmune disease. Due to differing experimental setup and the large number of variables that affect MS risk, along with the limited quantity of established facts about the etiology of the disease, the mechanism for gender-based risk and pathogenesis remains poorly understood. It has been very well

established that a female bias exists and that this gender dimorphism is largely due to endogenous effects of biological sex, as opposed to environmental factors associated with gender, though these environmental factors may further exacerbate risk differential. Reproductive hormones, sex chromosomes, or both potentially drive this biologically-induced increase in female MS susceptibility.

1.3.3 Multiple Sclerosis GWAS

Ideal MS treatments would target the causal cells and pathways necessary in establishing and maintaining disease, rather than broadly suppressing immune function. Understanding the genetic variants that predispose individuals to MS provides a clear pipeline to discovering the genes, pathways and cell types implicated in MS.

The methodology of GWAS, for MS or other common polygenic disease, has been well studied and established. In general, a large group of case and control individuals are drawn from the same population and genotyped on a particular platform, which contains a set of tag SNPs chosen based on the recombination landscape and on regions of particular interest — for example, the Immunochip platform was developed for immune-related diseases because it is useful in genotyping areas important in immune function (Mayes et al., 2014). Then, genotypes are correlated with case/control status using a logistic regression approach, including covariates that capture ancestry, using principal components analysis (Price et al., 2006, 2008), and other relevant biological or epidemiological variables. In partic-

ular, a Bayesian approach to genetic association studies has been implemented in the software SNPTEST (Stephens and Balding, 2009). For analysis of variants that have not been directly genotyped, software has been developed to impute the genotype at variants not present on the genotyping platform based on linkage disequilibrium within a haplotype (Marchini and Howie, 2010).

As a result of multiple large-scale efforts to find genetic associations with MS, a large number of loci have been identified. Many of these loci are in regions important in immune function (International Multiple Sclerosis Genetics Consortium et al., 2011; Sawcer et al., 2014) and many of those are shared between different immune-mediated diseases (Parkes et al., 2013). These results point convincingly to pursuing immune function, rather than abnormal neural proteins, in the search for precise mechanisms of and treatments for MS. GWAS results have highlighted both the major histocompatibility complex (MHC) and non-MHC immune loci (Ebers et al., 1996; International M.H.C. and Autoimmunity Genetics Network (IMAGEN) et al., 2009). The MHC intuitively makes sense in MS etiology; it presents peptides to lymphocytes, first to select against self-reactive immune responses and then to select for effective immune response to foreign antigens. Thus, it plays an important role in determining immune tolerance versus activation. Arguably more interesting, however, are the dozens of regions outside the MHC that are associated with MS. As of 2013, 110 variants in 103 extra-MHC regions were associated with MS (International Multiple Sclerosis Genetics Consortium et al., 2013). For most of these regions, unlike the MHC (Lang et al., 2002), it is difficult to understand how the variant is linked to disease risk through a known biological pathway.

1.3.4 Gender in GWAS

Because sex can play such a crucial role in shaping the immune response — and other biological processes not discussed here — it must be incorporated into many GWAS analysis pipelines in some way. Most GWAS for complex traits use the cohort as a whole, with a covariate included for gender, rather than stratifying the analysis, because separating males and females reduces power substantially (Magi et al., 2010). There have also been some attempts at understanding differential genetic effects based on sex. Two general approaches are used to deal with sex differences in genetic contribution to disease risk. The most straightforward approach is a modification of the traditional GWAS procedure, in which a logistic model is developed with sex as a covariate because of its potential to confound case-control analyses; if sex contribution is of interest, assessing the significance of the sex indicator variable or of a sex:genotype interaction term in the model allows for insight into the sex-specificity of genetic effects and the disease phenotype. Second, it is possible to examine the differential effect of genetic variants on disease phenotype based on sex by stratifying a cohort by sex, conducting separate GWA analyses on males and females, and comparing the effect size at each variant. One study looking at meta-analyses of several GWAS studies for sex-biased traits showed that stratified analyses of females and males was better than sex-combined analyses (Magi et al., 2010). When conducting a single sex-stratified GWAS, as opposed to a meta-analysis, it is possible to use, for example, the Breslow-Day statistic for homogeneity to compare odds ratios computed sep-

arately for males and females (Aragam et al., 2011). One main limitation of sex-stratified GWAS, however, is the reduced power that necessarily results from dividing a cohort by sex, making it difficult to detect significant associations and to replicate findings (Ober et al., 2008). Another group used an EM algorithm to assess whether certain haplotypes contributed risk differently between males and females (Wang et al., 2008).

More recently, work has been done to assess sex-specific genomic effects, which has been possible with increasingly large transcriptional datasets enabling in-depth eQTL analyses. These approaches are similar in principal and practice to previous work studying differential genetic contribution to disease risk, though they assess a quantitative trait rather than a dichotomous trait of interest in case-control studies; the methodologies to understand sex bias in biology could be applied almost interchangeably between eQTL studies and case-control studies. Sex-interacting eQTLs have been found by fitting a linear model and assessing the significance of a sex:genotype interaction term. These results were followed up by testing the type of heterogeneity in sex-interacting eQTLs in order to understand whether effects differed in magnitude or direction or were present in only one sex (Yao et al., 2014). Alternatively, sex-interacting eQTLs have been explored by dividing the cohort into males and females, performing separate eQTL analyses and comparing the effects in the groups using a mixed model (Jansen et al., 2014).

1.4 Approach

In order to explore how gender plays a role in MS risk in humans, I wanted to leverage large datasets from human cohorts to integrate genetic and functional

data. Although this type of data can be noisier than mouse models or cell lines, the causal cells and pathways are not yet elucidated in MS, so I hoped to use human case/control data to generate hypotheses about mechanism of disease, using sex as a lens through which to assess variation in disease risk and immune function. Because sex has many effects from early development to puberty and beyond, and effects are driven by both sex chromosomes and hormonal regulation, it is a particularly difficult variable to dissect using cell lines. Interrogating how gender and genetics intersect to inform MS risk was a problem particularly suited for integration of human datasets. Genetic data from MS cases and controls (International Multiple Sclerosis Genetics Consortium et al., 2013) was integrated with whole blood gene expression data from another cohort (Battle et al., 2014).

I hypothesized that functional genomic differences between males and females modify genetic susceptibility to multiple sclerosis. To investigate this hypothesis, I pursued two aims. First, I used genetic case-control data to model the interaction between gender and genetic risk of MS. A Bayesian framework was used to understand the differences in GWAS results between males and females at the SNPs that have been identified as highly correlated with MS risk genome-wide in both males and females. Second, I characterized transcriptional and eQTL differences between males and females in the blood genome-wide in order to understand differential and immunologically relevant gene regulation. I also used the transcriptional data to find eQTLs and sex-biased eQTLs among the SNPs associated with MS risk.

Together, these analyses offer methodology to analyze both genetic case-control

data and transcriptomic data through the lens of sexual dimorphism with an integrated approach that focuses on disease relevance. Historically, sample sizes, particularly for functional genomic datasets, have not been large enough to stratify by gender, but as technology improves, cost declines, and sample size becomes less of an analytical limitation, analyses like this, aimed at uncovering biologically and clinically significant differences between strata of a study population, will become increasingly feasible. Sex is a particularly fascinating parameter to analyze because of its clear impact on disease risk. With increasing appreciation of the differences between males and females in immune function and susceptibility to disease, it is important to explore robust methodology to analyze important, if subtle, differences in genetics and functional genomics that contribute to differential risk for disease.

Of note, in the framework of genetic variants having impact on disease risk, modulated by both environmental factors and genetic background, biological sex can be considered as an environmental or a genetic factor. It is clear that sex is determined genetically based the complement of X and Y chromosomes. In this analysis, sex is considered a binary categorical variable for which we have essentially perfect measurement. The genetically determined state of male or female then results in myriad effects, some of which are genetically based, for example expression of X chromosome genes, and others that are associated with sex but not entirely genetically based, such as hormone exposure over time; hormone exposure over a lifetime in females may be determined in part by having an X chromosome and some heritable components but also on age of menarche, influenced significantly by environmental variables unrelated to sex, the use of hormonal con-

traceptives, and/or parity. Although sex is genetically determined, therefore, the collective differences between males and females, including the hormonal environment and transcriptional landscape, can be considered environmental attributes, grouped into "male" and "female" categories. Within each sex, there can be further environmental variability, such as variation in progesterone within females or variation in testosterone level within males. Some of this variance can certainly be captured through genetics; conversely, biological sex correlates with many purely environmental variables (such as contraceptive use), and these variables are often captured by using the term "gender." For the purpose of this analysis, I use "gender" and "biological sex" interchangeably, because, with these datasets, I am dealing with a binary categorical variable that is the composite of a multitude of genetic and environmental factors.

1.5 Thesis overview

This thesis is divided by the two aims. The first chapter explores sex bias in genetic determinants of MS risk. I present a finding that effect size conferred by genetic variants for MS risk is systematically smaller in females than in males, and I also investigate variant-specific interactions with sex. In the second chapter, I investigate both differential gene expression and differential eQTL effects genome-wide between males and females; then I focus on MS risk variants that are also eQTLs to highlight genes that are likely important in MS pathogenesis, having been highlighted both as eQTLs and by differential expression.

2 Gender-biased genetic risk of MS

2.1 Introduction

To understand how sex impacts genetically-determined risk for MS, I first took a top-down approach: understanding how particular variants implicated in MS susceptibility may be cause differential risk between men and women.

Because MS is a complex polygenic disease with higher prevalence in females, established risk variants were investigated for differential contribution to risk in males and females. In a previous GWAS analysis (International Multiple Sclerosis Genetics Consortium et al., 2011), based on the Wellcome Trust Case Control Consortium 2 dataset, the authors investigated gender-specific effects and heterogeneity in effect sizes. They tested for association between each variant and MS in separate groups of males and females, and then used the following statistic to detect difference in effect size between males and females genome-wide: $\frac{(b_M - b_F)^2}{se_M^2 + se_F^2}$, where b is the estimated effect size and se is the standard error. In this dataset and using this approach, they found just eight variants with evidence for a sex-specific association across the genome. We were curious about whether we could more deeply investigate sex-bias effects in a dataset collected for a later GWAS analysis (International Multiple Sclerosis Genetics Consortium et al., 2013). Rather than looking for sex-specific or sex-biased effects genome-wide, I wanted to investigate the possibility of more subtle sex-based effects at confirmed MS susceptibility loci. This approach had two main advantages. First, the later GWAS identified many more variants associated with MS population-wide (International Multiple Sclerosis Genetics Consortium et al., 2013), allowing for a analysis encompassing a

large number of loci without necessitating a genome-wide scan. Already, over one hundred loci have been associated with MS; the present task was not to find additional loci but instead to understand the pathogenic mechanisms that are associated with these variants, and in particular the influence of sex on pathogenicity. Second, by investigating these highly associated variants alone, our analysis was not as penalized for the loss of power necessitated by conducting a sex-stratified analysis. We were therefore able to examine sex effects closely, including subtle sex biases.

The present analyses were based on data collected on the Immunochip platform; the genome-wide results from the MS GWAS have already been published (International Multiple Sclerosis Genetics Consortium et al., 2013). Of note, the analysis included neither MHC variants nor variants on sex chromosomes. The top variants from the Immunochip analysis included neither MHC nor sex chromosome variants, though data from both MHC regions and sex chromosomes is available. MHC variants account for the strongest risk for MS, and they were analyzed in a separate publication (Moutsianas et al., 2015). Analysis of the MHC and of sex chromosomes generally requires different considerations in methodology than non-MHC autosomal analysis, so I limited the analysis to autosomal non-MHC variants highlighted in the previous study (International Multiple Sclerosis Genetics Consortium et al., 2013).

In order to cast a sufficiently wide net of variants to examine, the 150 variants highlighted in the discovery phase of the GWAS were assessed. First, differences in variant effect size for MS risk based on gender were examined. Then, to more

deeply understand the sex effect on genetic risk, a framework of different genetic-gender interaction effects was developed, and each SNP was tested for its fit for each model of sex-SNP relationship. Third, the proportion phenotypic variance explained by genetic variance was characterized.

2.2 Methods

To conduct association studies of the variants found in the original Immunochip paper in males and females separately, I subsetted the genotype files by SNP ID using plink (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell et al., 2007). Of the 150 SNP ID's published in supplementary table 1 of the original paper, 144 were matched in the genotyped SNP file, and I continued with these SNPs.

Association testing was carried out using SNPTEST (Marchini et al., 2007). A Bayesian test was conducted using the method "score." Seven continuous covariates were included, representing the first seven principal components that represent ancestry. PCA had already been performed on a subset of 2963 women (891 cases and 1859 controls) and 1171 men (268 cases and 834 controls) via FLASHPCA (Abraham and Inouye, 2014). These individuals had been chosen by a set of previously-established filtering and quality control criteria. Association analysis was performed for the 144 SNPs in these individuals. It is worth noting that the sample size for this study is substantially smaller than in the original cohort due to filtering by males and females, because many were dropped based on not having a recorded sex. This reduced sample size made it especially useful

to have restricted our analysis to the top associated SNPs, rather than conducting a genome-wide scan.

I next wanted to compare the effect sizes for each variant between males and females. If there were no difference in genetic effect on MS risk between males and females, I expected effect sizes estimated in males and females to be highly correlated and to have a 1:1 relationship. I therefore conducted a simple linear regression to assess the strength of correlation and the numerical relationship between the male and female effect sizes. Secondly, aware that a linear regression assumes one independent and one dependent variable, I employed principal components analysis to understand the relationship between the two independent variables. By finding the slope of the first principal component, I computed an alternative value for a linear relationship between the male and female effect sizes. The slope was calculated by dividing the female component of the first PC's eigenvector by the male component of the first PC's eigenvector.

Next, to understand the relationship between gender and genetic risk in greater depth, a framework was developed to understand the extent of gender-biased effect at each SNP. Each SNP was tested for how well the observed case/control genotype data fit each of five proposed models. In each model, effect sizes for males and females are assumed to be from a multivariate normal distribution centered at zero, and correlation structure between male and female effect sizes differed between models as following, with σ set to 0.2 in all cases:

Model 1: Fixed effects

$$\beta_F = \beta_M \neq 0 \tag{1}$$

$$\begin{pmatrix} \beta_M \\ \beta_F \end{pmatrix} \sim MVN \left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \sigma^2 \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix} \right]$$

Model 2: Independent effects

$$\beta_F \neq 0; \beta_M \neq 0 \tag{2}$$

$$\begin{pmatrix} \beta_M \\ \beta_F \end{pmatrix} \sim MVN \left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \sigma^2 \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \right]$$

Model 3: Male-specific effect

$$\beta_F = 0; \beta_M \neq 0 \tag{3}$$

$$\begin{pmatrix} \beta_M \\ \beta_F \end{pmatrix} \sim MVN \left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \sigma^2 \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix} \right]$$

Model 4: Female-specific effect

$$\beta_F \neq 0; \beta_M = 0 \tag{4}$$

$$\begin{pmatrix} \beta_M \\ \beta_F \end{pmatrix} \sim MVN \left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \sigma^2 \begin{pmatrix} 0 & 0 \\ 0 & 1 \end{pmatrix} \right]$$

Model 5: Correlated effects

$$\beta_F = c\beta_M; \beta \neq 0; \beta_M \neq 0 \tag{5}$$

$$\begin{pmatrix} \beta_M \\ \beta_F \end{pmatrix} \sim MVN \left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \sigma^2 \begin{pmatrix} 1 & c \\ c & c^2 \end{pmatrix} \left(\frac{4}{(1+c)^2} \right) \right]$$

In Model 5, correlated effets, the scaling factor $\frac{4}{(1+c)^2}$ is used to ensure that the variance of the mean effect between male and female estimates is consistent with the variance assumed in the fixed-effects meta-analysis. I used the Immunochip data to calculate c, the coefficient of correlation between male and female effect sizes in Model 5. Based on the earlier analysis (Figure 2), I thought that a reasonable value of c would fall between 0 and 1. To find the best value using a maximum likelihood approach, I iterated over values of c from 0 to 1 in intervals of 0.01. For each value of c and each SNP, the priors for Model 5 were calculated and the log likelihood surface from the Immunochip data was used to find the Bayes factor (BF). Then, the $\log_{10}$ Bayes factors were summed over the 144 SNPs in the Immunochip data and the value of c that produced the maximum $\Sigma \log_{10}$BF was considered the best value for the model. As described in the results section and in Figure 3, the optimal value was $c = 0.42$, and therefore $c = 0.42$ was used

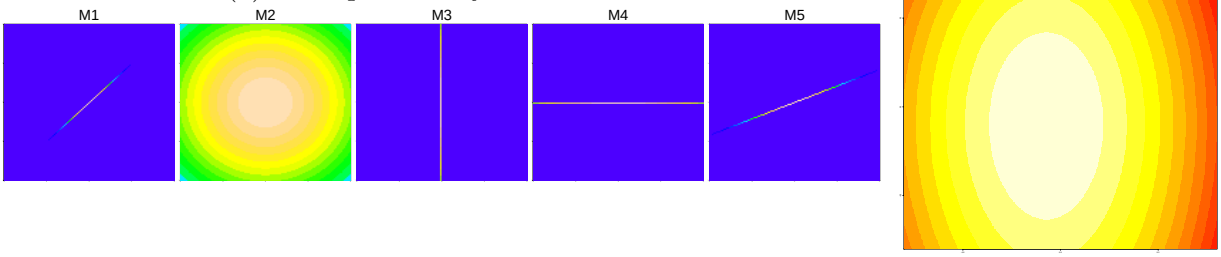
when this model framework was employed in genetic case/control data.

I then tested these five models in the genotype data for the 144 variants. An illustration of the model being used on an example SNP, rs3748817, is shown in Figure 1. First, prior probabilities were calculated for each model. In Figure 1a, the priors are graphed in a 2D plane, with axes corresponding to βs for females and for males. Then, genotype data from cases and controls in each group was used to create a likelihood surface, with axes again representing the β for each group (Figure 1b). The likelihood surface was generated under a general genetic model with logistic risk. The 2D graph represents the likelihood function for β_M and β_F given the genotype data. Next, the prior probabilities for each model were integrated with the likelihood surface to find Bayes factors and posterior probabilities for each model along a range of values of β_M and β_F (Figure 1c). Bayes factors for each model, across all values of β_M and β_F, were also calculated and used to calculate posterior probabilities for each model given the data (Figure 1d).

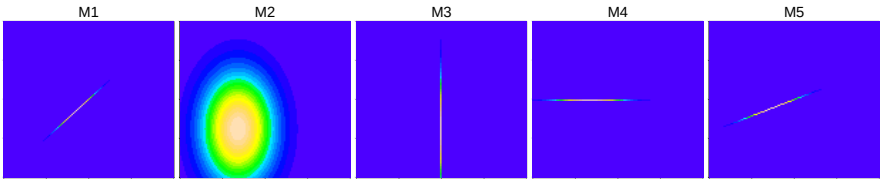
In order to assess how much phenotypic variance in disease risk was accounted for by genetic variance, I used GCTA (Genome-wide Complex Trait Analysis) (Yang et al., 2011). Starting with all genotyped SNPs in the Immunochip dataset, I used the PLINK command dup -var to remove 8 SNPs that GCTA saw as duplicates based on redundant position. 160311 SNPs remained in the analysis. Next, the GCTA command –make-grm was used to make GRM files for females and for males. With GCTA's –reml command, restricted maximum likelihood analysis was performed. Disease prevalence in the population was set to 0.00262 for females and 0.001 or males, based on prevalence estimates from East Scotland (Pugliatti et al., 2006).

(b) Log likelihood

(a) Prior probability distributions



(c) Posterior probability distributions



(d) Model posteriors

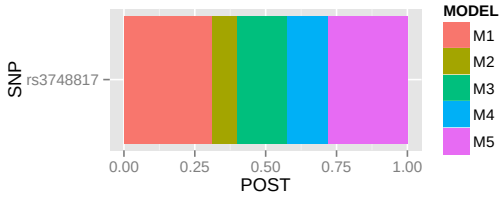


Figure 1: Illustration of the model employed for each of the 144 immunochip SNPS. These surfaces represent distributions of prior probability, log likelihood, and posterior probability for an example SNP: rs3748817. Five hypotheses of gender-gene interactions were tested. M1: Fixed effects, M2: Independent effect, M3: Male-specific effect, M4: Female-specific effect, M5: Correlated effects (with c=0.42). Shown are (A) the prior probability distribution for values of β_F and β_M under each gender-gene hypothesis and (B) log likelihood surface based on SNP genotype and case-control data in males and females. (C) Priors and log likelihood were integrated to find the posterior probability distribution under each hypothesis. (D) Posterior probabilities of the example SNP, rs3748817, following each of five models of gender-genetic interaction

	FEMALE		MALE	
	Variance	SE	Variance	SE
genetic variance V(G)	0.0877	0.109	0.108	0.0193
residual variance V(e)	0.139	0.00899	0.0868	0.0153
phenotypic variance Vp	0.226	0.00637	0.195	0.00894
ratio of genetic to phenotypic variance V(G)/Vp	0.388	0.0431	0.554	0.0852

Table 3: GCTA-derived statistics for genetic [V(G)] and environmental variance [V(e)] contributing to phenotypic variance [Vp], calculated separately in males and females. Variants across the genome (160311 SNPs) were included in the analysis, and GCTA parameters of disease prevalence in males and females were taken from prevalence estimates in East Scotland, as published by Pugliatti et al. (2006): 0.00262 for females and 0.001 for males.

2.3 Results

To get a sense of the gender differential in genetic risk for MS in the Immunochip data and cohort, I conducted separate association studies in the men and women of the cohort. Because restricting the association analysis to only one gender reduces sample size, and because previous work had not found compelling single-gender associations genome-wide, I started with 144 SNPs found in the discovery phase of the Immunochip analysis. Output from the stratified association analysis is visualized in Figure 2, with one point per variant, plotted by male and female variant effect size (β) for MS, along with error bars representing the standard errors on β of the B allele, as computed by SNPTEST (Marchini et al., 2007).

The male and female β's were correlated with an R-squared of 0.263 and p-value of 5.22e-11. The βs did not have a 1:1 relationship; in the linear model the coefficient for male β was 0.471. Because a linear model is best suited for data with an independent and dependent variable, whereas each variable is independent in this relationship, I also used principal components analysis (PCA) to quantify the

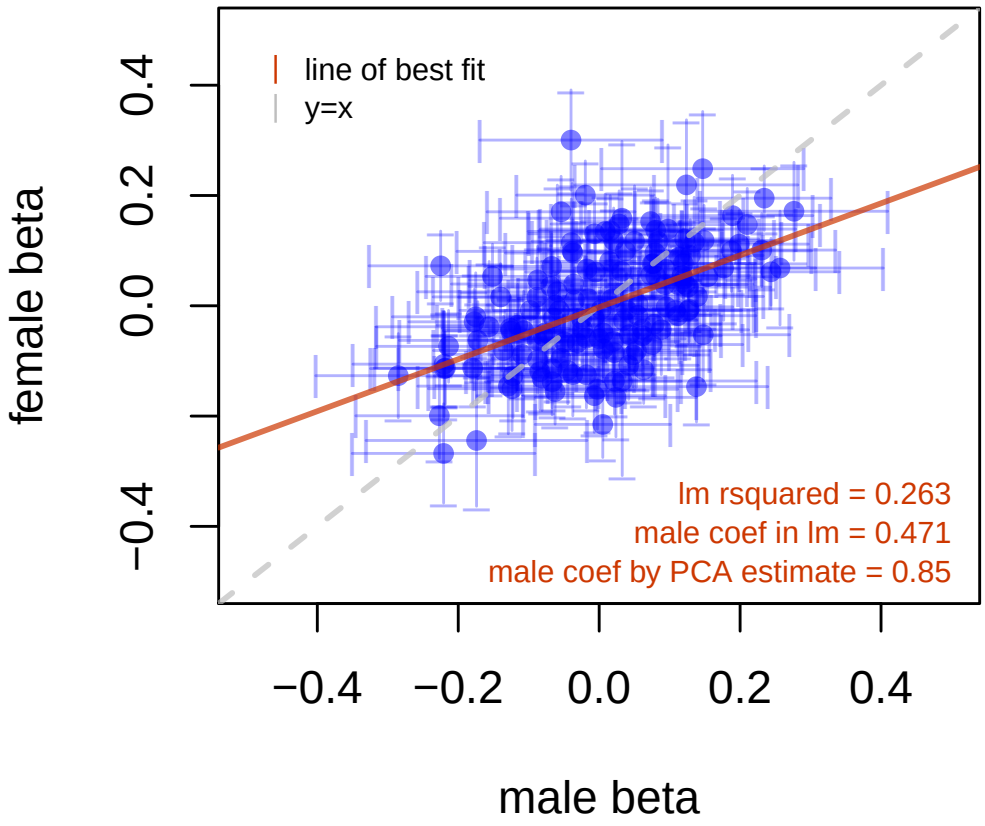


Figure 2: Effect sizes for risk of MS per variant, plotted by value of β in males and females, with error bars representing the standard error around the β estimate from SNPTEST. The result of a linear regression of $\beta_{female} \sim \beta_{male}$ is graphed as the line of best fit, along with the line $y = x$ for reference, which would imply a one-to-one relationship between male and female βs. The numeric results of the linear model (lm) are also presented by the r-squared for the line of best fit and the coefficient for male effect size in the model; slope for a line of best fit was also estimated using principal components analysis (PCA), dividing the rotation for female effect size by the rotation for male effect size in the first eigenvector.

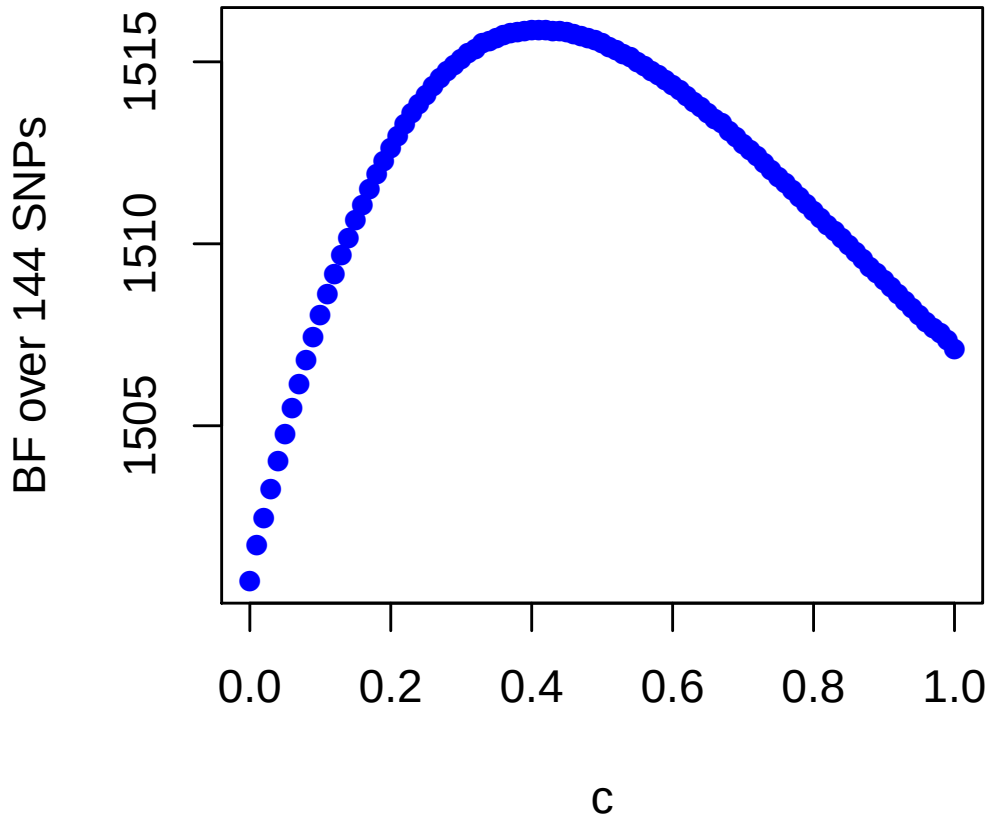
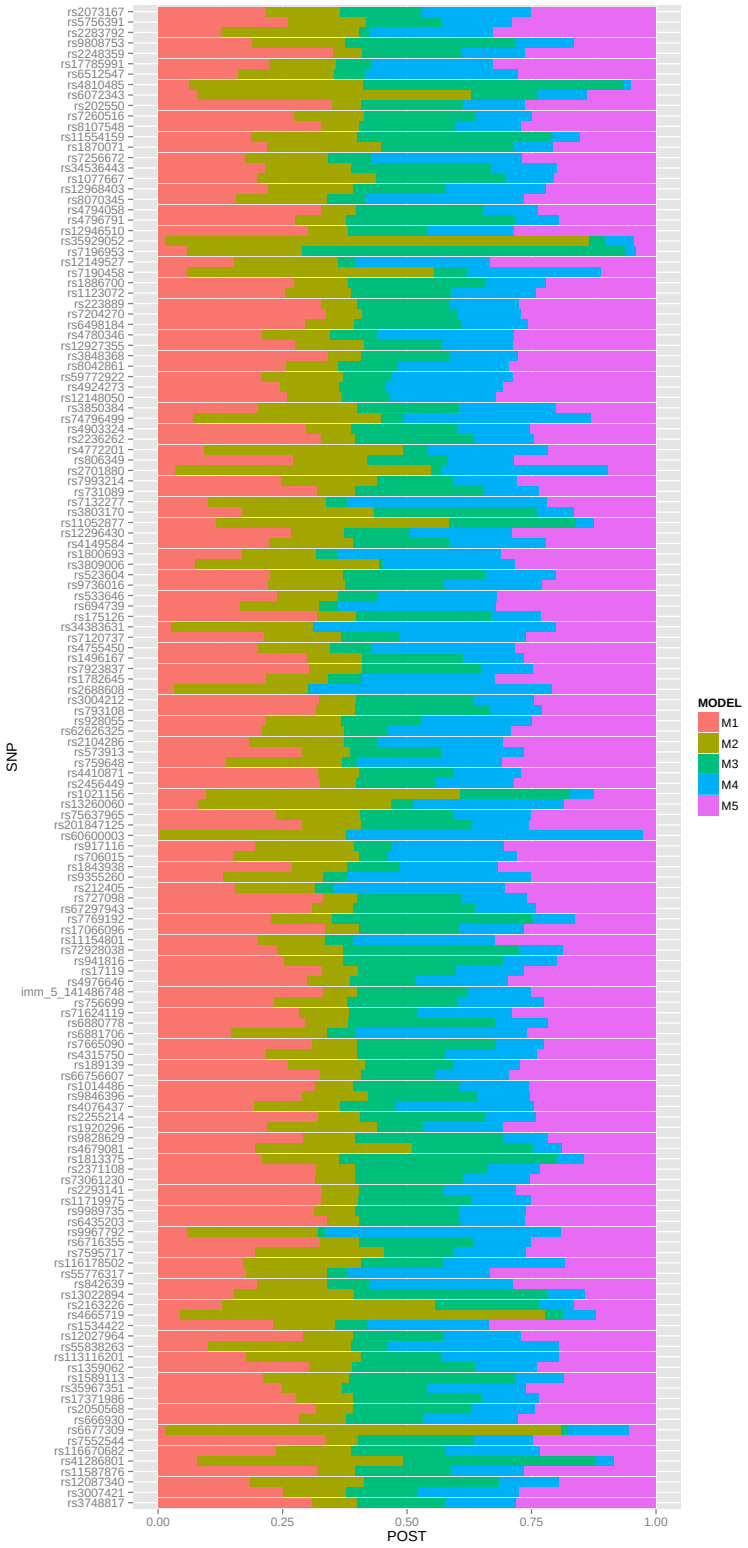


Figure 3: Sum of $\log_{10}$ Bayes factors for 144 immunochip SNPs under Model 5, correlated effects, with values of the correlation coefficient c ranging from 0 to 1 in increments of 0.01. The value of c with greatest $\Sigma \log_{10}$BF was 0.42.

Figure 4: Per-SNP results for each of the 144 SNPs in the Immunochip data previously highlighted for their association with MS. Here, each color represents a different model for gender-genetic interaction, and the x-axis shows posterior probability of the variant following one of the five models. M1: fixed effects, M2: independent effects, M3: male-specific effect, M4: female-specific effect, M5: correlated effects, with $c = 0.42$



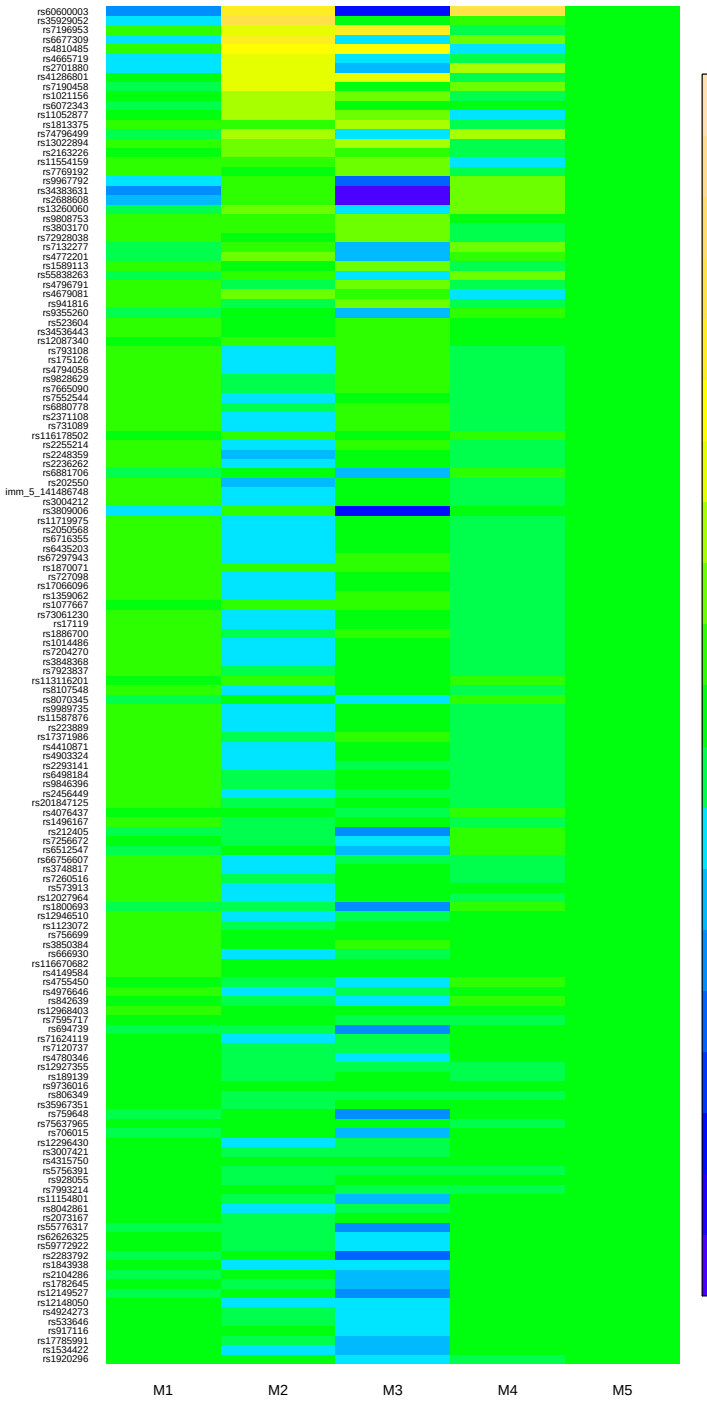


Figure 5: On a per-SNP basis, difference in model evidence to evidence for M5. Color represents the difference in $\log_{10}$BF to $\log_{10}$BF for M5 for each SNP and each model. Thus, for each SNP, a positive value (yellow box) represents a model having greater evidence than model 5, while negative values (blue) represent models with less evidence than model 5. The SNPs are ordered by greatest evidence for a model other than M5. Because difference is calculated using the $\log_{10}$BFs, the difference statistic plotted here represents an orders of magnitude of difference in Bayes factors, i.e. a statistic of -2 or +2 represents 100-fold lesser/greater evidence for a model versus model 5. The last column, M5, is shown simply for reference, as all values are 0 because these values were computed by subtracting by the $\log_{10}$BF of M5 for each SNP.

relationship. PC1 explained 75.9% of the variance in the data, and its slope was 0.850. It is likely that the slope calculated by PCA was so much greater than the linear model slope because of the influence of more extreme values in Figure 2 on the PCA slope estimate.

Next, I wanted to quantify the deviation from the 1:1 relationship of variant effect size between males and females. I also thought that looking for a systematic shift in odds ratio by sex could have been an over-simplification of the variant-gender interaction, so a per-variant analysis was conducted. A framework of five models was developed, each corresponding to a distribution of effect sizes for males and females. The steps of the model are visualized for one SNP in Figure 1. In order to calculate a realistic correlation coefficient, values of c between 0 and 1 were tested. For each value of c, a Bayes factor was calculated for each SNP under Model 5. The sum of the SNP $\log_{10}$ Bayes factors using each value of c is presented in Figure 3. The value of c that maximized the sum of the $\log_{10}$ Bayes factors, $c = 0.42$, was considered the best fit for the model of correlated effect sizes between males and females. The five models — including M5, correlated effects with $c = 0.42$ — were tested in all 144 SNPs associated with MS in the Immunochip data and the resulting posterior probabilities for each model at each variant are presented in Figure 4.

To test which of the five models would best fit all 144 SNPs, the sum of the $\log_{10}$BFs for each model over all SNPs was taken. In this case, Model 5, or the correlative model with a coefficient of 0.42, fits this data best, with approximately 10^8 times greater evidence than the next-best model, Model 1, based on the Bayes

factors over all top SNPs. The $\Sigma \log_{10}$ BF was 1515.885 for M5, versus 1507.106 for M1, 1493.759 for M4, 1490.354 for M2 and 1482.256 for M3.

Although, based on the variants considered all together, M1 (fixed effects) appears to be the second-best model to fit the data after M5 (correlated effects with $c = 0.42$), when I looked at the SNPs for which M5 was not the best fit (Figure 5), there is a cluster of SNPs that fit M2 (independent effects) better than they do M5. Notably, when assessed from this perspective, it is clear that are no SNPs that have strong evidence for M1, M3 (male-specific effect) or M4 (female-specific effect) that do not also have strong evidence for M2 or M5. It is likely that the high $\Sigma \log_{10}$ BF for M1 can be explained by its largely similar evidence to M5, as apparent in Figure 5; there are no SNPs with substantially ($\geq$ 1 order of magnitude) greater evidence for M1 than M5. It is understandable that the evidence would be similar between M1 and M5 because M1 (fixed effects, $c = 1$) and M5 (correlated effects with $c = 0.42$) are both correlative models. Thus, while M5 fits the data best when all SNPs are considered to follow a single model, a subset of SNPs appear to follow the model of independent effects (M2).

To understand the relationship between genetics and disease risk in females and males, GCTA was used to analyze genome-wide SNPs in the Immunochip dataset. Genetic variance accounts for a greater proportion of phenotypic variance in males (0.554, SE 0.0852) than in females (0.388, SE 0.0431) (Table 3).

2.4 Discussion

2.4.1 Discussion of genotype-sex analysis at MS risk variants

Together, these gender-based analyses of genetic risk for MS suggest that gender plays an important role in modulating genetic risk factors. First, the effect size of genetic risk variants are generally smaller in females than in males. We used several methods to estimate how the female and male effect sizes were related and variously computed the relationship as female βs being between 0.42 (maximum likelihood) and 0.850 (principal components analysis) of the male effect size. Regardless of the method used, female effect size was convincingly less than male effect size across the SNPs. Perhaps the best estimate of the relationship was 0.42, which was found by learning a model in which female effect size was systematically correlated with male effect size; Bayes factors for several values of the correlation coefficient were computed, and 0.42 was the correlation coefficient that had the highest Bayes factors across the SNPs analyzed, suggesting that it was the best fit model given the effect sizes were linearly correlated.

While this finding of a systematically lower effect size in females compared to males was intriguing, approaching this set of genetic variants all together may have been an overly broad approach. It is possible that some variants are sensitive to gender and others are not. For example, one could imagine a variant whose biological effect on MS risk was dependent on progesterone level, resulting in a gender-biased effect, but it would not necessarily follow that every variant associated with MS had an important interaction with gender. Therefore, we developed a framework of five hypotheses, each modeling the relationship between male and

female effect size for a single variant. The results, displayed in Figure 4, show that while there may not be a single model that fits any given variant perfectly, some models fit certain variants better than others, without complete uniformity across the SNPs tested.

However, if one model were to be used to summarize the sex-genotype relationship for these MS risk variants, Model 5, in which the effect sizes are correlated with a correlation coefficient of 0.42, would be the best model, with 10^8 greater evidence than any other model for all the variants together. Notably, Model 1, in which the βs for males and females are the same, was not strongly supported compared to Model 5 for any single variant. There do appear to be a subset of SNPs that follow Model 2 better than Model 5, suggesting that some variants have unrelated effect sizes in males and females 5. It is important to note that Model 5 can be understood as a generalized version of three other models: 1,3 and 4; these models could be derived by using extreme values of c. In the most simplistic model, one could imagine a set of models comprised of a very flexible Model 5 plus Model 2 (independent effects). However, the current breakdown is acceptable in a Bayesian analysis that aims to identify differences between these five models. Additionally, the impact of choosing σ at 0.2 should have been more thoroughly explored in order to establish the sensitivity of the results to changes in this parameter, which is fixed across all models in this analysis.

This analysis has several caveats that preclude a definitive conclusion. Most importantly, this exploratory analysis relied on a single estimate from a single cohort. To have real confidence in the conclusions, it would first be necessary

to carry out permutations of possible data distributions in order to empirically assess how unusual it is to have such a relationship between male and female effect sizes. Second, using a replication cohort would be crucial to show that this finding is not an artifact of the discovery cohort. The issue of the "winner's curse" plagues this analysis because I performed the gender-based analysis in the same dataset in which MS associations were originally discovered, and I looked only at those SNPs that were previously highlighted. The winner's curse, with respect to GWAS, suggests that the discovery cohort, in finding the few SNPs meeting the significance threshold necessary to be reported as associated with disease, will also overestimate the effect sizes; therefore, investigating *effect size* in this same cohort as a readout of male-female difference may have overstated the differential effect.

Despite these caveats these results together hint that there may be an overall effect of female sex on lower variant effect sizes for MS risk. Based on our per-SNP analysis, however, there may be certain SNPs with particular evidence of a gender-specific effect — for example example, rs60600003 (female-specific), rs7196953 (male-specific), and rs4810485 (male-specific), which all have more than an order of magnitude greater evidence for a sex-specific effect than for a correlated effect. These variants are worth further investigation for their specific interaction with sex.

The systematically lower effect size of genetic risk variants in females was perplexing given the higher disease prevalence in females, so I wanted to assess how well genetics accounts for disease state in males and females. To do this, I an-

alyzed SNPs across the genome using GCTA and found that genetics accounts for a higher proportion of phenotypic variance in males than it does in females. This suggests that the environment plays a larger role in MS risk females. This analysis would have been improved by the incorporation of power calculations. This is particularly evident in the GCTA analysis, in which the small sample size, particularly in males, likely affected the power of the analysis substantially; the differences in genetic, residual and the ratio of genetic to phenotypic variance are very small between males and females in the current analysis, so confidence in true difference would have been augmented by a power calculation or another enhanced measurement of statistical significance.

2.4.2 A liability threshold model of genotype-sex interaction

I had been assessing genotype-phenotype data using a logistic model of disease but wondered whether a liability threshold model could be used to understand the relationship between sex and disease risk. In its simplest form, liability variance can be decoupled into genetic variance and environmental variance. Because I found a difference in the contribution of genetic variance and environmental variance to phenotypic variance between males and females using GCTA, I created two models of gene-environment interaction that could differ between genders and cause a greater disease burden in females than males: either females have a lower threshold for disease or females have greater liability variance but the same threshold for disease. I assumed that disease liability was normally distributed and calculated the parameters of the distributions to fit observed disease prevalence:

Liability Model 1: Different liability threshold for disease in males and females

$$L_M \sim N(0,1); L_F \sim N(0,1) \tag{6}$$

$$T_M = 3.09; T_F = 2.79$$

Liability Model 2: Different liability variance between males and females

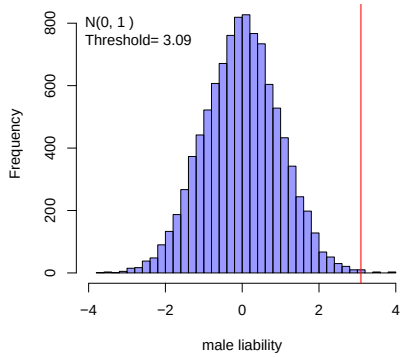
$$L_M \sim N(0,1); L_F \sim N(0,1.107) \tag{7}$$

$$T_M = T_F = 3.09$$

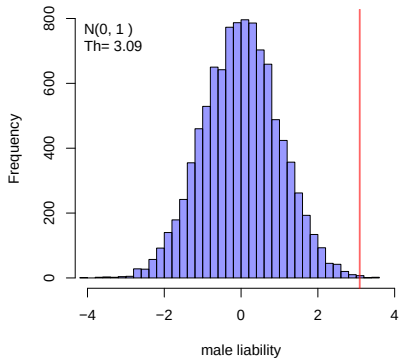
L, liability score; T, liability threshold for disease.

For liability model 1, in which both genders have the same variance in disease liability, I set the distribution in liability to $N(0,1)$ and calculated the liability threshold for disease in males and females based on the prevalence estimates in East Scotland: 0.001 in males and 0.00262 in females (Pugliatti et al., 2006). Because the distribution of liability was assumed to be $N(0,1)$, under the liability threshold model, prevalence is equivalent to the area under the distribution curve from the threshold to infinity. To calculate thresholds, I used the R package qnorm, which returns the boundary value given area (1-prevalence) and distribution parameters (mean=0 and standard deviation=1). Liability threshold was

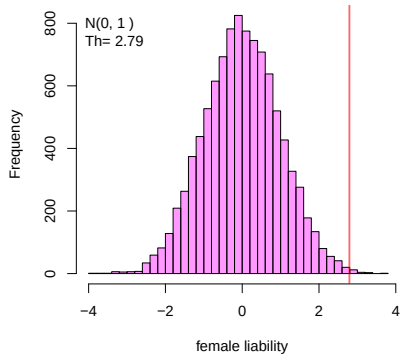
(a) Male liability under liability model 1 (constant variance)



(b) Male liability under liability model 2 (constant threshold)



(c) Female liability under liability model 1 (constant variance)



(d) Female liability under liability model 2 (constant threshold)

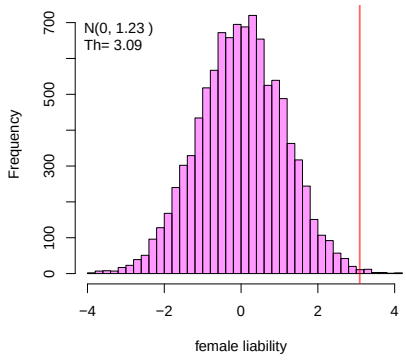


Figure 6: Simulations to demonstrate the two liability models that can explain the difference in MS prevalence between males and females using a liability threshold model. Each plot shows the distribution of liability scores in a population of 10000 males or females, with distribution shown in the upper left corner of the plot. (A) and (C) depict liability model 1, in which females (C) have a lower liability threshold than males (A) for disease, and males and females have the same liability variance. Liability threshold is calculated based on epidemiological prevalence estimates in males and females. (B) and (D) illustrate liability model 2: in which a greater liability variance in females (D) than males (B) explains the higher prevalence, but there is a constant liability threshold between males and females. Again, a reasonable variance is calculated based on sex-based prevalence estimates.

found to be 3.09 for males and 2.79 for females.

For liability model 2, in which the liability threshold for disease is the same between genders but variance differs, I again assumed both distributions were normally distributed around 0. I set the liability threshold to be the threshold calculated for males in model 1, 3.09. I set the liability variance in males to 1, so again male liability was distributed as $N(0, 1)$. It was necessary to find the appropriate liability variance in females, given a liability threshold of 3.09 and prevalence of 0.00262. The integral of the normal function from the liability threshold to infinity was set to 0.00262, prevalence in females, so it was possible to solve for variance using the Gauss error function (also known as erf). The erf function is coded as: $2 \cdot pnorm(x\sqrt{2}) - 1$ (as described in the *pnorm* documentation in R). Then, the roots of the following function, in the interval [0,10], were taken to find standard deviation (square root of variance):

$$0 = 0.5 - 0.5 \cdot erf(\tfrac{threshold}{SD \cdot \sqrt{2}}) - prevalence$$

By solving for standard deviation, I concluded that female liability should be distributed as $N(0, 1.23)$ in this liability model.

To simulate the odds ratio (OR) of a variant in males and females, effect size was set to 0.15 and minor allele frequency was set to 0.2. The male and female ORs were calculated by assuming a normal distribution of liability, shifted by the effect size for the minor allele. Then the female to male ratio of the $\log_{10}$OR was calculated (Figure 6).

In this abstract model of how disease risk is distributed in the population, if an individual's "liability" (comprised of genetic and environmental factors) passes some threshold value, an individual develops disease. Under model 1, with fixed variance but lower liability threshold in females, the female to male ratio of $\log_{10}$ORs was 0.918. Under model 2, with fixed threshold but higher liability variance in females, the female to male ratio of $\log_{10}$ORs was 0.831. Compared to the ratio observed in the data (calculated as the coefficient for male effect size: 0.42, 0.471, or 0.850, depending on the method), the model with greater liability variance in females, rather than lower liability threshold in females, came closer to the observed ratio.

A greater liability variance in females than males is consistent with the GCTA results, because even when analyzing females alone, genetics accounted for a relatively low proportion of phenotypic variance. These results — starting with an observation of correlated effects with weaker effect size in females than males, then the GCTA results of a lower genetic explanation for disease in females, and finally a modeling approach using a liability threshold model — all point to a greater environmental contribution in females than males in determining MS susceptibility.

2.4.3 Conclusions from genotype-sex analyses

This multi-pronged analysis of variant-sex interaction in MS risk suggests, first, a systematic decrease in effect size for risk variants in females compared to males,

with females effect size being about 0.42 as strong as the male effect size. There are a few SNPs, notably rs60600003, rs7196953, and rs4810485, which merit greater investigation for their strong evidence for a gender-specific effect. Second, the environment may play a greater role in determining disease risk, relative to genetics, in females compared to males. This can be understood in a liability threshold model, in which the liability of disease, comprehensive of genetic, behavioral, and other environmental factors, could be distributed with greater variance in females compared to males, resulting in more females being above the threshold for disease and presenting as MS cases. This model, combined with the genetic analysis presented here, suggests that the sex difference in MS prevalence is not due to a step change in disease risk that comes with being female. Instead, variance in sex-associated factors, such as hormone levels or X chromosome-based gene expression, might cause increased variance in MS liability in females. The initially perplexing observation that genetic risk variants contribute a lower effect size in females, despite females experiencing an increased risk of disease, could be explained by risk factors other than genetic risk variants having higher variance in females than in males. Environmental risk playing a greater role in MS in females than males is consistent with the epidemiological observation that MS risk has been increasing, more profoundly in females than males, over the last century (Koch-Henriksen and Sorensen, 2010; Orton et al., 2006). The population-wide increase in risk is better explained by environment than genetics, as one hundred years is too short a time frame for genetic risk to have changed; in parallel, if females are more strongly affected by environmental risk factors, it is consistent that women have experienced an even greater increase in MS prevalence. One way to follow up on this model of high-variance risk factors contributing to additional

disease risk in females would be to further examine sex-associated environmental variables in a female-only cohort. This experiment is difficult because so many variables are associated with sex, but, for simplicity, estrogen, progesterone or testosterone exposure could be interesting variables to assess. It is possible that some of these variables, in isolation or together in combination, play a major role in determining MS risk, diluting a genetic effect.

These analyses are all preliminary and exploratory, as discussed above. The analysis is limited by the winner's curse of exploring effect size differences in the same dataset in which the SNPs were discovered. Additional permutation analyses to empirically establish statistical significance, as well as confirming these results in a second cohort would be crucial steps to gain confidence in the hypotheses generated here. Beyond those concrete confirmatory next steps, another future experiment would be to use existing transcriptional data in order to understand functional genomic dimorphism between males and females. I thought that in order to support the idea that abstract environmental variables altered risk in males and females, it would be important to analyze gene expression in males and females, because these environmental variables could also have a differential impact on the regulatory landscape. Investigating transcriptional differences between the sexes could offer clues to the pathways implicated in differential disease risk.

3 Gender-based transcriptional variation in the blood

3.1 Introduction

Based on the results of sex-genotype interaction in MS risk, I wondered whether biological sex might correlate with transcriptional variation that could explain differential disease risk. MS is far from the only gender-biased disease, and significant work has been done in understanding basic biological differences between genders. In animal models, it has been well documented that genotype and sex interact in determining eQTL effects (Ober et al., 2008). More recently, a human study in cell lines found that >10% of eQTLs are sex-specific, and that these genes are not differentially expressed (Dimas et al., 2012), suggesting that differential gene expression and differential eQTL effect are meaningfully different. It has also been shown that significant and broad transcriptional and regulatory differences exist between the sexes when whole peripheral blood is examined, from the perspective of gene expression, gender-specific or gender-biased eQTL effects, or DNA methylation (Jansen et al., 2014; Zhang et al., 2011; Yao et al., 2014). eQTL analysis of MS risk variants has also been conducted, and dozens SNPs and genes have been highlighted for an eQTL relationship (Handel et al., 2010; Lin et al., 2015). To go beyond either the differential expression analysis or the eQTL analysis, I wanted to coordinate these two analyses: the intersection of genetic and gender-based MS risk with genetic and gender-based variation in gene expression could triangulate important pathways in MS pathogenesis.

I studied both differential gene expression and sex-specific eQTL effects with a specific focus on variants and pathways that might be relevant in MS. Gene expression analyses were conducted on raw gene counts taken from RNAseq of whole blood collected from 922 individuals enrolled in a depression cohort (Battle et al., 2014). I had three aims: first, to characterize differential gene expression, with the goal of confirming that broad differential gene expression is observed in this dataset (and see whether differentially expressed genes could be characterized by any known pathways that might be relevant in MS); second, to see whether established *cis*-eQTLs for this cohort were sex specific; and third, to find MS risk variants that showed evidence of being eQTLs based on this dataset and, more specifically, to find those MS risk variant eQTLs for genes that were also implicated by differential regulation based on gender.

3.2 Methods

3.2.1 Preliminary data processing

RNAseq data was preprocessed by Battle et al. (2014) and raw gene count data was obtained. Data was representative of whole blood RNA sequencing in 922 individuals, comprised of 275 males and 647 females. Included with the genotype and gene count data was a set of biological and technical covariates for each of the 922 participants, split into technical and biological covariates. Correlation of technical covariates with sex is shown in Table 4.

For the raw counts of each of the 15231 genes in the dataset, I regressed out technical covariates: sequencing depth, percent hemoglobin, individual-specific GC, individual-specific exon length, RNA yield, globin flag (technician), percent duplicated reads, number of coding bases, number of intergenic bases, number of intronic bases, median 3prime bias, median 5prime bias, median 5prime to 3prime bias, median CV coverage, percent coding bases, percent intergenic bases, percent intronic bases, percent mRNA bases, percent usable bases, percent UTR bases, number of PF aligned bases, number of PF bases, number of UTR bases, time of day, and the first 5 principal components of genotype (Battle et al., 2014). Biological covariates were included in subsequent steps of the analysis.

3.2.2 Differential transcription characterization

Next, I set out to see which genes were differentially expressed between males and females. I considered the following biological covariates: current alcohol use, age at interview, current BMI, cigarettes per day, whether the participant smoked before, whether the participant exercised before, whether the participant used cannabis in the prior week, whether the participant ate before, or whether the participant used any of the following drugs: ACE inhibitor, beta blocker, diuretic, blood pressure medication, oral birth control pills, anticholinergic medication, decongestant medication, oral hypoglycemic medication, cholesterol lowering medication, NSAIDs, thyroid medication, proton pump inhibitors, opiates, anti-histamines, and stimulant decongestant. For each gene, I built a linear model using forward stepwise regression. For reference, the correlation of these biological

Technical covariate	**p-value, Correlation with sex**
sequencing depth	0.006371
% hemoglobin	0.9301
individual-specific GC	0.1905
individual-specific exon length	0.1083
RNA yield	0.007459
globin flag (technician)	0.07494
% duplicated reads	0.004559
number of coding bases	0.003489
number of intergenic bases	0.002726
number of intronic bases	0.0005169
median 3prime bias	0.5167
median 5prim bias	0.1541
median 5prime to 3prime bias	0.2987
median CV coverage	0.01843
% coding bases	3.94E-07
% intergenic bases	3.37E-05
% intronic bases	3.63E-07
% mRNA bases	4.08E-06
% usable bases	4.08E-06
% UTR bases	0.000364
number of PF aligned bases	0.2988
number of PF bases	0.2993
number of UTR bases	0.02455
time of day	0.6582
genotype PC1	0.1184
genotype PC2	0.8946
genotype PC3	0.2078
genotype PC4	0.3654
genotype PC5	0.439

Table 4: For reference, correlation between technical covariates and sex, as computed using a univariate linear model. Significance for time of day was computed using a chi-square test because it is coded as a factor for am/pm.

covariates with sex is displayed in Table 5. The basic model was:

$$\text{gene expression residual} \sim \text{intercept} + \text{sex}$$

The full model additionally included all biological covariates, which were added in using a stepwise formula. The effect size for sex in the final model, along with its standard error and the p-value testing whether the effect size was significantly nonzero, were all recorded. P-values were corrected for multiple comparisons by using the Benjamini and Hochberg method, also known as FDR.

3.2.3 Pathway analysis of differentially expressed genes

After seeing that there were 5377 genes that were differentially expressed at an FDR<0.05, I wanted to examine the underlying structure of the gene expression data to elucidate pathways with great gender dimorphism. I used the gene expression residuals (after regressing out technical covariates) for these 5377 genes in 922 individuals and conducted principal components analysis (PCA) using the prcomp package in R. I used the resulting scores matrix, with a value corresponding to each individual, to investigate which of the top 10 principal components, which together explained 85.3% of inter-individual variation, were most highly correlated with sex. To do this, I made another multivariate linear model. The base model was, again:

$$\text{gene expression residual} \sim \text{intercept} + \text{sex}$$

and the full model included, again, all biological covariates, as above. I used the p-value associated with sex in the model to understand how closely each principal component correlated with sex.

Biological covariate	**p-value Correlation with sex**
current alcohol use	0.00163
age at interview	0.2087
current BMI	0.6756
cigarettes per day	0.9062
participant smoked before	0.9311
participant exercised before	0.5756
participant used cannabis in the prior week	0.006228
participant ate before	0.004081
Medication use:	
ACE inhibitor	0.05706
beta blocker	0.5141
diuretic	0.2982
blood pressure medication	0.06187
oral birth control pills	3.81E-09
anticholinergic medication	1
decongestant medication	0.9276
oral hypoglycemic medication	0.358
cholesterol lowering medication	0.003159
NSAIDs	0.1693
thyroid medication	4.08E-05
proton pump inhibitors	0.2546
opiates	0.3773
anti-histamines	0.811
stimulant decongestants	0.9983

Table 5: Correlation between biological covariates and sex. Univariate linear regression was used for continuous variables (current alcohol use, age at interview, current BMI, cigarettes per day, blood pressure medicines — presented as number of medications); all other variables were assessed using a chi-square test.

Next, I looked at which genes had the highest magnitude loadings in each of the top 10 principal components. I extracted the genes with the 1% highest absolute value loadings (i.e. 54 genes with highly positive loadings and 54 genes with highly negative loadings). These genes were inputted into the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang da et al., 2009a,b) and results for DAVID's cluster 1 output were manually summarized.

3.2.4 Sex interaction analysis of established *cis*-eQTLs

Cis-eQTLs established from the original Battle study were evaluated across the Battle cohort in males and females separately. The process for determining cis-eQTLs was slightly different than that used by Battle et al. and was somewhat more conservative. I took the list of 10914 gene-SNP pairs published as *cis*-eQTLs in the original analysis and employed a new analysis. In this analysis, I regressed out technical covariates, as described above, but in two separate analyses, after splitting the cohort by males and females. Because of missing data, the number of SNP-gene pairs was reduced to 10865.

Next, the per-gene residuals from the first regression were included in a second multivariate regression. For each SNP-gene pair from the published *cis*-eQTL list, a forward stepwise regression was conducted. The minimal model was gene residual $\sim$ SNP and the biggest model included the SNP and, as before, the following biological covariates: current alcohol use, age at interview, current BMI, cigarettes

per day, smoke before, exercise before, ate before, cannabis use in the past 2 weeks, ACE inhibitor, beta blocker, diuretic, blood pressure medications, birth control medication, anticholinergic medicines, decongestant medicines, oral hypoglycemic medicines, cholesterol lowering drugs, thyroid medicines, PPI, opiates, anti histamines, and stimulant decongestant. Notably, gender was not included in this model. The coefficient for the SNP, its standard error, and the associated p-value were recorded.

3.2.5 eQTL and sex interaction analysis of MS variants

At this point, I had characterized the genes whose transcripts were quantified in Battle et al. (2014) for differential transcription, and I had also assessed sex-specific effects in established *cis*-eQTLs across the genome. I wanted to combine these approaches in looking specifically at MS risk variants: I wanted to assess which variants were eQTLs, which genes implicated by these eQTLs were differentially expressed between males and females, and whether there were any interaction effects between sex and genetic variant on gene expression.

To examine the MS risk variants in the data from Battle et al. (2014), these SNPs needed to be imputed in the Battle genotype dataset. Haplotypes were inferred using SHAPEIT (Delaneau et al., 2013); untyped SNPs were imputed using IMPUTE version 2 and all samples from phase 3 of the 1000 Genomes Project as a reference (Howie et al., 2009). 147 SNPs were successfully imputed in the Battle dataset. A histogram of imputation scores is presented in Figure 7. I considered

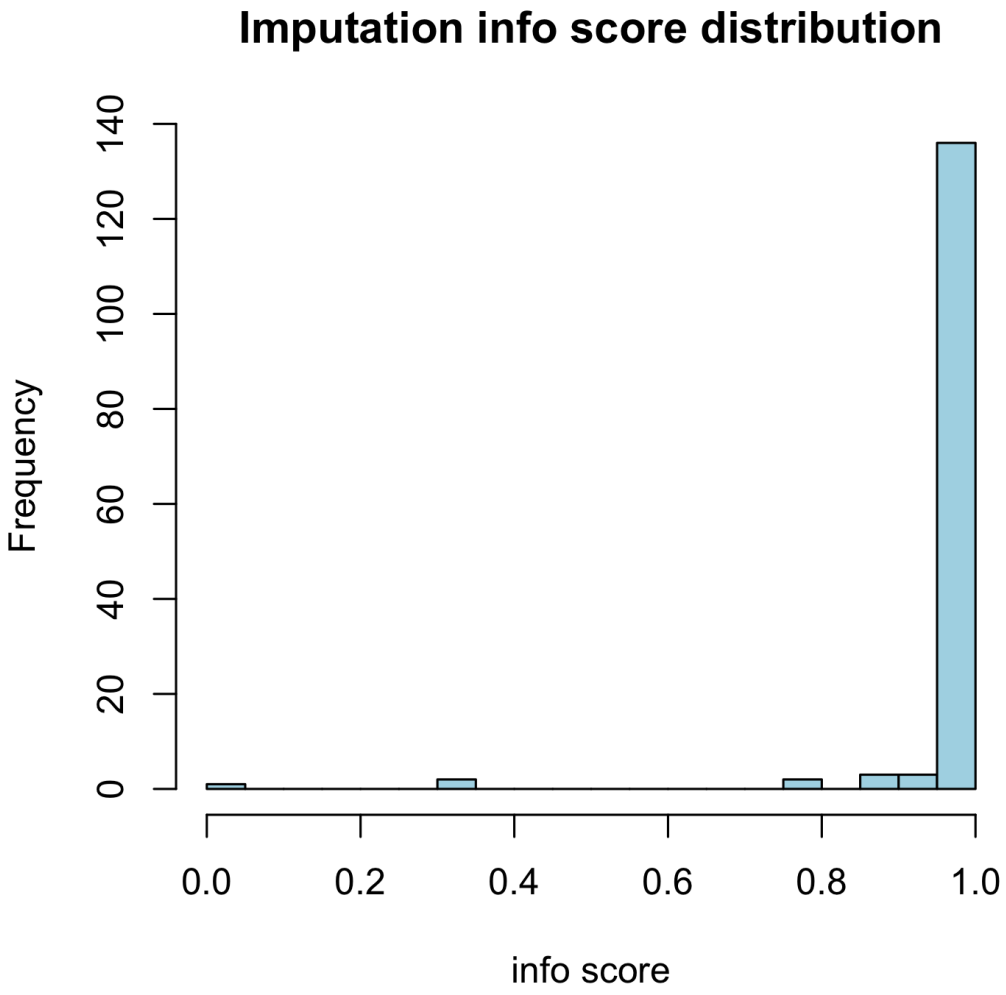


Figure 7: Imputation accuracy for 147 MS risk variants imputed in the Battle dataset. Imputation accuracy is assessed by the info score, which is reported for the imputation at every SNP. This histogram shows the distribution of info scores for the imputed MS risk variants. Variants with an info score less than 0.75 were considered to have too low imputation certainty and were not included in later analyses.

variants with info scores less than 0.75 to have too low imputation certainty to be reliable and were therefore excluded from subsequent analyses. Of the 150 variants listed as the top hits in the discovery phase of the MS GWAS (International Multiple Sclerosis Genetics Consortium et al., 2013), six were removed at this point: rs116670682, rs116178502, and rs371522651 (also known as imm_5_141486748) due to low imputation info score; and rs201847125, rs201202118, and rs523604 due to no match in imputation. The analysis continued with 144 variants.

Simultaneously, I had curated a list of genes surrounding each SNP that could be considered for *cis*-eQTL effects. To do this, I used a file included alongside the Battle et al. (2014) dataset that included genes and their chromosomes and positions to ensure that the position and naming schemes matched as closely as possible to the genotype and gene expression datasets we were using. I looked for genes with a start position within one megabase of the SNP position, creating a gene list for each SNP.

Then, I used the gene expression residuals calculated above, by regressing out technical covariates, as the dependent variable in our next linear model analysis. Using the entire cohort of males and females, I created a linear model for each gene-SNP pair, using all genes in all gene lists, which resulted in 2319 genes being tested with each SNP. (Because enrichment in *cis* effects is expected, computing statistics for *trans* effects in parallel could act as an internal control.) I again considered the following biological covariates: current alcohol use, age at interview, current BMI, cigarettes per day, whether the participant smoked before, whether the participant exercised before, whether the participant used cannabis in the

prior week, whether the participant ate before, or whether the participant used any of the following drugs: ACE inhibitor, beta blocker, diuretic, blood pressure medication, oral birth control pills, anticholinergic medication, decongestant medication, oral hypoglycemic medication, cholesterol lowering medication, NSAIDs, thyroid medication, proton pump inhibitors, opiates, anti-histamines, and stimulant decongestant. For each gene-SNP pair, I started with a null model (gene expression residual $\sim \beta_0$) and used forward stepwise regression to build up the model to include biological covariates, up to a model that included all biological covariates if necessary. From this base model of gene expression residual, I then built three linear models. In the first, just the SNP genotype was added; in the second, SNP and sex were added to biological covariates; in the third, SNP, sex and a sex:SNP interaction term were added to biological covariates.

3.3 Results

3.3.1 Differential transcription characterization and pathway analysis

In the broad differential expression analysis of whole blood RNA-seq data, it was clear that there were large transcriptional differences between males and females. I used a two-step linear model approach, first regressing out technical covariates and then building a model with the gene expression residual and sex, plus biological covariates added through forward stepwise regression. Using this method, of the 15231 genes analyzed, 5377 genes were differentially expressed at an FDR $p<0.05$ (Figure 8). The most extreme outlying genes are labeled in Figure 8.

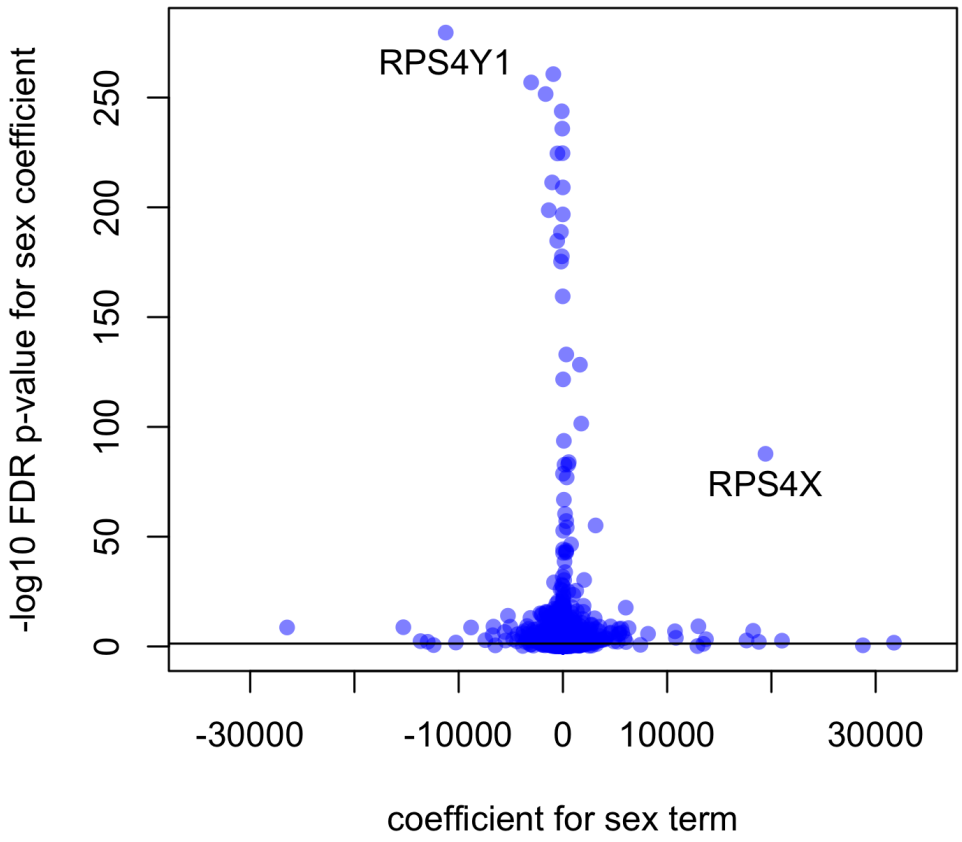


Figure 8: Differential gene expression between males and females, with data from whole blood, collected by Battle et al. (2014). Raw counts were first used in a linear regression with technical covariates, and the gene expression residuals were included in a second linear regression, which included an intercept term and sex in the basic model and added in biological covariates using a forward stepwise regression. The coefficient for the sex term in the final model corresponds to fold change in gene expression based on being female as compared to male and is graphed on the x-axis. The p-value for the coefficient being nonzero is graphed on the y-axis, after correction for multiple comparisons using the Benjamini and Hochberg method. Extreme values, both in significance and in magnitude of change, are labeled by gene name.

% variance explained	Multivariate p-value for sex	PC genes	DAVID Cluster 1 summary	Cluster 1 score
		PC1 bottom	plasma membrane	3.031
35.93	4.54E-04			
		PC1 top	cytoplasmic organelles and acetylation	2.953
		PC2 bottom	plasma membrane; signal peptide; disulfide bonds and glycoproteins	3.171
14.52	3.60E-08			
		PC2 top	translation: cytosolic ribosome, RNA binding	26.124
		PC3 bottom	translation: cytosolic ribosome, RNA binding	19.697
7.98	7.94E-09			
		PC3 top	actin cytoskeleton	5.972
		PC4 bottom	plasma membrane	4.386
7.07	0.3418			
		PC4 top	translation: cytosolic ribosome, RNA binding	13.577
		PC5 bottom	plasma membrane	2.124
4.84	1.08E-03			
		PC5 top	translation: cytosolic ribosome, RNA binding	9.382
		PC6 bottom	plasma membrane	3.043
4.41	0.1472			
		PC6 top	translation: cytosolic ribosome, RNA binding	20.515
		PC7 bottom	signal peptide; disulfide bonds, glycosylation and glycoproteins	4.796
3.4	4.03E-13			
		PC7 top	translation: cytosolic ribosome, RNA binding	17.533
		PC8 bottom	defense, inflammatory and wound response	1.592
2.94	2.29E-04			
		PC8 top	translation: cytosolic ribosome, RNA binding	14.308
		PC9 bottom	cell, leukocyte migration; cell motility	3.435
2.57	0.1141			
		PC9 top	translation: cytosolic ribosome, RNA binding	7.705
		PC10 bottom	translation: cytosolic ribosome, RNA binding	12.743
1.69	0.8094			
		PC10 top	defense, inflammatory and wound response	5.378

Table 6: DAVID pathway analysis of the top 10 principal components from PCA of differentially expressed genes between males and females. The multivariate p-value for sex represents the p-value of the coefficient for the sex term being nonzero. The multivariate regression was computed using a stepwise approach, from a basic model that included just intercept and sex terms, with biological covariates added in to make the full model. Genes with the highest magnitude loadings — the top 1% and the bottom 1% — were then included in separate pathway analyses via DAVID, and DAVID annotation results for cluster 1 are manually summarized, along with enrichment score from DAVID.

RPS4Y1, which was found to be much more highly expressed in males, is Ribosomal Protein S4, Y-Linked; RPS4X, which was found to be more highly expressed in females, is Ribosomal Protein S4, X-Linked, but, despite its location on the X chromosome, is not inactivated. The genes are homologs and both encode ribosomal protein 40S, but from different sex chromosomes (Fisher et al., 1990). It is reassuring that these sex chromosome linked genes were the genes most extremely differentially expressed.

I then wanted to dissect patterns in this vast number of differentially expressed genes. Using the residuals of gene expression found by regressing out technical covariates, we conducted principal components analysis. For the top 10 principal components, we assessed which were most highly correlated with sex by using a stepwise multivariate linear regression. As shown in Table 6, PCs 7, 3, 2, 1, 8, and 5 were all significantly correlated with sex (in order of lowest p-value). The genes that were most important in these 10 PCs, as assessed by highest 1% and lowest 1% loadings, were then included in pathway analysis via DAVID. A summary of the DAVID output for cluster 1 annotation is presented in Table 6. In general, fundamental cellular pathways and processes were highlighted in this analysis, as evidenced by the frequent annotations of, for example, translation components or cytoplamic organization.

3.3.2 Sex interaction analysis of established *cis*-eQTLs

To explore the links that triangulate gender, genetics and transcription, I then wanted to investigate whether established *cis*-eQTLs in the Battle data exhibited gender dimorphism in effect size, as MS variants did. I used a more conservative approach to detect eQTLs than was used in the published study but limited the analysis to *cis*-eQTLs that were found in that study.

I split the cohort by sex and conducted our eQTL analysis on each group separately, which necessarily resulted in a loss of power. To ensure that the method was detecting similar eQTLs as in the Battle study, I compared p-values of each SNP-gene between the female and male analyses to corresponding p-values in the original study (Figure 9). As expected, p-values were systematically higher (lower $-\log_{10}$P) in each of the present analyses versus the original study, explained by splitting the cohort and therefore substantially reducing sample size from the original 922 participants to subsets of 647 (females) and 275 (males). Power was likely further reduced by our conservative method of finding eQTLs. However, the correlation in p-values to Battle p-values was high, with an R-squared of 0.695 in the female analysis and 0.696 in the male analysis. Based on these results, I was fairly confident that the method had sufficient power to detect *cis*-eQTLs (and did not over-inflate significance) and was highlighting similar eQTL effects as the original analysis.

Next, I investigated SNP effect size between males and females. Interestingly, unlike the results for effect size at MS risk variants, genome-wide eQTL SNP coefficients were highly correlated between males and females, with an R-squared

of 0.901 and p-value <2.2E-16. Variant effect size was also very similar between males and females, as demonstrated by the line of best fit having a slope of 1.02. As indicated in Figure 10, some eQTLs were highlighted as significant in both males and females, in males only, in females only, or neither group. eQTLs that were significant in neither group were expected because all pairs were included from the original Battle analysis, including those that did not reach significance using this method with the stratified cohort.

Based on this result, it appeared that genome-wide *cis*-eQTLs did not generally have differential effect size between males and females, nor did there appear to be outliers from the $y = x$ line that might follow a model of sex-specific effect size. I still wondered how MS risk variants, which exhibited differential effect on disease risk between males and females, behaved in the sexually dimorphic transcriptional landscape.

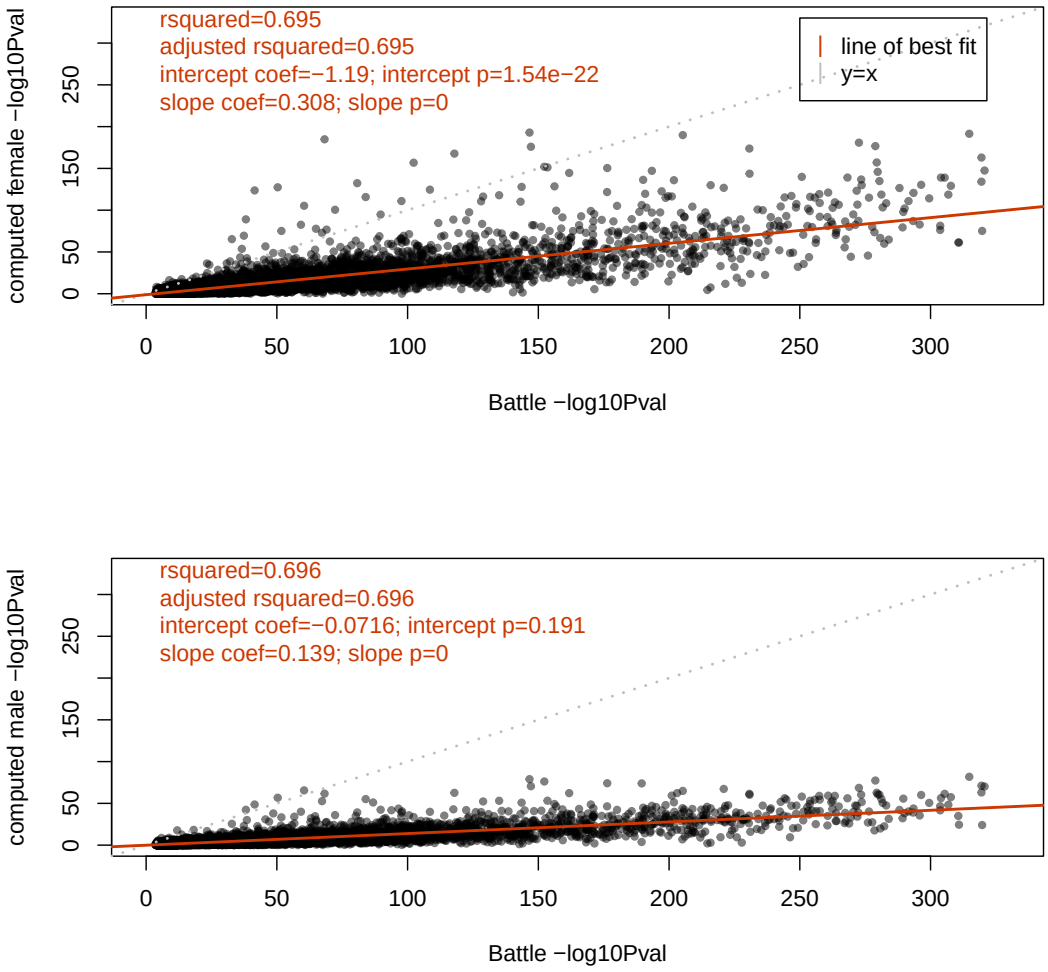


Figure 9: *cis*-eQTL SNP-gene pair p-value comparison between computed p-values and p-values reported in Battle et al. (2014). Shown is both the line $y = x$ and the line of best fit, determined by linear regression, along with the statistics accompanying the linear regression. Because log0 produces infinite values, any infinite values were set to 500 before being included in the linear regression. Of note, the original p-values were computed using the whole cohort, while each of these represent a process of finding eQTLs in the cohort split between females (n=647) and males (n=275), consistent with systematically higher p-values, evidence of the reduced power in these analyses.

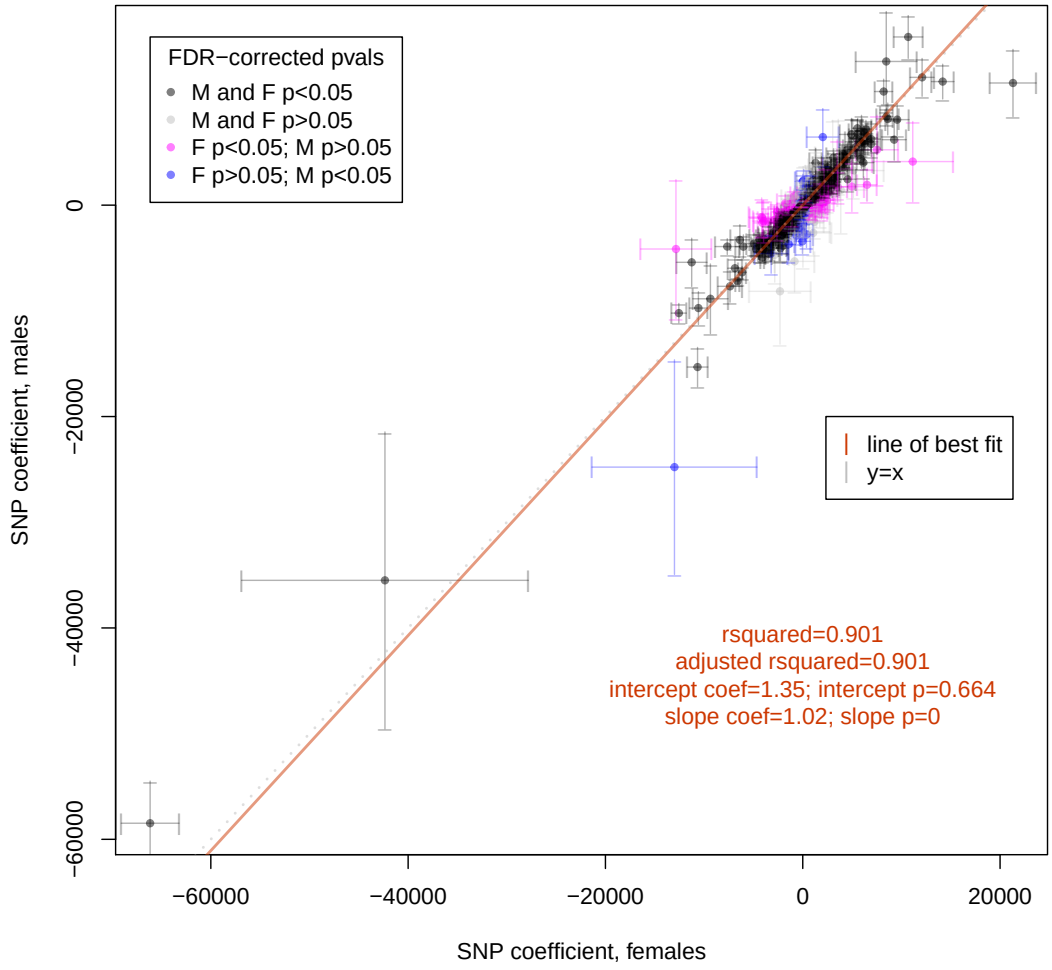


Figure 10: SNP coefficients in *cis*-eQTL analysis of SNP-gene pairs highlighted in the original analysis by Battle et al. (2014). Each point represents an established SNP-gene pair that exhibited an eQTL relationship in the original analysis. Error bars represent standard error around the estimate of SNP coefficient effect size. Points and error bars are colored by significance; in this analysis, there were some SNPs that were significant only in females or only in males, along with some SNPs that were significant in both groups or neither group.

3.3.3 eQTL and sex interaction analysis of MS variants

After building a set of linear models for the relationship between gene expression residual and SNP, sex and sex-SNP interaction, p-values on the SNP term were corrected for multiple comparisons using the Benjamini and Hochberg method, also known as FDR. A SNP-gene pair was considered to be significant if the FDR-corrected p-value on the SNP β from the model:

$$\text{gene expression residual} \sim \text{biological covariates (selected via forward stepwise regression)} + \text{SNP} + \text{sex}$$

was less than 0.05. P-values for sex in the above model were also FDR-corrected for multiple comparisons. In addition, the p-value on the β for the interaction term in the third model was also FDR-corrected:

$$\text{gene expression residual} \sim \text{biological covariates (selected via forward stepwise regression)} + \text{SNP} + \text{sex} + \text{sex:SNP}$$

QQ plots for each set of p-values is presented in Figure 11, showing the most skew from expected p-values under the null model in SNP effect sizes. The subset of gene-SNP pairs that met the criteria for a significant eQTL were then further assessed for the significance of sex and the sex:SNP interaction term.

333936 SNP-gene pairs were assessed, 2828 of which were potential *cis*-eQTLs and 331108 potential *trans*-eQTLs (i.e. 0.847% *cis*-eQTL). Just 108 met the criteria for a significant eQTL (Figure 12a. Of these, 91 were *cis*-eQTLs and 17 were *trans*-eQTLs (i.e. 84.3% *cis*-eQTLs), showing that the analysis was enriching for *cis* effects, as expected.

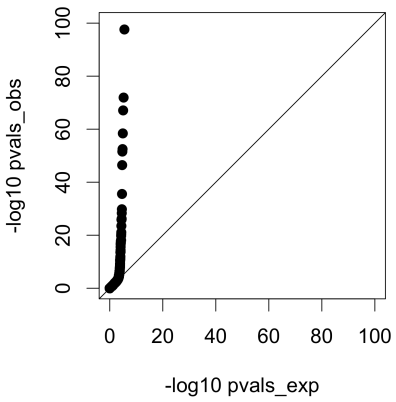
I had fitted both sex and SNP as fixed effects; of the SNP-gene pairs that exhibited an eQTL relationship, sex had a significantly (FDR < 0.05) non-zero effect size in 32 pairs (Figure 12b). These SNP-gene pairs were considered important because sex and MS variant both have effects on expression of the same gene. These 32 pairs comprised 24 different SNPs affecting 32 different genes (Table 7).

A few gene-SNP pairs are particularly noteworthy in this set of 32. First, some pairs have the same direction of effect for the MS risk allele and for female sex; I focused on a few with high magnitude effect sizes. rs9808753 stands out as a *cis*-eQTL for IFNGR2, the IFN-γ receptor. The SNP coefficient of effect size on the IFNGR2 expression residual is 695 (FDR-corrected p-value 0.000369) and the sex coefficient is 468 (FDR-corrected p-value 0.00407) (Figure 13a). Another pair of interest is rs7260516, a *cis*-eQTL for FPR1; the coefficient for the MS risk allele's effect on the FPR1 expression residual is 5440 (FDR-corrected p-value 0.00135) and the coefficient for being female is 7881 (FDR-corrected p-value 6.37E-06) (Figure 13b). Second, there were some pairs with opposite effect directions between the MS risk allele and being female. An example is shown in Figure 13c, in which rs11554159 is a *cis*-eQTL for IL12RB1; the MS risk allele, G, has an effect size on the IL12RB1 expression residual of 151 (FDR-corrected p-value 4.45E-05), while being female has an effect size of -121 (FDR-corrected p-value 0.00449) (see Table 7).

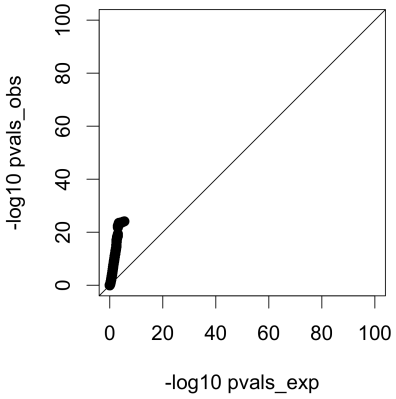
As evident from Figure 12c, in the set of significant eQTLs, there were no pairs with a significant sex-SNP interaction term. There were five pairs that were sig-

nificant at a significance level of FDR < 0.05 in the dataset overall. They were all *trans* effects; *trans* effects were included mainly as negative controls, but it is possible that there were true signals in the set of *trans* SNP-gene pairs. First, in order to check whether the interaction term was significant simply because of overfitting, I validated that p-values for the effect size of the interaction term were correlated with the p-values of the likelihood ratio test between the model including the interaction and the model including both SNP and sex but no interaction term (not shown). Then, I manually inspected the genotype data for these five pairs. In all cases, the signal was driven by outlying genotype:sex pairs, with an outlying genotype:sex state defined by at most 5 individuals belonging to a particular sex and a particular genotype class. Thus, I conclude that there were no significant sex:SNP eQTL interactions in this set of MS variants and this transcriptional dataset.

(a) QQ plot of pvals for SNP significance in eQTL analysis



(b) QQ plot of pvals for sex significance in eQTL analysis



(c) QQ plot of pvals for sex:SNP interaction significance in eQTL analysis

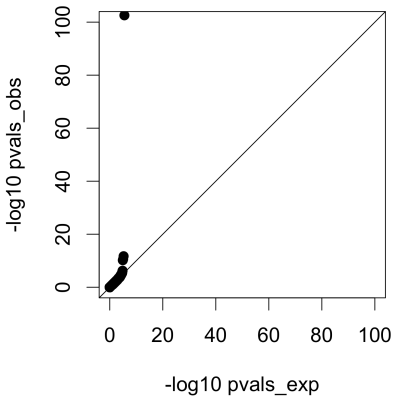
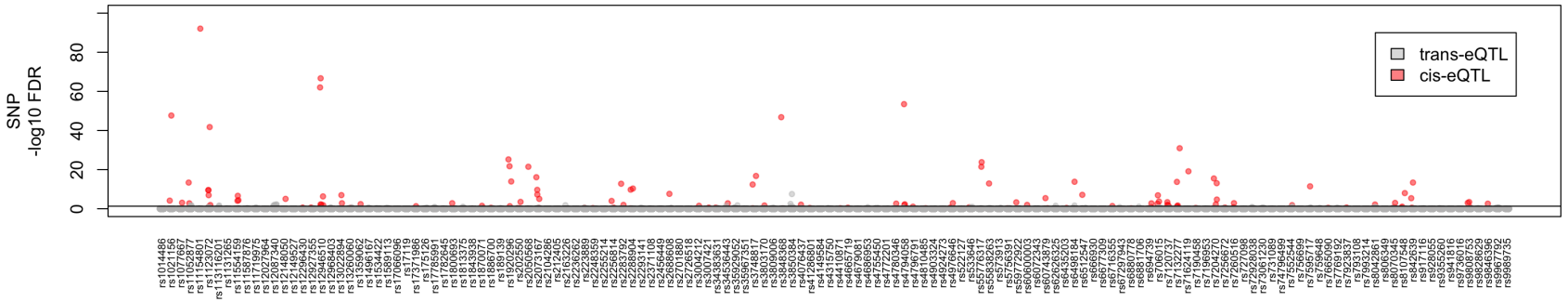


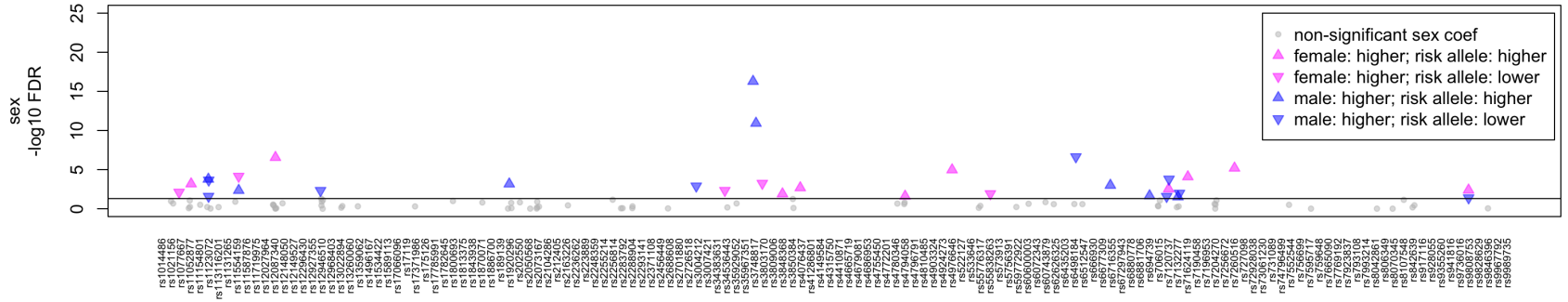
Figure 11

Figure 11: (Previous page.) QQ plots of expected and observed p-values for each of three sets of p-values: (A) SNP effect size being unequal to 0, (B) sex effect size being unequal to 0, and (C) sex:SNP interaction effect size being unequal to 0.

(a) eQTL significance for MS SNPs; model: gene expresion residual ∼ covariates + sex + SNP



(b) sex significance for MS SNPs that are eQTLs; model: gene expresion residual ∼ covariates + sex + SNP



(c) sex:SNP interaction significance for MS SNPs that are eQTLs; model: gene expresion residual ∼ covariates + sex + SNP + sex:SNP

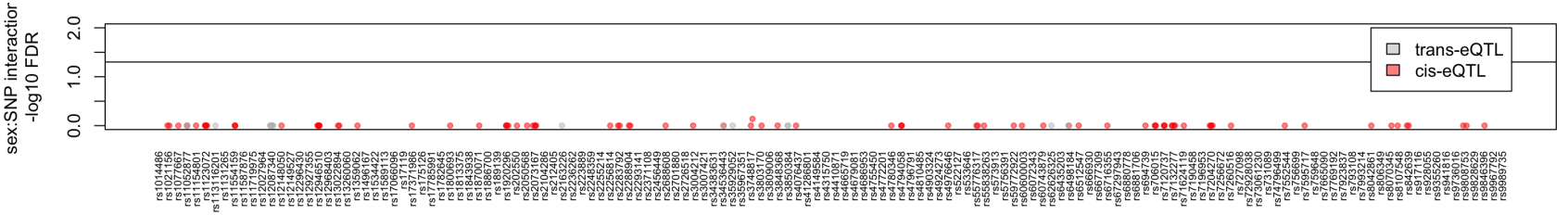
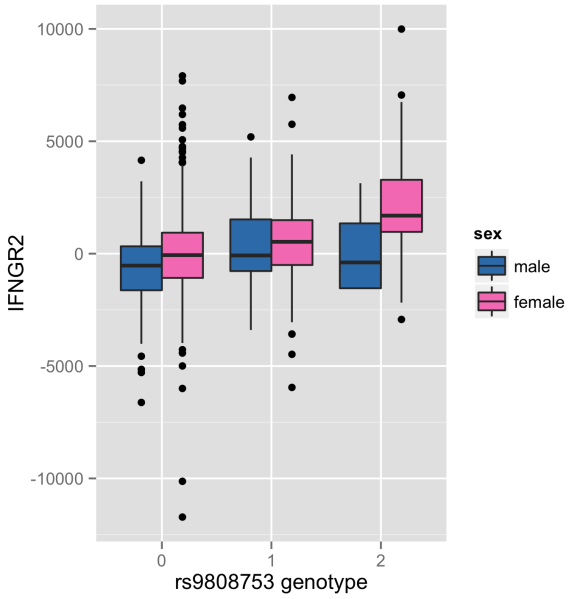


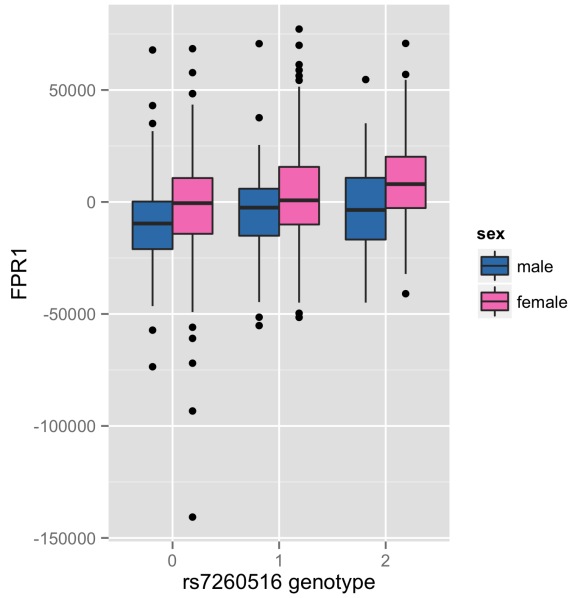
Figure 12

Figure 12: (Previous page.) eQTL analysis of MS variants imputed in the Battle et al. (2014) dataset. (A) FDR-corrected p-values for the SNP effect size on gene expression residual not being equal to 0; the 108 SNP-gene pairs meeting the criterion of FDR < 0.05 were considered significant eQTLs. (B) FDR-corrected p-values for the sex effect size not being equal to 0; of the 108 SNP-gene eQTL pairs, 32 gene expression residuals had a significant sex effect. Colors and symbols refer to the direction of effect based on gender and MS risk allele in genes with significant eQTL and sex effects (FDR < 0.05). (C) FDR-corrected p-values for the sex:SNP interaction term not being equal to 0; none of the 108 SNP-gene eQTL pairs met the threshold of FDR < 0.05 for a significant eQTL interaction term.

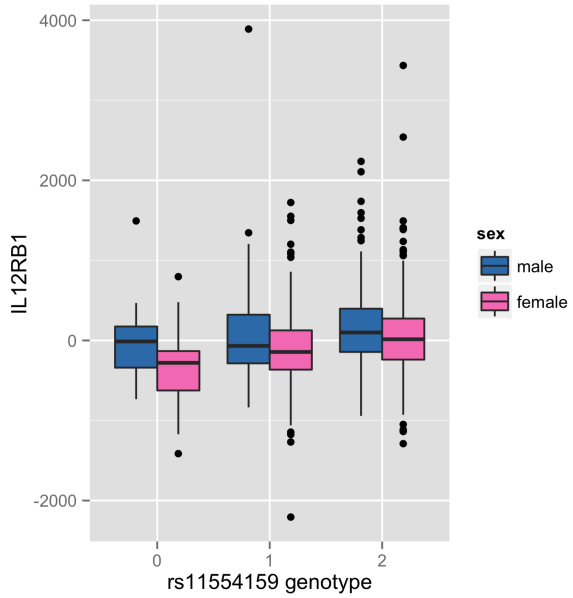


(a) Boxplot of rs9808753 genotype and sex factors along with gene expression residual for IFNGR2, after regressing out technical covariates.

Figure 13



(b) Boxplot of rs7260516 genotype and sex factors along with gene expression residual for FPR1, after regressing out technical covariates.



(c) Boxplot of 11554159 genotype and sex factors along with gene expression residual for IL12RB1, after regressing out technical covariates.

Figure 13

Figure 13: (Previous two pages.) These gene-SNP pairs, (A) rs9808753/IFNGR2, (B) rs7260516/FPR1 and (C) rs11554159/IL12RB1, are highlighted in the group of 32 SNPs that, after correcting for multiple comparisons, both showed a significant eQTL relationship and had a significant sex term. A genotype of "2" represents being homozygous for the MS risk allele: GG in rs9808753/IFNGR2, TT in rs7260516/FPR1, and GG in rs11554159/IL12RB1. In all cases the MS risk variant correlates with increased gene expression. In (A) rs9808753/IFNGR2 and (B) rs7260516/FPR, being female correlates with gene expression change in the same direction as the risk variant — an increase in gene expression residual. In (C), the effects act in opposite directions; being female correlates with a decreased IL12RB1 expression residual. Individuals are split by their genotype, on the x-axis, ranging from 0 to 2 based on dosage of the MS risk allele, and by sex, as indicated by color. Because these SNPs were imputed in this dataset, many individuals are not cleanly 0, 1 or 2, but imputed genotypes are rounded for the purpose of visualizing.

snp	gene	eqtl type	risk allele	protective allele	risk allele freq from GWAS, USA	risk allele freq, Battle	sex FDR	sex coefficient	sex coefficent SE	snp FDR	risk allele coefficient	risk allele coefficient SE	sex and risk allele direction	best genetic model
rs3748817	C1orf93	cis	T	C	0.659	0.656	5.19E-17	-148	15.8	1.74E-17	103	10.5	opposite	M1
rs3748817	MMEL1	cis	T	C	0.659	0.656	1.18E-11	-13.1	1.73	4.1E-13	9.92	1.15	opposite	M1
rs6498184	KLRC1	trans	C	T	0.836	0.835	2.31E-07	-41.2	7.08	0.0239	-28.1	6.21	same	M1
rs12087340	CNN1	trans	T	C	0.0825	0.0803	2.75E-07	9.01	1.56	0.00938	8.44	1.77	same	M3
rs7260516	FPR1	cis	T	G	0.355	0.361	6.37E-06	7.88E+03	1.54E+03	0.00135	5.44E+03	1.05E+03	same	M1
rs4976646	RGS14	cis	C	T	0.349	0.331	1.06E-05	636	128	0.00128	449	86.1	same	M1
rs11554159	KIAA1683	cis	G	A	0.723	0.729	7.25E-05	53.6	11.8	2.43E-07	-56.9	8.45	opposite	M3
rs71624119	ANKRD55	cis	G	A	0.754	0.767	8.14E-05	52.6	11.7	7.35E-20	93.5	8.94	same	M5
rs7120737	ACP2	cis	G	A	0.128	0.147	0.000165	-82.2	19.1	0.00043	-98	18	same	M5
rs1123072	C16orf86	cis	G	A	0.947	0.953	0.000166	-14.5	3.38	2.79E-10	39.2	5.06	opposite	M1
rs1123072	DUS2L	cis	G	A	0.947	0.953	0.000221	-76.2	18	1.72E-42	-410	26.8	same	M1
rs3803170	TMEM116	cis	A	G	0.797	0.794	0.000573	23.3	5.88	0.0215	-21.4	4.7	opposite	M3
rs11052877	CDC42BPG	trans	G	A	0.378	0.385	0.000654	16.2	4.14	0.0239	12.2	2.69	same	M2
rs1920296	FBXO40	cis	C	A	0.612	0.606	0.000673	-2.01	0.513	1.14E-14	3.15	0.346	opposite	M5
rs6716355	UBE2E3	cis	G	T	0.304	0.316	0.000998	-35.6	9.35	0.0405	28	6.39	opposite	M1
rs3004212	RET	cis	C	T	0.733	0.729	0.00128	-4.76	1.28	0.0313	-4.13	0.928	same	M1
rs4076437	RAB43	cis	C	T	0.117	0.119	0.00207	44	12.3	0.00822	59.9	12.5	same	M4
rs7120737	PTPRJ	cis	G	A	0.128	0.147	0.00293	479	139	0.00941	611	129	same	M5
rs9808753	IFNGR2	cis	G	A	0.133	0.138	0.00407	468	140	0.000369	695	127	same	M3
rs11554159	IL12RB1	cis	G	A	0.723	0.729	0.00449	-121	36.5	4.45E-05	151	25.8	opposite	M3
rs34536443	CTLA4	trans	G	C	0.957	0.952	0.00461	20.3	6.14	0.0272	-41.7	9.29	opposite	M3
rs12946510	PNMT	cis	T	C	0.453	0.466	0.00466	-1.63	0.492	0.00652	-1.52	0.313	same	M1
rs1077667	TNFSF14	cis	C	T	0.801	0.774	0.00783	133	42.5	0.000827	-179	33.7	opposite	M3
rs7132277	CDK2AP1	cis	T	C	0.191	0.184	0.0106	-47.7	15.8	1.85E-14	-115	12.7	same	M4
rs55838263	GPR25	cis	A	G	0.723	0.73	0.0121	25.6	8.66	1.22E-13	-57	6.5	opposite	M4
rs3848368	TMEM8A	cis	T	G	0.474	0.456	0.0124	382	129	1.57E-47	1.37E+03	84.4	same	M1
rs694739	CCDC88B	cis	A	G	0.629	0.619	0.0218	-706	258	0.00165	871	169	opposite	M5
rs4794058	TBKBP1	cis	T	C	0.511	0.508	0.0248	168	62.4	3.29E-54	701	40.1	same	M1
rs1123072	ZDHHC1	cis	G	A	0.947	0.953	0.0262	-13	4.88	1.16E-07	-50.4	7.35	same	M1
rs7120737	NR1H3	cis	G	A	0.128	0.147	0.0269	-17	6.41	0.0183	-27.7	6.03	same	M5
rs7132277	SETD8	cis	T	C	0.191	0.184	0.0298	-14.3	5.46	1.14E-31	59.1	4.49	opposite	M4
rs9808753	ITSN1	cis	G	A	0.133	0.138	0.0408	-11.8	4.77	0.00101	-23.4	4.44	same	M3

Table 7: Summary of SNPs and genes highlighted through an intersection of MS risk variants and differential transcriptional analysis by sex. The SNP ID is presented, along with the gene for which it is an eQTL and the type of eQTL. Effects are presented for the MS risk allele relative to the protective allele; the risk allele frequency is presented from the original Immunochip GWAS in the cohort from the USA for reference (International Multiple Sclerosis Genetics Consortium et al., 2013), as compared with the frequency of the risk allele in the Battle dataset. Next, the results for sex correlation with gene expression residual and SNP correlation with gene expression residual are presented. For some SNP/gene pairs, the risk allele acts in the same direction on gene expression as being female does, but in some the two act in opposite directions. Finally, for reference, the genetic model with the greatest evidence for sex-SNP interaction in influencing MS risk is listed. (M1: fixed effects, correlated with $c = 1$, M2: independent effects, M3: male-specific effect, M4: female-specific effect, M5: correlated effects with $c = 0.42$.)

3.4 Discussion

This analysis first showed that there were significant differences in expression of thousands of genes between males and females in the blood. Importantly, these results supported earlier studies in different datasets and using different protocols that showed differential gene expression in the blood. However, the incredible number of genes that were significantly expressed made it difficult to develop an *a priori* conception for which of these differences may be important in creating differential immune regulation that may in turn impact MS risk differently in men and women. Despite efforts to delve further into the differentially expressed genes using principal components analysis and gene ontology pathway analysis, it was not possible to establish a clear link between patterns in differential expression and specific immune function that might link to MS risk.

As highlighted by the examples of ribosomal genes on the X and Y chromosomes in Figure 8, some of the differentially expressed genes in this analysis are contributed by X/Y chromosomal differences. I did not conduct a separate analysis of differentially expressed genes limited to the autosomes, though it would be interesting to see what proportion of the differentially expressed genes were actually attributable to differences in sex chromosome complement. Looking at sex chromosomes alone would also allow some understanding of the extent to which dosage compensation mediates differences in the X chromosome complement between males and females regarding gene expression. For this preliminary analysis, however, my main goal was to understand how global transcriptional differences – on sex chromosomes or autosomes – might mediate MS susceptibility, rather than dissecting the contributions of autosomal vs sex chromosome genes. Separating

the analyses would be a fascinating future study.

An analysis of gender-specific eQTLs was necessary to see how genetics and gender might intersect to inform different biology in males and females and therefore differential disease risk. The effect size for eQTL variants was fairly equal between males and females, as supported by the line of best fit having a slope of 1.02 and by the high R-squared, 0.901, but I was still interested in understanding how MS risk variants affect gene expression, potentially influenced by or in line with sex effects.

To combine these two approaches — variant-based differences in gene expression and sex-based differences in gene expression — with MS, the top SNPs associated with MS were examined. First, SNPs that were eQTLs were highlighted; from this list, it was particularly interesting, first, to see which genes had differential expression based not only on genetic variant but also on sex, and second, to look for sex-genotype interaction effects on gene expression.

Although there were no variants with a significant interaction between sex and variant genotype, there were 32 SNP-gene pairs that have been significantly correlated with MS, were eQTLs and were differentially expressed between males and females.

Likely most promising investigation of MS risk are the genes that are affected in the same direction by the MS risk variant and being female. The principle of eQTL studies is to uncover how disease-associated variants act through biological pathways, perhaps by affecting gene expression, to increase disease risk. If an-

other risk factor, sex, is also associated with the same direction of gene expression change, it is reasonable to triangulate between disease state, genetics, sex and gene expression to implicate that gene in MS pathogenesis. A few SNPs stood out for their significance and magnitude of effect size, with the same direction of effect between being female and having the MS risk variant.

First, rs9808753, a *cis*-eQTL for IFNGR2, reinforces the importance of the IFN-γ pathway in multiple sclerosis. Both the risk allele, G, and being female correlate with increased expression of IFNGR2, the gene that encodes part of the IFN-γ receptor. It has been known for many decades that IFN-γ makes multiple sclerosis patients worse; recombinant IFN-γ was once thought to be a potential therapy through immune modulation but then was shown to have a negative effect clinically in MS patients (Panitch et al., 1987). Increased expression of a receptor component may cause increased responsiveness to IFN-γ, potentially mimicking the effect of treating with recombinant IFN-γ. In line with this pathogenic mechanism, anti-IFN-γ has shown to be therapeutically successful for another autoimmune disease, rheumatoid arthritis (Sigidin et al., 2001). Based mostly on EAE models, some have argued that IFN-γ is protective and anti-IFN-γ deleterious in MS risk (Duong et al., 1992). Not only are the IFNGR2 results presented here biological plausible, the analysis implies that IFN-γ signaling may be implicated in MS pathogenesis, so anti-IFN-γ may be useful in MS patients.

As a second example, rs7260516 is a *cis*-eQTL for FPR1. As in the first example, both being female and having the MS risk allele, T, correlate with an increased FPR1 expression residual. This gene is less clearly implicated in MS pathogenesis

than IFNGR2 is. FPR1, formyl peptide receptor 1, encodes a G-protein coupled receptor important in the innate immune response; it is a pattern recognition receptor that, when activated, causes activation of neutrophils (Mantovani et al., 2011). In multiple sclerosis patients, neutrophils have been found to be primed for activation, with higher expression of FPR1 (Naegele et al., 2012). The approach presented here provides an unbiased approach that highlights FPR1 and, by extension, likely neutrophil activation, in MS; further work on the significance of FPR1 in MS pathogenesis could investigate its potential as a therapeutic target.

While, on first glance, the most interesting SNPs may be those with a consistent direction of effect between the two risk factors of sex and MS risk variant, it is not unreasonable that some would show an opposite effect; multiple factors contribute to MS risk, and genes act through complicated pathways, so there is no need for the MS risk variant and being female to correlate with gene expression in the same direction in every case. One particularly interesting example of gene expression effect acting in opposite directions by variant and sex is rs11554159. This SNP is a *cis*-eQTL for IL12RB1, a gene that encodes a protein in both the IL12 and IL23 receptors. The MS risk variant correlates with increased expression of IL12RB1, while being female correlates with decreased expression of IL12RB1. T cells from patients with a recessive knockout of IL12RB1 produce lower amounts of IL17, suggesting an impaired Th17 subset (de Beaucoudrey et al., 2008). In contrast, a robust Th17 response has been implicated in several autoimmune diseases, including MS; based on biopsies of MS lesions, Th17 cells and IL17A gene expression are both elevated in these brain lesions (Noack and Miossec, 2014). In recent years, it has become clear that different autoimmune diseases share some

of the same risk loci, suggesting some common pathways of pathogenesis (Anaya et al., 2006; Parkes et al., 2013). For example, Th17 cells are also important in rheumatoid arthritis (Noack and Miossec, 2014). Th17 cells have been implicated ankylosing spondylitis, but, important for these results, only in males (Gracey et al., 2016). It is possible to imagine that, in this particular case, rs11554159 acts through IL12RB1 to affect the Th17 autoimmune response: rs11554159- or male-related increase in IL12RB1 expression produces a more robust Th17 response, which produces greater risk for several autoimmune diseases, including MS. There is no need for all variants to have a more profound effect in females. The genetic modeling approach shows some support for a male-specific effect at this SNP: for rs11554159, M3, a male-specific effect, has the best evidence, with a 4.12 times better evidence than model 5, the correlated effects model; impressively, M4, a female-specific effect, has the the least evidence for this SNP, with 4.22-fold worse evidence than model 5.

It should be noted that multiple analyses were performed and statistical correction for multiple comparisons was performed using the FDR method. There are obviously many ways of correcting for multiple statistical tests, and the point may be made that FDR correction is not as stringent as other approaches, such as Bonferroni. However, because this is an exploratory analysis, I decided that a less stringent correction for multiple comparisons would be appropriate, hence the use of FDR. An improvement to the analysis would have been to normalize each gene expression based on mean gene expression across the cohort to standardize effect sizes across genes. The gene expression values were standardized by controlling for a variety of potentially confounding biological and technical covariates; some

of these covariates correlated with sex, but all were included in the model to be as conservative as possible in controlling for spurious effects that might yield artifact. Ideally, this analysis would include a sensitivity analysis to test how important inclusion/exclusion of these variables truly is in the final result, though I anticipate the exact selection of these covariates would have little impact. Also, it would have been good to have conducted a power analysis to assess the strength to detect any signal in this dataset before embarking on the project to get a sense of the power to observe significant differences in gene expression by genotype and by sex. It is worth reiterating that this was an exploratory analysis not intended to conclusively implicate the pathways important in sex-biased MS risk but instead to generate hypotheses about the genes, and therefore pathways, that might be implicated in disease risk. Because, as discussed in the introduction, this problem of sex-biased MS risk has been so difficult to study, it made sense to conduct this set of exploratory analyses simply to explore whether an integrated approach using both genetic and sex-based clues about the heterogeneity of disease could help uncover interesting genes.

This analysis does highlight the merit of an intersectional approach to understanding MS pathogenesis through genomics. Differential gene expression between males and females uncovers too many genes to make clear sense of disease implications. There were no significant sex differentials in eQTL effects either genome-wide or at MS risk variants; this does not rule out the possibility of important sex-genotype interaction effects in some phenotypes. However, by combining a list of MS risk variants with eQTL analysis and differential gene expression analysis, 32 SNP-gene pairs (24 SNPs correlated with 32 genes) were both significant

eQTLs and had a significant sex effect on expression. Examination of three of these pairs, with risk allele and female sex acting in the same or opposite directions, highlights the biological plausibility and significance of these pathways in regulating sex- and genotype-based risk of MS. This approach could be extended to other immune-mediated diseases in order to more fully understand sex-biased immune function and how some of these sexually dimorphic pathways go awry in autoimmune disease.

4 Discussion and conclusions

In this set of analyses, I have taken a two-pronged approach to explore MS susceptibility variants and their interaction with sex to inform risk of disease. First, I carried out an in-depth exploratory characterization of sex bias in genetic effect size for MS risk. I suggest that genetic risk variants have, in general, an effect size that is lower in magnitude in females than males, by a factor of 0.42. I have not ruled out the possibility of sex-specific variants that contribute to MS risk, but it is not surprising that this analysis did not highlight sex-specific SNPs because I started with a set of variants found genome-wide in a cohort that included both males and females. Second, I found that environmental variance, compared to genetic variance, may play a greater role in determining MS in females than males. In a model of liability, in which liability for disease encompasses both genetic and environmental factors, it appears more likely that females have greater liability variance, likely attributable to environmental factors, rather than a lower threshold for disease compared to males. Beyond the conclusions important for MS derived from this analysis, the statistical modeling of sex bias in genetic determinants of disease is potentially applicable to a wider range of diseases with a genetic component and a gender bias.

Next, I carried out an analysis of differential gene regulation between males and females. This analysis had three parts. First, differential gene expression across the genome showed that over 5000 genes were significantly expressed in the blood, highlighting the substantial differences in gene regulation between males and females. Second, I investigated whether *cis*-eQTLs across the genome exhibited

sex-biased effect sizes, as in effect size for MS risk; there was no evidence of systematic sex-based skew of effect sizes. Third, I used these analysis frameworks to investigate MS risk variants. I found MS risk variants that were also eQTLs and from this list particularly focused on genes whose expression was affected by an MS risk variant and by sex. Again, there were no sex-genotype interaction effects on gene expression. The 32 SNP-gene pairs that satisfied the criteria of an MS risk variant affecting expression of a gene that was also differentially expressed between males and females serves as a good list of candidate genes in MS etiology. These genes have evidence for MS involvement through both eQTL and sex-based analyses.

These two arms of the analysis both yielded important hints about sex bias in MS susceptibility. The relevance of the intersection between the results is not entirely clear. In some cases, for example the IL12RB1 case, in which the risk allele and being male both increase expression, it makes sense that the male-specific model has greatest evidence. In the other cases highlighted, the sex-genotype model did not make as much biological sense on first impression: for FPR1, correlated effects with c=1 was the best model, and for IFNGR2, the male-specific model fit best, despite female sex aligning with the risk variant in effect on IFNGR2 expression. It is possible that the two analyses are describing slightly different phenomena. The purpose of using both sex and genotype to pinpoint genes of interest is to highlight pathways that are important in MS etiology. SNPs with greatest evidence for a male- or female-specific effect from the modeling approach might be consistent with sex-genotype interacting eQTLs, but no such eQTLs were found. For genes that are differentially expressed by sex and also have an

eQTL, there is no reason that the SNP would necessarily have a sex-biased effect, because this analysis shows that the SNP has indistinguishable effects in males and females but that both SNP and sex are important factors in gene expression independently. Therefore, both sets of analyses make sense independently and in parallel. The first set of analyses highlights a magnified environmental role in MS susceptibility in females, while the second highlights genes that may be important in the etiology of MS, based both on genetic and sex-based evidence.

This set of findings is intended to be exploratory and hypothesis-generating. As discussed previously, in particular the genetic analysis was conducted in the same dataset in which the genetic associations with MS were initially highlighted, bringing the issue of the "winner's curse" to the fore in this analysis. These results should be considered preliminary and should be validated using an independent cohort. The hypotheses generated here, for example the impact of environmental variability vs genetic variability in females compared to males, the relationship between effect sizes, and the particular genes triangulated by sex-differential and MS risk SNP-differential gene expression can all be examined in a fairly straightforward manner in another MS GWAS and/or RNAseq dataset (using imputation if two separate datasets are used, as in the present analysis). This step is necessary before these results can be considered conclusive.

This set of analyses may also help lay the foundation for further additional analyses based on the hypotheses generated here. An additional analysis that might further link the two branches of the present study would be to assess variance in gene expression between males and females. Because, based on the genetic

analyses, I have suggested that females likely experience greater liability variance, it may be useful to examine differential gene expression variance between males and females as one metric of differential environmental variance.

One limitation of the present study was using transcriptional data from whole blood, without data from specific cell populations. For example, this analysis highlighted the IFN-γ, and IFN-γ reduces apoptosis in neutrophils (Naegele et al., 2012), a cell type that is also implicated through FPR1, another gene marked for its importance in MS based on this analysis. It would be interesting to see whether differential transcriptional effects, via genotype or sex, were enriched in neutrophils or other cell populations. Cell-specific analyses could help pinpoint the particular pathways in certain cells important in disease etiology.

A caveat that will be difficult to overcome in any transcriptional study in humans aimed at exploring MS is the inability to examine the site of pathology in patients or healthy controls. Blood is a necessary proxy for the immunological environment of the CNS and at MS lesions, and is likely an imperfect proxy. However, studying specific cell types could achieve a more granular and focused set of conclusions that may lead to real understanding of MS pathology and drug targets, even in the absence of full understanding of CNS inflammation.

This analysis stratified men and women using sex as a binary categorical variable. Based on our set of genetic analyses, it appears that there is greater environmental variance in females that influences disease risk, in addition to genetic risk. It would be useful to investigate variables that have a greater variance in

females than males, for example plasma progesterone, estrogen or testosterone concentration. I did not have access to a dataset that comprised both hormone level data and transcriptional data. Many hormones, particularly those that cycle in women, are affected by a multitude of other variables, such as pregnancy and use of contraceptives. An ideal study on the effects of the hormonal environment on gene expression would involve collection of longitudinal hormone levels and concomitant gene expression data, so both inter-individual and intra-individual variation with respect to hormone level.

This data mining approach has uncovered multiple potentially interesting candidates for MS therapeutics. As described here, anti-FPR1 and anti-IFN-γ may be promising therapies that merit further study. IL12RB1 is also promising, particularly in males, and could be an interesting target for other autoimmune diseases. IL12RB1 forms part of the IL12 receptor and the IL23 receptor, and, as further evidence of its potential significance in autoimmunity, an anti-IL23R antibody, secukinumab, has already been shown to be effective in ankylosing spondylitis (Baeten et al., 2013). Other genes on the list of SNP-gene pairs influenced both by MS variant and by sex are also worth investigation as potential drug targets in MS. Following up these targets with further biological study, starting in mouse models, with special attention to sex-biased and/or hormone-biased effects, may pave the way for new therapies, some potentially targeted preferentially to one sex.

With increasing appreciation of the differences between male and female immune regulation, combined with increasingly large genetic and transcriptional datasets, it is now possible to investigate in depth how differential immune regulation might

relate to differential risk of immune-mediated disease. It is important to develop methodology that allows for analysis of disease risk along multiple axes of heterogeneity. Analysis of genetic variation as a determinant of disease susceptibility has been well developed. Bringing sexual dimorphism into genetic and genomic analyses, as presented in this analysis, allows for further interrogation of disease risk variants, which is particularly important in polygenic diseases like MS, with over 100 susceptibility loci associated with disease, each of which must be interrogated to uncover a potential drug target. In some ways, adding sexual dimorphism to the analysis pipeline in an effective way can be considered a high-throughput way of highlighting genes and pathways that are particularly important in disease etiology. With further work in cell-specific datasets, it may be possible to use a combination of genetics and transcriptomics, in the context of sexual dimorphism, to understand pathways involved in disease risk more deeply, including particular cell types implicated. It may also be possible to understand how these pathways interact with variables associated with sex, such as reproductive hormones, in order to develop therapeutics that are more specifically tailored to individual patients.

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References

G. Abraham and M. Inouye. Fast principal component analysis of large-scale genome-wide data. *PLoS One*, 9(4):e93766, 2014.

J. M. Anaya, L. Gomez, and J. Castiblanco. Is there a common genetic basis for autoimmune diseases? *Clin Dev Immunol*, 13(2-4):185–95, 2006.

N. Aragam, K. S. Wang, and Y. Pan. Genome-wide association analysis of gender differences in major depressive disorder in the netherlands nesda and ntr population-based samples. *J Affect Disord*, 133(3):516–21, 2011.

L. Arruvito, M. Sanz, A. H. Banham, and L. Fainboim. Expansion of cd4+cd25+and foxp3+ regulatory t cells during the follicular phase of the menstrual cycle: implications for human reproduction. *J Immunol*, 178(4):2572–8, 2007.

H. Babbe, A. Roers, A. Waisman, H. Lassmann, N. Goebels, R. Hohlfeld, M. Friese, R. Schroder, M. Deckert, S. Schmidt, R. Ravid, and K. Rajewsky. Clonal expansions of cd8(+) t cells dominate the t cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med*, 192(3):393–404, 2000.

D. Baeten, X. Baraliakos, J. Braun, J. Sieper, P. Emery, D. van der Heijde, I. McInnes, J. M. van Laar, R. Landewe, P. Wordsworth, J. Wollenhaupt, H. Kellner, J. Paramarta, J. Wei, A. Brachat, S. Bek, D. Laurent, Y. Li, Y. A. Wang, A. P. Bertolino, S. Gsteiger, A. M. Wright, and W. Hueber. Anti-interleukin-17a monoclonal antibody secukinumab in treatment of ankylosing spondylitis: a randomised, double-blind, placebo-controlled trial. *Lancet*, 382 (9906):1705–13, 2013.

A. Battle, S. Mostafavi, X. Zhu, J. B. Potash, M. M. Weissman, C. McCormick, C. D. Haudenschild, K. B. Beckman, J. Shi, R. Mei, A. E. Urban, S. B. Montgomery, D. F. Levinson, and D. Koller. Characterizing the genetic basis of transcriptome diversity through rna-sequencing of 922 individuals. *Genome Res*, 24(1):14–24, 2014.

Jr. Bebo, B. F., E. Zelinka-Vincent, G. Adamus, D. Amundson, A. A. Vandenbark, and H. Offner. Gonadal hormones influence the immune response to plp 139-151 and the clinical course of relapsing experimental autoimmune encephalomyelitis. *J Neuroimmunol*, 84(2):122–30, 1998.

A. G. Beiske, E. Svensson, I. Sandanger, B. Czujko, E. D. Pedersen, J. H. Aarseth, and K. M. Myhr. Depression and anxiety amongst multiple sclerosis patients. *Eur J Neurol*, 15(3):239–45, 2008.

M. J. Chao, B. M. Herrera, S. V. Ramagopalan, G. Deluca, L. Handunetthi, S. M. Orton, M. R. Lincoln, A. D. Sadovnick, and G. C. Ebers. Parent-of-origin effects at the major histocompatibility complex in multiple sclerosis. *Hum Mol Genet*, 19(18):3679–89, 2010.

M. J. Chao, S. V. Ramagopalan, B. M. Herrera, S. M. Orton, L. Handunnetthi, M. R. Lincoln, D. A. Dyment, A. D. Sadovnick, and G. C. Ebers. Mhc transmission: insights into gender bias in ms susceptibility. *Neurology*, 76(3):242–6, 2011.

Jeffrey A Cohen, Frederik Barkhof, Giancarlo Comi, Hans-Peter Hartung, Bhupendra O Khatri, Xavier Montalban, Jean Pelletier, Ruggero Capra, Paolo Gallo, Guillermo Izquierdo, Klaus Tiel-Wilck, Ana de Vera, James Jin, Tracy Stites, Stacy Wu, Shreeram Aradhye, Ludwig Kappos, and TRANSFORMS Study Group. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *New England Journal of Medicine*, 362(5):402–415, February 2010.

C. Confavreux, M. Hutchinson, M. M. Hours, P. Cortinovis-Tourniaire, and T. Moreau. Rate of pregnancy-related relapse in multiple sclerosis. pregnancy in multiple sclerosis group. *N Engl J Med*, 339(5):285–91, 1998.

J. Correale and M. F. Farez. The impact of parasite infections on the course of multiple sclerosis. *J Neuroimmunol*, 233(1-2):6–11, 2011.

J. Correale, M. Fiol, and W. Gilmore. The risk of relapses in multiple sclerosis during systemic infections. *Neurology*, 67(4):652–9, 2006.

D. A. Cottrell, M. Kremenchutzky, G. P. Rice, W. J. Koopman, W. Hader, J. Baskerville, and G. C. Ebers. The natural history of multiple sclerosis: a geographically based study. 5. the clinical features and natural history of primary progressive multiple sclerosis. *Brain*, 122 (Pt 4):625–39, 1999.

B. A. Cree. Multiple sclerosis genetics. *Handb Clin Neurol*, 122:193–209, 2014.

L. de Beaucoudrey, A. Puel, O. Filipe-Santos, A. Cobat, P. Ghandil, M. Chrabieh, J. Feinberg, H. von Bernuth, A. Samarina, L. Janniere, C. Fieschi, J. L. Stephan, C. Boileau, S. Lyonnet, G. Jondeau, V. Cormier-Daire, M. Le Merrer, C. Hoarau, Y. Lebranchu, O. Lortholary, M. O. Chandesris, F. Tron, E. Gambineri, L. Bianchi, C. Rodriguez-Gallego, S. E. Zitnik, J. Vasconcelos, M. Guedes, A. B. Vitor, L. Marodi, H. Chapel, B. Reid, C. Roifman, D. Nadal, J. Reichenbach, I. Caragol, B. Z. Garty, F. Dogu, Y. Camcioglu, S. Gulle, O. Sanal, A. Fischer, L. Abel, B. Stockinger, C. Picard, and J. L.

Casanova. Mutations in stat3 and il12rb1 impair the development of human il-17-producing t cells. *J Exp Med*, 205(7):1543–50, 2008.

O. Delaneau, J. F. Zagury, and J. Marchini. Improved whole-chromosome phasing for disease and population genetic studies. *Nat Methods*, 10(1):5–6, 2013.

A. S. Dimas, A. C. Nica, S. B. Montgomery, B. E. Stranger, T. Raj, A. Buil, T. Giger, T. Lappalainen, M. Gutierrez-Arcelus, Ther Consortium Mu, M. I. McCarthy, and E. T. Dermitzakis. Sex-biased genetic effects on gene regulation in humans. *Genome Res*, 22(12):2368–75, 2012.

T. T. Duong, J. St Louis, J. J. Gilbert, F. D. Finkelman, and G. H. Strejan. Effect of anti-interferon-gamma and anti-interleukin-2 monoclonal antibody treatment on the development of actively and passively induced experimental allergic encephalomyelitis in the sjl/j mouse. *J Neuroimmunol*, 36(2-3):105–15, 1992.

G. C. Ebers and A. D. Sadovnick. The geographic distribution of multiple sclerosis: a review. *Neuroepidemiology*, 12(1):1–5, 1993.

G. C. Ebers, K. Kukay, D. E. Bulman, A. D. Sadovnick, G. Rice, C. Anderson, H. Armstrong, K. Cousin, R. B. Bell, W. Hader, D. W. Paty, S. Hashimoto, J. Oger, P. Duquette, S. Warren, T. Gray, P. O'Connor, A. Nath, A. Auty, L. Metz, G. Francis, J. E. Paulseth, T. J. Murray, W. Pryse-Phillips, R. Nelson, M. Freedman, D. Brunet, J. P. Bouchard, D. Hinds, and N. Risch. A full genome search in multiple sclerosis. *Nat Genet*, 13(4):472–6, 1996.

G. C. Ebers, A. D. Sadovnick, D. A. Dyment, I. M. Yee, C. J. Willer, and N. Risch. Parent-of-origin effect in multiple sclerosis: observations in half-siblings. *Lancet*, 363(9423):1773–4, 2004.

G Edan, D Miller, M Clanet, C Confavreux, O Lyon-Caen, C Lubetzki, B Brochet, I Berry, Y Rolland, J C Froment, E Cabanis, M T Iba-Zizen, J M Gandon, H M Lai, I Moseley, and O Sabouraud. Therapeutic effect of mitoxantrone combined with methylprednisolone in multiple sclerosis: a randomised multicentre study of active disease using MRI and clinical criteria. *Journal of neurology, neurosurgery, and psychiatry*, 62(2):112–118, February 1997.

J. S. El-Sayed Moustafa and P. Froguel. From obesity genetics to the future of personalized obesity therapy. *Nat Rev Endocrinol*, 9(7):402–13, 2013.

E. N. Fish. The x-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol*, 8(9):737–44, 2008.

E. M. Fisher, P. Beer-Romero, L. G. Brown, A. Ridley, J. A. McNeil, J. B. Lawrence, H. F. Willard, F. R. Bieber, and D. C. Page. Homologous ribosomal protein genes on the human x and y chromosomes: escape from x inactivation and possible implications for turner syndrome. *Cell*, 63(6):1205–18, 1990.

D. Furman, B. P. Hejblum, N. Simon, V. Jojic, C. L. Dekker, R. Thiebaut, R. J. Tibshirani, and M. M. Davis. Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. *Proc Natl Acad Sci U S A*, 111(2):869–74, 2014.

S. Giatti, R. Rigolio, S. Romano, N. Mitro, B. Viviani, G. Cavaletti, D. Caruso, L. M. Garcia-Segura, and R. C. Melcangi. Dihydrotestosterone as a protective agent in chronic experimental autoimmune encephalomyelitis. *Neuroendocrinology*, 101(4):296–308, 2015.

Gavin Giovannoni, Giancarlo Comi, Stuart Cook, Kottil Rammohan, Peter Rieckmann, Per Soelberg Sørensen, Patrick Vermersch, Peter Chang, Anthony Hamlett, Bruno Musch, Steven J Greenberg, and CLARITY Study Group. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. *New England Journal of Medicine*, 362(5):416–426, February 2010.

Ralf Gold, Ludwig Kappos, Douglas L Arnold, Amit Bar-Or, Gavin Giovannoni, Krzysztof Selmaj, Carlo Tornatore, Marianne T Sweetser, Minhua Yang, Sarah I Sheikh, and Katherine T Dawson. Placebo-Controlled Phase 3 Study of Oral BG-12 for Relapsing Multiple Sclerosis. *New England Journal of Medicine*, 367 (12):1098–1107, September 2012.

S. M. Gold and R. R. Voskuhl. Estrogen treatment in multiple sclerosis. *J Neurol Sci*, 286(1-2):99–103, 2009.

S. M. Gold, S. Chalifoux, B. S. Giesser, and R. R. Voskuhl. Immune modulation and increased neurotrophic factor production in multiple sclerosis patients treated with testosterone. *J Neuroinflammation*, 5:32, 2008.

E. Gracey, Y. Yao, B. Green, Z. Qaiyum, Y. Baglaenko, A. Lin, A. Anton, R. Ayearst, P. Yip, and R. D. Inman. Sexual dimorphism in the th17 signature of ankylosing spondylitis. *Arthritis Rheumatol*, 68(3):679–89, 2016.

J. M. Greer and P. A. McCombe. Role of gender in multiple sclerosis: clinical effects and potential molecular mechanisms. *J Neuroimmunol*, 234(1-2):7–18, 2011.

D. Haghmorad, A. A. Amini, M. B. Mahmoudi, M. Rastin, M. Hosseini, and M. Mahmoudi. Pregnancy level of estrogen attenuates experimental autoimmune encephalomyelitis in both ovariectomized and pregnant c57bl/6 mice through expansion of treg and th2 cells. *J Neuroimmunol*, 277(1-2):85–95, 2014.

A. E. Handel, A. J. Williamson, G. Disanto, L. Handunnetthi, G. Giovannoni, and S. V. Ramagopalan. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. *PLoS One*, 5(9), 2010.

H. P. Hartung, O. Aktas, and A. N. Boyko. Alemtuzumab: a new therapy for active relapsing-remitting multiple sclerosis. *Mult Scler*, 21(1):22–34, 2015.

S Hauser and D Goodin. Multiple sclerosis and other demyelinating diseases, 2012.

B. N. Howie, P. Donnelly, and J. Marchini. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet*, 5(6):e1000529, 2009.

W. Huang da, B. T. Sherman, and R. A. Lempicki. Systematic and integrative analysis of large gene lists using david bioinformatics resources. *Nat Protoc*, 4 (1):44–57, 2009a.

W. Huang da, B. T. Sherman, and R. A. Lempicki. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*, 37(1):1–13, 2009b.

G. C. Hughes, S. Thomas, C. Li, M. K. Kaja, and E. A. Clark. Cutting edge: progesterone regulates ifn-alpha production by plasmacytoid dendritic cells. *J Immunol*, 180(4):2029–33, 2008.

G. C. Hughes, E. A. Clark, and A. H. Wong. The intracellular progesterone receptor regulates cd4+ t cells and t cell-dependent antibody responses. *J Leukoc Biol*, 93(3):369–75, 2013.

International M.H.C. and Autoimmunity Genetics Network (IMAGEN), J. D. Rioux, P. Goyette, T. J. Vyse, L. Hammarstrom, M. M. Fernando, T. Green, P. L. De Jager, S. Foisy, J. Wang, P. I. de Bakker, S. Leslie, G. McVean, L. Padyukov, L. Alfredsson, V. Annese, D. A. Hafler, Q. Pan-Hammarstrom, R. Matell, S. J. Sawcer, A. D. Compston, B. A. Cree, D. B. Mirel, M. J. Daly, T. W. Behrens, L. Klareskog, P. K. Gregersen, J. R. Oksenberg, and S. L. Hauser. Mapping of multiple susceptibility variants within the mhc region for 7 immune-mediated diseases. *Proc Natl Acad Sci U S A*, 106(44):18680–5, 2009.

International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium, S. Sawcer, G. Hellenthal, M. Pirinen, C. C. Spencer, N. A. Patsopoulos, L. Moutsianas, A. Dilthey, Z. Su, C. Freeman, S. E. Hunt, S. Edkins, E. Gray, D. R. Booth, S. C. Potter, A. Goris, G. Band, A. B. Oturai, A. Strange, J. Saarela, C. Bellenguez, B. Fontaine, M. Gillman, B. Hemmer, R. Gwilliam, F. Zipp, A. Jayakumar, R. Martin, S. Leslie, S. Hawkins, E. Giannoulatou, S. D'Alfonso, H. Blackburn, F. Martinelli Boneschi, J. Liddle, H. F. Harbo, M. L. Perez, A. Spurkland, M. J. Waller, M. P. Mycko, M. Ricketts, M. Comabella, N. Hammond, I. Kockum, O. T. McCann, M. Ban, P. Whittaker, A. Kemppinen, P. Weston, C. Hawkins, S. Widaa, J. Zajicek, S. Dronov, N. Robertson, S. J. Bumpstead, L. F. Barcellos, R. Ravindrarajah, R. Abraham, L. Alfredsson, K. Ardlie, C. Aubin, A. Baker, K. Baker, S. E. Baranzini, L. Bergamaschi, R. Bergamaschi, A. Bernstein, A. Berthele, M. Boggild, J. P. Bradfield, D. Brassat, S. A. Broadley, D. Buck, H. Butzkueven, R. Capra, W. M. Carroll, P. Cavalla, E. G. Celius, S. Cepok, R. Chiavacci, F. Clerget-Darpoux, K. Clysters, G. Comi, M. Cossburn, I. Cournu-Rebeix, M. B. Cox, W. Cozen, B. A. Cree, A. H. Cross, D. Cusi, M. J. Daly, E. Davis, P. I. de Bakker, M. Debouverie, B. D'Hooghe M, K. Dixon, R. Dobosi, B. Dubois, D. Ellinghaus, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*, 476(7359):214–9, 2011.

International Multiple Sclerosis Genetics Consortium, A. H. Beecham, N. A. Patsopoulos, D. K. Xifara, M. F. Davis, A. Kemppinen, C. Cotsapas, T. S. Shah, C. Spencer, D. Booth, A. Goris, A. Oturai, J. Saarela, B. Fontaine, B. Hemmer, C. Martin, F. Zipp, S. D'Alfonso, F. Martinelli-Boneschi, B. Taylor, H. F. Harbo, I. Kockum, J. Hillert, T. Olsson, M. Ban, J. R. Oksenberg, R. Hintzen, L. F. Barcellos, Consortium Wellcome Trust Case Control, I. B. D. Genetics Consortium International, C. Agliardi, L. Alfredsson, M. Alizadeh, C. Anderson, R. Andrews, H. B. Sondergaard, A. Baker, G. Band, S. E. Baranzini, N. Barizzone, J. Barrett, C. Bellenguez, L. Bergamaschi, L. Bernardinelli, A. Berthele, V. Biberacher, T. M. Binder, H. Blackburn, I. L. Bomfim, P. Brambilla, S. Broadley, B. Brochet, L. Brundin, D. Buck, H. Butzkueven, S. J. Caillier, W. Camu, W. Carpentier, P. Cavalla, E. G. Celius, I. Coman, G. Comi, L. Corrado, L. Cosemans, I. Cournu-Rebeix, B. A. Cree, D. Cusi, V. Damotte, G. Defer, S. R. Delgado, P. Deloukas, A. di Sapio, A. T. Dilthey, P. Donnelly, B. Dubois, M. Duddy, S. Edkins, I. Elovaara, F. Esposito, N. Evangelou, B. Fiddes, J. Field, A. Franke, C. Freeman, I. Y. Frohlich, D. Galimberti, C. Gieger, P. A. Gourraud, C. Graetz, A. Graham, V. Grummel, C. Guaschino, A. Hadjixenofontos, H. Hakonarson, C. Halfpenny, G. Hall, P. Hall, A. Hamsten, J. Harley, T. Harrower, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet*, 45(11):1353–60,

2013.

R. Jansen, S. Batista, A. I. Brooks, J. A. Tischfield, G. Willemsen, G. van Grootheest, J. J. Hottenga, Y. Milaneschi, H. Mbarek, V. Madar, W. Peyrot, J. M. Vink, C. L. Verweij, E. J. de Geus, J. H. Smit, F. A. Wright, P. F. Sullivan, D. I. Boomsma, and B. W. Penninx. Sex differences in the human peripheral blood transcriptome. *BMC Genomics*, 15:33, 2014.

K P Johnson, B R Brooks, C C Ford, A Goodman, J Guarnaccia, R P Lisak, L W Myers, H S Panitch, A Pruitt, J W Rose, N Kachuck, and J S Wolinsky. Sustained clinical benefits of glatiramer acetate in relapsing multiple sclerosis patients observed for 6 years. Copolymer 1 Multiple Sclerosis Study Group. *Mult Scler*, 6(4):255–266, August 2000.

K. T. Jorgensen, K. Rostgaard, I. Bache, R. J. Biggar, N. M. Nielsen, N. Tommerup, and M. Frisch. Autoimmune diseases in women with turner's syndrome. *Arthritis Rheum*, 62(3):658–66, 2010.

O. H. Kantarci, L. F. Barcellos, E. J. Atkinson, P. P. Ramsay, R. Lincoln, S. J. Achenbach, M. De Andrade, S. L. Hauser, and B. G. Weinshenker. Men transmit ms more often to their children vs women: the carter effect. *Neurology*, 67 (2):305–10, 2006.

L. Kappos, D. Li, P. A. Calabresi, P. O'Connor, A. Bar-Or, F. Barkhof, M. Yin, D. Leppert, R. Glanzman, J. Tinbergen, and S. L. Hauser. Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. *Lancet*, 378(9805):1779–87, 2011.

Ludwig Kappos, Ernst-Wilhelm Radue, Paul O'Connor, Chris Polman, Reinhard Hohlfeld, Peter Calabresi, Krzysztof Selmaj, Catherine Agoropoulou, Malgorzata Leyk, Lixin Zhang-Auberson, Pascale Burtin, and FREEDOMS Study Group. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *New England Journal of Medicine*, 362(5):387–401, February 2010.

R. Khalid. Contributing factors in multiple sclerosis and the female sex bias. *Immunol Lett*, 162(1 Pt A):223–32, 2014.

E. Kingwell, F. Zhu, R. A. Marrie, J. D. Fisk, C. Wolfson, S. Warren, J. Profetto-McGrath, L. W. Svenson, N. Jette, V. Bhan, B. N. Yu, L. Elliott, and H. Tremlett. High incidence and increasing prevalence of multiple sclerosis in british columbia, canada: findings from over two decades (1991-2010). *J Neurol*, 262 (10):2352–63, 2015.

G. P. Knudsen, H. F. Harbo, C. Smestad, E. G. Celius, E. Akesson, A. Oturai, L. P. Ryder, A. Spurkland, and K. H. Orstavik. X chromosome inactivation in females with multiple sclerosis. *Eur J Neurol*, 14(12):1392–6, 2007.

N. Koch-Henriksen and P. S. Sorensen. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol*, 9(5):520–32, 2010.

H. L. Lang, H. Jacobsen, S. Ikemizu, C. Andersson, K. Harlos, L. Madsen, P. Hjorth, L. Sondergaard, A. Svejgaard, K. Wucherpfennig, D. I. Stuart, J. I. Bell, E. Y. Jones, and L. Fugger. A functional and structural basis for tcr cross-reactivity in multiple sclerosis. *Nat Immunol*, 3(10):940–3, 2002.

B. Levine, M. Packer, and P. Codogno. Development of autophagy inducers in clinical medicine. *J Clin Invest*, 125(1):14–24, 2015.

C. Libert, L. Dejager, and I. Pinheiro. The x chromosome in immune functions: when a chromosome makes the difference. *Nat Rev Immunol*, 10(8):594–604, 2010.

X. Lin, F. Y. Deng, X. B. Mo, L. F. Wu, and S. F. Lei. Functional relevance for multiple sclerosis-associated genetic variants. *Immunogenetics*, 67(1):7–14, 2015.

F. D. Lublin, S. C. Reingold, J. A. Cohen, G. R. Cutter, P. S. Sorensen, A. J. Thompson, J. S. Wolinsky, L. J. Balcer, B. Banwell, F. Barkhof, Jr. Bebo, B., P. A. Calabresi, M. Clanet, G. Comi, R. J. Fox, M. S. Freedman, A. D. Goodman, M. Inglese, L. Kappos, B. C. Kieseier, J. A. Lincoln, C. Lubetzki, A. E. Miller, X. Montalban, P. W. O'Connor, J. Petkau, C. Pozzilli, R. A. Rudick, M. P. Sormani, O. Stuve, E. Waubant, and C. H. Polman. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology*, 83(3): 278–86, 2014.

R. Magi, C. M. Lindgren, and A. P. Morris. Meta-analysis of sex-specific genome-wide association studies. *Genet Epidemiol*, 34(8):846–53, 2010.

A. Mantovani, M. A. Cassatella, C. Costantini, and S. Jaillon. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol*, 11(8):519–31, 2011.

J. Marchini and B. Howie. Genotype imputation for genome-wide association studies. *Nat Rev Genet*, 11(7):499–511, 2010.

J. Marchini, B. Howie, S. Myers, G. McVean, and P. Donnelly. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet*, 39(7):906–13, 2007.

Luca Massacesi, Irene Tramacere, Salvatore Amoroso, Mario A Battaglia, Maria Donata Benedetti, Graziella Filippini, Loredana La Mantia, Anna Repice, Alessandra Solari, Gioacchino Tedeschi, and Clara Milanese. Azathioprine versus Beta Interferons for Relapsing-Remitting Multiple Sclerosis: A Multicentre Randomized Non-Inferiority Trial. *PLOS ONE*, 9(11):e113371–13, November 2014.

M. D. Mayes, L. Bossini-Castillo, O. Gorlova, J. E. Martin, X. Zhou, W. V. Chen, S. Assassi, J. Ying, F. K. Tan, F. C. Arnett, J. D. Reveille, S. Guerra, M. Teruel, F. D. Carmona, P. K. Gregersen, A. T. Lee, E. Lopez-Isac, E. Ochoa, P. Carreira, C. P. Simeon, I. Castellvi, M. A. Gonzalez-Gay, Group Spanish Scleroderma, A. Zhernakova, L. Padyukov, M. Alarcon-Riquelme, C. Wijmenga, M. Brown, L. Beretta, G. Riemekasten, T. Witte, N. Hunzelmann, A. Kreuter, J. H. Distler, A. E. Voskuyl, A. J. Schuerwegh, R. Hesselstrand, A. Nordin, P. Airo, C. Lunardi, P. Shiels, J. M. van Laar, A. Herrick, J. Worthington, C. Denton, F. M. Wigley, L. K. Hummers, J. Varga, M. E. Hinchcliff, M. Baron, M. Hudson, J. E. Pope, D. E. Furst, D. Khanna, K. Phillips, E. Schiopu, B. M. Segal, J. A. Molitor, R. M. Silver, V. D. Steen, R. W. Simms, R. A. Lafyatis, B. J. Fessler, T. M. Frech, F. Alkassab, P. Docherty, E. Kaminska, N. Khalidi, H. N. Jones, J. Markland, D. Robinson, J. Broen, T. R. Radstake, C. Fonseca, B. P. Koeleman, and J. Martin. Immunochip analysis identifies multiple susceptibility loci for systemic sclerosis. *Am J Hum Genet*, 94(1):47–61, 2014.

P. McCrone, M. Heslin, M. Knapp, P. Bull, and A. Thompson. Multiple sclerosis in the uk: service use, costs, quality of life and disability. *Pharmacoeconomics*, 26(10):847–60, 2008.

D. P. McGovern, S. Kugathasan, and J. H. Cho. Genetics of inflammatory bowel diseases. *Gastroenterology*, 149(5):1163–1176 e2, 2015.

N M Milligan, R Newcombe, and D A Compston. A double-blind controlled trial of high dose methylprednisolone in patients with multiple sclerosis: 1. Clinical effects. *Journal of neurology, neurosurgery, and psychiatry*, 50(5):511–516, May 1987.

L. Moutsianas, L. Jostins, A. H. Beecham, A. T. Dilthey, D. K. Xifara, M. Ban, T. S. Shah, N. A. Patsopoulos, L. Alfredsson, C. A. Anderson, K. E. Attfield, S. E. Baranzini, J. Barrett, T. M. Binder, D. Booth, D. Buck, E. G. Celius, C. Cotsapas, S. D'Alfonso, C. A. Dendrou, P. Donnelly, B. Dubois, B. Fontaine, L. Lar Fugger, A. Goris, P. A. Gourraud, C. Graetz, B. Hemmer, J. Hillert, I. B. D. Genetics Consortium International, I. Kockum, S. Leslie, C. M. Lill, F. Martinelli-Boneschi, J. R. Oksenberg, T. Olsson, A. Oturai, J. Saarela, H. B. Sondergaard, A. Spurkland, B. Taylor, J. Winkelmann, F. Zipp, J. L. Haines,

M. A. Pericak-Vance, C. C. Spencer, G. Stewart, D. A. Hafler, A. J. Ivinson, H. F. Harbo, S. L. Hauser, P. L. De Jager, A. Compston, J. L. McCauley, S. Sawcer, G. McVean, and Consortium International Multiple Sclerosis Genetics. Class ii hla interactions modulate genetic risk for multiple sclerosis. *Nat Genet*, 47(10):1107–13, 2015.

M. Naegele, K. Tillack, S. Reinhardt, S. Schippling, R. Martin, and M. Sospedra. Neutrophils in multiple sclerosis are characterized by a primed phenotype. *J Neuroimmunol*, 242(1-2):60–71, 2012.

M. Noack and P. Miossec. Th17 and regulatory t cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev*, 13(6):668–77, 2014.

C. Ober, D. A. Loisel, and Y. Gilad. Sex-specific genetic architecture of human disease. *Nat Rev Genet*, 9(12):911–22, 2008.

Paul O'Connor, Jerry S Wolinsky, Christian Confavreux, Giancarlo Comi, Ludwig Kappos, Tomas P Olsson, Hadj Benzerdjeb, Philippe Truffinet, Lin Wang, Aaron Miller, Mark S Freedman, and TEMSO Trial Group. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. *New England Journal of Medicine*, 365(14):1293–1303, October 2011.

S. Oertelt-Prigione. The influence of sex and gender on the immune response. *Autoimmun Rev*, 11(6-7):A479–85, 2012.

S. M. Orton, B. M. Herrera, I. M. Yee, W. Valdar, S. V. Ramagopalan, A. D. Sadovnick, G. C. Ebers, and Group Canadian Collaborative Study. Sex ratio of multiple sclerosis in canada: a longitudinal study. *Lancet Neurol*, 5(11):932–6, 2006.

A. Palanichamy, L. Apeltsin, T. C. Kuo, M. Sirota, S. Wang, S. J. Pitts, P. D. Sundar, D. Telman, L. Z. Zhao, M. Derstine, A. Abounasr, S. L. Hauser, and H. C. von Budingen. Immunoglobulin class-switched b cells form an active immune axis between cns and periphery in multiple sclerosis. *Sci Transl Med*, 6(248):248ra106, 2014.

K. M. Palaszynski, H. Liu, K. K. Loo, and R. R. Voskuhl. Estriol treatment ameliorates disease in males with experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *J Neuroimmunol*, 149(1-2):84–9, 2004a.

K. M. Palaszynski, K. K. Loo, J. F. Ashouri, H. B. Liu, and R. R. Voskuhl. Androgens are protective in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *J Neuroimmunol*, 146(1-2):144–52, 2004b.

H. S. Panitch, R. L. Hirsch, A. S. Haley, and K. P. Johnson. Exacerbations of multiple sclerosis in patients treated with gamma interferon. *Lancet*, 1(8538): 893–5, 1987.

T. L. Papenfuss, C. J. Rogers, I. Gienapp, M. Yurrita, M. McClain, N. Damico, J. Valo, F. Song, and C. C. Whitacre. Sex differences in experimental autoimmune encephalomyelitis in multiple murine strains. *J Neuroimmunol*, 150(1-2): 59–69, 2004.

T. L. Papenfuss, N. D. Powell, M. A. McClain, A. Bedarf, A. Singh, I. E. Gienapp, T. Shawler, and C. C. Whitacre. Estriol generates tolerogenic dendritic cells in vivo that protect against autoimmunity. *J Immunol*, 186(6):3346–55, 2011.

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

D W Paty and D K Li. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. *Neurology*, 43(4):662–667, April 1993.

M. J. Polanczyk, B. D. Carson, S. Subramanian, M. Afentoulis, A. A. Vandenbark, S. F. Ziegler, and H. Offner. Cutting edge: estrogen drives expansion of the cd4+cd25+ regulatory t cell compartment. *J Immunol*, 173(4):2227–30, 2004.

Chris H Polman, Paul W O'Connor, Eva Havrdova, Michael Hutchinson, Ludwig Kappos, David H Miller, J Theodore Phillips, Fred D Lublin, Gavin Giovannoni, Andrzej Wajgt, Martin Toal, Frances Lynn, Michael A Panzara, Alfred W Sandrock, and AFFIRM Investigators. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *New England Journal of Medicine*, 354(9):899–910, March 2006.

C. Pozzilli, P. Falaschi, C. Mainero, A. Martocchia, R. D'Urso, A. Proietti, M. Frontoni, S. Bastianello, and M. Filippi. Mri in multiple sclerosis during the menstrual cycle: relationship with sex hormone patterns. *Neurology*, 53(3): 622–4, 1999.

A. L. Price, N. J. Patterson, R. M. Plenge, M. E. Weinblatt, N. A. Shadick, and D. Reich. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*, 38(8):904–9, 2006.

A. L. Price, M. E. Weale, N. Patterson, S. R. Myers, A. C. Need, K. V. Shianna, D. Ge, J. I. Rotter, E. Torres, K. D. Taylor, D. B. Goldstein, and D. Reich.

Long-range ld can confound genome scans in admixed populations. *Am J Hum Genet*, 83(1):132–5; author reply 135–9, 2008.

M. Pugliatti, G. Rosati, H. Carton, T. Riise, J. Drulovic, L. Vecsei, and I. Milanov. The epidemiology of multiple sclerosis in europe. *Eur J Neurol*, 13(7):700–22, 2006.

S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. de Bakker, M. J. Daly, and P. C. Sham. Plink: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*, 81(3):559–75, 2007.

S. Sawcer, R. J. Franklin, and M. Ban. Multiple sclerosis genetics. *Lancet Neurol*, 13(7):700–9, 2014.

C. Seillet, S. Laffont, F. Tremollieres, N. Rouquie, C. Ribot, J. F. Arnal, V. Douin-Echinard, P. Gourdy, and J. C. Guery. The tlr-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor alpha signaling. *Blood*, 119(2):454–64, 2012.

N. L. Sicotte, S. M. Liva, R. Klutch, P. Pfeiffer, S. Bouvier, S. Odesa, T. C. Wu, and R. R. Voskuhl. Treatment of multiple sclerosis with the pregnancy hormone estriol. *Ann Neurol*, 52(4):421–8, 2002.

Y. A. Sigidin, G. V. Loukina, B. Skurkovich, and S. Skurkovich. Randomized, double-blind trial of anti-interferon-gamma antibodies in rheumatoid arthritis. *Scand J Rheumatol*, 30(4):203–7, 2001.

D. L. Smith-Bouvier, A. A. Divekar, M. Sasidhar, S. Du, S. K. Tiwari-Woodruff, J. K. King, A. P. Arnold, R. R. Singh, and R. R. Voskuhl. A role for sex chromosome complement in the female bias in autoimmune disease. *J Exp Med*, 205(5):1099–108, 2008.

M. Sospedra and R. Martin. Immunology of multiple sclerosis. *Annu Rev Immunol*, 23:683–747, 2005.

L. Steinman. Immunology of relapse and remission in multiple sclerosis. *Annu Rev Immunol*, 32:257–81, 2014.

M. Stephens and D. J. Balding. Bayesian statistical methods for genetic association studies. *Nat Rev Genet*, 10(10):681–90, 2009.

S. Tiwari-Woodruff, L. B. Morales, R. Lee, and R. R. Voskuhl. Differential neuroprotective and antiinflammatory effects of estrogen receptor (er)alpha and erbeta ligand treatment. *Proc Natl Acad Sci U S A*, 104(37):14813–8, 2007.

C. Wang, Y. Cheng, T. Liu, Q. Li, R. B. Fillingim, M. R. Wallace, R. Staud, L. Kaplan, and R. Wu. A computational model for sex-specific genetic architecture of complex traits in humans: implications for mapping pain sensitivity. *Mol Pain*, 4:13, 2008.

T. C. Washburn, Jr. Medearis, D. N., and B. Childs. Sex differences in susceptibility to infections. *Pediatrics*, 35:57–64, 1965.

C. T. Watson, G. Disanto, F. Breden, G. Giovannoni, and S. V. Ramagopalan. Estimating the proportion of variation in susceptibility to multiple sclerosis captured by common snps. *Sci Rep*, 2:770, 2012.

B G Weinshenker, P C O'Brien, T M Petterson, J H Noseworthy, C F Lucchinetti, D W Dodick, A A Pineda, L N Stevens, and M Rodriguez. A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease. *Ann Neurol*, 46(6):878–886, December 1999.

A. Winkelmann, M. Loebermann, E. C. Reisinger, H. P. Hartung, and U. K. Zettl. Disease-modifying therapies and infectious risks in multiple sclerosis. *Nat Rev Neurol*, 12(4):217–33, 2016.

A. J. Wisdom, Y. Cao, N. Itoh, R. D. Spence, and R. R. Voskuhl. Estrogen receptor-beta ligand treatment after disease onset is neuroprotective in the multiple sclerosis model. *J Neurosci Res*, 91(7):901–8, 2013.

World Health Organization. Atlas multiple sclerosis resources in the world, 2008.

J. Yang, S. H. Lee, M. E. Goddard, and P. M. Visscher. Gcta: a tool for genome-wide complex trait analysis. *Am J Hum Genet*, 88(1):76–82, 2011.

C. Yao, R. Joehanes, A. D. Johnson, T. Huan, T. Esko, S. Ying, J. E. Freedman, J. Murabito, K. L. Lunetta, A. Metspalu, P. J. Munson, and D. Levy. Sex- and age-interacting eqtls in human complex diseases. *Hum Mol Genet*, 23(7): 1947–56, 2014.

F. F. Zhang, R. Cardarelli, J. Carroll, K. G. Fulda, M. Kaur, K. Gonzalez, J. K. Vishwanatha, R. M. Santella, and A. Morabia. Significant differences in global genomic dna methylation by gender and race/ethnicity in peripheral blood. *Epigenetics*, 6(5):623–9, 2011.

A. Zorgdrager and J. De Keyser. Menstrually related worsening of symptoms in multiple sclerosis. *J Neurol Sci*, 149(1):95–7, 1997.