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Genetic heterogeneity within the HLA region in three distinct clinical subgroups of myasthenia gravis

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ABSTRACT

This study aims to investigate genetic susceptibility to early-onset and late-onset anti-acetylcholine receptor antibody positive myasthenia gravis (EOMG and LOMG) and anti-muscle specific kinase antibody positive MG (MuSK-MG) at genome-wide level in a single population. Using a custom-designed array and imputing additional variants and the classical HLA alleles in 398 patients, we detected distinct associations. In EOMG, rs113519545 in the HLA class I region (OR = 5.71 [3.77–8.66], $P = 2.24 \times 10^{-16}$), *HLA-B*08:01* (OR = 7.04 [3.95–12.52], $P = 3.34 \times 10^{-11}$) and *HLA-C*07:01* (OR = 2.74 [1.97–3.81], $P = 2.07^{-9}$), in LOMG, rs111256513 in the HLA class II region (OR = 2.22 [1.59–3.09], $P = 2.48 \times 10^{-6}$) and in MuSK-MG, an intronic variant within *HLA-DQB1* (rs68081734, OR = 5.86, $P = 2.25 \times 10^{-14}$) and *HLA-DQB1*05:02* (OR = 8.56, $P = 6.88 \times 10^{-13}$) revealed the most significant associations for genome-wide significance. Differential genetic susceptibility within the HLA to EOMG, LOMG and MuSK-MG has been established in a population from Turkey.

1. Introduction

Acquired myasthenia gravis (MG) is a rare, organ specific autoimmune disease mediated by autoantibodies (ab). The disease is heterogeneous with respect to the presence of ab, age at disease onset, clinical features, thymic changes and sex distribution [1–4]. In 80–85% of patients, pathogenic ab directed against the nicotinic acetylcholine receptor (AChR) causes the disease (AChR-MG). In a small (~5%) subgroup of patients, ab against muscle specific kinase (MuSK) are detected (MuSK-MG) [5–7]. With recent discoveries of ab directed against low density lipoprotein receptor-related protein 4 (LRP4) [8] and agrin [9], mainly in patients with either anti-AChR or anti-MuSK ab, the proportion of seronegative MG patients is further reduced.

Differences between ab in MG subgroups reflect different pathogenic mechanisms: the AChR-specific ab are of immunoglobulin (Ig) IgG1 and IgG3 isotypes [10] and exert their effects mainly by activation of the complement cascade [11,12], whereas anti-MuSK ab are predominantly of the non-complement fixing IgG4 isotype [13]. Moreover, AChR-MG is also heterogeneous in terms of thymic changes and age of disease onset. Considering thymoma associated MG (TAMG) as a separate disease of paraneoplastic nature, non-thymomatous AChR-MG is separated into i) early onset MG (EOMG) that occurs predominantly in women with thymic follicular hyperplasia (TFH), and ii) late onset MG (LOMG) that is more prevalent in men and not associated with TFH [14].

Genetic susceptibility is implicated by the associations with the human leukocyte antigen (HLA) in this immunologically heterogeneous disease. Association of HLA A1-B8-DR3 haplotype is demonstrated in women with AChR-MG, thymic hyperplasia and early onset disease [2,15–18]. Further studies extended the association to *DQB1* alleles with other positive and negative associations in different subgroups of MG with respect to ab and thymic changes [19–22]. Recently, specific HLA associations were reported in LOMG and EOMG supporting the genetic differences also between these subgroups [23–26]. An immune-related gene screening in an extensive European EOMG cohort identified novel candidates outside of the HLA complex [27]. The first genome-wide association study (GWAS) in AChR-MG included only EOMG cases and

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confirmed variants in the major histocompatibility complex (MHC) class I and class II as susceptibility markers. Protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) and TNFAIP3 interacting protein 1 (*TNIP1*) loci have also been identified in subgroups of the disease [28]. A subsequent GWAS also in AChR-MG patients from two different cohorts demonstrated distinct pattern of susceptibility in MHC loci and identified cytotoxic T-lymphocyte–associated protein 4 gene (*CTLA4*) as another marker for the disease [29]. In the most recent GWAS in LOMG three distinct *HLA-DQA1* associations were identified [30]. On the other hand, distinct *HLA-DRB1* and -*DQB1* associations in the rare subgroup of MuSK-MG have been demonstrated in smaller groups of patients [31–33], revealing that MG phenotypes have different genetic backgrounds. However, risk loci outside the HLA region and their eventual association with known risk loci in the HLA region have not been reported yet in MuSK-MG patients.

We therefore sought to characterize the known loci and search for additional risk loci by genotyping three independent patient subgroups (EOMG, LOMG, and MuSK-MG) in parallel in a single population with a customized SNP array, the Immunochip, designed to interrogate 186 autoimmune disease loci primarily identified in prior GWAS of other autoimmune diseases [34].

2. Methods

2.1. Study population

The study population consisted of 472 individuals diagnosed with generalized MG at Istanbul University and 576 healthy controls (HC) from Turkey. A total of 386 patients were included as generalized AChR-MG with clinical diagnostic criteria, the presence of anti-AChR ab and no evidence of thymoma. Among AChR-MG patients, age of disease-onset was before 50 years in 255 patients (66.1%, EOMG), while disease presented at or after age 50 in the remaining 131 patients (33.9%, LOMG) [35]. An additional 86 patients had anti-MuSK ab (MuSK-MG). AChR-MG patients with ocular form of the disease and thymoma, as well as anti-AChR and anti-MuSK ab negative patients were not included in the study.

All donors were enrolled under the protocol approved by the Ethical Committee of the Istanbul Medical Faculty. All participating individuals provided written informed consent.

2.2. Genotyping and quality control

Genotyping was performed using the Illumina Human Immuno DNA Analysis BeadChip kit (Immunochip) which contains 196,524 genetic variants contained in loci previously associated with autoimmune diseases. Initially, we created a set of 29,332 linkage disequilibrium (LD) pruned markers by filtering raw genotyping data on the basis of genotype success rate (GSR> 0.9), minor allele frequency (MAF> 0.01), and Hardy-Weinberg equilibrium P value (HWE, P> 0.0001). LD-pruning was performed by pairwise removal of a single SNP among marker pairs with r-squared values> 0.2 within a 100 kb window. A total of 10 principal components were calculated from this set of 29,332 LD-pruned markers using Eigensoft 2.0. Samples were also removed on the basis of outlying heterozygosity and identity by descent using the LD-pruned marker set. Prior to analysis, a total of 16 individuals were removed as outliers on the basis of the first 3 principal components (±4SD). Additional 6 samples with increased heterozygosity (±2SD) and 32 samples with elevated identity by descent (pihat>; 0.4) were removed. Prior to statistical analysis, 55,946 genotyped markers were removed on the basis of MAF (<0.01), 4278 due to deviation from HWE (1276 markers cases HWP> 0.01, 3002 markers controls HWP> 0.0001), and 5492 markers due to GSR (<0.90). Markers were also filtered on the basis of differential missingness (P-value < 0.01). A total of 67 additional samples were then removed on the basis of GSR (<0.88) among the filtered set of 110,467 variants. Following quality control, a total of 398 MG cases and 541 HC were used in the final analysis. Of the 398 cases, there were 211 cases of EOMG, 109 cases of LOMG and 78 cases of MuSK-MG (Table 1).

2.3. Imputation of the HLA region

Imputation was performed using a multiethnic reference panel of 1092 individuals in IMPUTE2 [36]. Imputed genotype calls were inferred using a posterior probability of 0.9. Imputed markers were filtered on the basis of call rate (>90%), MAF (> 0.01), and HWE (P > 0.0001).

2.4. Imputation of classical HLA alleles

HLA*IMP software package was used to infer classical alleles in both HLA class I and class II regions (*HLA-A*, *HLA-B*, *HLA-C*, *HLA-DQA1*, *HLA-DQB1* and *HLA-DRB1*). A posterior probability of 0.9 was used to assign allele calls for each imputed HLA haplotype, and imputation results were analyzed only if both chromosomes were successfully imputed at each locus.

2.5. Statistical analysis

Statistical analysis was performed as previously described [37,38]. We performed logistic regression to examine genetic associations of single-nucleotide variants and imputed classical HLA alleles using PLINK [39]. Logistic regression models were constructed to examine the independence of single markers and classical HLA haplotypes. Analysis was performed separately among cases of EOMG, LOMG, and MuSK-MG using the same control population of 541 HC in each group. Conditional analysis was also performed within the HLA region using logistic regression. All logistic regression models, unless otherwise specified, were adjusted for 3 principal components and sex.

3. Results

We performed genetic association analysis among 196,524 genetic variants included on the Illumina Immunochip platform in 3 distinct subgroups of patients with MG from Turkey. As sex distribution within our AChR-MG case cohort followed a bimodal pattern, with EOMG being more common in women (3.8 fold) and LOMG in men (2.4 fold), patient groups with EOMG and LOMG, as well as MuSK-MG were analyzed separately and all analyses performed was adjusted for sex [29,40]. In each subgroup of MG, the genetic association signal is largely confined to the HLA region (Fig. 1). However, we observed significant differences in the distribution of genetic associations within the HLA region between MG subgroups (Fig. 2).

3.1. Early-onset myasthenia gravis (EOMG)

Among patients with EOMG, we observed significant genetic associations within the HLA region centered around the *HLA-B/MICA* loci. To fine-map and localize this genetic effect, we imputed additional genetic variants using the 1000 Genomes project data in the region extending

Table 1
Demographic characteristics of the patients and healthy controls (HC) included in this study. The distribution reflects only the samples used in the final analysis. HP/TX: Thymic hyperplasia detected in the thymectomized patients.

Groups	Women (W)	%	Men (M)	%	W/M	HP/TX	Total
EOMG	167	79.1	44	20.8	3.8	84/133	211
Onset age	23.7 ± 8.7		28 ± 10.9				
LOMG	32	70.6	77	294	0.4	2/7	109
Onset age	63.7 ± 7.2		62.7 ± 7.6				
MuSK-MG	52	667	26	33.3	2.0		78
Onset age	32.2 ± 13.8		40.9 ± 17.7				
HC	270	49.9	271	50.1			541
	32.5 ± 12		31.6 ± 11				