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Multiple sclerosis susceptibility alleles in African Americans

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Abstract

Multiple sclerosis (MS) is an autoimmune demyelinating disease characterized by complex genetics and multifaceted gene-environment interactions. Compared to whites, African Americans have a lower risk for developing MS, but African Americans with MS have a greater risk of disability. These differences between African Americans and whites may represent differences in genetic susceptibility and/or environmental factors. SNPs from 12 candidate genes have recently been identified and validated with MS risk in white populations. We performed a replication study using 918 cases and 656 unrelated controls to test whether these candidate genes are also associated with MS risk in African Americans. *CD6*, *CLEC16a*, *EVI5*, *GPC5*, and *TYK2* contained SNPs that are associated with MS risk in the African American dataset. *EVI5* showed the strongest association outside the MHC (rs10735781, OR = 1.233, 95% CI = 1.06–1.43, *P* value = 0.006). In addition, *RGS1* appears to affect age of onset whereas *TNFRSF1A* appears to be associated with disease progression. None of the tested variants showed results that were statistically in-consistent with the effects established in whites. The results are consistent with shared disease genetic mechanisms among individuals of European and African ancestry.

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Conflict of Interest

None of the authors have a conflict of interest.

Supplementary information

Supplementary information is available at Genes and Immunity's website.

Introduction

Multiple sclerosis (MS, OMIM 126200) is the most common cause of non-traumatic chronic neurological disability in young adults.1 The incidence of MS seems to have increased considerably over the last century,2 but ancestry remains an important modifier of the global burden of MS; disease prevalence is substantially higher in populations of northern European decent. Other populations, including Asians, Africans, and North and South Amerindians, have a pronounced lower frequency of MS.3 Although African Americans have a higher disease risk compared to black Africans, they have a lower relative risk compared to northern Europeans and white Americans (relative risk of 0.64).4 On the other hand, African Americans with MS appear to have a greater risk of ambulatory disability and more often have symptoms restricted to the spinal cord and optic nerve compared to white patients.5–10 The differences in the clinical phenotype between African Americans and whites with MS may be due to the influence of genetic and/or environmental factors.

MS is a prototypic multifactorial disease with a complex genetic component. Multiple studies yielded convincing evidence for the presence of a major susceptibility gene or genes in the MHC locus on chromosome 6p21.3, but also indicated a polygenic mode of inheritance with each non-MHC gene contributing only a modest effect to the overall risk.11 Spearheaded by the recent remarkable progress in high-throughput genotyping technologies, hypothesis-neutral genome-wide association studies (GWAS) in MS led to the identification of true susceptibility genes and loci of interest, including CD25 Antigen (*CD25*), CD58 Antigen (*CD58*), C-type lectin domain family 16, member A (*CLEC16a*). Interleukin-2 receptor alpha chain (*IL2RA*). Interleukin-7 receptor (*IL7R*), Glypican 5 (*GPC5*), Regulator of G protein signaling 1 (*RGS1*) and Tyrosine kinase 2 (*TYK2*).12–14 A meta-analysis of GWAS data identified additional susceptibility SNPs in or next to Tumor necrosis factor receptor superfamily member 1A (*TNFRSF1A*), Interferon regulatory factor 8 (*IRF8*) and CD6 antigen (*CD6*).15 The distribution and penetrance of these genetic variations in non-white MS groups is unknown.

In African Americans, previous studies have demonstrated the association of MHC class II *HLA-DRB1*15* alleles with disease susceptibility and progression.16, 17 In addition, the presence of *HLA-DRB1*15* alleles in African American patients correlates with the disseminated MS phenotype compared to opticospinal MS.18 However, the *HLA* effect in disease risk may not be as strong as in whites, suggesting a more prominent role for non-*HLA* genes. To better describe the MS susceptibility genetic profile in African Americans, the objectives of this study were to confirm in this population the previously identified susceptibility genes in whites affected with MS and to test whether these genes are associated with disease phenotypes. We tested, in 918 well characterized cases and 656 controls, the allele frequencies of 19 SNPs in 12 MS loci.13–15, 19, 20 *CD6*, *CLEC16a*, Ecotropic viral integration site 5 (*EVI5*), *GPC5*, and *TYK2* contained SNPs that are significantly associated with MS in African Americans. In addition, a preliminary analysis suggests that *RGS1* may be associated with age of onset, whereas *TNFRSF1A* may affect disease severity.

Results

The African American dataset used in this study is comprised of 918 cases and 656 controls. Baseline clinical characteristics of this dataset are listed in Table 1. The female to male ratio in cases was 3.7:1 and 2.4:1 in controls. The gender ratios are significantly different (χ^2, P = 0.0002). Gender, therefore, is fit into the model for all subsequent analyses, thus minimizing the effect of this difference. The average age of onset of MS was 32.4 years and the mean age at time of analysis was 44 years for cases and 43 years for controls (*t* test, P = 0.081). The majority of the cases (85%) had either relapsing remitting or secondary progressive MS. We genotyped all individuals for the presence of *DRB1*1501* or **1503*, since these alleles are associated with MS in African Americans.16, 17 *DRB1*1501* and **1503* are in Hardy-Weinberg equilibrium (Pearson's χ^2 test, P =0.26).

We used logistic regression analysis to test 19 SNPs in 12 genes, previously identified in MS datasets of northern European ancestry, for association with MS in African Americans (Table 2). The average genotyping failure rate was 0.43% for all SNPs tested. None of the SNPs deviated from Hardy-Weinberg equilibrium ($p > 10^{-2}$) in controls. Seven of the SNPs in five genes were significantly associated with MS in the African American dataset: *CD6* rs11230563 (*P*= 0.012, OR= 1.203, 95% CI= 1.042–1.388), *CLEC16a* rs12708716 (*P*= 0.029, OR= 1.173, 95% CI= 1.016–1.357), *CLEC16a* rs6498169 (*P*= 0.028, OR= 1.142, 95% CI= 0.923–1.416), *EVI5* rs10735781 (*P*= 0.006, OR= 1.233, 95% CI= 1.063–1.431), *EVI5* rs6680578 (*P*= 0.025, OR= 1.185, 95% CI= 1.021–1.375), *GPC5* rs553717 (*P*= 0.007, OR= 1.281, 95% CI= 1.025–1.602), and *TYK2* rs34536443 (*P*=0.045, OR= 2.037). Due to the low minor allele frequency (0.01) of rs34536443, the results of this association may be inaccurate and warrant further testing. All *P* values are uncorrected, but we considered the present study as a replication of previously validated findings. Sex was not found to significantly interact with any of the SNPs tested. However, *DRB1* status was found to possibly interact with *EVI5* (rs10735781, *P*= 0.0493) and CD226 antigen (*CD226*) (rs763361, *P*= 0.0041) (Table 3). The minor alleles are susceptible for both SNPs in the *DRB1* non-carriers group. A recent GWAS study performed using cases from Australia and New Zealand also identified the *EVI5-DRB1* interaction.21

Although most of the tested SNPs failed to show significant association in this dataset, those that were significant had slightly larger odds ratios when compared to previous studies in whites (Table 2 and Figure S1). However, none of the tested SNPs showed significant differences in a Cochrane heterogeneity Q test, signifying no difference in the association between African Americans and whites. Global association between MS and each SNP across the 2 populations, are also shown in Table 2.

Because the major MS susceptibility gene *HLA-DRB1* was previously shown to affect some important aspects of the phenotype, we next tested the above 19 SNPs for association with age of onset and multiple sclerosis severity scale (MSSS) using linear regression analysis with relevant covariates placed in the model (Table 4). As expected, *DRB1*1501*/**1503* were significantly associated with age of onset ($P = 7.496 \times 10^{-7}$).18 Individuals with 0 copies of the *DRB1* risk allele had a mean age of onset of 33.3 years. One copy of the *DRB1* risk allele reduced the mean age of onset by 2.7 years and two copies of the *DRB1* risk allele

reduced the mean age of onset by 6.02 years. *RGS1* may also influence the age of onset (P = 0.006). Individuals with two copies of the rs2760524 minor allele had a reduced mean age of onset of 23.0 years from 32.1 years. *TNFRSF1A* appears to modify MSSS metrics (P = 0.014). One copy of the *TNFRSF1A* minor allele reduced the mean MSSS by 0.86. With the exception of the *HLA*, none of these modifier associations survive statistical correction for multiple comparisons. Given the low prior odds that these SNPs are associated with MS phenotypes and the failure of these SNPs to survive correction for multiple comparisons, we consider this data to be preliminary and further testing will be necessary to confirm the association of *RGS1* and *TNFRSF1A* to age of onset and MSSS, respectively.

Discussion

Previous studies have determined an association between *HLA* and MS in African Americans, albeit not as strong as in whites,16, 17 suggesting common immunological mechanisms underlying the diseases across ethnic backgrounds. In the present study, we report the first evidence of genetic association between non-*HLA* genes and MS risk in this population. *CD6*, *CLEC16a*, *EVI5*, *GPC5*, and *TYK2* convincingly replicated in the African American dataset. On the other hand, *CD226*, *CD58*, *IL7R*, *IL2R*, *IRF8*, *RGS1*, *TNFRSF1A* failed to show evidence of association. Two primary explanations exist for this difference: first the study may have been underpowered to detect these associations, and second it is possible the different linkage disequilibrium patterns across populations may render the selected SNPs less effective in tagging putative causative variants in African Americans. It is also conceivable that some of the genes that influence MS in whites do not do so in African Americans.

With a sample size of 1574 African Americans, one would expect only some of the genetically relevant loci to show statistically significant evidence of association, even if an association exists. Also for a majority of the markers, the minor allele frequencies were lower in this African American dataset compared to previous studies in whites. Therefore, this study may have indeed been underpowered to detect associations for some of the SNPs. For example, the minor allele frequencies for SNPs such as rs12044852 (*CD58*, MAF 0.07), rs2104286 (*IL2Ra*, MAF 0.07), rs17445836 (*IRF8*, MAF 0.05), rs2760524 (*RGS1*, MAF 0.06), rs1800693 (*TNFRSF1A*, MAF 0.08), and rs34536443 (*TYK2*, MAF 0.01) are lower than in whites (all MAFs are greater than 0.1 in CEU, www.HapMap.org). To illustrate this effect, power was plotted as a function of minor allele frequency (Supplementary Figure 2). For the SNPs, rs12720222 (*TYK2*), rs17445836 (*IRF8*), rs1800693 (*TNFRSF1a*), rs2816316 (*RGS1*), rs17424933 (*CD6*), rs34536443 (*TYK2*) and rs12044852 (*CD58*) the power to detect a true association in this dataset was low.

However, for SNPs rs2760524 (*RGS1*), rs2104286 (*IL2Ra*), rs6897932 (*IL7Ra*), rs763361 (*CD226*) and rs9533762 (*GPC5*) there appears to have been sufficient statistical power but an association was not identified. In addition, the linkage disequilibrium data in HapMap (www.HapMap.org) suggests that SNPs used to detect association may be less powerful in African Americans, and even if the association of a gene with MS is the same in African Americans and whites, significantly larger datasets may be needed. It should be noted, however, that very large datasets, currently only available for high-risk white populations,

may be necessary to categorically exclude or replicate MS susceptibility markers.13 Based on US Census figures and the relative risk of MS, the current dataset represents an estimated 5% sample of African Americans with MS. It is plausible nevertheless, that differences in genetic susceptibility SNPs between whites and African Americans may represent true differences in disease heritability and that these SNPs do not influence MS risk in African Americans.

Of the genes associated with MS risk in African Americans, *EVI5* displayed the most significant association outside the MHC. The association of *EVI5* with MS was first detected in a GWAS performed using 12,360 non-Hispanic whites from the US and the UK, (SNPs rs10735781; OR=1.14, $p = 3.35 \times 10^{-4}$ and rs6680578; OR= 1.11, $p = 5.00 \times 10^{-4}$).13 The association was confirmed through the analysis of 240 case-control individuals from a genetically isolated Dutch population (rs10735781; OR= 2.01, $p = 0.01$, and rs6680578 OR= 1.9, $p = 0.01$) and in a larger dataset comprised of 2825 individuals from multi-case Canadian families (rs10735781; OR= 1.15, $p = 0.03$; and rs6680578; OR= 1.15, $p = 0.04$).20 *EVI5* is an oncogene implicated in T cell lymphomas, is a common site of retroviral integration22, and facilitates cell septation during mitosis.23 EVI5 has been shown to physically bind the small GTPase binding protein RAB11 in cell culture.24, 25 RAB11 is required for the endocytic recycling of cell surface molecules26 including transferrin,27 IgA,28 and CXCR2 chemokine receptors,29 and has also been implicated in the regulation of cytokinesis,30, 31 neurite extension,32 and the formation of the immune synapse.33 EVI5 competes with RAB effector proteins to bind with RAB11, which suggests a role for EVI5 regulation of downstream RAB11 pathways.25 In CD4+ T cells, Uncoordinated 119 (UNC119) activates RAB11 to transport Lymphocyte cell-specific protein-tyrosine kinase (LCK) to the plasma membrane.33 Upon binding of the antigen-MHC complex from the antigen presenting cell, LCK initiates signaling from the T cell receptor leading to T cell activation.34, 35 Allelic differences in *EVI5* may contribute to altered function of RAB11 and altered formation of the immunological synapse, thus contributing to MS susceptibility. This hypothesis may explain the underlying genetic interaction between *EVI5* and *DRB1*. Further studies will be needed to determine the functional effect of EVI5 allelic variants on RAB11 and the immune synapse.

CD6, *CLEC16a*, and *GPC5* are also associated with MS risk in African Americans. *CD6* is a T cell surface protein involved in the activation of T cells.36 Allelic variants at rs11230563 may result in altered activation of T cells, thereby affecting an individual's susceptibility to MS. *CLEC16a* has been associated with disease susceptibility in white MS patients13, 15, 21, 37, 38 as well as other autoimmune diseases.38–40 Although the function of *CLEC16a* is unknown, *CLEC16a* is expressed on B cells, dendritic cells, and natural killer cells.41 *GPC5*, a member of the glypican family, is a cell surface molecule and is a type of heparan sulfate proteoglycan.42 These molecules are implicated in axon guidance and growth, and synapse formation.43, 44 Interestingly, heparan sulfate proteoglycans have been identified in active MS brain lesions where they may contribute to the sequestering of proinflammatory cytokines.45 *GPC5* is expressed in neurons46 and is known to interact with chemokines, extracellular matrix proteins, and growth factors.47 Allelic variants of *GPC5* may affect neuronal repair and contribute to differences in MS susceptibility.

The *RGS1* SNP, rs2760524, did not show evidence of association with MS risk in African Americans, but our preliminary analysis suggests an effect on age of onset that warrants follow-up in large northern-European datasets. RGS1 is involved in the trafficking of B cells into and out of lymph nodes.48 B cell-mediated antigen presentations and antibody responses appear to be necessary for the full development of demyelination, both in humans and in experimentally induced disease. B cells from *RGS1* knockout mice have increased homing to lymph nodes, increased adhesion to lymph nodes, and faster movement within lymph nodes compared to wild-type B cells.48 In humans with MS, polymorphisms in *RGS1* may conceivably lead to changes in B cell mobility, leading to altered recruitment of B cells to the central nervous system, thereby affecting the initiation of MS. Recent studies associated polymorphisms in *RGS1* to Celiac disease and type I diabetes, further supporting a key role for *RGS1* in the initiation and development of autoimmunity.49–51

The association of *TNFRSF1A*-rs1800693 with MS was recently identified through a GWAS meta-analysis.15 The present data suggests that TNFRSF1A, one of the major receptors for TNF-α, which is involved in apoptosis, inflammation, and under certain conditions immunosuppression,52, 53 may also affect disease progression. Polymorphisms in *TNFRSF1A* may cause altered signaling of TNF-α in the CNS, leading to increased activation of T cells, demyelination and inflammation, thereby affecting the severity of MS. 54–57

In conclusion, in a large African American dataset we have tested a selected number of polymorphisms in candidate genes recently shown to be associated with MS in white populations. SNPs in *CD6*, *CLEC16a*, *EVI5*, *GPC5*, and *TYK2* replicated the association with MS risk in African Americans. This is the first observation of an effect by non-*HLA* genes in a non-white MS group. The data is consistent with a commonality in disease mechanisms among individuals of European and African ancestry, and suggest the prominent role of environmental factors in explaining the paucity of MS in Africa.

Materials, subjects and methods

Study participants

The data set studied consisted of 1574 African American individuals, including 918 MS cases, and 656 unrelated control individuals. All study participants are self-reported African Americans, but European ancestry was documented in 985 of the individuals based on the genotyping of 186 SNPs highly informative for African versus European ancestry as previously described (mean European ancestry = 21%).58 All MS subjects met established diagnostic criteria.59 MS phenotypes were characterized by systematic chart review as described.7 Ascertainment protocols and clinical and demographic characteristics were summarized elsewhere.7, 17 Eight individuals with Aquaporin-4 seropositivity were excluded from this study as they have met new diagnostic criteria for neuromyelitis optica. 60 Informed consent was obtained from all study participants prior to participation in the study.

Genotyping

Genotyping of SNPs was performed using validated TaqMan® SNP genotyping assays (Applied Biosystems Inc., Foster City, CA) for all SNPs except rs9523762, which was performed using a custom TaqMan® SNP genotyping assay (Applied Biosystems Inc., Foster City, CA). Each PCR reaction contained 10ng DNA, 1x; TaqMan® Genotyping Master Mix (Applied Biosystems Inc., Foster City, CA), and 1xSNP assay (Applied Biosystems Inc., Foster City, CA). Amplification was performed in an ABI 9700 GeneAmp® PCR system (Applied Biosystems Inc., Foster City, CA). The PCR program consisted of 95°C for 10min, followed by 50 cycles of 95°C for 15s and 62°C for 1min. The plates were then read on an ABI prism 7900HT Sequence Detection System using SDS 2.0 software (Applied Biosystems Inc., Foster City, CA). For *DRB1*, a PCR locus-specific amplification was used as previously described.16 The average genotyping failure rate was 0.43% for all SNPs tested.

Statistical analysis

Summary statistics in table 1 were performed using R v2.8.1 (www.r-project.org). All SNP genotypes were tested for deviation from Hardy-Weinberg equilibrium in cases and controls using SNP Stats (http://bioinfo.iconcologia.net/index.php?module=Snpstats).61 To determine SNP associations with MS risk, logistic regression was performed using SAS v. 9.1.3 (SAS Institute, Inc., Cary, NC) with sex and *DRB1* status as covariates in the regression model. Positive *DRB1* status was defined as at least one copy of either the *DRB1*1501* or **1503* allele. Two statistical models were tested for each SNP and *DRB1*1501*/**1503*. The genotypic model tested the dominant/recessive/additive allele model and the trend model tested additive allelic effects for each SNP. The model that best fit the data is reported. Since this is a replication study, uncorrected *P* values are reported. SNP interactions with gender and *DRB1* status were tested using SNP Stats (http://bioinfo.iconcologia.net/index.php?module=Snpstats).61 The Cochrane Heterogeneity Q test was performed using the rmeta package in R. The Q test measures the existence of differences between the individual study effects and the pooled effect across studies. The global odds ratio was calculated under a fixed effect model using the inverse variance weighting method.

Genotype-phenotype correlations were tested for age of onset and MSSS. Linear regression was performed using SAS v.9.1.3 (SAS Institute, Inc., Cary, NC). For age of onset, the data followed a normal distribution and was not transformed. For all SNPs tested, gender and *DRB1* status were covariates in the linear regression model. For *DRB1*, only gender was fit into the model. For MSSS, the data was transformed using the “normal score transformation” ($y_i=\phi^{-1}(r_i-3/8)/(n+1/4)$) to yield a normally distributed dependent variable for regression analysis.62 Gender, age of onset, and treatment status (Y/N/unknown) were fit into the linear regression model for all SNPs and *DRB1*. Uncorrected *P* values are reported.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical characteristics of study participants.

	Cases	Controls
Number	918	656
Sex		
Female, n	724	463
Male, n	196	193
Female:Male Ratio, *P*=0.0002	3.7:1	2.4:1
Age (years)		
Age of onset of disease	(n=867)	
Mean	32.4	
Range	7 – 69	
Age at time of analysis	(n=831)	(n=635)
Mean, *P*=0.081	44	43
Range	14 – 75	18 – 82
Disease course	(n=918)	
Relapsing remitting	542 (59.0%)	
Secondary progressive	239 (26.0%)	
Primary progressive	69 (7.5%)	
Progressive relapsing	20 (2.2%)	
Clinically isolated syndrome	20 (2.2%)	
No information available	28(3.1%)	
EDSS	(n=870)	
<3	318 (36.6%)	
3 to <6	219 (25.2%)	
6–6.5	222 (25.5%)	
≥7	110 (12.6%)	
MSSS	(n=846)	
Average	5.24	
Range	0.01 – 9.90	
DRB1 status, n	(n=918)	(n=656)
1 copy of *1501 and/or*1503 allele	320 (34.9%)	185 (28.2%)
2 copies of *1501 and/or*1503 allele	40(4.4%)	17 (2.6%)
No copies of *1501 and/or*1503 allele	558(60.8%)	454(69.2%)

EDSS, expanded disability status scale; MSSS, multiple sclerosis severity scale. *DRB1* alleles are in Hardy-Weinberg equilibrium (Pearson's χ^2 test, $P = 0.26$).

Table 2

association analysis of MS risk.

Results from African Americans in this study									Results from whites in previous studies							Meta analysis	
SNP	**Location**	**Minor/Major Allele**	**MAF**	**Risk Allele**	**Model**	**OR of Risk Allele**	**95% CI**	***P***	**MAF**	**Risk Allele**	**OR of Risk Allele**	**95% CI**	**Corrected *P* value**	**Ref**	**HET *P*[a]**	**Global Odds Ratio**	**Global 95% CI**
rs2300747	1p13	G/A	.23G	A	Trend	1.06	(.90–1.27)	0.478	0.12G	A	1.30	(1.13–1.47)	3.10×10^{-10}	15	0.065	1.22	(1.1–1.34)
rs12044852	1p13	A/C	.07A	C	Trend	1.01	(75–1.35)	0.961	0.08A	A	1.24	(1.12–1.37)	1.90×10^{-5}	13	0.186	1.21	(1.1–1.33)
rs10735781	1p22.1	C/G	.35C	G	Trend	1.23	(1.06–1.43)	0.006*	0.38G	G	1.11	(1.05–1.18)	1.68×10^{-4}	13	0.376	1.14	(1.09–1.19)
									0.38G	G	1.16	(1.06–1.26)	7.55×10^{-6}	15			
rs6680578	1p22.1	A/T	.36A	T	Trend	1.19	(1.02–1.38)	0.025*	0.38T	T	1.11	(1.04–1.17)	2.08×10^{-4}	13	0.417	1.12	(1.06–1.17)
rs2760524	1q31	A/G	.06A	G	Trend	1.13	(.86–1.50)	0.386	0.15A	G	1.12	(1.04–1.21)	2.88×10^{-3}	13	0.938	1.13	(1.06–1.2)
									0.16A	G	1.15	(1.03–1.30)	9.77×10^{-6}	15			
rs2816316	1q31	C/A	.29C	A	Genotypic	1.02	(.82–1.26)	0.660									
rs6897932	5p13	T/C	.13T	C	Genotypic	1.044	(.82–1.33)	0.612	0.25T	C	1.18	(1.11–1.26)	2.94×10^{-7}	13	0.477	1.15	(1.1–1.22)
									0.25T	C	1.12	(1.02–1.23)	1.67×10^{-6}	15			
	6p21			*1501/*1503		Trend	1.421	(1.18–1.71)	0.0002*								
rs2104286	10p15-p14	C/T	.07C	T	Trend	1.18	(.90–1.55)	0.224	0.25C	T	1.19	(1.11–1.26)	5.92×10^{-8}	13	0.843	1.18	(1.12–1.24)
									0.24C	T	1.15	(1.04–1.27)	9.33×10^{-8}	15			
rs17824933	11q13	G/C	.06G	G	Genotypic	1.16	(.84–1.60)	0.24	0.25G	G	1.18	(1.07–1.30)	3.79×10^{-9}	15	0.905	1.18	(1.07–1.29)
rs11230563	11q13	C/T	.48C	C	Trend	1.20	(1.04–1.39)	0.012*	0.21A	C	1.01	(0.90–1.12)	5.23×10^{-4}	15	0.052	1.07	(0.99–1.17)
rs1800693	12p13.2	C/T	.08C	C	Trend	1.23	(.93–1.64)	0.153	0.45C	C	1.20	(1.10–1.31)	1.59×10^{-11}	15	0.867	1.2	(1.11–1.31)
rs553717	13q32	T/C	.19T	T	Genotypic	1.28	(1.03–1.60)	0.007*									
rs9523762	13q32	A/G	.38A	G	Genotypic	1.15	(.92–1.43)	0.154	0.35A	A	1.361	(1.19–1.56)	1.20×10^{-6}	14	0.198	1.3	(1.16–1.46)

Results from African Americans in this study										Results from whites in previous studies							
Gene	**SNP**	**Location**	**Minor/Major Allele**	**MAF**	**Risk Allele**	**Model**	**OR of Risk Allele**	**95% CI**	***P***	**MAF**	**Risk Allele**	**OR of Risk Allele**	**95% CI**	**Corrected *P* value**	**Ref**	**HET *P*** [a]	**Glob**
CLEC16a	rs12708716	16p13.3	G/A	.39G	A	Genotypic	1.17	(1.02–1.36)	0.029*	0.35G	A	1.15	(1.05–1.27)	1.49×10^{-6}	15	0.826	
CLEC16a	rs6498169	16p13.3	G/A	.27G	A	Genotypic	1.14	(.92–1.42)	0.028*	0.37G	G	1.14	(1.08–1.21)	3.83×10^{-6}	13	0.988	
IRF8	rs17445836	16q24.1	A/G	.05A	G	Trend	1.33	(98–1.80)	0.071	0.19A	G	1.25	(1.12–1.39)	3.73×10^{-9}	15	0.724	
CD226	rs763361	18q22.3	C/T	.33C	T	Genotypic	1.04	(.838–1.289)	0.570	0.48T	T	1.07	(0.98–1.16)	0.0429	15	0.808	
TYK2	rs34536443	19p13.2	C/G	.01C	G	Trend	2.04	(1.015–4.082)	0.045*	0.047C	G	1.32	(1.17–1.47)	2.7×10^{-6}	19	0.227	
TYK2	rs12720222	19p13.2	T/C	.17T	T	Trend	1.15	(95–1.39)	0.166								

Results from association analysis of MS risk in African Americans are reported in the left panel. *DRB1* was adjusted for gender and all SNPs were adjusted for gender and *DRB1* status. Where data is available, results from previous genome wide association studies of MS risk in whites is reported in the middle panel. Results of the meta analysis are reported in the right panel. Global odds ratios were estimated under the fixed effect model, using inverse variance weights. MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval; Ref, reference number;

*significant p values;

[a]Cochrane Heterogeneity Q test *P* value.

Table 3

*DRB1*15* stratified odds ratios for interacting loci.

	***EVI5*-*DRB1* interaction (*P*=0.0493)**			***CD226*-*DRB1* interaction (*P*=0.0041)**	
	***DRB1* non-carriers**	***DRB1* carriers**		***DRB1* non-carriers**	***DRB1* carriers**
G/G	1.00	0.90 (0.65–1.24)	T/T	1.00	0.68 (0.50–0.94)
G/C	1.31 (1.00–1.72)	0.84 (0.61–1.16)	T/C	1.33 (0.87–2.04)	0.83 (0.61–1.14)
C/C	1.96 (1.33–2.88)	0.75 (0.43–1.29)	C/C	1.33 (0.87–2.04)	0.32 (0.17–0.60)

EVI5 (rs10735781) and *CD226* (rs763361) show a statistical interaction with *DRB1* status. The odds ratios are given for each genotype. 95% confidence intervals are shown in parentheses. *DRB1* non-carriers have no copies of *DRB1*1501* and **1503*. *DRB1* carriers have at least one copy of the **1501* or **1503* allele.

Table 4

Genotype-Phenotype analysis in African Americans.

		Age of Onset		MSSS	
Gene	**SNP**	**Model**	***P* value**	**Model**	***P* value**
CD58	rs2300747	Genotypic	0.205	Trend	0.181
CD58	rs12044852	Genotypic	0.526	Genotypic	0.241
EVI5	rs10735781	Trend	0.325	Genotypic	0.568
EVI5	rs6680578	Trend	0.469	Genotypic	0.853
RGS1	rs2760524	Genotypic	0.006*	Trend	0.125
RGS1	rs2816316	Trend	0.614	Trend	0.826
IL7R	rs6897932	Trend	0.951	Trend	0.080
DRB1	*1501/*1503	Trend	7.50×10^{-07} *	Genotypic	0.504
IL2Ra	rs2104286	Trend	0.589	Trend	0.308
CD6	rs17824933	Trend	0.357	Trend	0.663
CD6	rs11230563	Trend	0.792	Trend	0.299
TNFRSF1A	rs1800693	Trend	0.211	Genotypic	0.014*
GPC5	rs553717	Trend	0.505	Genotypic	0.798
GPC5	rs9523762	Trend	0.340	Genotypic	0.275
CLEC16a	rs12708716	Trend	0.480	Genotypic	0.614
CLEC16a	rs6498169	Genotypic	0.074	Trend	0.712
IRF8	rs17445836	Trend	0.164	Trend	0.490
CD226	rs763361	Trend	0.737	Genotypic	0.771
TYK2	rs34536443	Trend	0.243	Genotypic	0.656
TYK2	rs12720222	Trend	0.820	Genotypic	0.047

For age of onset, gender and *DRB1* status are fit into the linear regression model, except for *DRB1* (only gender used). For MSSS, gender, age of onset, and treatment status are fit into the linear regression model. MSSS, multiple sclerosis severity scale

* significant *P* values.