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The multiple sclerosis risk allele within the *AHI1* gene is associated with relapses in children and adults

Jennifer S. Graves, MD, PhD, MAS[1,*], **Lisa F. Barcellos, PhD, MPH**[2], **Steve Simpson Jr., PhD**[5], **Anita Belman, MD, FAAN**[4], **Rui Lin, MD, PhD**[5,6], **Bruce V Taylor, MD, PhD**[5], **Anne-Louise Ponsonby, PhD**[7], **Terence Dwyer, MD**[7], **Lauren Krupp, MD, FAAN**[4], **Emmanuelle Waubant, MD, PhD, FAAN**[1], and **Ingrid A F van der Mei, PhD**[5]

[1]UCSF Pediatric MS Center, San Francisco, CA, USA

[2]Genetic Epidemiology and Genomics Lab, School of Public Health, and California Institute of Quantitative Biosciences, UC Berkeley, Berkeley, CA, USA

[4]National Pediatric MS Center, Stonybrook, NY, USA

[5]Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia

[6]Guangxi Center for Disease Prevention and Control, Nanning, China

[7]Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia

[8]School of Medicine, University of Tasmania, Hobart, Australia

Abstract

Background—While common variant non-human leukocyte antigen alleles have been associated with MS risk, their role in disease course is less clear. We sought to determine whether established multiple sclerosis (MS) genetic susceptibility factors are associated with relapse rate in children and an independent cohort of adults with MS.

Methods—Genotyping was performed for 182 children with MS or clinically isolated syndrome with high risk for MS from two Pediatric MS Centers. They were prospectively followed for

[*]Corresponding author: Jennifer Graves MD, PhD, Address: 675 Nelson Rising Lane, Suite 221 Box 3206, San Francisco, CA, Telephone: (415) 297-2344; Fax: (415) 514-2170.

Author Contributions:

Dr. Graves designed the study, collected and cleaned data, performed the analyses and wrote and revised the manuscript.
Dr. Waubant contributed to study design, data collection, result interpretation and manuscript writing.
Dr. Barcellos contributed to study design, provided genetic data, contributed to data interpretation and analysis, and revised the manuscript.
Dr. Simpson was involved in the conception and implementation of the analysis and revision of the manuscript.
Drs. Belman and Krupp contributed genetic samples and clinical outcome data, contributed to data interpretation and revised the manuscript.
Dr. Lin contributed to data preparation, analysis, and interpretation and revised the manuscript.
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relapses. Fifty-two non-HLA MS susceptibility single nucleotide polymorphisms (SNPs) were evaluated for association with relapse rate. Cox regression models were adjusted for sex, genetic ancestry, disease-modifying therapy (DMT), 25-OH vitamin D level and *HLA-DRB1*15:01/03* status. Investigation of pediatric subject SNP results was performed using a second cohort of 141 adult MS subjects of Northern European ancestry from the Southern Tasmanian Multiple Sclerosis Longitudinal Study.

Results—For pediatric subjects, 408 relapses were captured over 622 patient-years of follow-up. Four non-HLA risk SNPs (rs11154801, rs650258, rs12212193, rs2303759) were associated with relapses ($p<0.01$) in the pediatric subjects. After adjustment for genetic ancestry, sex, age, vitamin D level, DMT use and *HLA-DRB1*15* status, having two copies of the MS risk allele within *AHI1* (rs11154801) was associated with increased relapses among children (HR=1.75, 95%CI=1.18–2.48, p=0.006) and this result was also observed among adults (HR=1.81, 95%CI=1.05–3.03, p=0.026).

Conclusions—Our results suggest that the MS genetic risk variant within the gene *AHI1* may contribute to disease course in addition to disease susceptibility.

Keywords

multiple sclerosis; genetics

1. INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system and a leading cause of disability amongst young adults. Both genetic and environmental factors play a role in the risk for the disease. The strongest known genetic risk factor for pediatric and adult MS is human leukocyte antigen (*HLA*)-*DRB1*15:01*.[1 2] More recently, a range of non-HLA single nucleotide polymorphisms (SNPs) have been associated with MS through genome wide association studies (GWAS).[3 4]

The potential role of these non-HLA risk alleles on MS course is not yet clear. Some SNPs have been shown to be associated with MRI lesion distribution, clinical attack location and attack severity, but studies of cross-sectional metrics of disability, MRI lesion load, and atrophy have not shown clear associations.[5–9] Few studies of genetic risk factors have included relapse rate as a longitudinal outcome, though this is the most treatment relevant outcome. Pediatric patients are ideal subjects for studying the association of risk factors with relapses, as they have higher relapse rates than adults.[10]

The goal of this study was to determine whether established non-HLA MS genetic risk variants are associated with relapse rate in children with MS and whether these significant findings can also be observed in adults with MS.

2. PATIENTS AND METHODS

2.1 Pediatric Patient Dataset

Children presenting for clinical care at two Pediatric MS Centers of Excellence at Stonybrook University, New York and University of California, San Francisco were offered

enrollment as previously described.[11] To be included, children had to have diagnoses of either MS or clinically isolated syndrome with abnormal MRI consistent with early MS based on published criteria with symptom onset at less than 18 years of age.[12] Institutional Review Board approval was obtained from the two clinical centers and UC Berkeley. Written informed consent was obtained from parents and assent from children. Blood samples were collected upon enrollment and subjects followed for clinical course. Relapses were included from January 2006 to December 2011 during which time largely only injectable MS medications were used.[11] Despite being tertiary referral centers, these pediatric MS centers deliver care for patients from wide geographic regions and provide care for children both with and without health insurance.

2.2 Clinical, demographic and vitamin D level assessments

To be defined as relapses, clinical events had to meet the following criteria: new or significantly worsened neurological symptoms lasting greater than 24 hours without evidence of infection or fever or exposure to extreme heat (pseudo-exacerbation excluded by the neurologist). Disease-modifying therapy dates of initiation and cessation were recorded for all medications used per patient and therapy use was treated as a time-varying covariate. Race and ethnicity information, recorded by the NIH guidelines, were reported by the parents of each child and genetic ancestral markers were also obtained as below. Batched 25(OH) vitamin D levels were determined by chemoluminescence assay as previously described (Heartlands, ARUP).[11 13]

2.3 Genotyping

Whole blood collected from patients was processed and extracted for DNA using standard procedures. For all participants, *HLA-DRB1* typing was performed as previously described.[14] As the pediatric subjects had diverse ancestry, both *HLA-DRB1*15:01* and *15:03* were characterized and if either allele was present, this was noted as positive for *HLA-DRB1*15*. The Infinium 660K BeadChip or HumanOmniExpress BeadChip was used to genotype each study participant as previously described.[3 15] The Illumina GenomeStudio software was used to assess the overall sample and SNP call rates (<90%), sex discrepancies, reproducibility of replicates and checks for Mendelian inheritance using CEPH control trios, as well as performance of internal Illumina controls for each BeadChip. Stringent quality control (QC) measures and comparison of sample genotypes across the two Illumina platforms described above were performed using PLINK v.1.07[16].

Briefly and as previously described[11], for the Illumina 660K BeadChip, Mendelian consistency based on CEPH trio genotype results was >99.95%. SNPs that deviated from HWE ($p<0.0001$) (1.7%), had a MAF <0.01 (5.9%) or call-rate <90% (5.9%) were excluded from the final data set. For the OmniExpress BeadChip, Mendelian consistency based on CEPH trio genotype results was >99.97%. As by standard protocol, SNPs that deviated from HWE ($p<0.0001$) (0.4%), had a MAF <0.01 (7.3%) or call-rate <90% (0.25%) were dropped from the data set. Ten study replicates had >99.99% genotype matches among replicates of successfully typed SNPs. A total of 324,294 SNPs overlapped between the two Illumina BeadChips. For additional stringency in QC, a group of 21 samples were genotyped on both BeadChips with ~99% agreement. Discrepancies in sex were not observed. All case samples

were successfully genotyped. Imputation and determination of genetic ancestry for each was performed as previously described.[11]

2.4 Adult Patient Dataset

The Southern Tasmanian Multiple Sclerosis Longitudinal Study was conducted from 2002–2005, initially as a prospective study of the role of UV exposure and 25-OH vitamin D in the clinical course of MS. Of the prevalent population with clinically definite MS in southern Tasmania at the time, 203 participants were recruited and 198 were included in analyses, the difference being due to changes in diagnosis or other loss to follow-up. A total of 145 subjects (73%) with relapsing-remitting MS completed at least one follow-up visit, while genetic data were available for 141 (97%) of these. The study was approved by the Southern Tasmanian Human Research Ethics Committee and informed consent obtained by all participants. This study has yielded a number of significant research findings, including the dose-dependent protective effect of increased 25-OH vitamin D against relapse[17] and the associations of MS risk associated SNPs and vitamin D pathway SNPs including protein kinase C in modulating both relapse and the 25-OH vitamin D-relapse associations.[18 19]

At each biannual review (summer and winter), participants were queried for a range of behavioral and environmental covariates of relevance to MS, in light of potential and established risk factors in the literature of the time. Participants also had blood taken at biannual review, for subsequent measure of serum 25-OH vitamin D and viral serological parameters, while genotyping was done using whole blood. As described in more detail previously[18], genotyping of samples from 164 MS cases were done using the Illumina Infinium Hap370CNV Array[20], while an additional 29 MS cases were genotyped using the Illumina HumanOmniExpress-12v1_A array. All the samples were previously identified as being from persons of European descent [20], and a conservative quality control was conducted with PLINK [21]: individuals with call rates less than 0.90, SNPs with call rates less than 0.95 or in Hardy-Weinberg equilibrium ($p<10^{-7}$), or duplicates were excluded, leaving 97.9% (189/193) 189 cases with 290,536 SNPs. Of these, 141 were of RRMS course followed beyond baseline and thus could be included in this relapse investigation.

2.5 Statistical analysis

Descriptive statistics, including frequencies, means and medians as appropriate, were used to compare the two groups of MS subjects. The associations between genetic risk factors and relapse rate were evaluated with repeated events analyses, specifically, Cox regression models using time to relapse and adjustment in standard errors to reflect multiple relapses per patient. To confirm the proportional hazards assumption, the Schoenfeld residuals test was used for all models. *A priori* emphasis was placed on multivariable models with and without interaction terms with vitamin D level.[13 22] Potential confounders, sex, 25-OH vitamin D level, genetic ancestry, *HLA-DRB1*15* status, DMT use and age were also included in the models. Disease-modifying therapy use was treated as a time-varying covariate. Multi-dimensional scaling components were used to adjust for genetic ancestry. Given prior known association of vitamin D level with relapse activity, we also examined potential modification of this association by MS genetic risk variants. Genetic risk factor-vitamin D level interactions with p-values of p=0.1 or less were considered of interest. For

the independent adult patient dataset, all subjects were of Northern European ancestry and genetic ancestry was not included in models. As EDSS in these adults was shown to be associated with relapse activity (as opposed to the children who all had low EDSS), models were also adjusted for the covariate of EDSS category (0–2.5, 3.0–5.0, 5.5–7.0, 7.5–9.0). Adjustment for multiple comparisons was made using the Benjamini-Hochberg False Discovery Rate (FDR). In this method p values for the SNPs evaluated were ranked. Given the expected range of p-values an FDR of 10% was selected prior to analysis. To calculate the FDR q-value for a given model the rank was divided by the number of tests and multiplied by 0.10. The null hypothesis was considered rejected if the original p-value was less than the q-value. By this method false positives would be expected to be 10% or less of SNPs evaluated with raw p-values less than 0.05. Analyses were performed using STATA v12.0 (Statacorp, TX).

3. RESULTS

Analyses were performed for 182 subjects with pediatric MS or CIS. Characteristics of this cohort have been previously published[11] and are similar to other reported cohorts of pediatric MS subjects (Table 1). The majority of subjects (n=131; 71%) were exposed to disease-modifying therapy during the follow-up period. Of these, 105 (80%) had exposure to interferon medications. A total of 408 relapses were captured over 622 person-years of follow-up (0.66 relapse/person-year). The median number of relapses per patient was one, but ranged from 0 to 12.

The independent adult dataset consisted of prevalent MS cases with median disease duration since first symptoms of 11.6 years (Table 1). Of the 141 subjects, 70 experienced 122 relapses over 330.2 person-years (0.37 relapses/person-year). The range of vitamin D levels and frequency of *HLA-DRB1*15* positivity was similar to the pediatric subjects, though the latter were of more diverse racial and ethnical backgrounds.

3.1 Non-HLA risk alleles main effect models for relapse rate

In multivariable models adjusting for sex, genetic ancestry, DMT use, 25-OH vitamin D level, and *HLA-DRB1*15* status, four MS risk alleles were associated with clinically relevant changes in relapse hazard in children ($p<0.01$; Table 2). Two (rs11154801, rs12212193) were significant after a correction for multiple comparisons with 10% false discovery rate (FDR, Table 2). For rs11154801, the association with relapses was also observed with near identical magnitude of effect in the adult subjects (Table 2). Having two copies of the A risk allele at rs11154801 within the *Abelson Helper Integration Site 1* (*AHI1*) gene was associated with a 1.75-fold (95% CI 1.18–2.48, p=0.006) higher hazard to relapse in children and 1.81-fold (95%CI=1.05–3.03, p=0.026) higher hazard to relapse in adults.

3.2 Non-HLA risk alleles and modification of vitamin D effects on relapse rate

We found that several non-HLA susceptibility alleles (associated with genes *IL22RA2, TNFRSF1A, EVI5, EOMES, CYP27B1*,) modified the association of vitamin D level ($p_{ixn}<0.1$) with relapse hazard in the pediatric subjects (Table 3). There was suggestion of

replication for the interaction between the risk SNP downstream of the gene *IL22RA2* and vitamin D level in the adult subjects (Table 4), but it did not reach statistical significance (p_{ixn}=0.10). Having a GG genotype at this locus was associated with 1.6 fold (95%CI 1.15–2.29, p=0.006) in children and 1.3 fold (95%CI 1.07–1.55, p=0.007) in adults increase in relapse hazard per 10 ng/ml lower vitamin D level. There was no association of vitamin D level with relapse hazard in the GA and AA genotypes (Tables 3 and 4).

4. DISCUSSION

In our well-characterized clinical cohort of pediatric MS subjects, we have found that being a homozygote for the MS risk allele within *AHI1* is associated with an increased hazard to relapse. This finding was also observed in an independent dataset of adult patients with a very similar effect size. AHI1 is associated with brainstem and cerebellar development, in particular the neurons that give rise to the crossing axons of the corticospinal tract, and mutations within *AHI1* cause the recessive disease Joubert's syndrome.[23 24] Joubert's syndrome is a rare genetic disorder that affects the brainstem and cerebellum, areas of the brain that control balance and coordination. In addition, loss of function mutations in *AHI1* have been associated with depressive behaviors in mice[25] and variants within *AHI1* are being studied in relation to neuropsychiatric illnesses.[26 27] *AHI1* has also been strongly associated with leukemia development and as a provirus integration site and has been linked to the c-myb proto-oncogene.[28] Level of AHI1 expression has been shown to be associated with hematopoietic cell differentiation and proliferation.[28] Dysregulation of AHI1 expression has been associated with chronic myelogenous leukemia and cutaneous T-cell lymphoma. While not direct evidence, these data support the potential for AHI1 expression levels to affect immune cell differentiation and proliferation in MS.

The SNP rs11154801 on chromosome 6 analyzed in our study is located within an intron of *AHI1* and has been previously annotated as a functional variant with eQTL *cis*-effects.[29] The SNP is within a putative transcription factor binding site and falls within an active regulatory region relevant to multiple cell lines.[29] Differential expression of *AHI1* in MS cases and controls has been observed in peripheral blood mononuclear cells (PBMC) and CD34+ hematopoietic progenitor cells (HPC).[29]

In the pediatric subjects we found several individual SNPs that may modify the association of vitamin D with relapse rate. For the polymorphism associated with *IL22RA2*, there was a strong magnitude of the effect of the statistical interaction in children, and in adults the point estimates demonstrated similar evidence for interaction, though the statistical evidence was less robust in the smaller adult cohort. Lower vitamin D levels may confer more risk for relapse in those homozygous for the minor variant G allele. The SNP rs17066096 is located 14 kilobases downstream of *IL22RA2*. [30] Its effect on the gene product is not clear, but *IL22RA* through expression of interleukin 22 binding protein is known to influence autoimmunity.[31] Recent results from a murine model of experimental autoimmune encephalitis demonstrate beneficial effects on disease severity of deleting the ortholog of *IL22RA*.[32]

For the polymorphism rs1800693 (gene candidate *TNFRSF1A*), found to have potential interaction with vitamin D level in children, a previous study demonstrated a modest association for this allele with time to second relapse in adults, but an interaction with vitamin D level was not reported.[7] Previous work has demonstrated that the relationship between tumor necrosis factor alpha and relapse rate may be modified by carrier allele status at rs1800693.[33]

Another consideration in the interpretation of our results is whether genetic polymorphisms have direct effects on intrinsic relapse rate of an individual or instead affect treatment response. While our study was not powered to look for interactions between individual MS treatments and risk alleles, this is an important consideration for future studies of genotype associations with MS disease course.

We acknowledge limitations within this investigation. Our sample size was modest and constrained by onset of MS in childhood occurring in only 5% of MS cases. While smaller sample sizes pose risk for false positives and negatives, the follow-up duration and capture of 408 relapse events in the children with MS strengthened the statistical power of the study. Furthermore, several alleles of interest were rare within our sample group. While we rigorously applied the Schoenfeld test to detect violations of the proportional hazard assumption, this test may be insensitive in restricted sample sizes. Our study was strengthened by the addition of an independent set of adult subjects; however, the adult patients had a greater disease duration and exhibited fewer relapses.

The strengths of our study include the longitudinal characterization of relapse activity in both sets of subjects, rigorous statistical modeling, and demonstration that MS risk genes may predict relapse rate. Prior studies examining genetic risk factors for association with measures of disease severity have not yielded strong findings, but most have been limited by cross-sectional designs and have not been directed toward longitudinal relapse activity, the outcome against which all therapies have been targeted.[3] Our results from both pediatric and adult MS subjects motivate the need for large, prospective studies of gene effects on disease activity in the context of environmental risk factors.

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Highlights

- Several genetic common variant susceptibility alleles were associated with relapses in children with MS.
- One of these alleles within the gene *Abelson Helper Integration Site 1* was also associated with relapses in an independent set of adults with MS with near identical effect size.
- This variant rs11154801 on chromosome 6 is located within an intron of *AHI1* and has been previously annotated as a functional variant.

Table 1

Subject Characteristics

	Pediatric RRMS or CIS* (n=182)	Adult RRMS (n=141)
Age at baseline, mean yrs (± SD)	13.1 (± 4.2)	45.8(±10.4)
Median follow-up time, months (range)	34 (2–174)	30 (7–36)
Median relapses (range)	1 (0–12)	0 (0–5)
White (%)	120 (65.1)	141 (100)
Hispanic (%)	55 (30.3)	0 (0)
Female (%)	124 (65.8)	106 (75.2)
Median baseline 25(OH) vitamin D (range)	23(2–63) ng/ml	16 (6–49) ng/ml
*HLA-DRB1*15:01/03 positive* (%)	74 (41.4)	53 (37.6)
DMT exposure during follow-up period (%)	131 (72)	116 (82.3)

*Subjects had clinically definite MS or CIS at study entry and then were followed prospectively. DMT = disease modifying therapy.

Table 2

Association of MS genetic risk variants with hazard to relapse in children and adults

	Pediatric Subjects[a]					Adult Subject[b]		
Risk variant	**MS risk allele/frequency**	**Genotype**	**Adjusted HR for relapse (95% CI)**	**P-value**	**FDR[c] Q-value**	**MS risk allele/frequency**	**Adjusted HR for relapse (95% CI)**	**P-value**
rs11154801 (*AHI1*)	A 0.361	AA/ AG +GG	1.75* (1.18–2.48)	0.0056	0.0058	A 0.348	1.81* (1.05–3.03)	0.026
rs650258 (*CD6*)	G 0.722	GA+GG/ AA	1.83 (1.21–2.77)	0.0042	0.0038	G 0.649	0.70 (0.40–1.23)	0.21
rs12212193 (*BACH2*)	G 0.379	AA/ GA + GG	1.65 (1.16–2.35)	0.0062	0.0077	A 0.465	1.04 (0.61–1.79)	0.88
rs2303759 (*DKKL1*)	C 0.272	CC/ CA + AA	0.40 (0.21–0.73)	0.0033	0.0019	C 0.298	0.88 (0.44–1.78)	0.72

[a] Adjusted for sex, 25-OH vitamin D level, DMT use, age of onset, *HLA-DRB1*15* status and multidimensional scaling components representing population substructure (ancestry).

[b] Adjusted for sex, 25-OH vitamin D level, DMT use, age of onset, *HLA-DRB1*15* status and EDSS category.

[c] FDR- False discovery rate; q-value for 10% FDR presented. The null hypothesis is considered rejected if p-value less than the q-value.

Table 3

MS genetic risk variants, 25-OH vitamin D level and association with hazard to relapse in pediatric subjects: multiplicative interaction.

Risk variant (Gene candidate)	MS risk allele/frequency	Alleles	Unadjusted HR for relapse (95% CI, p value)	Adjusted HR for relapse (95% CI, p value)	Vit D $p_{interaction}$	HR for −10U vit D Genotype Pos (95% CI, p value)	HR for −10U vit D Genotype Neg (95% CI, p value)
rs17066096 ***IL22RA2***	G 0.238	GG/ GA + AA	0.91(0.54–1.53) 0.72	4.73(1.79–12.5) 0.002	0.015	1.62(1.15–2.29) 0.006	1.03(0.90–1.17) 0.71
rs1800693 ***TNFRSF1A***	G 0.423	GG/ AG + AA	1.07(0.74–1.54) 0.73	3.13(1.61–6.08) 0.001	0.021	1.42(1.10–1.82) 0.007	1.02 (0.89–1.16) 0.81
rs11810217 ***EVI5***	A 0.224	AA +AG/ GG	0.85(0.66–1.11) 0.24	1.80(0.93–3.50) 0.083	0.042	1.40(1.04–1.87) 0.026	1.02(0.91–1.14) 0.78
rs11129295 ***EOMES***	A 0.533	AA + AG/ GG	1.03(0.78–1.38) 0.81	0.62(0.36–1.09) 0.096	0.055	0.99(0.86–1.14) 0.89	1.24(1.03–1.50) 0.023
rs12368653 ***CYP27B1***	A 0.494	AA+AG/ GG	0.85(0.66–1.09) 0.21	1.51(0.88–2.60) 0.14	0.061	1.18(1.00–1.39) 0.048	0.97(0.84–1.12) 0.67

[a]Adjusted for sex, age at onset, genetic ancestry, interaction with vitamin D level, DMT use and *HLA-DRB1*15* status. Models are presented for genotypes with p values <0.1 for interaction with vitamin D level. To illustrate the interaction, stratified results are shown for the hazard ratio for 10U lower 25-OH D level in presence or absence of the risk genotype.

Table 4

MS genetic risk variants, 25-OH vitamin D level and association with hazard to relapse in adult dataset: multiplicative interaction.

Risk variant (Gene candidate)	MS risk allele/frequency	Alleles	Unadjusted HR for relapse (95% CI, p value)	Adjusted HR for relapse (95% CI, p value)	Vit D $p_{interaction}$	HR for −10U vit D Genotype Pos (95% CI, p value)	HR for −10U vit D Genotype Neg (95% CI, p value)
rs17066096 ***IL22RA2***	G 0.270	GG/ GA + AA	1.15(0.45–2.93) 0.77	1.79(0.53–6.02) 0.34	0.10	1.29(1.07–1.55) 0.007	1.10(1.02–1.19) 0.018
rs1800693 ***TNFRSF1A***	G 0.397	GG/ AA + AG	0.86(0.49–1.52) 0.61	0.82(0.22–3.06) 0.76	0.62	1.03(0.88–1.21) 0.71	1.13(1.04–1.22) 0.004
rs11810217 ***EVI5***	A 0.312	AA +AG/ GG	0.71(0.46–1.08) 0.11	0.47(0.21–1.06) 0.07	0.91	1.14(1.02–1.27) 0.025	1.13(1.03–1.24) 0.012
rs11129295 ***EOMES***	A 0.369	AA+ AC/ CC	0.91(0.58–1.41) 0.66	0.77(0.34–1.73) 0.52	0.13	1.05(0.97–1.14) 0.26	1.20(1.06–1.36) 0.004
rs12368653 ***CYP27B1***	A 0.479	AA + AG/ GG	0.72(0.46–1.13) 0.15	0.68(0.29–1.62) 0.39	0.76	1.07(0.98–1.17) 0.14	1.05(0.93–1.19) 0.40

[a] Adjusted for sex and interaction with 25-OH vitamin D level and stratified by *HLA-DRB1**15 status, EDSS category. Unadjusted for multiple comparisons.