MHC transmission

Insights into gender bias in MS susceptibility

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ABSTRACT

Objective: Major histocompatibility complex (MHC) genes dominate genetic susceptibility factors in multiple sclerosis (MS). Given the general consensus that incidence and prevalence of MS has been rising and specifically in women, we evaluated MHC–gender interactions.

Methods: In a large family-based cohort consisting of 7,093 individuals (2,127 affected individuals) from 1,055 MS families, we examined MHC transmission by family structure and gender stratified by genetic distance of affected relatives from the MS proband.

Results: We found that affected individuals with *HLA-DRB1*15*-positive genotypes have higher female-to-male ratios as compared with affected individuals with *HLA-DRB1*15*-negative genotypes ($\chi^2 = 9.97$, $p = 0.0015$) with the exception of multiplex families with 3 or more affected across 2 generations. Transmission disequilibrium test results show that *HLA-DRB1*15* transmission was more distorted in collateral families vs nuclear families ($\chi^2 = 8.030$, $p = 0.0046$), exclusively in affected female-female pairs ($\chi^2 = 7.81$, $p = 0.0051$), but not in mixed gender pairs ($\chi^2 = 1.58$, $p = 0.21$) or matched male pairs (Fisher $p = 0.21$).

Conclusions: These observations implicate the MHC as the site of interactions and modifications mediating the female-to-male gender ratio in MS and its progressive increase. They further suggest this occurs via gene–environment interactions and epigenetic modifications in this region. The difference between collateral and nuclear families provides some insight into the inheritance, decay, and gender specificity of putative epigenetic marks. **Neurology® 2011;76:242–246**

GLOSSARY

ASP = affected sibling pair; **AUNN** = aunt/uncle–niece/nephew; **HLA** = human leukocyte antigen; **MHC** = major histocompatibility complex; **MPX** = multiplex; **MS** = multiple sclerosis; **MZ** = monozygotic; **PC** = parent-child; **SNP** = single nucleotide polymorphism; **TDT** = transmission disequilibrium test.

The etiology of multiple sclerosis (MS) remains unclear; however, several lines of evidence suggest that MS is an acquired autoimmune disease and is triggered by environmental factors in genetically susceptible individuals.[1]

The main genetic contribution in MS comes from the major histocompatibility complex (MHC), and more specifically the human leukocyte antigen (HLA) Class II genes.[2,3] Additional epistatic interactions and suppressor effects have also been observed within this locus.[4,5]

There is general consensus that the incidence and prevalence of MS has been rising with an increased penetrance among women,[6] and excess monozygotic (MZ) twins concordance for MS is almost entirely female specific.[7] Moreover, there is a maternal parent-of-origin effect[8] with higher number of affected mother-daughter pairs and few father-son pairs.[9]

There is little known about how the MHC might interact with the observed gender bias in MS. Since HLA alleles carry the strongest genetic susceptibility factors, we reasoned that the reported gender predisposition could be MHC-mediated.[10] Here we have used a large family-

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based cohort containing a range of relative pairs concordant for MS, which are characterized by their genetic distance from the MS proband. These affected relative pairs share a common genetic background, and are more homogeneous regarding exposure to environmental factors, which loom large in MS risk. They can also be differentiated by gender combinations that are either matching (female-female or male-male) or mixed (female-male), which are useful for dissecting gender-specific effects in MS susceptibility.

METHODS

Subjects. We selected a large family-based cohort (family n = 1,055, total individual n = 7,093, affected individual n = 2,127) consisting of 6 different types of MS families (based on their family structure and affected relatives of MS proband), for which the methodology has been described previously.[11] The 6 different types of MS families included affected sibling pair (ASP: family n = 358, total individual n = 2,935, affected individual n = 716), parent-child (PC: family n = 172, total individual n = 806, affected individual n = 344), sporadic (family n = 73, total individual n = 171, affected individual n = 73), aunt/uncle–niece/nephew (AUNN: family n = 217, total individual n = 1,671, affected individual n = 434), cousin (family n = 198, total individual n = 1,202, affected individual n = 396), and multiplex (MPX: family n = 37, total individual n = 308, affected individual n = 164). All families were Canadian and of European descent.

Parents, children, and full siblings share approximately 50% of their genes and are referred to as first-degree relatives. Aunts, uncles, nieces, and nephews share, on average, 25% of their genes and are referred to as second-degree relatives. First cousins share around 12.5% of their genes and are referred to as third-degree relatives. In this study, the selected families can be categorized into 2 types by the affected relative of the MS proband. They include nuclear families (n = 603) with affected first-degree relatives consisting of ASP, PC, and sporadic families, and collateral families (n = 452) with affected second- and third-degree relatives consisting of AUNN, cousin, and MPX families.

Nuclear (n = 530) and collateral (n = 415) families can be further stratified by the gender combinations of affected relative pairs; however, because of the family structure of sporadic and MPX families, they were excluded. Female-female pairs include affected daughter-daughter pairs in ASP families, affected mother-daughter pairs in PC families, affected aunt-niece pairs in AUNN families, and affected female-female cousins. Female-male pairs consist of affected daughter-son pairs in ASP families, affected mother-son pairs and father-daughter pairs in PC families, affected aunt-nephew pairs and uncle-niece pairs in AUNN families, and affected female-male cousins. Male-male pairs include affected son-son pairs in ASP families, affected father-son pairs in PC families, affected uncle-nephew pairs in AUNN families, and affected male-male cousins.

Table 1 Gender ratios of affected offspring with MS positive and negative for *HLA-DRB1*15* from 6 different types of MS families

MS families (n = 1,055)	*HLA-DRB1*15*-positive genotype			*HLA-DRB1*15*-negative genotype		
	Female	Male	Female:male	Female	Male	Female:male
ASP (n = 358)	309	103	3.00	205	99	2.070
PC (n = 172)	158	49	3.22	103	34	3.03
Sporadic (n = 73)	36	7	5.14	21	9	2.33
AUNN (n = 217)	187	52	3.60	138	57	2.42
Cousin (n = 198)	159	49	3.24	125	63	1.98
MPX (n = 37)	70	42	1.67	34	18	1.89

Abbreviations: ASP = affected sibling pair; AUNN = aunt/uncle–niece/nephew; HLA = human leukocyte antigen; MPX = multiplex; MS = multiple sclerosis; PC = parent-child.

Standard protocol approvals, registrations, and patient consents. This study obtained ethical approval from the relevant institutional review boards and written informed consent was received from all subjects.

HLA typing. Genotyping of *HLA-DRB1* was performed using either low- or high-resolution allele-specific PCR amplification method.[4]

Statistical methods. The family pedigrees were first tested using the PEDCHECK program for the presence of any inconsistencies in Mendelian transmission.[12] Transmission disequilibrium test (TDT) was performed using the TDTPHASE program of the UNPHASED software package.[13]

RESULTS

Gender ratio analysis. The gender ratio results of affected individuals either positive or negative for *HLA-DRB1*15* are presented in table 1. The total ratio for all 6 types of MS families show that of cases with *HLA-DRB1*15*-positive genotypes, 919 were female and 302 were male (female:male = 3.04). Of cases with *HLA-DRB1*15*-negative genotypes, 626 were female and 280 were male (female:male = 2.24). The female-to-male ratio is higher in cases with *HLA-DRB1*15* vs those without (χ^2 = 9.97, p = 0.0015).

***HLA-DRB1*15* transmission.** TDT analyses of *HLA-DRB1*15* in nuclear (ASP, PC, and sporadic) and collateral (AUNN, cousin, and MPX) families are presented in table 2. The combined transmission of *HLA-DRB1*15* in nuclear families was 497 times transmitted and 222 times not transmitted (χ^2 = 94.22, $p = 2.82 \times 10^{-22}$). The pooled transmission of *HLA-DRB1*15* in collateral families was 263 times transmitted and 81 times not transmitted (χ^2 = 96.29, $p = 9.92 \times 10^{-23}$). Transmission of *HLA-DRB1*15* was more distorted in collateral families vs nuclear families (χ^2 = 6.27, p = 0.012).

***HLA-DRB1* transmissions (from *HLA-DRB1*15*-negative parents).** To remove the confounding effect of the dominant *HLA-DRB1*15* association, which results in secondary undertransmissions of other *HLA-DRB1* alleles from the heterozygous parents, transmissions of other *HLA-DRB1* alleles were examined from parents negative for *HLA-DRB1*15*. Because of the rarer nature of some of the *HLA-DRB1* alleles in comparison with the more frequent *HLA-*

Table 2 TDT of *HLA-DRB1*15* in nuclear (ASP, PC, and sporadic: n = 603) and collateral (AUNN, cousin, and MPX: n = 452) families

	TR	NT	OR	χ^2	*p*
Nuclear families					
ASP (n = 358)	356	172	2.10	65.49	5.85×10^{-16}
PC (n = 172)	81	34	2.38	19.78	8.68×10^{-06}
Sporadic (n = 73)	42	16	2.63	12.08	0.00051
Total (n = 603)	497	222	2.16	94.22	2.82×10^{-22}
Collateral families					
AUNN (n = 217)	118	29	4.07	57.78	2.93×10^{-14}
Cousin (n = 198)	100	36	2.78	31.34	2.16×10^{-08}
MPX (n = 37)	45	16	2.81	14.36	0.00015
Total (n = 452)	263	81	3.25	96.29	9.92×10^{-23}

Abbreviations: ASP = affected sibling pair; AUNN = aunt/uncle–niece/nephew; HLA = human leukocyte antigen; MPX = multiplex; NT = nontransmitted; OR = odds ratio; PC = parent-child; TDT = transmission disequilibrium test; TR = transmitted.

*DRB1*15* allele, their combined transmissions in the pooled nuclear families vs pooled collateral families were presented. No significant differences in the transmissions of other *HLA-DRB1* alleles in the absence of *HLA-DRB1*15* were observed between the nuclear vs collateral families (table e-1 on the *Neurology*® Web site at www.neurology.org).

***HLA-DRB1* transmissions by gender of affected relative pairs.** The *HLA-DRB1* transmission analyses by affected relative pairs are presented in table 3. Difference of *HLA-DRB1*15* transmission was observed between nuclear vs collateral families for the total affected relative pairs ($\chi^2 = 8.030$, $p = 0.0046$). This difference was most evident in affected female-female pairs ($\chi^2 = 7.81$, $p = 0.0051$), but not in affected female-male ($\chi^2 = 1.58$, $p = 0.21$) and affected male-male pairs (Fisher $p = 0.21$). Transmissions of other *HLA-DRB1* alleles from *HLA-DRB1*15*-negative parents were also examined (table e-2).

DISCUSSION In high prevalence areas, some 20% of MS cases have at least one affected relative.[14] Risks are increased in first-degree relatives including parents, offspring, and siblings (2.0%–5.7% for relatives of female index cases; 2.5%–5.1% for relatives of male index cases), and also in distant relatives such as aunts, uncles, nieces, nephews, and cousins (1.0%–2.9% for relatives of female index cases; 1.5%–3.3% for relatives of male index cases).[14] MS risk for half-siblings who have one parent in common and share a quarter of their genome is 1.9%.[8] The risk for offspring of conjugal MS (both parents have MS) is as much as 200 times more than that of the general population,[15] while the risk for adoptees is no different from that of the general population.[16] The inheritance pattern of MS shows that PC risk (3.5%) is roughly equal to that of sibling risk (3.5%); however, the risks are diluted thereafter, with the half-sibling risk approximately half of the full-sibling risk. These observations show a pseudodominant inheritance pattern with almost no dominant variance. The rapid dilution of risk in descendants of patients with MS has suggested epigenetic marks and their subsequent decay in vertical transmission as one possible explanation.

The female-to-male ratio was near unity in North America at the turn of the 20th century,[17] and today, a ratio of 2:1 is common despite regional variations. In Canada this ratio has been increasing for at least 60 years, and now surpasses 3.2:1,[6] while in Scotland a change in sex ratio from unity to more than 3:1 has taken place since the 1950s.[18] The exact cause of this increase remains unknown, but given the short duration over which this rise occurs, genetic factors can be ruled out and environmental changes would be the likely candidate, perhaps resulting from gene–environment interactions.[6] Since HLA genes are the main genetic contributor to MS susceptibility, we hypothesized that gender-discrepant HLA-associated effects are possible. By using a large family-based cohort, we were able to address this question.

Gender ratio analysis revealed that affected individuals with *HLA-DRB1*15* have a significantly

Table 3 Comparisons of *HLA-DRB1*15* transmission stratified by gender of affected relative pairs in nuclear (ASP and PC: n = 530) and collateral (AUNN and cousin: n = 415) families

Gender of affected relative pairs	Nuclear affected relative pairs					Collateral affected relative pairs					Comparison	
	TR	NT	OR	χ^2	*p*	TR	NT	OR	χ^2	*p*	χ^2	*p*
Female-female	249	106	2.35	57.60	3.21×10^{-14}	129	29	4.45	63.29	1.78×10^{-15}	7.81	0.0051
Female-male	155	80	1.94	23.94	9.96×10^{-07}	75	28	2.68	21.45	3.64×10^{-06}	1.58	0.21
Male-male	33	20	1.65	3.19	0.074	14	8	1.75	1.64	0.20	—[a]	0.21
Total	437	206	2.12	82.99	8.26×10^{-20}	218	65	3.35	82.72	9.47×10^{-20}	8.030	0.0046

Abbreviations: ASP = affected sibling pair; AUNN = aunt/uncle–niece/nephew; HLA = human leukocyte antigen; NT = nontransmitted; OR = odds ratio; PC = parent-child; TR = transmitted.

[a] Fisher exact test was used when the expected transmissions in any of the cells of the table were below 10.

higher female-to-male ratio as compared to those without. Most of the 6 types of MS families seem to share the same trend of HLA-associated female predominance in MS susceptibility. MPX families appear to be an anomaly, in which the gender ratio is actually lower in affected individuals positive for *HLA-DRB1*15* as compared with those negative (1.67 vs 1.89, not significant). The reason for this discrepancy is yet to be determined but indicates genetic heterogeneity in these families. In MS, families with more than 3 or 4 affected cases and pedigrees with more than 2 or 3 consecutive affected generations are uncommon.[19] Future research is needed with a special focus on these large multigenerational family pedigrees containing multiple affected individuals.

TDT results found that transmission of *HLA-DRB1*15* is significantly more distorted in collateral families vs nuclear families. This finding suggests that differential transmission of the same haplotype in families with affected first-degree relatives vs those consisting of second- and third-degree relatives reflects the inheritance of putative epigenetic marks. In families with extended relatives concordant for MS, haplotype-specific epigenetic modifications could be more conserved (vertical vs collinear transmission). Differential risk carried by other *HLA-DRB1* haplotypes could also exist. However, because of the rarer nature of these haplotypes and perhaps their effect size as compared with the more frequent *HLA-DRB1*15*, it will require much larger numbers of typed families to assess the patterns.

We further stratified the nuclear and collateral families by gender of affected relative pairs, which not only takes into account gender-specific effects, but also the degree of genetic sharing and environmental exposures. We found a significant increase of risk carried by *HLA-DRB1*15* in collateral pairs vs nuclear pairs, which was female-female pair specific, but not in mixed gender pairs or matched male pairs. We have previously shown gender-specific intergenerational differences of *HLA-DRB1*15* frequency in AUNN pairs, supported by the finding that MS nieces were more numerous and had a higher *HLA-DRB1*15* allele frequency than their own affected aunts, clearly localizing gene–environment interactions mediated by putative epigenetic mechanisms to the MHC region.[20] The results here further suggest that the molecular explanation for the increased penetrance of MS among women will have roots in female gender-specific epigenetic modifications of HLA Class II haplotypes.

How environmental factors interact with the genome to influence MS risk is being determined. There are now 5 discrete insights relevant to this interaction which include the maternal parent-of-origin effects,[21] month of birth effects,[22] presence of vitamin D responsiveness in a susceptibility gene,[23] and transgenerational differences in allele frequency.[20] Here, we add a fifth interaction with gender ratio. All of these localize to the MHC.

The MHC has been associated with nearly every autoimmune disease,[24] and female predominance has been recognized for the majority of them.[25] An earlier example reported gender ratios in HLA-related Sjögren syndrome with a strong association of *HLA-DRB1*03* in female cases.[26] Although studies have proposed the role of sex hormones in this bias,[27] results here implicate epigenetics within the MHC as central to this propensity.

This study investigated gender-dependent inheritance of a genetic locus, but did not examine gender-dependent inheritance of epigenetic marks within the MHC region. An epigenetic mark that directly modifies the DNA is methylation, which has been associated with human autoimmunity.[28] The determination of methylation differences in the MHC has been challenging because of single nucleotide polymorphisms (SNPs) being present every 5 to 10 base pairs. Furthermore, the relationship between genetic and epigenetic polymorphisms remains unclear. Two studies searched for possible links between SNPs and DNA methylation, and found that DNA sequence differences rarely affect methylation.[29,30] However, a more recent study reported that the presence of CpGs at SNPs influences local DNA methylation status in cis.[31] Given that the MHC is the most polymorphic region in the human genome plus the known effects of DNA methylation on mutation rate,[32,33] there could be a unique interdependence between genetics and epigenetics within this region underlying disease susceptibility.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Michael Chao.

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DISCLOSURE

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