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## Genetic risk factors for pediatric–onset multiple sclerosis

Milena A Gianfrancesco, MPH, PhD<sup>1</sup>, Pernilla Stridh, PhD<sup>2</sup>, Xiaorong Shao, MA<sup>1</sup>, Brooke Rhead, BS<sup>3</sup>, Jennifer S. Graves, MD, PhD<sup>4</sup>, Tanuja Chitnis, MD<sup>5</sup>, Amy Waldman, MD<sup>6</sup>, Timothy Lotze, MD<sup>7</sup>, Teri Schreiner, MD<sup>8</sup>, Anita Belman, MD<sup>9</sup>, Benjamin Greenberg, MD<sup>10</sup>, Bianca Weinstock–Guttman, MD<sup>11</sup>, Gregory Aaen, MD<sup>12</sup>, Jan M Tillema, MD<sup>13</sup>, Janace Hart, BA<sup>14</sup>, Stacy Caillier, BS<sup>14</sup>, Jayne Ness, MD<sup>15</sup>, Yolanda Harris, NP<sup>15</sup>, Jennifer Rubin, MD<sup>16</sup>, Meghan Candee, MD<sup>17</sup>, Lauren Krupp, MD<sup>9</sup>, Mark Gorman, MD<sup>18</sup>, Leslie Benson, MD<sup>18</sup>, Moses Rodriguez, MD<sup>13</sup>, Soe Mar, MD<sup>19</sup>, Ilana Kahn, MD<sup>20</sup>, John Rose, MD<sup>21</sup>, Shelly Roalstad, MSc, BS<sup>22</sup>, T. Charles Casper, PhD<sup>22</sup>, Ling Shen, PhD, MPH<sup>23</sup>, Hong Quach, BA<sup>1</sup>, Diana Quach, BA<sup>1</sup>, Jan Hillert, MD<sup>24</sup>, Anna Hedstrom, MD<sup>24</sup>, Tomas Olsson, MD<sup>2</sup>, Ingrid Kockum, PhD<sup>2</sup>, Lars Alfredsson, PhD<sup>24,25</sup>, Catherine Schaefer, PhD<sup>23,26</sup>, Lisa F Barcellos, PhD, MPH<sup>1,3,23,\*</sup>, Emmanuelle Waubant, MD, PhD<sup>4,\*</sup>, for the Network of Pediatric Multiple Sclerosis Centers<sup>#</sup>

<sup>1</sup>Division of Epidemiology, School of Public Health, University of California, Berkeley, CA

<sup>2</sup>Department of Clinical Neuroscience and Center for Molecular Medicine, Karolinska Institutet at Karolinska University Hospital, Stockholm, Sweden

<sup>3</sup>Computational Biology Graduate Group, University of California, Berkeley, CA

<sup>4</sup>Department of Neurology, University of California, San Francisco, CA

<sup>5</sup>Partners Pediatric Multiple Sclerosis Center, Massachusetts General Hospital for Children, Boston, MA

<sup>6</sup>Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA

<sup>7</sup>Blue Bird Circle Multiple Sclerosis Center, Baylor College of Medicine, Houston, TX

Corresponding Author: Dr. Emmanuelle Waubant, Multiple Sclerosis Center, University of California, San Francisco, 675 Nelson Rising Lane, San Francisco, CA 94158; Emmanuelle.Waubant@ucsf.edu; Phone: 415-514-8199.

\*These authors contributed equally to the manuscript

<sup>#</sup>Authors and their institutions as part of the Network of Pediatric Multiple Sclerosis Centers are listed in the supplemental material (Appendix 1)

### DECLARATION OF CONFLICTING INTERESTS

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- <sup>8</sup>Children's Hospital Colorado, University of Colorado, Denver, CO
- <sup>9</sup>Lourie Center for Pediatric MS, Stony Brook Children's Hospital, Stony Brook, NY
- <sup>10</sup>Department of Neurology, University of Texas Southwestern, Dallas, TX
- <sup>11</sup>Pediatric Multiple Sclerosis Center, Jacobs Neurological Institute, SUNY Buffalo, NY
- <sup>12</sup>Pediatric MS Center at Loma Linda University Children's Hospital, Loma Linda, CA
- <sup>13</sup>Mayo Clinic's Pediatric MS Center, Rochester, MN
- <sup>14</sup>University of California, San Francisco, Regional Pediatric MS Center, Neurology; San Francisco, CA
- <sup>15</sup>University of Alabama Center for Pediatric-onset Demyelinating Disease, Children's Hospital of Alabama, Birmingham, AL
- <sup>16</sup>Department of Pediatric Neurology, Northwestern Feinberg School of Medicine, Chicago, IL
- <sup>17</sup>University of Utah, Primary Children's Hospital, Salt Lake City, UT
- <sup>18</sup>Boston Children's Hospital, Boston, MA
- <sup>19</sup>Pediatric-onset Demyelinating Diseases and Autoimmune Encephalitis Center St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO
- <sup>20</sup>Children's National Medical Center, Washington, DC
- <sup>21</sup>Department of Neurology, University of Utah School of Medicine, Salt Lake City, UT
- <sup>22</sup>Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT
- <sup>23</sup>Kaiser Permanente Division of Research, Oakland, CA
- <sup>24</sup>Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
- <sup>25</sup>Centre for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden
- <sup>26</sup>Research Program on Genes, Environment and Health, Kaiser Permanente, Oakland, CA

## Abstract

**Background**—Strong evidence supports the role of both genetic and environmental factors in pediatric-onset multiple sclerosis (POMS) etiology.

**Objective**—We comprehensively investigated the association between established MHC and non-MHC adult MS-associated variants and susceptibility to POMS.

**Methods**—Cases with onset <18 years (n=569) and controls (n=16,251) were included from the U.S. and Sweden. Adjusted logistic regression and meta-analyses were performed for individual risk variants and a weighted genetic risk score (wGRS) for non-MHC variants. Results were compared to adult MS cases (n=7,588).

**Results**—*HLA-DRB1\*15:01* was strongly associated with POMS ( $OR_{meta} = 2.95$ ,  $p < 2.0 \times 10^{-16}$ ). Further, 28 of 104 non-MHC variants studied (23%) were associated ( $p < 0.05$ ); POMS

cases carried, on average, a higher burden of these 28 variants compared to adults ( $OR_{avg}=1.24$  vs. 1.13, respectively), though the difference was not significant. The wGRS was strongly associated with POMS ( $OR_{meta}=2.77$ , 95% CI 2.33, 3.32,  $p<2.0 \times 10^{-16}$ ) and higher, on average, when compared to adult cases. Additional class III risk variants in the MHC region associated with POMS were revealed after accounting for *HLA-DRB1\*15:01* and *HLA-A\*02*.

**Conclusion**—Pediatric and adult MS share many genetic variants suggesting similar biological processes are present. MHC variants beyond *HLA-DRB1\*15:01* and *HLA-A\*02* are also associated with POMS.

### Search terms

multiple sclerosis; pediatrics; genetics; epidemiology

## INTRODUCTION

Multiple sclerosis (MS) disease onset occurs on average around 34 years of age, although approximately 5% of all MS patients have symptom onset before 18 years.<sup>1,2</sup> Environmental factors established in adult-onset MS, such as remote infection with Epstein-Barr virus and exposure to cigarette smoking, have also been strongly implicated in pediatric-onset disease susceptibility.<sup>3</sup> However, unlike adult-onset MS, pediatric cases almost exclusively have a relapsing-remitting course, and, in North America, are less likely to be of white/European ancestry.<sup>1,2</sup>

While limited evidence supports a role for similar genetic risk factors, such as *HLA-DRB1*, in pediatric MS susceptibility,<sup>4,5</sup> to date, the 110 non-MHC adult MS susceptibility loci identified through a genome-wide association study,<sup>6</sup> as well as the remainder of the MHC, have not been studied in pediatric-onset MS (POMS). Sorting out which adult MS risk variants are associated with onset in childhood will help determine whether the genetic contributions to the development of MS pathology occur irrespective of age. In addition, identifying variants with larger effects in POMS could point to critical biological pathways determining an earlier onset. The goal of this study was to: (1) determine whether established MS risk variants from GWAS in adults are also associated with POMS and have higher effect sizes; (2) to study *HLA-DRB1\*15*, *HLA-A\*02* and the MHC region in POMS using high density SNP genotyping; (3) to directly compare results from pediatric-onset and adult cases for differences in overall genetic burden and effect size; and (4) to determine whether genetic risk variants are associated with age of onset in POMS cases.

## METHODS

### U.S. Participants

POMS cases were enrolled through pediatric MS centers established across the U.S. between January 2006 and December 2014 (Appendix 2). Consecutive patients with onset of MS or clinically isolated syndrome suggestive of early MS before age 18 seen at some of these pediatric MS clinics were offered participation as previously described.<sup>7</sup> In addition, we utilized adult cases from Kaiser Permanente Northern California (KPNC) with reported age of first symptom onset <18 years.<sup>8</sup> Age of onset of MS was determined as year of first

self-reported clinical symptom, based on the following questions: “How old were you when you had your first clinical symptoms of MS?”, “What were your first clinical symptoms of MS?”. Year of disease onset was calculated using date of birth and was verified in the medical record when possible. KPNC MS cases with age of onset > 18 years were included for comparison in the study (n=1,103).<sup>8</sup> Established diagnostic criteria were used for all pediatric (n=738)<sup>9</sup> and adult-onset cases (n=1,103).<sup>10,11</sup>

Control individuals without a diagnosis of MS or related condition (optic neuritis, transverse myelitis, or demyelination disease) were recruited from Northern California,<sup>8</sup> including individuals from the Genetic Epidemiology Research on Adult Health and Aging cohort who participated in the KPNC Research Program on Genes, Environment, and Health (RPGEH) (dbGaP phs000674.v2.p2), and controls recruited as part of a pediatric MS case-control study (total n=12,300).<sup>12</sup>

### Swedish Participants

Data were collected from two population based case-control studies in Sweden of incident (Epidemiological Investigation of MS [EIMS]) and prevalent (Genes and Environment in MS [GEMS]) MS patients. The EIMS study (2005–2014) inclusion criteria were: age 16–70 years, diagnosed MS according to the McDonald criteria<sup>10,11</sup> within two years, and ability to understand the Swedish language. GEMS study participants were identified from the Swedish National MS registry, fulfilled the McDonald criteria, and were recruited during 2009–2011. For both studies, controls were randomly chosen from the population register and matched to cases by sex, age at inclusion in the study, and region of residence. Two controls were matched to each case in the EIMS study and one control per case in the GEMS study. All participants in the EIMS study were distinct from those in the GEMS study. Details of the study design have been described elsewhere.<sup>13</sup> Age of onset of MS was determined as year of first self-reported clinical symptom and confirmed when possible, as described above. Data for 175 POMS cases (reported age of onset <18 years), 6,485 adult-onset MS cases, and 5,376 controls were available for the current study.

### Standard Protocol Approvals

Study protocols were approved by the Institutional Review Boards for Human Subjects at UCSF, Stony Brook, Children’s Hospital of Philadelphia, Texas Children’s Hospital, University of Colorado School of Medicine, University of Texas Southwestern, State University of New York Buffalo, Loma Linda University, Mayo Clinic, University of Alabama at Birmingham, Ann & Robert Lurie Children’s Hospital of Chicago, University of Utah, Boston Children’s Hospital, Massachusetts General Hospital, Washington University St. Louis, Children’s National Medical Center, Kaiser Permanente Division of Research, UC Berkeley, Regional Ethical Review Board in North Stockholm, and Karolinska Institutet. Informed consent or assent (children) was obtained for all study participants and their parents when appropriate.

### Statistical Analyses

Genotyping and quality control procedures are outlined in the supplemental materials (Appendix 2). Characterization of *HLA-DRB1\*15* was assessed for each study participant;

for the U.S. study, a tag SNP (rs3135388) was used.<sup>14</sup> Whole genome data were used to test the previously established 110 MS GWAS SNP genotypes<sup>6</sup> for association with POMS. Each SNP genotype was considered as zero, one, or two minor alleles (additive effect) for analysis. Further, a weighted genetic risk score (wGRS) that combines the weighted odds ratio (OR) from each of 110 non-MHC MS susceptibility loci was calculated for each pediatric-onset case and control by multiplying the number of risk alleles for each locus by the weight for that variant and then taking the sum across the 110 loci.<sup>15</sup> The weight for each locus is the natural log of the OR for each allele. Three of 110 SNPs were missing in the U.S. study (rs201202118, rs201847125 and imm\_5\_141486748), and one SNP failed Hardy-Weinberg equilibrium, rs2744148. Three of 110 SNPs were missing from the Swedish data (rs2028597, rs4679081, and imm\_5\_141486748). Adequate SNP tagging proxies were not identified; therefore, these SNPs were not included in the individual SNP meta-analyses (n=6) or each cohort's respective wGRS.

Logistic regression modeling was used adjusting for genetic ancestry and other covariates when noted for case-control analyses. 95% confidence intervals (CI), allelic OR (unless otherwise noted) and two-sided p-values were reported. For the MHC region, individual variants were analyzed spanning 6p22.1 to 6p21.3 (~29Mb to 33Mb on hg19 assembly). Meta-analyses were performed under a random effects model. Linear regression was performed in case-only analyses with age of onset as the outcome; values were transformed to the fifth power ( $x^5$ ) to normalize the negatively skewed distribution. All analyses were performed in PLINK v.1.07 or R v.3.2.2. Because the study of candidate SNPs and wGRS was strongly hypothesis-based, we used  $p < 0.05$  as a type I error rate for reporting significance. For the MHC region analyses (beyond *HLA-DRB1\*15:01* and *HLA-A2*), the number of independent MHC haplotype blocks was used to control for multiple comparisons and guide interpretation of results.

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Funding sources listed below (see Acknowledgements) had no involvement in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

## RESULTS

### Subject Characteristics

We restricted our study sample to white non-Hispanic individuals (European ancestry), the largest group in our dataset, to ensure a genetically homogenous sample and avoid the possibility of confounding by genetic ancestry (Supplementary Figure 1). After excluding population outliers, the final dataset was comprised of 394 POMS cases and 10,875 controls from the U.S., as well as 175 POMS cases and 5,376 controls from Sweden (total N=16,820). Demographic features and clinical characteristics of participants are shown in Table 1. Mean age at symptom onset for POMS cases was 14.05 years (+/- 3.30 years) in the U.S. study and 14.91 (+/- 2.67) in the Swedish study. We also included 1,103 and 6,485 adult-onset MS cases from the U.S. and Sweden, respectively (total N=7,588), for comparison of genetic associations observed in POMS (Supplementary Table 1).

## Established Non–MHC MS Genetic Risk Variants

Results from the meta-analysis of MS variants identified in recent GWAS demonstrated that 28 of 104 established MS SNPs tested were significantly associated with pediatric-onset (Table 2; Supplementary Table 2). POMS cases carried, on average, a higher burden of the 28 variants compared to adult MS cases carrying the same polymorphisms (Figure 1) though the difference was not significant; the average OR of the 28 significant variants was 1.24 (95% CI 1.09, 1.41) in POMS cases compared to 1.13 (95% CI 1.08, 1.19) in adult MS cases, using estimates published by the International Multiple Sclerosis Genetics Consortium, with confidence intervals overlapping.<sup>6</sup>

A significantly higher wGRS was observed in POMS cases compared to controls in both populations even after controlling for *HLA-DRB1\*15* carrier status ( $OR_{meta}=2.77$ , 95% CI 2.33, 3.32,  $p<2.0 \times 10^{-16}$ ), indicating strong, independent effects of both wGRS and *HLA-DRB1* on disease susceptibility. No association was observed between wGRS and age of onset in POMS cases (data not shown). Further, we compared pediatric-onset with adult MS cases for differences in wGRS. Results showed the mean wGRS was higher in pediatric-onset compared to adult MS cases; this difference was significant in the Swedish dataset ( $p=0.01$ ); similar results were observed in the U.S. dataset, but were not significant ( $p=0.31$ ).

## Major Histocompatibility Complex

*HLA-DRB1\*15:01* was strongly associated with POMS cases compared to controls ( $OR_{meta}=2.95$ , 95% CI 2.33, 3.73,  $p<2.0 \times 10^{-16}$ ; Figure 2A). Evidence for a protective effect of *HLA-A2* was also present ( $OR_{meta}=0.73$ , 95% CI 0.54, 0.98,  $p=0.04$ ). Adjustment for both *HLA-DRB1\*15:01* and *HLA-A2* associations revealed more than 300 MHC variants associated with POMS ( $p<0.05$ ) (Figure 2B; Supplementary Table 3); the top 20 SNPs are shown in Table 3 ( $p < 10^{-3}$ ) and suggest both class II and III effects, independent of *HLA-A2* and *HLA-DRB1\*15:01*, are present. No association was observed between *HLA-DRB1\*15:01* and age of onset in POMS cases (data not shown). When we compared pediatric-onset with adult MS cases, *HLA-DRB1\*15:01* allele distribution differences were present in the Swedish dataset ( $p=0.02$ ); 68% of pediatric-onset Swedish cases carried one or two risk alleles, compared to 58% of adult-onset cases. No significant difference in *HLA-DRB1\*15:01* allele distribution between pediatric and adult-onset MS cases were found in the U.S. dataset (data not shown).

## DISCUSSION

This study comprehensively investigated the genetic component in MS cases for whom first clinical disease symptoms appeared before age 18. *HLA-DRB1\*15* was associated with an effect estimate ( $OR_{meta}=2.95$ ) similar to previous smaller studies of this age group ( $OR=2.28-3.30$ ).<sup>4,5,16</sup> Additional MHC variants have been implicated in previous studies of adult MS patients, including a protective effect for *HLA-A\*02*.<sup>17-20</sup> Through fine mapping of the MHC region in the current study, we observed only modest evidence for *HLA-A\*02* effect in POMS.

Strong evidence for independent class II and III effects were detected, similar to findings from a previous study of adult MS cases and controls for which 11 independent associations within the MHC were reported.<sup>18</sup> In the current study, several SNPs within/near *NOTCH4* in the class III region provided the strongest evidence for association after accounting for *HLA-DRB1\*15:01* and correction for multiple comparisons, with one SNP (rs438475) demonstrating high evidence of functionality as an expression quantitative trait locus and transcription factor binding site. Nominal evidence for class II effects was also present, including two SNPs (rs3129888 and rs2395182) identified as expression quantitative trait loci and transcription factor binding sites. The human MHC class III region is the most gene-dense region of the human genome.<sup>21</sup> Variation within *NOTCH4* has been shown recently to decrease the risk for developing MS in Japanese adults,<sup>22,23</sup> similar to our finding, but in contrast to another.<sup>24</sup> Taken together, the involvement in POMS susceptibility of *HLA-DRB1\*15:01* and one or more gene variants within the class III MHC region is strongly supported. MHC region and classical HLA loci genotyping is needed in a larger data set to fully characterize the MHC contribution to POMS, which, similar to adult studies, appears to be complex.<sup>18,25</sup>

For the first time, we studied individual and collective associations between 104 of the 110 established non-MHC adult MS risk variants<sup>6</sup> and susceptibility to POMS. Twenty-eight variants were significantly associated at  $p < 0.05$ ; several variants had larger effect estimates compared to published results from adult MS studies. Top SNPs included rs74796499 within *GALC* and rs9828629 in *FOXPI*; both are involved in nervous system development. We found a significant inverse association between a SNP in *BACH2* and POMS, which has been shown to increase the risk of MS in adults; further work and replication is needed to confirm the difference in directionality between the two populations. A significantly higher wGRS was also observed in POMS cases compared to controls in both U.S. and Swedish datasets after adjusting for genetic ancestry and *HLA-DRB1\*15:01*, in agreement with a small study that used a subset of the 110 MS risk variants to construct a 57 SNP wGRS.<sup>26</sup> We did not find an association between wGRS and age of pediatric disease onset, which has been previously reported for adult-onset MS.<sup>27</sup> One reason why an association with age of onset in children may not be found is that the range is limited (age 4–17 years included). Nevertheless, these findings warrant further investigation.

Whether *HLA-DRB1\*15:01* is associated with earlier age of symptom onset in adult MS has been the subject of several investigations. Strong evidence supports the hypothesis that *HLA-DRB1\*15:01* is indeed associated with an earlier onset in adults;<sup>28–30</sup> though conflicting findings have been reported, including one large<sup>6</sup> and two small studies with < 100 patients.<sup>4,31</sup> In the current study, we did not observe a relationship between *HLA-DRB1\*15:01* and age of onset in POMS cases. When we compared the cases with symptom onset < 18 years of age to cases with symptom onset > 18 years of age, *HLA-DRB1\*15:01* allele distribution differences were present in the Swedish but not U.S. dataset.

Our study had several important strengths. Rigorously diagnosed and clinically well-characterized POMS cases with symptom onset ~ 20 years earlier than most adult MS cases were studied. In addition, a well-powered analysis of *HLA-DRB1\*15:01* and 104 established MS risk variants was considered for the first time in POMS, and a comparison

with adult cases was included. We studied the MHC region with high density SNP mapping in POMS which has not been previously attempted. Genetic diversity among POMS cases is well established, and the potential for population stratification in a case–control study leading to false positive findings is high. Our initial patient group included representatives from non–white populations (n=320; 43% of cases). Comprehensive methods, including multi–dimensional scaling, were used to identify these subgroups, and we restricted the current study to white/European, non–Hispanic individuals for increased genetic homogeneity (Appendix 2). We also adjusted for genetic ancestry within Europeans in our models when necessary.

Some limitations of this study must also be acknowledged. POMS cases were identified through tertiary clinics and may not represent all MS cases with onset in childhood. It is possible that individuals with a more benign disease or without access to healthcare may have been missed. While many of our POMS cases were diagnosed as children in a clinical setting (n=130), some reported the onset of their first disease symptoms in childhood and/or data were extracted from medical records regarding timing of first symptoms but were ascertained as adults. We stratified these groups for comparison to detect any clinical or genetic differences that may have biased our findings (Supplementary Table 4). Notably, MS patients diagnosed as children in the clinic (U.S.) had a slightly lower mean age of symptom onset compared to patients with pediatric symptom onset who were recruited as adults (U.S. and Sweden); this patient group also had a lower frequency of *HLA–DRB1\*15:01* and average wGRS compared to other groups; though as reported, the patient group as a whole (<18 years of age at onset) had a significantly higher wGRS and *HLA–DRB1\*15:01* frequency when compared to controls. The possibility of two genetically distinct MS patient groups – one with symptom onset in early childhood – and one with symptom onset in late teen/early adulthood is one to strongly consider, given the findings presented in the current study. We were unable to complete well–powered stratified analyses based on adolescence due to the limited sample size of cases. Other factors may have a stronger influence on disease onset in younger MS patients, such as yet unidentified genetic loci or environmental factors, as previously suggested.<sup>3</sup> Due to the unavailability of environmental exposures for a substantial portion of our control group, we were unable to additionally adjust for this information. These factors may be more influential in triggering symptoms of MS in early childhood, given that prenatal, perinatal and early childhood windows represents a period of vulnerability to exposures.

MS onset in childhood is a rare occurrence, and thus our case sample was small compared to genetic studies in adult disease. It is possible that very modest effects for less common alleles may have been missed due to our sample size. While this study had >85% power to detect associations with ORs >1.25 and <0.80 for the 110 candidate SNP analysis in the combined meta–analysis (minor allele frequency of 0.20, p=0.05), certain candidates may have been missed due to lack of power. The results presented here will need to be replicated in a larger data set. Finally, our study was restricted to white/European, non–Hispanics. Examination of rare variants and established European MS genetic risk variants in non–white patient groups is needed to further the understanding of MS pathogenesis, as well as focused studies to identify population– specific risk variants.



In conclusion, we confirmed a role for several previously identified adult-onset MS genetic risk factors in the development of pediatric-onset disease and identified stronger effects of some variants compared to adult-onset MS. Comprehensive assessment of genome-wide associations in POMS, as well as interaction between genetic and environmental exposures, will be important to further characterize POMS susceptibility and are currently underway.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

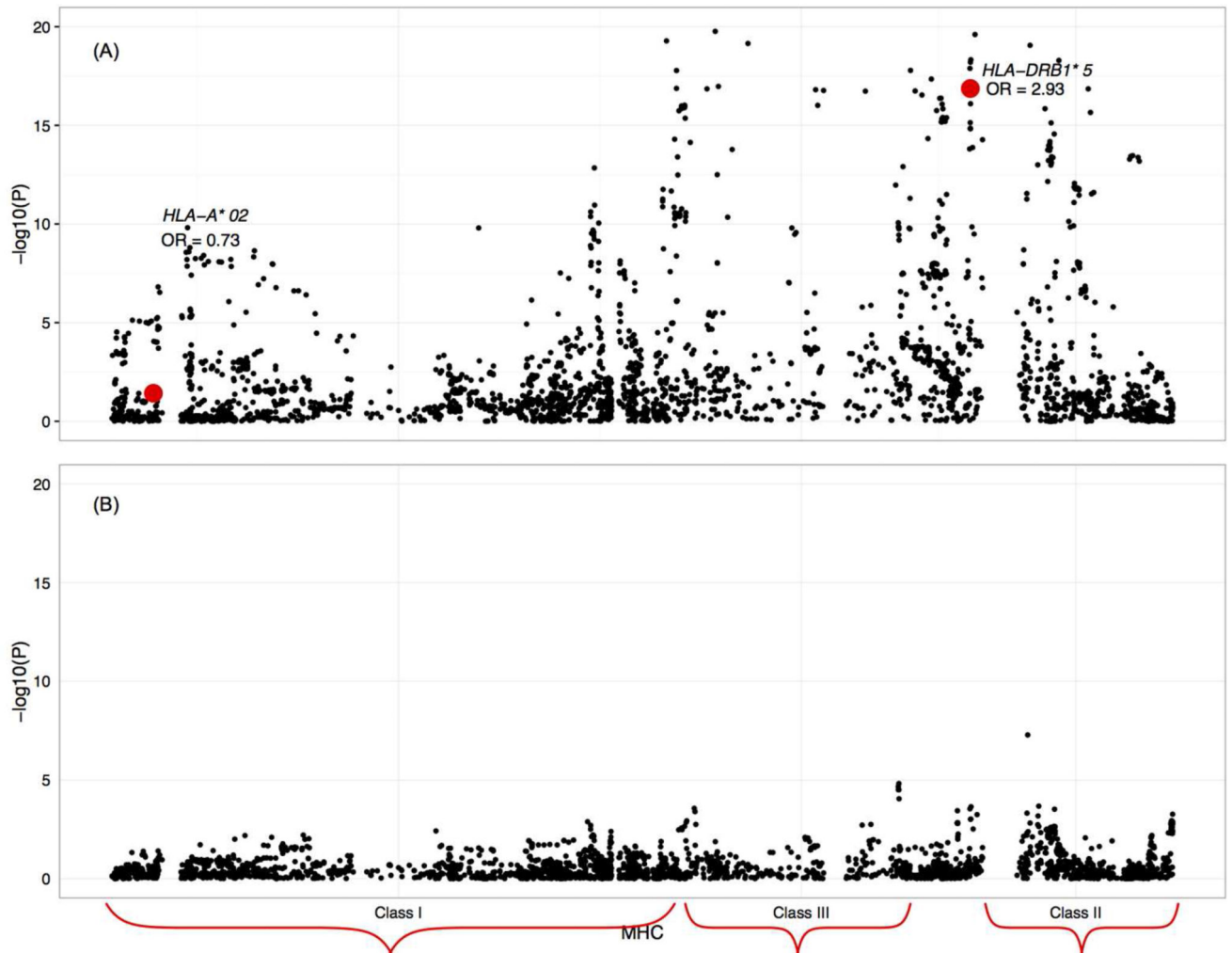
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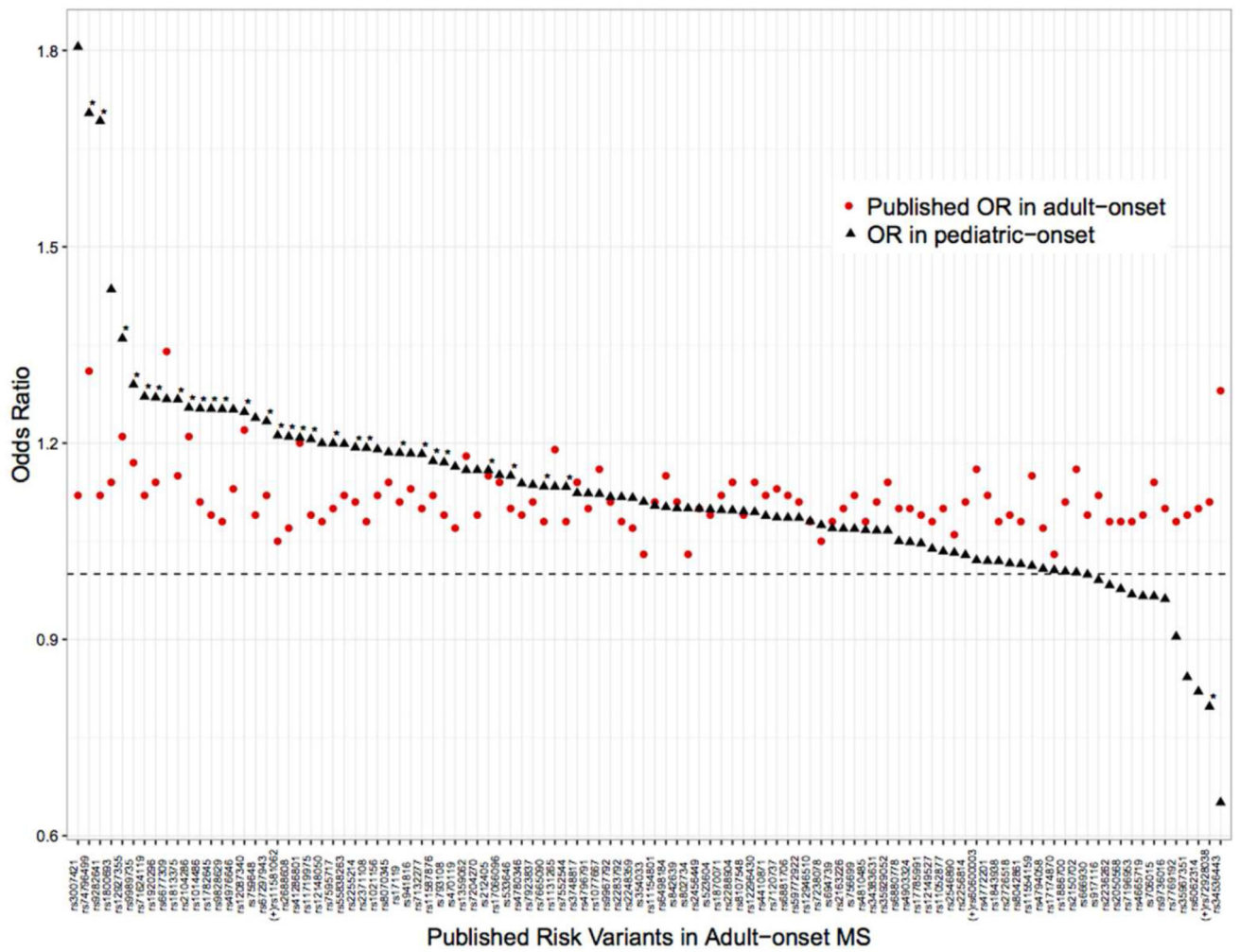
## References

1. Chitnis T, Krupp L, Yeh A, et al. Pediatric multiple sclerosis. *Neurol Clin.* 2011; 29:481–505. [PubMed: 21439455]
2. Yeh EA, Chitnis T, Krupp L, et al. Pediatric multiple sclerosis. *Nat Rev Neurol.* 2009; 5:621–31. [PubMed: 19826402]
3. Waubant E, Ponsonby A, Pugliatti M, et al. Environmental and genetic factors in pediatric inflammatory demyelinating diseases. *Neurol.* 2016; 87(Suppl 2):S20–7.
4. Boiko AN, Gusev EI, Sudomoina MA, et al. Association and linkage of juvenile MS with HLA-DR2(15) in Russians. *Neurol.* 2002; 58(4):658–60.
5. Banwell D, Bar-Or A, Arnold DL, et al. Clinical, environmental, and genetic determinants of multiple sclerosis in children with acute demyelination: a prospective national cohort study. *Lancet Neurol.* 2011; 10(5):426–45.
6. Beecham AH, Patsopoulos NA, Xifara DK, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet.* 2013; 45:1353–60. [PubMed: 24076602]
7. Waubant E, Mowry EM, Krupp L, et al. Common viruses associated with lower pediatric multiple sclerosis risk. *Neurol.* 2011; 76:1989–95.
8. Gianfrancesco MA, Glymour MM, Walter S, et al. Causal effect of genetic variants associated with body mass index on multiple sclerosis susceptibility. *Am J Epidemiol.* 2017; 185(3):162–171. [PubMed: 28073764]
9. Krupp LB, Tardieu M, Amato MP, et al. International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. *Mult Scler.* 2013; 19(10):1261–7. [PubMed: 23572237]
10. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol.* 2001; 50:121–7. [PubMed: 11456302]
11. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol.* 2011; 69:292–302. [PubMed: 21387374]
12. Nourbakhsh B, Graves J, Casper TC, et al. Dietary salt intake and time to relapse in paediatric multiple sclerosis. *J Neurol Neurosurg Psychiatry.* 2016; 87(12):1350–3. [PubMed: 27343226]
13. Hedstrom AK, Hillert J, Olsson T, Alfredsson L. Smoking and multiple sclerosis susceptibility. *Eur J Epidemiol.* 2013; 28:867–74. [PubMed: 24146047]
14. Zivkovic M, Stankovic A, Dincic E, et al. The tag SNP for HLA-DRB1\*1501, rs3135388, is significantly associated with multiple sclerosis susceptibility: cost-effective high-throughput detection by real-time PCR. *Clin Chim Acta.* 2009; 406:27–30. [PubMed: 19433080]

15. De Jager PL, Chibnik LB, Cui J, et al. Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: a weighted genetic risk score. *Lancet Neurol.* 2009; 8:1111–9. [PubMed: 19879194]
16. Disanto G, Magalhaes S, Handel AE, et al. HLA-DRB1 confers increased risk of pediatric-onset MS in children with acquired demyelination. *Neurol.* 2011; 76(9):781–6.
17. Barcellos LF, Sawcer S, Ramsay PP, et al. Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. *Hum Mol Genet.* 2006; 15:2813–24. [PubMed: 16905561]
18. Patsopoulos NA, Barcellos LF, Hintzen RQ, et al. Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects. *PLoS Genet.* 2013; 9(11):e1003926. [PubMed: 24278027]
19. Yeo TW, De Jager PL, Gregory SG, et al. A second major histocompatibility complex susceptibility locus for multiple sclerosis. *Ann Neurol.* 2007; 61:228–36. [PubMed: 17252545]
20. Link J, Kockum I, Lorentzen AR, et al. Importance of human leukocyte antigen (HLA) class I and II alleles on the risk of multiple sclerosis. *PLoS One.* 2012; 7(5):e36779. [PubMed: 22586495]
21. Xie T, Rowen L, Begona A, et al. Analysis of the gene-dense major histocompatibility complex class III region and its comparison to mouse. *Genome Res.* 2003; 13(12):2621–36. [PubMed: 14656967]
22. Huang J, Yoshimura S, Isobe N, et al. A NOTCH4 missense mutation confers resistance to multiple sclerosis in Japanese. *Mult Scler.* 2013; 19(13):1696–1703. [PubMed: 23549433]
23. McElroy JP, Isobe N, Gourraud PA, et al. SNP-based analysis of the HLA locus in Japanese multiple sclerosis patients. *Genes Immun.* 2011; 12(7):523–30. [PubMed: 21654846]
24. Duvefelt K, Anderson M, Fogdell-Hahn A, Hillert J. A NOTCH4 association with multiple sclerosis is secondary to HLA-DR\*1501. *Tissue Antigens.* 2004; 63(1):13–20. [PubMed: 14651518]
25. Ramagopalan SV, Knight JC, Ebers GC. Multiple sclerosis and the major histocompatibility complex. *Curr Opin Neurol.* 2009; 22(3):219–25. [PubMed: 19387341]
26. van Pelt ED, Mescheriakova JY, Makhani N, et al. Risk genes associated with pediatric-onset MS but not with monophasic acquired CNS myelination. *Neurol.* 2013; 81(23):1996–2001.
27. Sorosina M, Esposito F, Guaschino C, et al. Inverse correlation of genetic risk score with age at onset in bout-onset and progressive-onset multiple sclerosis. *Mult Scler.* 2015; 21(11):1463–7. [PubMed: 25533292]
28. Bove R, Chua AS, Xia Z, et al. Complex relation of HLA-DRB1\*1501, age at menarche, and age at multiple sclerosis onset. *Neurol Genet.* 2016; 2(4):e88. [PubMed: 27504495]
29. Ramagopalan SV, Byrnes JK, Dymant DA, et al. Parent-of-origin of HLA-DRB1\*1501 and age of onset of multiple sclerosis. *J Hum Genet.* 2009; 54:547–9. [PubMed: 19629136]
30. Masterman T, Ligers A, Olsson T, et al. HLA-DR15 is associated with lower age at onset in multiple sclerosis. *Ann Neurol.* 2000; 48:211–9. [PubMed: 10939572]
31. Anagnostouli MC, Manouseli A, Artemiadis AK, et al. HLA-DRB1\* allele frequencies in pediatric, adolescent, and adult-onset multiple sclerosis patients, in a hellenic sample. Evidence for new and established associations. *J Mult Scler.* 2014; 1:104.



**Figure 1.** Individual 104 risk variant comparisons between POMS findings and published IMSGC adult MS odds ratios.



**Figure 2.** Meta-analyses of the major histocompatibility complex (MHC) in POMS cases and controls: (A) unadjusted, and (B) adjusted for *HLA-DRB1\*15:01* and *HLA-A\*02*. Individual variants were analyzed spanning 6p22.1 to 6p21.3. Evidence for MHC involvement, particularly in the Class III HLA region is shown. U.S. analyses additionally adjusted for genetic ancestry.

**Table 1**

## Characteristics of POMS Case and Control Participants

	U.S. POMS Cases	U.S. Controls	Sweden POMS Cases	Sweden Controls
N	394	10,875	175	5,376
Females:Males	3:0:1	1:6:1	2:5:1	3:2:1
Age of onset	14.05 (3.30)	—	14.91 (2.67)	—
wGRS	12.50 (0.66)	12.06 (0.68)	12.73 (0.66)	12.22 (0.69)
<i>HLA-DRB1*15:01</i>				
0	193 (49)	7,973 (72)	57 (33)	3,806 (71)
1-2	201 (51)	2,902 (28)	118 (67)	1,570 (29)

MS= multiple sclerosis; wGRS=weighted genetic risk score

Table values are mean (SD) for continuous variables or No. (%) for categorical variables.

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**Table 2**  
Significant meta-analysis results for 104 non-MHC MS risk loci in POMS cases and controls (p<0.05)

CHR	SNP	GENE	U.S.			Sweden			Meta-Analysis <sup>d</sup>			Published Estimate in Adults			
			A	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
1	rs11581062	<i>SLC30A7</i>	G	1.20	1.03, 1.39	0.02	1.25	0.99, 1.59	0.06	1.21	1.06, 1.38	<0.01	1.12	1.07, 1.17	<0.01
1	rs12087340	<i>BCL10</i>	A	1.32	1.05, 1.67	0.02	1.06	0.72, 1.55	0.78	1.24	1.02, 1.52	0.03	1.22	1.15, 1.29	<0.01
1	rs41286801	<i>EVF5</i>	A	1.26	1.04, 1.51	0.02	1.10	0.82, 1.47	0.53	1.21	1.04, 1.41	0.01	1.20	1.15, 1.25	<0.01
2	rs7595717	<i>PLEK</i>	A	1.24	1.06, 1.44	0.01	1.12	0.88, 1.41	0.37	1.20	1.05, 1.36	0.01	1.10	1.06, 1.14	<0.01
2	rs9989735	<i>SPI40</i>	C	1.30	1.10, 1.54	<0.01	1.27	0.98, 1.65	0.07	1.29	1.12, 1.49	<0.01	1.17	1.12, 1.22	<0.01
3	rs9282641	<i>CD86</i>	G	1.44	1.08, 1.93	0.01	2.21	1.33, 3.66	<0.01	1.25	1.10, 1.43	0.01	1.12	1.05, 1.19	<0.01
3	rs11719975	<i>SATB1</i>	C	1.18	1.01, 1.38	0.04	1.26	1.00, 1.58	0.05	1.21	1.06, 1.38	<0.01	1.09	1.05, 1.13	<0.01
3	rs1014486	<i>IL12A</i>	G	1.14	0.99, 1.32	0.06	1.42	1.14, 1.76	<0.01	1.25	1.01, 1.54	0.04	1.11	1.07, 1.14	<0.01
3	rs2255214	<i>CD86</i>	G	1.20	1.04, 1.39	0.01	1.18	0.95, 1.46	0.13	1.19	1.06, 1.34	<0.01	1.11	1.08, 1.15	<0.01
3	rs2371108	<i>EMES</i>	A	1.23	1.06, 1.42	0.01	1.12	0.90, 1.39	0.31	1.19	1.06, 1.34	<0.01	1.08	1.05, 1.12	<0.01
3	rs9828629	<i>FOXPI</i>	G	1.27	1.09, 1.47	<0.01	1.22	0.97, 1.53	0.09	1.70	1.14, 2.53	<0.01	1.08	1.05, 1.12	<0.01
3	rs1920296	<i>IQCB1</i>	C	1.26	1.08, 1.46	<0.01	1.30	1.03, 1.65	0.03	1.27	1.12, 1.45	<0.01	1.14	1.11, 1.18	<0.01
3	rs1813375	<i>CMCI</i>	A	1.29	1.11, 1.48	<0.01	1.23	0.99, 1.52	0.06	1.27	1.13, 1.42	<0.01	1.15	1.12, 1.19	<0.01
4	rs7665090	-	G	1.11	0.96, 1.28	0.15	1.18	0.96, 1.47	0.12	1.14	1.01, 1.28	0.03	1.08	1.05, 1.12	<0.01
5	rs71624119	<i>ANKRD55</i>	G	1.17	0.98, 1.40	0.08	1.47	1.10, 1.96	0.01	1.27	1.03, 1.57	0.03	1.12	1.08, 1.17	<0.01
6	rs67297943	-	T	1.25	1.02, 1.52	0.03	1.21	0.93, 1.58	0.15	1.23	1.05, 1.44	0.01	1.12	1.07, 1.16	<0.01
6	rs72928038	<i>BACH2</i>	A	0.77	0.61, 0.97	0.03	0.85	0.62, 1.15	0.29	0.80	0.66, 0.96	0.02	1.11	1.07, 1.16	<0.01
6	rs17119	<i>JARID2</i>	A	1.19	0.99, 1.44	0.07	1.17	0.87, 1.58	0.30	1.18	1.00, 1.39	0.05	1.11	1.06, 1.15	<0.01
6	rs212405	<i>TAGAP</i>	T	1.15	0.99, 1.35	0.07	1.17	0.93, 1.48	0.18	1.16	1.02, 1.32	0.03	1.15	1.11, 1.19	<0.01
10	rs2104286	<i>IL2RA</i>	T	1.22	1.03, 1.45	0.02	1.34	1.03, 1.73	0.03	1.26	1.09, 1.41	<0.01	1.21	1.16, 1.26	<0.01
10	rs1782645	<i>ZMIZ1</i>	T	1.19	1.02, 1.39	0.03	1.37	1.11, 1.70	<0.01	1.25	1.09, 1.43	<0.01	1.09	1.05, 1.13	<0.01
10	rs793108	<i>ZNF438</i>	A	1.14	0.99, 1.32	0.07	1.24	1.00, 1.53	0.05	1.17	1.04, 1.31	0.01	1.09	1.06, 1.13	<0.01
10	rs2688608	-	A	1.23	1.06, 1.42	0.01	1.16	0.89, 1.50	0.28	1.20	1.05, 1.38	0.01	1.07	1.03, 1.10	<0.01
11	rs533646	-	G	1.13	0.96, 1.32	0.14	1.20	0.96, 1.51	0.12	1.15	1.01, 1.31	0.04	1.10	1.06, 1.14	<0.01
12	rs7132277	<i>PITPNM2</i>	A	1.26	1.06, 1.49	0.01	1.05	0.81, 1.37	0.70	1.18	1.00, 1.40	0.05	1.10	1.06, 1.15	<0.01
14	rs74796499	<i>GALC</i>	C	1.59	1.04, 2.44	0.03	2.10	0.99, 4.46	0.05	1.71	1.18, 2.49	0.01	1.31	1.21, 1.42	<0.01

CHR	SNP	GENE	U.S.			Sweden			Meta-Analysis <sup>a</sup>			Published Estimate in Adults			
			A	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
16	rs4780346	<i>CLECT16A</i>	A	1.15	0.98, 1.35	0.09	1.11	0.89, 1.40	0.36	1.14	1.00, 1.29	0.05	1.09	1.05, 1.13	<0.01
16	rs12927355	<i>CLECT16A</i>	C	1.33	1.14, 1.56	<0.01	1.43	1.11, 1.84	<0.01	1.36	1.19, 1.56	<0.01	1.21	1.17, 1.26	<0.01

CHR=chromosome; SNP=single nucleotide polymorphism; A= allele; OR=odds ratio; CI=confidence interval

<sup>a</sup>Analyses adjusted for genetic ancestry

**Table 3**  
Top twenty meta-analysis results of MHC region in POMS cases and controls adjusted for *HLA-DRB1\*15:01* and *HLA-A\*02*

CHR	BP	SNP	GENE	SUBREGION	OR	p-value
6	32595063	rs28720766	-	Class II	2.16	5.19×10 <sup>-8a</sup>
6	32186245	rs438475	<i>NOTCH4</i>	Class III	0.59	1.51×10 <sup>-5a</sup>
6	32184345	rs404860	<i>NOTCH4</i>	Class III	0.63	2.18×10 <sup>-5a</sup>
6	32184574	rs384247	<i>NOTCH4</i>	Class III	0.64	3.11×10 <sup>-5a</sup>
6	32186193	rs431541	<i>NOTCH4</i>	Class III	0.64	3.14×10 <sup>-5a</sup>
6	32186872	rs2256594	<i>NOTCH4</i>	Class III	0.65	8.95×10 <sup>-5</sup>
6	32629868	rs1049088	<i>HLA-DQB1</i>	Class II	0.67	2.11×10 <sup>-4</sup>
6	32415109	rs3135387	<i>HLA-DRA</i>	Class II	1.55	2.27×10 <sup>-4</sup>
6	31537606	rs2857706	<i>LTA</i>	Class III	1.36	2.74×10 <sup>-4</sup>
6	32411726	rs3129888	<i>HLA-DRA</i>	Class II	1.54	2.88×10 <sup>-4</sup>
6	32679992	rs12527228	-	Class II	1.78	3.03×10 <sup>-4</sup>
6	32372032	rs73400865	<i>BTNL2</i>	Class III	1.55	3.60×10 <sup>-4</sup>
6	31539767	rs2844482	<i>LTA</i>	Class III	1.36	4.02×10 <sup>-4</sup>
6	32592737	rs28421666	-	Class II	1.86	4.80×10 <sup>-4</sup>
6	33054457	rs1042544	<i>HLA-DPB1</i>	Class II	1.26	5.43×10 <sup>-4</sup>
6	32434314	rs12529318	-	-	1.75	5.69×10 <sup>-4</sup>
6	32627700	rs6689	<i>HLA-DQB1</i>	Class II	0.73	6.66×10 <sup>-4</sup>
6	32414420	rs7382085	<i>HLA-DRA</i>	Class II	1.44	9.57×10 <sup>-4</sup>
6	32413317	rs2395182	<i>HLA-DRA</i>	Class II	1.43	9.83×10 <sup>-4</sup>
6	33051683	rs9277411	<i>HLA-DPB1</i>	Class II	1.25	1.15×10 <sup>-3</sup>

CHR=chromosome; BP=base pair; SNP=single nucleotide polymorphism; OR=odds ratio

<sup>a</sup>Significant after multiple comparisons correction