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Genomewide admixture study in Mexican Mestizos with multiple sclerosis

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ABSTRACT

Background: Multiple sclerosis (MS) is a complex immune-mediated disease. It has been suggested that genetic factors could explain differences in the prevalence among ethnic groups. To know whether genetic ancestry is a potential risk factor for MS in Mexican patients and to identify candidate genes for the susceptibility to the disease we conducted an initial trial of genome-wide analysis.
Methods: 29 patients with diagnosis of definitive MS and 132 unrelated healthy controls were genotyped using the Affymetrix human 6.0 array. After QC procedures, ancestry determination and a preliminary case–control association study were performed.
Results: We identified significant differences in the European ancestry proportion between MS cases and controls (33.1 vs 25.56, respectively; $p = 0.0045$). Imputation analysis in the MHC region on chromosome 6 showed a signal with a significant level ($p < 0.00005$) on the HLA-DRB region. Additionally, a preliminary association analysis highlighted the *ASF1B* as novel candidate gene participating in MS.
Conclusion: Our data suggest that European ancestry is a risk factor to develop MS in Mexican Mestizo population. Conversely, indigenous ancestry of Asian origin seems to confer protection. Further studies with more MS cases are needed to confirm these findings.

1. Introduction

Genetic factors play a crucial role for individual susceptibility to multiple sclerosis (MS). It has been suggested that ethnic differences, together with geographical distribution, participate in the prevalence of MS [1]. The high incidence of MS occurs in Caucasians from the northern hemisphere, mostly in Europe and North-America [2,3]. Mexico is located below the parallel 40 and has been traditionally considered as a low incidence area for MS (12/100,000 population) [1,3,4]; however, over the last years a steady rise in incidence and prevalence has been reported [3,5].

During the last four decades numerous studies have tried to identify genetic loci involved either in risk or protection for the development of MS. So far, more than 100 loci MS associated have been identified, being the human leukocyte antigen (HLA) region, located on chromosome 6, the strongest susceptibility locus linked to MS [6]. Genome-wide association studies (GWAS) have confirmed the participation of DRB*15:01 allele as the primary susceptibility allele for MS, as well as non-HLA *loci* like the type 1 interferon (IFN1) pathway (IMSGC) [7]. Recently the International Multiple Sclerosis Genetics Consortium (IMSGC) has reported a total of 110 established non-MHC risk variants, 48 new and 49 previously known non-MHC variants associated with multiple sclerosis increasing the catalog of MS risk variants; thus, opening a better comprehension of the implicated networks involved in MS disease [8]. However, many initial findings have not been confirmed in similar studies and their real implication as risk factors is still unclear [7,9,10].

Likewise, several studies have identified the population ancestry as a risk factor not only for MS susceptibility but also for its clinical progression and disability grade. On this regard, in some endemic areas with relevant integration of different ethnic groups the disease outcomes are more severe in patients with African than Caucasian or Asian ancestry [11,12]. Nevertheless, genetic and genomic studies of ancestry in Latin American patients with MS are notoriously scarce. These investigations could be relevant due to the genetic admixture of most inhabitants of Latin American countries composed mainly by European and Amerindian genes.

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The modern Mexican population is composed mostly of mestizos with a genetic background derived primarily from the original Native American inhabitants (51%) and European settlers (45%), with minimal contribution of West African population (3.7%) [13]. Demographic studies of MS in Mexico have shown the absence of the disease in indigenous population with no European ancestry [14]. Also, it has been shown that in Mexico MS affects preponderantly subjects from the most affluent educational and economical strata of society, which also coincide with mestizo groups with higher European ancestry [3,15]. The above factors indicate that genomic studies based on ancestry estimations in the Mexican population could shed important information on etiological and risk factors that participate in the development of MS.

Genetic studies in populations from geographical areas with low prevalence of a given disease are a valuable tool for etiological studies [3]. The main value of these studies lies in the fact that the comparatively few individuals with the disease might be exposed to precipitating factors that could not be self-evident in the geographical areas with endemic frequency of the disease. Mexican Mestizos share a genetic combination from populations with a high and low (European and Asian) incidence of MS, which might participate, together with environmental risk factors (viral agents, smoked tobacco, vitamin D, etc.) in the etiopathogenesis of MS [5,16,17].

We conducted an initial trial of genome-wide analysis in Mexican Mestizos to study genetic ancestry as a potential risk factor of MS; we also implemented a preliminary case–control study to identify candidate genes associated to MS in a cohort of Mexican patients.

2. Methods

2.1. Populations

A total of 161 Mestizo subjects were enrolled in the study, recruited from two public tertiary level institutions of health, located in Mexico City. Twenty-nine were patients that fulfilled the McDonald criteria for the diagnosis of MS [18] and 132 were blood bank donors, included as healthy controls. Patients were drawn from the Instituto Nacional de Neurología y Neurocirugía and controls from the Instituto Nacional de Pediatría. Both patients and controls were subjects born and living in Mexico, like their parents and grandparents and no reference of recent European ancestry contribution. Mestizo ethnicity was assessed through analysis of whole genome, based on markers included in the Affymetrix human 6.0 array. This study was approved by the respective local ethics and research committees and all individuals signed an informed consent.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the GenePure Genomic Blood DNA Purification kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Genotyping was performed at the Instituto Nacional de Medicina Genómica. Samples were genotyped using the Affymetrix human 6.0 array (Affymetrix, Santa Clara, CA, USA). The initial dataset including cases and controls consisted of 907,164 SNPs.

2.3. Reference panels

Genotypes from 118 unrelated individuals from the Human Genome Diversity Project [19] were used as reference panels for admixture and local ancestry inference. This reference panel was used because it is the only one having Native Americans (Pima and Maya), in addition to southern Europeans (Basque, French and Italians) and Africans (Yorubas and Mandenkas).

2.4. Quality control

The QC procedures were made using PLINK software [20] with previously established thresholds [21]. Only samples passing a call rate threshold of 97% and gender concordance were considered. SNPs that had <1% minor allele frequency and called <97%, or with genotyping fails significantly different between cases and controls or with highly departures from Hardy–Weinberg equilibrium ($p < 0.001$) were removed for the analysis. Finally, we discarded sample relatives using pairwise identity-by-descent (IBD).

2.5. Population structure

We compared the genetic structure of samples from cases and controls using ADMIXTURE software [22], for $k = 3$, based on a dataset containing 545,347 SNPs, merged from HGPD panel populations and our dataset. We also estimate the local ancestry of each individual chromosome. The significance of the difference in the average proportion of Native American, European and African ancestry between cases and controls were tested using the non-parametric Mann–Whitney U test. To address multiple testing in the chromosome specific ancestry comparison, Bonferroni's correction was used considering 22 independent tests and statistical significance was set when $p \leq 0.002$.

2.6. Local ancestry estimation

Local ancestry estimation was performed using LAMP-LD version 1.0 [23], using HGDP reference panels. The panels were then jointly phased using SHAPEIT version 1.53 [24].

2.7. Association study

After performing stringent quality control, the association analysis between SNP genotyped with MS was evaluated using PLINK. To minimize spurious associations, the analysis was adjusted by sex and population structure via PCA, accounting 707,757 SNPs. Also, statistical power was estimated using QUANTO version 1.2 (http://biostats.usc.edu/software), considering a case–control rate 1:4.2, 0.00012% of prevalence [1], under the additive model using MAF in controls and OR detected.

We used principal component analysis to summarize the genetic variation of cases and controls as compared to other continental samples (Native Americans, Africans and Southern Europeans). Otherwise we performed a comparative analysis in the frequency of the associated SNPs between our data and those described in the public database.

2.8. Imputation

Due to the relatively low number of SNPs included in A*ffymetrix*® human 6.0 array for screening of the MHC region, and the high linkage disequilibrium in that locus, a regional imputation was done using IMPUTE2 software [25], with the 1000 genomes Phase I integrated variant set as reference panel and prephasing genotypes of cases and controls on the MHC region. Only SNPs passing quality metrics above 0.9 were considered for association analysis.

3. Results

MS cases: all 29 patients had definitive diagnosis of relapse-remitting MS, the mean ($X \pm$ SD) age at diagnosis was 30 ± 8 years old, 20 (69%) were females and 9 (31%) were males; the duration of