Association of the *CBLB* gene with multiple sclerosis: new evidence from a replication study in an Italian population

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ABSTRACT

Background The T allele of rs9657904 within the *CBLB* gene was recently found to be significantly associated with multiple sclerosis (MS) in a genome-wide association study in Sardinia.

Objective To replicate this association in an independent population with a different genetic background.

Methods The rs9657904 variant was typed in a sample of 1435 cases and 1466 controls from the Italian mainland.

Results It was found that in this sample also, the common allele T of rs9657904 is significantly positively associated (one-tailed $p=7.35\times10^{-5}$) and with a comparable effect size with MS (OR=1.31, 95% CI 1.14 to 1.52).

Conclusion These data provide further evidence of the association of MS disease with variation within *CBLB*.

INTRODUCTION

Multiple sclerosis (MS) is a multifactorial neuroinflammatory and autoimmune disorder characterised by a progressive demyelination of axons of the central nervous system and neuronal cell degeneration, resulting in a severe disabling condition. Interactions between unknown environmental factors and alleles of many susceptibility loci across the genome contribute together to the development of the disease.[1] Until recently, the only genes consistently associated with MS mapped to the human major histocompatibility complex or HLA (human leucocyte antigen) region.

Study of the genetics of MS, after years of difficulty, is undergoing a period of rapid development. This is owing to the use of large datasets as well as improved genotyping techniques that have allowed the first genome-wide association scans (GWAS).[2–6] Once those significant associations are replicated in different populations, they acquire a profound importance for understanding a disease and for focusing lines of investigation for functional and bioinformatics analysis.[7 8]

Thus far, the major GWAS findings have come from analyses of populations with northern European origin in which MS is particularly common. Recently, a novel association with risk for MS of some markers within the *CBLB* gene (Cas-Br-M (murine) ecotropic retroviral transforming sequence b, 3q13.11) was observed in the island population from Sardinia.[6] To assess the associated variant in an independent sample set from the Italian mainland is cogent for several reasons. First, while sharing a very similar environment with much of continental Italy, Sardinians are, by most measures, genetically different from continental Italians, although to a lesser extent than populations from northern Europe.[9] Furthermore, Sardinia has an MS prevalence at least three times higher than that of the Italian mainland.[10] Also, the main genetic risk factor for MS at the HLA class II *DRB1-DQB1* loci shows a very different allelic distribution in Italy than in Sardinia. In the Sardinian population, the HLA association is mainly accounted for by the *HLA-DRB1*03:01* allele,[11] included within an extended or ancestral HLA haplotype—namely, *HLA-A*30, B*18, Cw*5, DRB1*03:01*, which is very rare elsewhere. Conversely, in continental Italy the MS association, as in most other populations, is marked by the *HLA-DRB1*15:01* allele.[12]

Hence we tested the positive association observed with the T allele of the top associated variant (rs9657904) observed in the Sardinian study in a large cohort of continental patients with MS and controls.

PATIENTS AND METHODS

The SNP rs9657904 C→T in intron 1 of the *CBLB* gene was genotyped in 1435 patients with MS and 1466 regionally matched controls from continental northern–central Italy.

Patients with MS had a female:male ratio of 2:1, a mean age of onset of 31.6±10.3 years, a mean expanded disability status scale 3.10±2.23 and a mean multiple sclerosis severity score 3.91±2.72.[13] Ninety per cent of the patients presented a relapsing remitting while 10% presented a primary progressive disease course. The controls (female:male 1.3:1) were blood donors who shared the same ethnicity background with cases. Individuals with Sardinian origin were selectively excluded. All the samples were collected after informed consent and appropriate ethical approval.

Genotyping was performed with a Taqman genotyping assay (assay ID C_1499397_10, Applied Biosystems, Foster City, CA). The genotype success rate of this Taqman assay was 97%.

The statistical significance of the difference of allele and genotype frequencies between patients with MS and controls was evaluated using the χ^2 test with Yates's correction. The strength of association was evaluated by the odds ratio (OR) and its 95% CIs.

Table 1 Association results of *CBLB* rs9657904

	Cases N (%)	Controls N (%)	OR (95% CI)
Genotype			
TT	1054 (73.4)	974 (66.4)	1.40 (1.19 to 1.64)
TC	347 (24.2)	451 (30.8)	0.72 (0.61 to 0.85)
CC	34 (2.4)	41 (2.8)	0.84 (0.52 to 1.37)
Allele			
T	2455 (85.5)	2399 (81.8)	1.31 (1.14 to 1.52)
C	415 (14.5)	533 (18.2)	

The frequency of the T allele was significantly increased in the patients (one-tailed $p = 7.35\times10^{-5}$).
N indicates the number of individuals or alleles.

RESULTS AND DISCUSSION

The same allele (T) of rs9657904 single nucleotide polymorphism (SNP), positively associated with MS in Sardinian population, was significantly associated with MS risk (one-tailed $p=7.35\times10^{-5}$), and even showed a comparable effect size (OR=1.31) also in the Italian mainland (table 1).

The genotype frequencies did not deviate from Hardy–Weinberg equilibrium either in cases (p=0.39) or controls (p=0.19). The association of the T allele seems to be consistent with a recessive model, since it showed a significant increase in patients with MS only in homozygosis (table 1). Moreover, no significant interaction was detected in a case-only analysis with *HLA-DRB1*15:01* allele, with no difference of allele frequencies between *HLA-DRB1*15:01* positive (n=361) and negative (n=825) patients (minor allele frequency: 0.15 vs 0.14 p = 0.67). This is consistent with the reported lack of interaction with *HLA-DRB1*03:01* in the Sardinian study[5]and indicates that the same *CBLB* variant is associated with MS in two populations with distinct HLA associations. Moreover, the associated variant exhibits nearly overlapping frequencies in Sardinia[5] and in the Italian mainland; it is thus unlikely that variation at *CBLB* explains the higher disease prevalence seen in Sardinia.

Conversely, the *CBLB* gene does not appear in the list of MS-associated loci that satisfy the genome-wide significance threshold from previous GWAS, despite this SNP is tagged ($r^2\geq0.9$) by at least one SNP in the different platforms used so far.[1 2–4] This could suggest that in these populations, mainly of northern European origin, rs9657904 SNP shows a weaker association with MS. This observation supports the hypothesis that the tested SNP in the *CBLB* gene is not the primarily associated variant and may indicate that the linkage disequilibrium structure of populations from southern Europe might favour the detection of this association.

Since the disease association might be affected by many variables, in particular by the linkage disequilibrium between marker allele and causal allele, further cross-population comparisons using samples from more distantly related populations along with additional resequencing/fine mapping work appear necessary to reduce the MS association to its essential, potentially causal elements.

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Competing interests None.

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