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# Oligoclonal bands and age at onset correlate with genetic risk score in multiple sclerosis

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## Abstract

**Background—**Many genetic risk variants are now well established in multiple sclerosis (MS), but the impact on clinical phenotypes is unclear.

**Objective—**To investigate the impact of established MS genetic risk variants on MS phenotypes, in well-characterized MS cohorts.

**Methods—**Norwegian MS patients ($n$ = 639) and healthy controls ($n$ = 530) were successfully genotyped for 61 established MS-associated single nucleotide polymorphisms (SNPs). Data including and excluding Major Histocompatibility Complex (MHC) markers were summed to a MS Genetic Burden (MSGB) score. Study replication was performed in a cohort of white American MS patients ($n$ = 1997) and controls ($n$ = 708).

**Results—**The total human leukocyte antigen (HLA) and the non-HLA MSGB scores were significantly higher in MS patients than in controls, in both cohorts ($P << 10^{-22}$). MS patients, with and without cerebrospinal fluid (CSF) oligoclonal bands (OCBs), had a higher MSGB score than the controls; the OCB-positive patients had a slightly higher MSGB than the OCB-negative patients. An early age at symptom onset (AAO) also correlated with a higher MSGB score, in both cohorts.



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**Notes** HFH initiated the project and participated in the study design, contributed to the establishment and funding of the Oslo dataset, performed the experiments, contributed to the statistical analysis, and drafted and edited the manuscript. NI participated in experiments, statistical analysis and edited the manuscript. PBH contributed to establishment of the Oslo dataset, clinical analyses, drafting and editing of the manuscript. SDB contributed to the statistical analysis, drafting and editing of the manuscript. SJC assisted in sample preparation, genotyping and management. MWG and ILM contributed to establishment of the Oslo dataset, sample preparation and management. EGC contributed to establishment, funding and clinical analyses of the Oslo dataset, and editing of the manuscript. SLH contributed to the design, as well as phenotypic characterization, of the UCSF study participants and critically revised the manuscript. JRO contributed to the initiation and study design of the project, supervised the study and edited the manuscript. PAG contributed to the initiation and study design of the project, supervised the experiments and statistical analyses, and edited the manuscript.

**Conclusion—**The MSGB score was associated with specific clinical MS characteristics, such as OCBs and AAO. This study underlines the need for well-characterized, large cohorts of MS patients, and the usefulness of summarizing multiple genetic risk factors of modest effect size in genotype-phenotype analyses.

### Keywords

Age of onset; cerebrospinal fluid; genetic association; genetic risk; genotype; multiple sclerosis; oligoclonal bands; Multiple Sclerosis Genetic Burden

## Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS),[1] caused most likely by the interaction between genetic and environmental factors.[2] Recent advances in single nucleotide polymorphism (SNP) array methodologies and improved analytic capabilities, combined with a cooperative global effort to clarify the genetic underpinnings of MS, have succeeded in elucidating over 50 common DNA variants that are unequivocally associated with this complex disorder.[2–5] The majority of risk markers are located nearby or within genes that are known to have roles in the immune system, arguing for a primary immune, and likely autoimmune, etiology for MS.

Another principle of the genetic landscape of MS is that, with the sole exception of the MHC region, each variant confers only a very small contribution to MS risk (odds ratio (OR) < 1.2). Given the small effect sizes, even in aggregate, the identified genetic MS risk variants confer only a fraction of the total inherited risk, as estimated from family studies including twin studies, and at an individual level they are insensitive for risk assessments; however, at a population level, genetic risk factors could represent important new tools to better understand the clinical biology of MS, including heterogeneity. For example, we recently identified genetic networks that are associated with different patterns of MS lesion distribution in the CNS.[6]

The MS Genetic Burden (MSGB) score is a useful statistic that sums the aggregate genetic risk of MS, based on validated association signals derived from genetic studies of MS.[7] Beyond the association with susceptibility, this score provides a simple metric to explore the influence, if any, of genetic burden on disease expression.

A registry for MS was created in the high-risk population of Oslo, Norway, with a population of more than 500,000 inhabitants, that supports population-based investigations of this well-characterized and longitudinally monitored cohort of MS patients,[8] facilitating standardization of data collection in this region. For example: systematic lumbar punctures are obtained, enabling population-wide assessments of cerebrospinal fluid (CSF) parameters, like oligoclonal bands (OCBs). The current study's aim was to investigate the associations of the MSGB score with clinical and paraclinical data in this highly characterized Oslo MS cohort. To replicate our findings, we used an independent well-characterized dataset from the University of California San Francisco (UCSF), US, representing a large sample of white American MS patients and controls recruited at that site.

## Materials and methods

The Oslo MS registry established in 1992 contains clinical data from 1648 patients, all collected at the Oslo University Hospital, Ullevål, Oslo, Norway. The Oslo MS clinic diagnoses and prospectively follows closely all MS cases within the Oslo population. Approximately one-half of these patients have donated blood for genetic studies. In this study, 639 Norwegian MS patients and 530 healthy controls obtained from the Norwegian Bone Marrow Registry were successfully genotyped (Table 1(a)) for 61 well-established MS-associated SNPs, using TaqMan® OpenArray® genotyping technology (Life Technologies, Carlsbad, CA, US) (Supplementary Table 1). For the MSGB calculation we used 61 successfully genotyped SNPs, 3 SNPs from the intended 64 SNPs showed call rates below 90% (rs2028597, rs2150702 and rs10411936) and were excluded before MSGB calculation. From the HLA region, we included rs3129889 tagging HLA-DRB1*15:01 and rs2523393, tagging HLA-B*44.[9] The samples had not been genotyped for this SNP panel in previous studies. Classical HLA typing data from the HLA-A, -B, -C and -DRB1 loci were available for a subgroup of the included study participants (Table 1(b)). The Oslo MS samples are described in further detail, in earlier studies.[10]

By using the OpenArray® Genotyping Analysis Software, we visually inspected genotype plots and we were able to exclude samples in overlapping or questionable clusters from the analyses. We excluded samples from individuals with an average call rate below 90%. The resulting sample call rate was 98.1% in the Oslo MS cases and 98.6% in the Oslo controls.

By using identical criteria for quality control as in the Norwegian dataset, the replication sample collected at UCSF consisted of 1997 MS cases and 708 healthy controls (Table 1(a)). These were genotyped for the same SNP panel, using the same technology mentioned above. The UCSF replication sample is an extension of the dataset that was described in detail previously.[7] The sample call rates for the UCSF replication dataset were 97.9% in MS patients and 98.7% in controls.

We performed the clinical and paraclinical examinations in both cohorts according to established international guidelines and practices. All MS patients were diagnosed according to the updated McDonald diagnostic criteria.[11] Gender, disease course, age at symptom onset (AAO), Expanded Disability Status Scale (EDSS),[12] multiple sclerosis severity score (MSSS),[13] OCB in CSF and information about MS in the family was recorded. OCB positivity was determined either by isoelectric focusing or agarose gel electrophoresis, both in the Oslo[14] and UCSF cohorts. The IgG index was not available for all patients, so was therefore not included in the analysis.

We calculated the MSGB score per sample using a log-additive model, as described by Gourraud et al.,[7] and it was repeated after excluding the SNPs of the MHC region (6p21.3), in order to evaluate the contribution of non-HLA MS risk variants. In the rare event that an individual's genotype for a SNP was missing, we used the risk allele frequency in the healthy control population for that SNP, to estimate the complete MSGB score for that individual. We analyzed the phenotype-MSGB correlations using Chi-square statistics, *t*-test and linear logistic regression, including MSGB scores as a predictor and gender as a

covariate. We performed analyses using IBM SPSS 20.0 and R statistical software. The *P*-values provided were not corrected.

## Results

Clinical and demographic characteristics are shown, in Table 1(a), of the total of 1648 Oslo MS cases, the 639 Oslo MS cases and 530 Oslo controls that were successfully genotyped, as well as the UCSF replication set of 1997 MS cases and 708 controls. There were no significant differences between all Oslo MS cases and the genotyped cases from the Oslo cohort regarding sex, AAO, disease course nor disability. As expected, classical HLA data available for a large subset of the Oslo MS cases and controls that were included in the MSGB analysis confirmed that there was a strong association of MS to HLA-DRB1*15:01. The strongest associations for HLA class I alleles were to HLA-A*02, HLA-B*7, HLA-B*44 and HLA-C*07 (Table 1(b)).

The clinical characteristics of the white American replication cohort are shown for comparison in Table 1(a). The relative proportion of relapsing–remitting MS patients was higher in the UCSF cohort; also, they were on average somewhat younger at examination and had slightly lower EDSS and MSSS scores. This may be explained by the differences in recruitment to the two MS clinics: candidates recruited at UCSF are often referred for evaluation of FDA-approved immune modulatory therapies, whereas MS patients in the Oslo clinic represent the whole MS population in Oslo.

The total MSGB score in the Oslo MS cases, ranged from 6.59–11.54, whereas the non-HLA MSGB ranged from 6.25–10.02. Oslo MS patients had a significantly higher total MSGB score as compared to controls, respectively: mean MSGB (standard error (SE)) 8.97 (0.03) versus 8.18 (0.04), $P = 5.44*10^{-50}$ and non-HLA MSGB score (mean (SE) 7.98 (0.02) versus 7.64 (0.03), $P = 3.63*10^{-23}$, as shown in Figure 1(a) and Figure 1(b). This finding was replicated in the UCSF cohort (MSGB score mean (SE) 8.94 (0.02) versus 8.19 (0.03), $P = 4.72*10^{-98}$; non-HLA MSGB score mean (SE) 8.02 (0.01) versus 7.76 (0.02), $P = 8.19*10^{-25}$ (Figure 1(c) and Figure 1(d)). In the Oslo cohort, there were no statistical differences in the total MSGB scores between MS cases reporting any family history of MS ($n = 124$) and those reporting no family history ($n = 515$) ($P = 0.629$), in contrast to what was observed in the UCSF family studies earlier[7] and in the present UCSF dataset ($n = 433$ reported family history of MS, versus $n = 1143$ whom reported no familial history of MS; $P = 1.35*10^{-3}$). The Oslo MS subgroup with a family history of MS was too small to have sufficient power for analyses stratified for first-degree or second-degree relatives with MS in the family.

The OCB-positive MS patients from Oslo ($n = 504$) had a higher MSGB score than OCB-negative patients ($n = 64$), when including HLA in the score estimation (MSGB mean (SE) 8.96 (0.04) versus 8.83 (0.12), although this did not reach significance in the Oslo sample ($P = 0.251$). No difference was observed for the non-HLA MSGB for the OCB positives, versus OCB-negative Oslo MS patients (MSGB mean (SE) 7.96 (0.02) versus 7.96 (0.07), $P = 0.999$); however, there was a very significant difference between OCB-positive MS patients and controls, for both the total MSGB score and the non-HLA MSGB score ($P =$

1.71 $*10^{-8}$ and $P = 3.70 *10^{-5}$ (Figure 2(a) and 2(b)). In the UCSF samples, the difference in MSGB between OCB-positive ($n = 526$) and OCB-negative MS patients ($n = 234$) was significant, both when including and excluding the HLA region markers (MSGB mean (SE) 9.04 (0.04) versus 8.78 (0.06), $P = 3.58*10^{-4}$; non-HLA MSGB mean (SE) 8.07 (0.03) versus 7.96 (0.04), $P = 0.016$) (Figure 2(c) through Figure 2(d)).

MSGB score ($P = 0.035$) in the Oslo MS patients, and this was more pronounced for the non-HLA MSGB score ($P = 0.003$) (Figure 3(a) through 3(b)). An earlier age at onset was also observed for the UCSF patients with a higher total MSGB score ($P = 2.65*10^{-4}$); however, this association was observed only when including the HLA in the score calculation (Figure 3(c) and Figure 3(d)). No differences were observed between the disease courses of RRMS nor PPMS, nor female versus male gender (data not shown). Also, the total MSGB and non-HLA MSGB scores were not associated with the MS severity score (MSSS)[13] in the Oslo samples (Supplementary Figure 1(a) and Supplementary Figure 1(b)), and the MSSS did not differ between patients with and without OCBs in CSF (data not shown).

## Discussion

This study reports that the total MSGB and non-HLA MSGB scores were significantly higher in MS cases from the population-based Oslo MS registry, compared to healthy controls, as well as from a large white American sample collected at UCSF. Indeed, it is remarkable how similar the results from the two datasets were, despite the independent collections and the genetic architecture of the populations sampled. This observation strongly confirms the validity of the MSGB score in diverse MS cohorts.

The observation that the total MSGB score is higher in both OCB-positive and -negative MS patients, compared to controls, may indicate that these two MS phenotypes share the same degree of genetic risk factors. In the UCSF cohort, which was better powered than the Oslo cohort, we also confirmed a higher MSGB score in OCB-positive versus OCB-negative MS patients. This concurs with our recent Scandinavian study, which reported a stronger association with HLA-DRB1*15:01 in OCB-positive than OCB-negative MS patients.[14] As the total MSGB score represented an aggregated risk score that was strongly influenced by the effect of HLA-DRB1*15:01, it is possible that the MSGB-OCB relationship could be due to an effect of HLA-DRB1*1501, independent of the other 60 loci comprising the MSGB estimation in our analysis. However, in the larger UCSF sample, the difference between OCB-positive and OCB-negative MS patients remained significant, even when excluding the HLA markers in the MSGB estimation. This novel finding indicates that non-HLA genes also have impact on the OCB-positive phenotype. OCBs in CSF are present in the majority of MS patients.[15,16] Of note, the proportion of OCB-negative MS patients was larger in the white American than the Norwegian cohort, which may be due to population-specific differences.[17,18] We cannot rule out some differences in laboratory methods between the centers, although both clinics evaluated OCB in CSF with sensitive, modern techniques. Earlier, inconsistent reports on the clinical differences between MS patients with and without OCB in the CSF were published.[19–24] In our study, we did not identify

differences in MSGB scores in the patients with phenotypes like gender, disease course and severity, but we did identify a lower AAO leave “occurred” away with higher MSGB scores.

Interestingly, the higher MSGB scores observed in patients with lower AAO were identified in both populations. A possible interpretation is that the accumulation of genetic risk factors is lowering the threshold for the expression of the disease thereby lowering AAO. A lower age at onset in MS patients carrying the MS-associated HLA-DRB1*15:01 allele was previously reported.[3,25–28] In agreement with earlier observations in the UCSF cohort,[7] the MSGB score was not associated with disease course (RRMS versus PPMS) in the Oslo dataset. In addition, we could not find an association between MSGB and disability trajectories, as measured by MSSS.

The MSGB score is helpful for summarizing the per-patient genetic burden, although this score cannot be used for disease risk prediction in the clinic for an individual patient. Our study illustrates the usefulness of this score in the assessment of differences between groups of patients, by being able to show differences in genetic impact on phenotypes. Genotyping costs are reduced by estimating the summarized genetic risk only by genotyping the established risk loci; however, aggregate scores for disease risk loci are vulnerable for missing SNP genotypes, especially in the absence of proxy markers (as in a genome-wide screening), that may be used to impute genotypes. We applied a conservative approach after strict quality control, by replacing the few missing genotypes in both patients and controls with the risk allele frequencies in the healthy control population, for that specific locus, when estimating the MSGB scores. The MSGB score is not affected much by this replacement, except reducing the power to detect deviations on an individual level, but it saves power to detect the difference between the groups, by keeping as many individuals as possible in the study.

By conducting discovery and replication studies in two independent MS centers, recruitment biases or potential differences in the manner in which clinical measurements are obtained can be overcome, and results are thus likely to be widely applicable across different practice settings. Evaluations of AAO were assessed in the same manner at the time of the initial visit, in both MS centers. OCB status didn’t prove amenable to standardization across different laboratories; and thus, it was particularly encouraging that similar associations were identified in our two study populations. Analyses of other phenotypic parameters, such as metrics derived from magnetic resonance imaging (MRI) of brain and spinal cord, will likely require prospective studies using standardized MRI protocols across the recruiting clinics. Establishment of well-defined protocols applicable both in clinical practice and in research across different clinics and continents are needed for well-powered genotype-phenotype analyses, but the current data indicate that at least for some parameters, large sample sizes coupled with the use of discovery and replication cohorts can overcome the variability inherent in comparison of data from different cohorts.

In conclusion, analysis of a population-based MS cohort from Oslo, Norway and a replication study in a MS cohort from San Francisco revealed that aggregated MS genetic burden scores, calculated from the current list of MS-associated genetic variants, is associated with OCB positivity as well as AAO. The MSGB summary scoring methods open

a new metric for assessment of differences that may prove useful in exploring how genetic risk contributes to clinical phenotypes, environmental risk and underlying immuno-pathogenic heterogeneity in MS.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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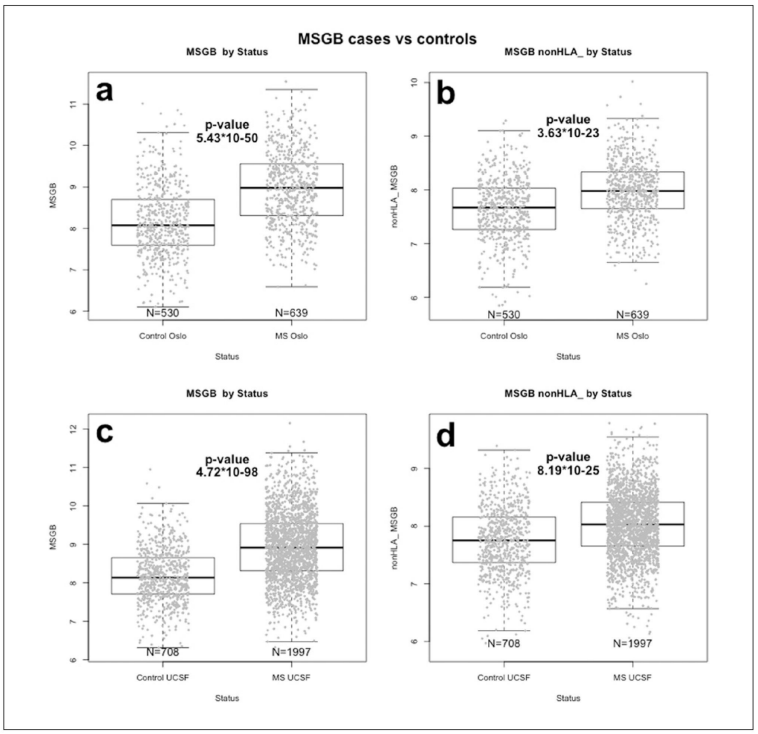


**Figure 1.**

Comparison of MSGB according to disease status.

(a) Total MSGB boxplots for patients and controls in the Oslo cohort.

(b) Total MSGB excluding HLA contribution (MSGB non-HLA) boxplots for patients and controls in the Oslo cohort.

(c) Total MSGB boxplots for patients and controls collected at UCSF.

(d) Total MSGB excluding HLA contribution (non-HLA MSGB) boxplots for patients and controls collected at UCSF.

HLA: Human leukocyte antigen; MS: multiple sclerosis; MSGB: MS genetic burden; UCSF: University of CA, San Francisco.

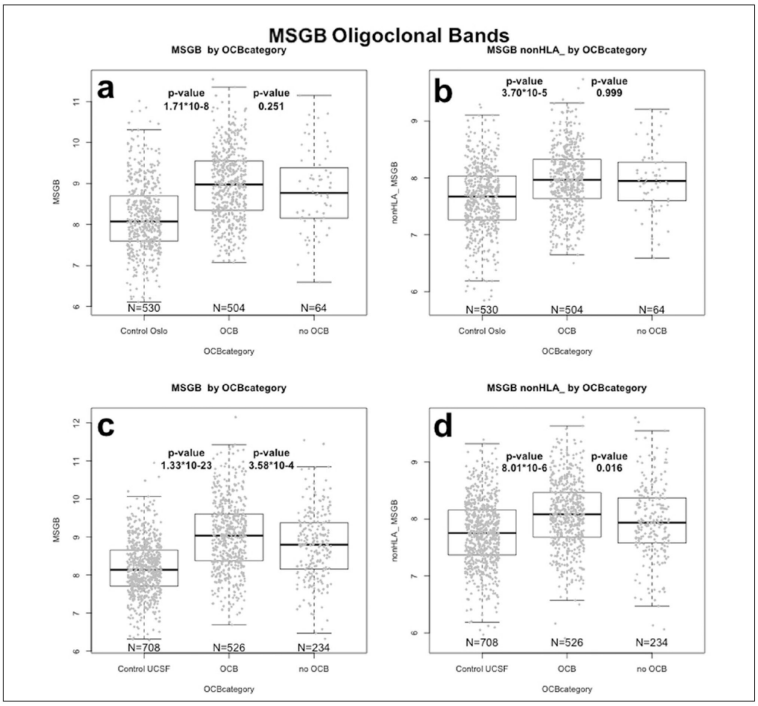


**Figure 2.**

Comparison of MSGB, according to OCB positivity.

(a) Total MSGB boxplots for OCB-negative and OCB-positive MS patients, and controls, in Oslo cohort.

(b) Total MSGB excluding HLA contribution (non-HLA MSGB) boxplots for OCB-negative and OCB-positive MS patients, and controls, in Oslo cohort.

(c) Total MSGB boxplots for OCB-negative and OCB-positive MS patients, and controls, in UCSF cohort.

(d) Total MSGB excluding HLA contribution (non-HLA MSGB) boxplots for OCB-negative and OCB-positive MS patients in the UCSF cohort.

HLA: Human leukocyte antigen; MS: multiple sclerosis; MSGB: MS genetic burden; OCB: oligoclonal band; UCSF: University of CA, San Francisco.

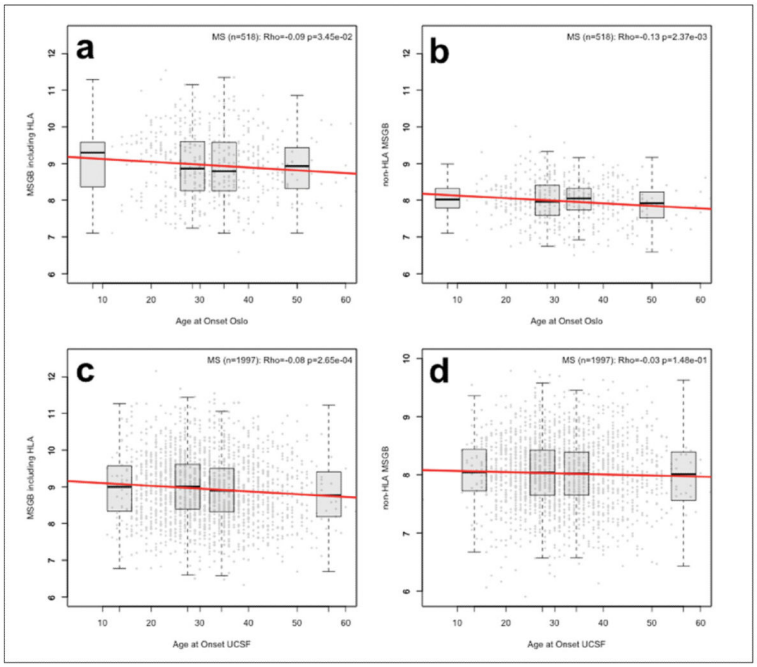


**Figure 3.**

Association between MSGB and AAO, illustrated as scatterplots, showing MSGB versus AAO in each cohort. A linear regression line is drawn in red. We provide Rho estimates and *p*-values from the Spearman's non-parametric correlation test. Boxplots for each quartile are displayed and located at the mean value of quartile group on the x-axis.

**(a)** Oslo cohort scatterplot of MSGB versus AAO.

**(b)** Oslo cohort scatterplot of non-HLA MSGB versus AAO. **(c)** UCSF sample scatterplot of MSGB versus AAO.

**(d)** UCSF sample scatterplot of non-HLA MSGB versus AAO.

AAO: Age at onset of MS; HLA: human leukocyte antigen; MS: multiple sclerosis; MSGB: MS genetic burden; UCSF: University of CA, San Francisco.

## Table I

(a) Clinical data of our study's included MS and control cohorts. (b) HLA data of our study's included MS and control cohorts.

**1(a)**

| **Clinical data of the Oslo MS-control cohort** | **MS patients from the Oslo MS registry** | **Oslo MS patients genotyped** | **Oslo controls genotyped** | **UCSF MS patients genotyped** | **UCSF controls genotyped** |
|---|---|---|---|---|---|
| | ***N* = 1648** | ***N* = 639** | ***N* = 530** | ***N* = 1997** | ***N* = 708** |
| Female, % | 68.5 | 73.0 | 68.6 | 73.0 | 52.7 |
| RRMS, % | 79.5[a] | 82.6 | | 90.4 | |
| PPMS, % | 20.5[a] | 17.4 | – | 8.5 | |
| AAO, mean (SE) | 32.4 (0.27)[b] | 32.4 (0.42) | – | 31.6 (0.21) | |
| Age at EDSS exam, mean (SE) | 49.1 (0.42)[c] | 50.2 (0.55) | – | 43.6 (0.24) | |
| OCB positive, % | 84.8[d] | 88.7[d] | | 69.3[d] | |
| EDSS, mean (SE) | 4.5 (0.09)[c] | 4.6 (0.12) | – | 3.49 (0.05) | |
| MSSS, mean (SE) | 4.76 (0.11)[e] | 4.80 (0.14) | – | 4.75 (0.06) | |

1(b)

| Associated HLA and DRB1 alleles in Oslo MS cohort subset[f] | Allele | Allele frequency in MS (%) | Allele frequency in Control (%) | *p*-value |
|---|---|---|---|---|
| | | 2*n* = 1156 | 2*n* = 698 | |
| HLA-A | *02 | 326 (28.2) | 236 (33.8) | 0.0092 |
| | *03 | 228 (19.7) | 108 (15.5) | 0.0251 |
| | | 2*n* = 1140 | 2*n* = 699 | |
| HLA-B | *07 | 285 (25) | 102 (14.6) | 0.0001 |
| | *44 | 58 (5.1) | 92 (13.2) | 0.0001 |
| | | 2*n* = 1211 | 2*n* = 625 | |
| HLA-C | *07 | 467 (39.3) | 178 (28.5) | 0.0001 |
| | *03 | 207 (17.4) | 133 (21.3) | 0.0428 |
| | *06 | 63 (5.3) | 52 (8.3) | 0.0146 |
| | | 2*n* = 1183 | 2*n* = 700 | |
| HLA-DRB1 | *04 | 181 (15.3) | 156 (22.3) | 0.6119[g] |
| | *15 | 460 (38.8) | 103 (14.8) | $1*10^{-26}$ |
| | *01 | 103 (8.7) | 88 (12.6) | 0.7536[g] |
| | *13 | 100 (8.4) | 86 (12.3) | 0.7506[g] |
| | *03 | 128 (10.8) | 83 (11.9) | 0.0824[g] |
| | *07 | 68 (5.7) | 79 (11.3) | 0.0277[g] |

[a] From 1485 patients.

[b] From 1504 patients.

[c] From 927 patients.

[d] Based on 1293 patients in the Oslo MS registry, 568 genotyped patients from the Oslo MS registry and 760 patients from UCSF. OCB measured by isoelectric focusing and agarose gel electrophoresis.

[e] From 735 patients.

[f] Available classical HLA typing data for the included MS cases and controls, from the Oslo cohort.

[g] HLA-DRB1*15 was excluded from the analysis.

AOO: Age at onset; EDSS: Expanded Disability Status Scale; HLA: human leukocyte antigen; MS: multiple sclerosis; MSSS: MS severity score; OCB: oligoclonal bands; PPMS: primary progressive MS; RRMS: relapsing–remitting MS; SE: standard error; UCSF: University of CA - San Francisco.