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ABSTRACT

Objective: We investigated the association between 52 risk variants identified through genome-wide association studies and disease severity in multiple sclerosis (MS).

Methods: Ten unique MS case data sets were analyzed. The Multiple Sclerosis Severity Score (MSSS) was calculated using the Expanded Disability Status Scale at study entry and disease duration. MSSS was considered as a continuous variable and as 2 dichotomous variables (median and extreme ends; MSSS of ≤ 5 vs >5 and MSSS of <2.5 vs ≥ 7.5, respectively). Single nucleotide polymorphisms (SNPs) were examined individually and as both combined weighted genetic risk score (wGRS) and unweighted genetic risk score (GRS) for association with disease severity. Random-effects meta-analyses were conducted and adjusted for cohort, sex, age at onset, and *HLA-DRB1*15:01*.

Results: A total of 7,125 MS cases were analyzed. The wGRS and GRS were not strongly associated with disease severity after accounting for cohort, sex, age at onset, and *HLA-DRB1*15:01*. After restricting analyses to cases with disease duration ≥ 10 years, associations were null (*p* value ≥ 0.05). No SNP was associated with disease severity after adjusting for multiple testing.

Conclusions: The largest meta-analysis of established MS genetic risk variants and disease severity, to date, was performed. Results suggest that the investigated MS genetic risk variants are not associated with MSSS, even after controlling for potential confounders. Further research in large cohorts is needed to identify genetic determinants of disease severity using sensitive clinical and MRI measures, which are critical to understanding disease mechanisms and guiding development of effective treatments. *Neurol Genet* 2016;2:e87; doi: 10.1212/NXG.0000000000000087

GLOSSARY

CI = confidence interval; **EAE** = experimental autoimmune encephalomyelitis; **EDSS** = Expanded Disability Severity Scale; **GRS** = unweighted genetic risk score; **GWAS** = genome-wide association studies; **KPNC** = Kaiser Permanente Medical Care Plan in the Northern California Region; **MHC** = major histocompatibility complex; **MS** = multiple sclerosis; **MSSS** = Multiple Sclerosis Severity Score; **OR** = odds ratio; **SNP** = single nucleotide polymorphism; **UCSF** = University of California at San Francisco; **wGRS** = weighted genetic risk score.

Multiple sclerosis (MS) is a severe autoimmune inflammatory disease of the central nervous system. Neurologic damage in MS is caused by irreversible demyelination of axons and lesion formation. Although early disease may manifest as attacks with full recovery, over time MS is extremely debilitating for the majority of patients. Only 37% of individuals with mild MS are employed, and within 15 years of diagnosis, 50%–60% of patients will require assistance with walking, posing tremendous economic and societal burden.[1,2]

Evidence suggests that both genetic and environmental components contribute to the risk of MS. The strongest genetic risk factor is within the human leukocyte antigen (*HLA*)-*DRB1* locus, specifically the *15:01* allele,[3] and studies support the presence of additional independent susceptibility alleles within the major histocompatibility complex (MHC) Class I and Class II regions.[4] However, these genes have not been convincingly associated with progression.[3,5]

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See editorial

Supplemental data
at Neurology.org/ng

Environmental risk factors for MS include infection with Epstein-Barr virus, tobacco smoke exposure, low serum levels of vitamin D, and childhood/adolescent obesity.[6,7] With the exception of tobacco smoke and low vitamin D,[8,9] environmental influences on MS disease severity are unknown.

Through international collaboration, genome-wide association studies (GWAS) followed by replication have identified a large number of non-MHC MS risk variants.[10,11] We hypothesized that MS risk variants might also influence disease severity. We investigated the association of the Multiple Sclerosis Severity Score (MSSS) with both a weighted genetic risk score (wGRS) and unweighted genetic risk score (GRS) comprising 52 established risk variant alleles, and each susceptibility variant alone in 7,125 MS cases from 10 independent cohorts.

METHODS **Study populations.** Ten independent and well-characterized MS case data sets were analyzed (table 1). The analysis included 1,079 white non-Hispanic MS patients recruited from Kaiser Permanente Medical Care Plan in the Northern California Region (KPNC).[12] The following additional cases were included: 1,019 MS white non-Hispanic patients recruited from 2 other clinical sites in the United States (US1 and US2)[5]; 422 MS patients recruited through a population-based study in Oslo, Norway (Norway)[13]; 2,348 MS patients recruited through a population-based study in Sweden (Sweden)[14]; 890 MS patients from a cohort in Denmark (Denmark)[11]; 485 white non-Hispanic MS patients from a University of California San Francisco (UCSF)[15] cohort; 678 MS patients from 2 cohorts recruited in Italy (Italy1 and Italy2)[10,16]; and 204 patients from a Tasmanian cohort study (Australia).[17,18] Each case included in the meta-analysis fulfilled disease criteria for MS.[19] Classical *HLA-DRB1*15:01* typing was used in the KPNC, Sweden, and UCSF studies. Validated tagging single nucleotide polymorphisms (SNPs) for *DRB1*15:01* were used in the US1 and US2, Italy1 and Italy2, and Australia (rs9271366), Norway (rs9270986), and Denmark (rs3135388) studies. Each of these tagging SNPs was tested against the classical high-resolution *HLA-DRB1* typing performed in the KPNC data set; the correlations (r^2) were 0.99, 0.91, and 0.95, respectively.

Standard protocol approvals, registrations, and patient consents. Each study protocol was approved by the appropriate Institutional Review Board of the participating academic institution. All participants provided written informed consent.

Table 1	Characteristics of non-Hispanic white MS cases in 10 cohorts								
	Mean (SD)/frequency (%)								
	KPNC	US	Norway	Sweden	Denmark	UCSF	Italy	Australia	Meta-analysis
No. of cases	1,079	1,019	422	2,348	890	485	678	204	7,125
MSSS continuous	3.37 (2.6)	3.28 (2.6)	4.59 (3.0)	4.61 (2.8)	4.48 (2.6)	2.98 (2.4)	4.57 (2.9)	4.12 (2.7)	4.09 (2.8)
MSSS binary (high)	255 (23.6)	261 (25.6)	180 (42.7)	1,045 (44.5)	370 (41.6)	101 (20.8)	295 (43.5)	73 (35.8)	2,580 (36.2)
MSSS extreme (high)	129 (19.6)	103 (17.3)	101 (40.2)	474 (41.2)	136 (36.8)	31 (10.5)	150 (40.7)	30 (28.6)	1,154 (30.4)
Female	868 (80.4)	779 (76.5)	309 (73.2)	1,702 (72.5)	614 (69.0)	333 (68.7)	436 (64.3)	147 (72.1)	5,188 (72.8)
Age at onset	31.94 (9.8)	31.03 (8.5)	32.66 (9.4)	34.60 (10.7)	30.92 (8.9)	33.47 (9.3)	32.50 (10.5)	35.25 (10.2)	32.85 (10.0)
HLA-DRB1*15:01 (positive)	581 (53.9)	567 (55.6)	243 (57.6)	1,366 (58.2)	534 (60.0)	224 (46.2)	189 (27.9)	120 (58.8)	3,824 (53.7)
Disease duration	12.23 (8.5)	12.05 (8.5)	16.16 (10.5)	9.71 (8.6)	12.23 (7.8)	9.54 (9.1)	11.38 (8.5)	15.67 (10.2)	11.44 (8.9)
wGRS	6.75 (0.5)	6.59 (0.5)	6.84 (0.5)	6.74 (0.5)	6.68 (0.5)	6.71 (0.5)	6.85 (0.5)	6.72 (0.5)	6.73 (0.5)
GRS	56.72 (4.4)	55.77 (4.4)	57.11 (4.4)	56.44 (4.5)	56.38 (4.5)	56.46 (4.3)	57.81 (4.5)	56.47 (4.6)	56.55 (4.5)
Genotyping platform	Affymetrix GeneChip Human Mapping 500K Array set	Affymetrix GeneChip Human Mapping 500K Array set	TaqMan OpenArray Genotyping Technology	Immunochip—Illumina Infinium HD Custom Array and Illumina Human Quad 660	Immunochip—Illumina Infinium HD Custom Array	Illumina HumanHap550 Beadchip circa 2006	Illumina Human Quad 660	Illumina Infinium Hap370CNV array	—

Abbreviations: GRS = unweighted genetic risk score; KPNC = Kaiser Permanente Medical Care Plan in the Northern California Region; MS = multiple sclerosis; MSSS = Multiple Sclerosis Severity Score; SNP = single nucleotide polymorphism; UCSF = University of California at San Francisco; wGRS = weighted genetic risk score.
MSSS is presented as a continuous variable and as 2 dichotomous variables. The first dichotomous variable was based on the median MSSS value, defined as MSSS ≤5 vs >5, with a smaller score indicating a more benign phenotype. The second dichotomous variable was based on extreme ends of the MSSS distribution, defined as MSSS <2.5 vs ≥7.5. *HLA-DRB1*15:01* tag SNP if classical HLA typing was not available: US and Italy cohorts used rs9271366 as a tag SNP, Norway used rs9270986 as a tag SNP, and Denmark used rs3135388 as a tag SNP. All cohorts had data for all 52 individual SNPs, except the US and Australian cohorts. The US cohort used the following SNPs as tagging SNPs: rs6685440 tags rs11581062; and rs8106574 tags rs1077667. The Australian cohort imputed the following SNPs: rs1323292, rs7522462, rs17174870, rs10201872, rs669607, rs12212193, rs17066096, rs13192841, rs354033, rs1520333, rs10466829, rs2119704, rs7200786, rs13333054, and rs2425752. The US cohort contains 2 cohorts—US1 and US2. The Italy cohort contains 2 cohorts—Italy1 and Italy2. They are combined in table 1, but analyzed separately in the meta-analysis.

Genotyping and imputation. In addition to *HLA-DRB1*15:01* (described above), a group of 52 independent non-MHC GWAS SNPs were chosen for the present study. They were selected because they were previously identified and further replicated through GWAS, and they demonstrated larger magnitudes of effect on disease risk. All participants were genotyped using separate platforms: Affymetrix platform using the GeneChip Human Mapping 500K Array set (KPNC and United States), TaqMan OpenArray Genotyping Technology (Norway), Illumina Infinium HD Custom Array and Illumina Human Quad 660 (Sweden, Denmark, and Italy), and Illumina HumanHap550 Beadchip ©2006 (UCSF). All cohorts, except United States and Australia, contained genotyping information for all 52 variants. In the United States, tagging SNPs were used as proxies for 2 missing candidate SNPs: rs6693456 tagged rs11581062 (r^2 = 0.95), and rs8106574 tagged rs1077667 (r^2 = 0.63), based on publicly available data. In the Australia data set, 15 SNPs were missing: rs1323292, rs7522462, rs17174870, rs10201872, rs669607, rs12212193, rs17066096, rs13192841, rs354033, rs1520333, rs10466829, rs2119704, rs7200786, rs13333054, and rs2425752. Genetic data within 1-MB regions around all missing SNPs were available for each patient; therefore, imputation was possible against the 1000 Genomes reference. After imputation, missingness was lower than 2% in all cohorts. However, to recover all missing genotypes, the risk allele frequency from each cohort was used to estimate the missing genotypes. The estimation for each individual was made using a multinomial distribution generated from 1,000 random samples from each respective cohort. The probability for each of the 3 possible genotypes was generated. When a single genotype was missing for an individual, one sample was drawn randomly from the generated distribution from that cohort. This was done for a total of 146 individuals (KPNC = 92, Norway = 19, Sweden = 22, Denmark = 9, UCSF = 15, and Italy = 8) and a total of 48 SNPs (KPNC = 44, Norway = 15, Sweden = 4, Denmark = 2, UCSF = 10, and Italy = 8).

Weighted and unweighted genetic risk scores. wGRS was calculated for each MS patient using the discovery odds ratios (ORs) as the weight for each of 52 non-MHC risk alleles derived from 9,772 MS cases and 17,376 controls,[10] as previously described. In brief, the number of risk alleles for each SNP was multiplied by the weight for that variant, and then the sum across all 52 variants was calculated (table 1).[10,20] GRS was calculated as the sum of risk allele copies for each SNP without weighting (table 1). Both wGRS and GRS were analyzed as continuous variables.

Multiple Sclerosis Severity Score. MSSS is a probabilistic algorithm that uses the Expanded Disability Severity Scale (EDSS) to calculate disease severity and duration of disease, which was defined as time between first symptom and EDSS assessment.[21] The MSSS for each patient in the current study was assigned using Global MSSS reference data derived from a large independent cohort of 9,892 patients with EDSS and disease duration ranging from 1 to >30 years. EDSS scores from a pooled reference patient cohort were ranked as previously described,[21] and the average of the lowest and highest ranks for each possible EDSS value was calculated, taking into account also, scores reported for 2 years before and after each designated time point. These averages were normalized to account for the number of available assessments for each year (disease duration) and multiplied by 10. MSSS was analyzed as a continuous variable and as 2 dichotomous variables, as previously described.[5] In brief, a binary MSSS variable was based on the median MSSS value, defined as MSSS ≤5 vs >5, with a smaller score indicating more benign or "mild" disease. The second variable was based on extreme ends of the MSSS distribution, defined as MSSS <2.5 (benign) vs ≥7.5 (severe) (table 1, figure 1).

Statistical analysis. All 10 data sets were included in a random-effects meta-analysis. Random-effects meta-analysis allows for heterogeneity across studies because of inherent differences and/or differential biases among each cohort, unlike fixed-effects models, which assume a single common effect underlies each study. A random-effects meta-analysis is generally more conservative, generating wider confidence intervals (CIs) and larger *p* values. Weighted and unweighted GRS, and all 52 non-MHC risk variants, were tested with the 3 MSSS outcomes in the meta-analysis.

In addition, analyses restricted to cases with a preestablished disease duration greater than or equal to 10 years were conducted to increase the likelihood that the MSSS measurement was stable. Both adjusted linear and logistic regression models were used to estimate adjusted beta values (β), ORs, and 95% CIs. The meta-analysis was adjusted for sex, age at onset, and *HLA-DRB1*15:01*. All analyses were conducted in STATA v13.1 (StataCorp, College Station, TX). The present study had sufficient power for all analyses (table e-1 at Neurology.org/ng).

RESULTS A total of 7,125 individuals were included in the meta-analysis of the 3 MSSS outcomes (table 1). The overall distributions of MSSS demonstrated similarities across individual cohorts (figure 1) and were comparable to those in other cohorts reported previously[21]; however, there were some notable

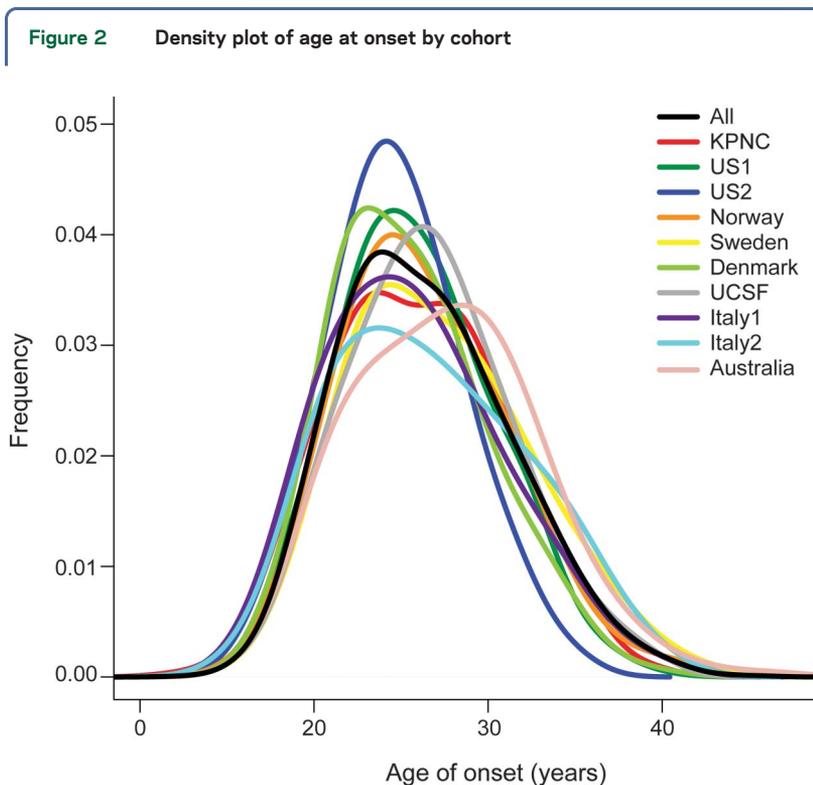**Figure 1** Density plot of MSSS by cohort
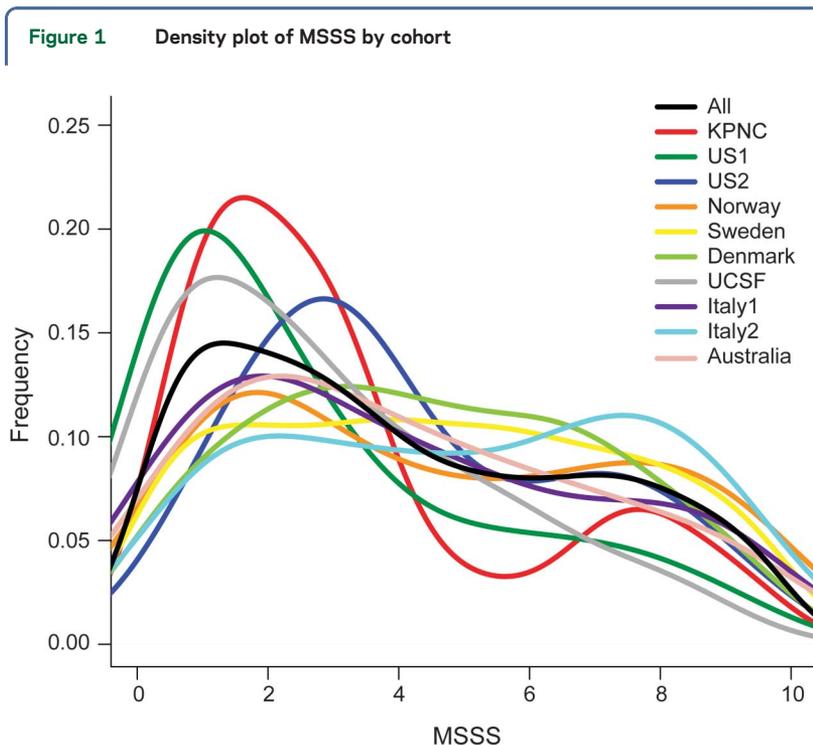
The density plot represents the frequency distribution of MSSS in each of the 10 cohorts and the total 7,125 individuals with MS. KPNC = Kaiser Permanente Medical Care Plan in the Northern California Region; MS = multiple sclerosis; MSSS = Multiple Sclerosis Severity Score. UCSF = University of California at San Francisco.

differences. Cohorts from Nordic countries and Italy had a larger proportion of cases with greater disease severity; disease duration, on average, was shorter in the Swedish and US (UCSF) cohorts (table 1). The sex distribution was also very similar across cohorts, with a 3:1 female to male ratio; KPNC had the highest proportion of females (80.4%), and Italy had the lowest proportion of females (64.3%) (table 1). Age at onset was similar among all cohorts and normally distributed, ranging from 30.9 years of age in Denmark to 35.3 years of age in Australia (table 1, figure 2). On average, disease duration since first symptom was 11.4 years across all cohorts and normally distributed, ranging from 9.5 years in UCSF to 16.2 years in Norway (table 1, figure e-1). Last, the distribution of *HLA-DRB1*15:01* was typical of established genetic patterns in the literature (table 1): MS participants of Northern European descent were more likely to be *HLA-DRB1*15:01* positive than participants in the Italy cohort.[22] The non-MHC wGRS and GRS were similar across cohorts (figures e-2 and e-3).

Meta-analyses accounting for the random effect of each cohort were used. We observed some evidence for association with wGRS and GRS for the MSSS outcomes (table 2). However, after restricting the data set to individuals who had a disease duration of 10 years or more, no significant associations remained (table e-2). Sex, age at onset, and *HLA-DRB1*15:01* status were all analyzed as fixed effects. Sex and age at onset were consistently associated with MSSS in all 3 models, even after restricting to individuals with 10 years or more of disease (all *p* values <0.001) (table 2 and table e-2). Male sex and a later age at onset were both associated with more severe disease. *HLA-DRB1*15:01* was not associated with MSSS in any of the models.

The 52 non-MHC risk variants were also tested individually in random-effects meta-analytic models adjusting for sex, age at onset, and *HLA-DRB1*15:01* across all MS cases (full cohort), and in MS cases with disease duration of 10 years or more (restricted cohort) (table 3, table e-3). In the full cohort, 2 variants showed evidence of association for all 3 MSSS outcomes: rs874628 and rs650258, within *MPV17L2* and 44-kb upstream of *CD6*, respectively. Similar results were observed in the restricted cohort (data not shown). All associations were in the same direction as the wGRS and GRS. However, after accounting for multiple testing, no single variant remained significant. When analyses were stratified by *DRB1*15:01*-positive and -negative carrier status in cases, results were similar (data not shown). We also restricted the above analyses to combined US, European, and Australian cohorts separately and did not observe any cohort-specific associations between MSSS and the wGRS or GRS (data not shown).

DISCUSSION The identification of disease-modifying genes in MS is critical to further our understanding of disease pathogenesis, given the phenotype heterogeneity observed in patients for clinical manifestations, temporal course, and disease severity. This important topic has been previously reviewed, and several challenges have been described.[23] Minimal progress has been made in the past decade owing mostly to the need for large, well-designed studies to detect presumably modest genetic effects, similar to what has been observed for GWAS, and clinical outcome data for large numbers of patients that accurately capture measures likely to be modified by genetic variation. Here, a meta-analysis of association between 52 established non-MHC MS genetic risk factors with larger effect sizes demonstrated in GWAS and disease severity was performed. Thus far, this is the largest combined MS patient cohort used to evaluate the relationship between established risk variants and disease severity. Ten independent and well-characterized data sets comprising 7,125 individuals with clinically definite MS were studied. The hypothesis that one or more known MS genetic risk variants are also associated with disease severity characterized by MSSS was comprehensively tested. Genetic factors that

Figure 2   Density plot of age at onset by cohort

Legend:
- All
- KPNC
- US1
- US2
- Norway
- Sweden
- Denmark
- UCSF
- Italy1
- Italy2
- Australia

The density plot represents the frequency distribution of age at onset in each of the 10 cohorts and the total 7,125 individuals with MS. KPNC = Kaiser Permanente Medical Care Plan in the Northern California Region; MS = multiple sclerosis; MSSS = Multiple Sclerosis Severity Score. UCSF = University of California at San Francisco.

Table 2 Meta-analysis results for wGRS and GRS and MSSS in 7,125 MS cases

	MSSS outcome					
	Continuous		**Binary**		**Extreme**[a]	
	β (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
wGRS (unadjusted)	0.08 (−0.1 to 0.2)	0.22	1.1 (1.0–1.2)	0.36	1.1 (1.0–1.3)	0.17
wGRS (adjusted)	0.1 (0.01 to 0.3)	0.03	1.1 (1.0–1.2)	0.09	1.2 (1.0–1.4)	0.02
Sex	−0.6 (−0.7 to −0.4)	<0.001	0.7 (0.6–0.8)	<0.001	0.5 (0.5–0.6)	<0.001
Age at onset	0.06 (0.05 to 0.07)	<0.001	1.0 (1.0–1.0)	<0.001	1.1 (1.1–1.1)	<0.001
*HLA-DRB1*15:01*	−0.02 (−0.1 to 0.1)	0.76	1.0 (0.9–1.1)	0.65	1.0 (0.8–1.1)	0.75
GRS (unadjusted)	0.01 (−0.004 to 0.02)	0.18	1.0 (1.0–1.0)	0.32	1.0 (1.0–1.0)	0.11
GRS (adjusted)	0.02 (0.002 to 0.03)	0.03	1.0 (1.0–1.0)	0.09	1.0 (1.0–1.0)	0.01
Sex	−0.6 (−0.7 to −0.4)	<0.001	0.7 (0.6–0.8)	<0.001	0.5 (0.5–0.6)	<0.001
Age at onset	0.06 (0.05 to 0.07)	<0.001	1.0 (1.0–1.0)	<0.001	1.1 (1.1–1.1)	<0.001
*HLA-DRB1*15:01*	−0.02 (−0.1 to 0.1)	0.77	1.0 (0.9–1.1)	0.66	1.0 (0.8–1.1)	0.76

Abbreviations: CI = confidence interval; GRS = unweighted genetic risk score; MS = multiple sclerosis; MSSS = Multiple Sclerosis Severity Score; OR = odds ratio; wGRS = weighted genetic risk score.
All βs and ORs are from the random-effects models. Unadjusted and adjusted results are shown. Adjusted models included the following covariates: sex, age at onset, and *HLA-DRB1*15:01*. All βs and ORs for the covariates in the adjusted models are also listed above.
[a] A total of 3,795 individuals with MS are included in this analysis.

influence disease susceptibility tested here were not shown convincingly to affect disease severity.

The individuals included in this analysis are representative of the international MS population with regard to sex distribution, average age at onset, and proportion of *HLA-DRB1*15:01* allele carriers. Male sex and older age at onset were associated with more severe disease, which is consistent with other reports.[24] While *HLA-DRB1*15:01* is an established risk factor for MS, it was not associated with MS disease severity here, as in previous studies that examined MSSS[3,5] and brain atrophy.[25]

Available MS family data show that familial factors do not substantially influence eventual disease severity. However, they may increase the probability of a progressive clinical course, either from onset or after a phase of relapsing remitting disease, and evidence suggests that genetic influences are involved.[26] Findings from studies of other neurodegenerative diseases, such as Alzheimer disease and Parkinson disease, have linked disease progression with specific genetic markers, also raising the possibility that MS progression might have a genetic component,[27,28] given evidence for both neurodegeneration and inflammation in MS. However, to date, there has been limited success identifying non-MHC genetic associations with disease severity or other clinical phenotypes in MS. One study showed that a small subset (n = 16) of risk variants investigated in the present study was not associated with clinical and MRI outcomes in 179 recently diagnosed MS patients.[29] However, the cumulative burden of non-MHC risk variants (n = 110) contributed modestly to the number of relapses in 842 MS patients.[30] Furthermore, some variants studied (n = 17) were associated with attack severity, recovery, or frequency in 503 recently diagnosed MS cases.[31] Relapse data were not available for the present study. These observations require further investigation and much larger samples sizes for confirmation. The most compelling evidence for genetic associations with cerebrospinal fluid antibody levels, both immunoglobulin G index and oligoclonal band positive status, was reported recently for MHC and immunoglobulin heavy-chain region genes in 6,950 patients.[32] Both antibodies are markers of more severe disease in MS. The results, in conjunction with the present study findings, underscore the need for new studies to identify or exclude genetic contributions to disease severity in MS.

There is evidence in animal models related to MS to suggest that risk alleles have an effect on progression. The experimental autoimmune encephalomyelitis (EAE) model, which mirrors an inflammatory autoimmune disease of the central nervous system in rats and mice, has offered numerous experimental insights into MS. When genetically dissected into high-resolution quantitative trait loci, *Eae25* and *Eae29* have been shown to influence both susceptibility and progression.[33,34] In addition, differential expression of an interleukin 2 (*IL2*) repressor in the gene *ZEB1* results in EAE severity changes.[35] Similarly, congenic rats with *Eae18b* locus have been

Table 3 Meta-analysis results for 52 SNPs and MSSS in 7,125 MS cases

			MSSS outcome					
			Continuous		Binary		Extreme[a]	
Chr	Gene	SNP	β (95% CI)[b]	p Value	OR (95% CI)[b]	p Value	OR (95% CI)[b]	p Value
1	MMEL1	rs4648356	−0.07 (−0.2 to 0.02)	0.14	1.0 (0.9–1.1)	0.45	0.9 (0.8–1.0)	0.14
	EVI5	rs11810217	0.06 (−0.03 to 0.2)	0.21	1.1 (1.0–1.1)	0.27	1.1 (1.0–1.2)	0.15
	VCAM1	rs11581062	0.05 (−0.05 to 0.1)	0.32	1.0 (1.0–1.1)	0.24	1.0 (0.9–1.2)	0.65
	CD58	rs1335532	0.07 (−0.07 to 0.2)	0.32	1.1 (0.9–1.2)	0.44	1.1 (0.9–1.3)	0.35
	RGS1	rs1323292	−0.1 (−0.2 to 0.01)	0.06	0.9 (0.8–1.0)	0.01	1.0 (0.8–1.1)	0.48
	C1orf106	rs7522462	−0.09 (−0.2 to 0.01)	0.09	0.9 (0.9–1.0)	0.08	0.9 (0.8–1.0)	0.08
2	No gene	rs12466022	0.05 (−0.05 to 0.2)	0.34	1.0 (1.0–1.1)	0.49	1.0 (0.9–1.2)	0.54
	PLEK	rs7595037	0.05 (−0.03 to 0.1)	0.23	1.0 (1.0–1.1)	0.55	1.1 (1.0–1.2)	0.16
	MERTK	rs17174870	−0.003 (−0.1 to 0.10)	0.94	1.0 (0.9–1.1)	0.71	1.0 (0.9–1.2)	0.80
3	SP140	rs10201872	0.01 (−0.1 to 0.1)	0.82	1.0 (0.9–1.1)	0.69	1.0 (0.9–1.2)	0.99
	EOMES	rs11129295	0.02 (−0.07 to 0.1)	0.64	1.0 (0.9–1.1)	0.98	1.0 (0.9–1.1)	0.93
	No gene	rs669607	0.09 (−0.003 to 0.2)	0.05	1.1 (0.9–1.1)	0.12	1.1 (1.0–1.2)	0.08
	CBLB	rs2028597	0.06 (−0.1 to 0.2)	0.49	1.0 (0.9–1.2)	0.68	1.0 (0.8–1.2)	0.88
	TMEM39A	rs2293370	−0.1 (−0.2 to 0.02)	0.09	0.9 (0.8–1.0)	0.07	0.9 (0.8–1.1)	0.18
	CD86	rs9282641	−0.05 (−0.2 to 0.1)	0.56	1.0 (0.9–1.1)	0.69	1.0 (0.8–1.2)	0.76
	IL12A	rs2243123	0.01 (−0.08 to 0.1)	0.82	1.0 (0.9–1.1)	0.69	1.0 (0.9–1.1)	0.91
5	IL7R	rs6897932	0.004 (−0.1 to 0.1)	0.93	1.0 (0.9–1.1)	0.61	1.0 (0.9–1.1)	0.76
	PTGER4	rs4613763	0.06 (−0.06 to 0.2)	0.31	1.1 (1.0–1.2)	0.08	1.1 (1.0–1.3)	0.15
	IL12B	rs2546890	0.05 (−0.03 to 0.1)	0.24	1.0 (0.9–1.1)	0.90	1.1 (1.0–1.2)	0.06
6	BACH2	rs12212193	0.03 (−0.06 to 0.1)	0.56	1.0 (0.9–1.1)	0.80	1.0 (0.9–1.2)	0.58
	THEMIS	rs802734	0.03 (−0.07 to 0.1)	0.58	1.0 (0.9–1.1)	0.67	1.1 (0.9–1.2)	0.40
	MYB	rs11154801	0.08 (−0.01 to 0.2)	0.08	1.1 (1.0–1.2)	0.10	1.1 (1.0–1.2)	0.15
	IL22RA2	rs17066096	0.09 (−0.01 to 0.2)	0.09	1.1 (1.0–1.2)	0.14	1.2 (1.0–1.3)	0.01
	No gene	rs13192841	−0.02 (−0.1 to 0.08)	0.71	1.0 (0.9–1.1)	0.77	1.0 (0.9–1.1)	0.52
	TAGAP	rs1738074	−0.03 (−0.1 to 0.06)	0.50	1.0 (0.9–1.1)	0.87	1.0 (0.9–1.1)	0.54
7	ZNF746	rs354033	−0.03 (−0.1 to 0.07)	0.56	1.0 (0.9–1.1)	0.86	0.9 (0.8–1.1)	0.31
8	IL7	rs1520333	−0.01 (−0.1 to 0.09)	0.89	1.0 (1.0–1.1)	0.30	1.0 (0.9–1.1)	0.72
	MYC	rs4410871	0.06 (−0.04 to 0.2)	0.25	1.1 (1.0–1.1)	0.28	1.0 (0.9–1.2)	0.54
	PVT1	rs2019960	0.05 (−0.05 to 0.2)	0.32	1.0 (1.0–1.1)	0.49	1.1 (0.9–1.2)	0.38
10	IL2RA	rs3118470	0.05 (−0.04 to 0.1)	0.30	1.0 (0.9–1.1)	0.62	1.1 (1.0–1.2)	0.07
	ZMIZ1	rs1250550	0.04 (−0.05 to 0.1)	0.37	1.1 (1.0–1.1)	0.24	1.1 (1.0–1.2)	0.14
	HHEX	rs7923837	0.02 (−0.07 to 0.1)	0.69	1.0 (1.0–1.1)	0.56	1.0 (0.9–1.1)	0.80
11	CD6	rs650258	0.1 (0.02 to 0.2)	0.02	1.1 (1.0–1.2)	0.02	1.2 (1.0–1.3)	0.01
12	TNFRSF1A	rs1800693	−0.02 (−0.1 to 0.1)	0.63	1.0 (0.9–1.1)	0.78	1.0 (0.9–1.1)	0.60
	CLECL1	rs10466829	0.02 (−0.06 to 0.1)	0.61	1.0 (0.9–1.1)	0.84	1.1 (1.0–1.2)	0.23
	CYP27B1	rs12368653	−0.04 (−0.1 to 0.05)	0.42	1.0 (0.9–1.1)	0.61	1.0 (0.9–1.1)	0.43
	ARL61P4	rs949143	0.07 (−0.03 to 0.2)	0.16	1.0 (1.0–1.1)	0.55	1.1 (1.0–1.2)	0.16
14	ZFP36L1	rs4902647	0.06 (−0.03 to 0.1)	0.19	1.0 (1.0–1.1)	0.29	1.1 (1.0–1.2)	0.15
	BATF	rs2300603	−0.05 (−0.2 to 0.05)	0.31	1.0 (0.9–1.0)	0.34	1.0 (0.9–1.1)	0.79
	GALC	rs2119704	−0.01 (−0.2 to 0.2)	0.93	1.0 (0.9–1.2)	0.77	0.9 (0.7–1.1)	0.44
16	CLEC16A	rs7200786	0.02 (−0.06 to 0.1)	0.59	1.0 (1.0–1.1)	0.67	1.1 (1.0–1.2)	0.28
	IRF8	rs13333054	0.1 (−0.01 to 0.2)	0.07	1.1 (1.0–1.2)	0.05	1.1 (1.0–1.3)	0.09

Continued

Table 3 Continued

Chr	Gene	SNP	MSSS outcome					
			Continuous		Binary		Extreme[a]	
			β (95% CI)[b]	p Value	OR (95% CI)[b]	p Value	OR (95% CI)[b]	p Value
17	STAT3	rs9891119	−0.02 (−0.1 to 0.07)	0.63	1.0 (0.9–1.1)	0.93	1.0 (0.9–1.1)	0.95
18	MALT1	rs7238078	−0.06 (−0.2 to 0.04)	0.24	1.0 (0.9–1.0)	0.27	1.0 (0.8–1.1)	0.44
19	TNFSF14	rs1077667	−0.04 (−0.2 to 0.07)	0.47	1.0 (0.9–1.0)	0.28	1.0 (0.9–1.2)	0.98
	TYK2	rs8112449	−0.01 (−0.1 to 0.09)	0.85	1.0 (0.9–1.1)	0.53	0.9 (0.8–1.0)	0.20
	MPV17L2	rs874628	0.1 (0.02 to 0.2)	0.02	1.1 (1.0–1.2)	0.03	1.2 (1.0–1.3)	0.01
	DKKL1	rs2303759	0.06 (−0.04 to 0.2)	0.24	1.1 (1.0–1.2)	0.05	1.0 (0.9–1.2)	0.51
20	CD40	rs2425752	0.03 (−0.1 to 0.06)	0.52	1.0 (0.9–1.0)	0.27	1.0 (0.9–1.1)	0.58
	CYP24A1	rs2248359	0.07 (−0.02 to 0.2)	0.13	1.1 (1.0–1.1)	0.20	1.1 (0.9–1.2)	0.39
22	MAPK1	rs2283792	0.06 (−0.03 to 0.1)	0.19	1.0 (1.0–1.1)	0.28	1.1 (1.0–1.2)	0.18
	SCO2	rs140522	−0.02 (−0.1 to 0.08)	0.71	1.0 (0.9–1.1)	0.99	1.1 (0.9–1.2)	0.36

Abbreviations: CI = confidence interval; MS = multiple sclerosis; MSSS = Multiple Sclerosis Severity Score; OR = odds ratio; SNP = single nucleotide polymorphism.
These marginal models tested the association between the 3 MSSS phenotypes and all 52 individual SNPs. Cohort was a random-effect variable in the models.
[a] A total of 3,795 individuals with MS are included in this analysis.
[b] All βs and ORs are from the adjusted models. Models are adjusted for sex, age at onset, HLA-DRB1*15:01, and cohort.

shown to develop milder disease, with decreased demyelination and reduced recruitment of inflammatory cells to the brain.[36] Evidence for multiple linked quantitative trait loci within the Tmevd2/Eae interval controlling disease severity in mice has also been reported.[37] However, these findings have not been replicated in humans.

The present study had many strengths. First, the MSSS has been favored over EDSS to capture disease severity in MS because it incorporates disease duration to account for time.[21] This approach builds on earlier work[38] and is advantageous for large studies where EDSS measurements may have only been recorded at a single time point. We used data from a large, independent cohort reference (Global MSSS) to assign MSSS for each case in the present study. Sparse data were therefore avoided for cells in the reference table and potentially much larger variances, and missing data, in general; both could result from small sample sizes if our cohorts, which vary greatly in size, had been characterized instead by local MSSS.[21] Furthermore, the large sample size used to create the Global MSSS made it possible to reduce the effect of stochastic fluctuations over time. Specifically, EDSS scores in the surrounding 2 years for each disease duration year are accounted for in the ranking; very large data sets are required to do this. It is important to note that the Global MSSS reference data set is at least ∼5 times larger than the largest individual cohort in the present study. The power of Global MSSS for genetic studies based on the large pooled reference data has also been previously demonstrated.[21]

Additional strengths included application of a statistical model for association testing that accounted for random (cohort) effects, and we adjusted for potential confounders such as age and sex. Moreover, use of MSSS extremes analysis reduced the possibility of phenotype misclassification because individuals with benign disease were compared with individuals with severe disease, and individuals in the middle of the MSSS spectrum who are most likely to be misclassified were excluded. While restricting analyses to extreme categories of MSSS reduced the overall number of individuals in our data set, given our overall large sample size, close to 4,000 individuals were still available for association testing.

Analyses were also restricted to individuals with 10 or more years of disease duration since symptom onset, to help ensure stability of the MSSS. At least 1 year of duration is required before the EDSS can be used to calculate the MSSS with the published algorithm.[21] Median time to requiring unilateral assistance ranges from 15 to 30 years in MS, based on the present estimates.[24] Conservatively, the present study used disease duration near the median value for the overall data set (table 1), but before the early end of the transition to requiring unilateral assistance (or an EDSS of 6). When analyses were restricted by disease duration (≥5, ≥10, ≥15, and ≥20 years), wGRS, GRS, and individuals SNPs (data not shown) were

not associated with any of the 3 MSSS outcomes (figures e-4 and e-5).

There were also several limitations in this study. The EDSS used to calculate the MSSS is heavily weighted by physical measurements of disability. While MSSS is a widely accepted measure of disability for MS research, there are concerns about its sensitivity, given that different combinations of disease duration and EDSS can result in similar measures of the MSSS. In addition, it is possible that population-specific EDSS rankings could differ from those derived using the Global MSSS. A genetic association might therefore only be uncovered when a population is examined independently. Even larger studies will be required to determine whether studies of disability based on locally derived values for MSSS reveal population-specific genetic effects. Furthermore, because subclinical disease can occur many years before clinical symptom onset, the measure of disease duration required to derive the MSSS may not be accurate. Recent work shows that age could potentially be used for ranking EDSS in future studies when disease duration is unknown, because the exact age of symptom onset may not be certain.[39] Longitudinal data on progression were not available for cases in the present study. There is also potential selection bias if individuals with mild disease are more willing to participate in research studies than those with severe disease. However, more than 1,100 participants were categorized in the highest or "most severe" MSSS phenotype in the present study (table 1), which provided a sufficient representation of cases with severe disease. Finally, our negative findings may have resulted from population or individual factors that could be not assessed in the present study. Additional genetic variants (untested), environmental exposures, or medication histories were not available and may distinguish MSSS differences in the presence or absence of the established genetic risk variants that were investigated.

The current data set includes 52 non-MHC MS genetic risk variants,[10] and a larger list of the most recently identified non-MHC MS risk variants through GWAS and follow-up studies is now available.[11] The 52 non-MHC MS risk variants used here were stronger genetic markers of MS susceptibility; the average OR based on GWAS for our 52 non-MHC variants is 1.19 vs average OR for new 48 non-MHC variants, which is 1.09.[10,11] GWAS of disease severity, to date, have not yet yielded new candidates at genome-wide significance[5,10]; and while disease risk and severity variants appear to differ in MS, this still remains a question. We have comprehensively pursued testing of the strongest established disease risk variants identified, thus far, for evidence of influence on MS severity. Larger studies of cases with additional genotype data for newly discovered GWAS variants are warranted. Finally, whole-genome data were not available to formally adjust for potential effects of population stratification. However, this was not a case-control study, each cohort was treated separately in the analysis, and consistent nonsignificant estimates for each of the 10 independent cohorts were observed. Our findings in this large, well-powered study are unlikely to result from population stratification.

Results derived from investigation of a large number of recently established GWAS variants in 7,125 MS cases suggest that the genetics underlying MS susceptibility and disease severity, as measured by MSSS, do not substantially overlap. Sensitive measures of severity and progression are needed, and for larger prospective cohort studies of incident cases. Comprehensive quantitative trait data derived from high-resolution brain MRIs can be used as dependent variables for a GWAS, as recently demonstrated with 284 MS patients,[40] or for whole-genome sequencing studies. Longitudinal studies will be informative for genetic investigations. However, such studies will be necessarily balanced by a lack of statistical power to detect modest genetic effects unless very large numbers of individuals can be assembled and followed for long periods of time.

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AUTHOR CONTRIBUTIONS

Dr. George: conception and design, analysis and interpretation of the data, drafting of the manuscript, and final approval of the version to be published. Dr. Briggs: conception and design, analysis and interpretation of the data, reviewing and critically revising the manuscript, and final approval of the version to be published. Ms. Shao and Ms. Gianfrancesco: analysis and interpretation of the data, reviewing and critically revising the manuscript, final approval of the version to be published. Dr. Kockum and Dr. Harbo: acquisition of the data, clinical evaluation of patients, collection of samples, evaluation of manuscript, and final approval of the version to be published. Dr. Celius: clinical evaluation of patients, collection of samples, evaluation of manuscript, and final approval of the version to be published. Dr. Bos, Dr. Hedström, and Dr. Shen: data preparation, evaluation of manuscript, and final approval of the version to be published. Dr. Bernstein: clinical evaluation of patients, evaluation of manuscript, and final approval of the version to be published. Dr. Alfredsson, Dr. Hillert, Dr. Olsson, Dr. Patsopoulos, Dr. De Jager, Dr. Oturai, Dr. Søndergaard, Dr. Sellebjerg, and Dr. Sorensen: acquisition of the data, collection of samples, evaluation of manuscript, and final approval of the version to be published. Ms. Gomez, Ms. Caillier, Dr. Cree, and Dr. Hauser: clinical evaluation of patients, evaluation of manuscript, and final approval of the version to be published. Dr. Oksenberg: acquisition of the data, collection of samples, evaluation of manuscript, and final approval of the version to be published. Dr. D'Alfonso: acquisition of the data, clinical evaluation of patients, collection of samples, evaluation of manuscript, and final approval of the version to be published. Dr. Leone: acquisition of the data, collection of samples, evaluation of manuscript, and final approval of the version to be published. Dr. Martinelli Boneschi: acquisition of the data, clinical evaluation of patients, collection of samples, evaluation of manuscript, and final approval of the version to be published. Dr. Sorosina: acquisition of the data, collection of samples, evaluation of manuscript, and final approval of the version to be published. Dr. van der Mei and Dr. Taylor: acquisition of the data, clinical evaluation of patients, collection of samples, evaluation of manuscript, and final approval of the version to be published. Mr. Zhou: data preparation, evaluation of manuscript, and final approval of the version to be published. Dr. Shaefer: conception and design, acquisition of the data, reviewing and critically revising the manuscript, and final approval of the version to be published. Dr. Barcellos: conception and design, acquisition of the data, analysis and interpretation of the data, drafting and writing of the manuscript, and final approval of the version to be published.

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