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

Lucia Corrado¹, Laura Bergamaschi¹, Nadia Barizzzone¹, Maria Edvige Fasano², Franca R Guerini³,
Marco Salvetti⁴, Daniela Galimberti⁵, Maria Donata Benedetti⁶, Maurizio Leone⁷, Sandra D'Alfonso¹

¹ Department of Medical Sciences and Interdisciplinary Research Center of Autoimmune Diseases (IRCAD),
University of Eastern Piedmont, Novara, Italy

² SCDU Transplantation Immunology, A.O.U. S. Giovanni Battista di Torino, Turin, Italy

³ Laboratory of Molecular Medicine and Biotechnologies, Don C. Gnocchi Foundation IRCCS, S. Maria
Nascente, Milan, Italy

⁴ Neurology and Center for Experimental Neurological Therapy (CENTERS), Università La Sapienza, Rome,
Italy

⁵ Università di Milano, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan , Italy

⁶ Section of Neurology, Department of Neurological and Vision Sciences, University of Verona, Policlinico G.
Rossi, Verona, Italy

⁷ Department of Neurology, A.O.U. Maggiore della Carità, and IRCAD, Novara, Italy

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Corresponding author:

Sandra D'Alfonso

Department of Medical Sciences

Eastern Piedmont University

Via Solaroli 17

28100 Novara, Italy

Telephone: 39 321 660606, Fax: 39 321 620421

Email: dalfonso@med.unipmn.it

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 (GWAS) in Sardinia.

Objective To replicate this association in an independent population with a different genetic background. **Methods** We typed the rs9657904 variant in a sample of 1435 cases and 1466 controls

from the Italian mainland. **Results** We found that also in this sample, the common allele T of rs9657904 is significantly positively associated (one tailed $p = 7.35 \times 10^{-5}$) and with a comparable effect size with MS (OR = 1.31, 95%CI: 1.14-1.52). **Conclusion** These data provide further evidence of MS disease association with variation within *CBLB*.

Introduction

Multiple sclerosis (MS) is a multifactorial neuroinflammatory and autoimmune disorder characterized by a progressive demyelination of axons of the central nervous system (CNS) 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 (MHC) or **HLA (Human Leukocyte Antigens)** region.

The study of the genetics of MS, after years of difficulty, is undergoing a period of rapid development. This is due 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 disease understanding 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 of all, 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 at least 3 times higher MS prevalence than 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 versus Sardinia. **In the Sardinian population, the HLA association is mainly accounted for 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*, that 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 MS patients and controls.

Patients and Methods

The SNP rs9657904 **C>T** in intron 1 of the *CBLB* gene was genotyped in 1435 MS cases and 1466 regionally matched controls from continental Northern-Central Italy.

MS patients showed Female:Male ratio of **2:1**, a mean age of onset of 31.6 ± 10.3 **years**, a mean Expanded Disability Status Scale (EDSS) 3.10 ± 2.23 and a mean Multiple Sclerosis Severity Score (MSSS) 3.91 ± 2.72 . [13] Ninety percent of the patients presented a Relapsing Remitting while 10% a Primary Progressive disease course. The controls (Female: Male 1.3:1) were blood donors who shared the same ethnicity background of cases. Individuals with Sardinian origin were selectively excluded.

All the samples were collected after informed consent and appropriate ethical approvals.

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

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

Results and Discussion

The same allele (T) of rs9657904 SNP, positively associated with MS in Sardinian population, was significantly associated with MS risk (one tailed $p = 7.35 \times 10^{-5}$), 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 ($p = 0.39$) and controls ($p = 0.19$). The association of the T allele seems to be consistent with a recessive model, since it shows a significant increase in MS patients 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 observed in Sardinia.

Conversely, *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 ($r^2 \geq 0.9$) by at least one SNP in the different platforms utilized so far.[1,2-4] This could suggest that in these populations, mainly from northern European origin, rs9657904 SNP shows a weaker association with MS. This observation can support the hypothesis that the tested SNP in *CBLB* gene is not the primarily associated variant and may indicate that the linkage disequilibrium structure of populations from southern Europe might favor 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 re-sequencing/fine mapping

work appear necessary to reduce the MS association to its essential, potentially causal elements.

Table 1: Association results of *CBLB* rs9657904

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

Notes to Table 1:

The frequency of the T allele was significantly increased in the patients (one tailed $p = 7.35 \times 10^{-5}$)

N indicates the number of individuals or alleles

OR: Odds ratio

95% C.I: 95% confidence intervals

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Competing financial interests

The authors declare no competing financial interests.

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