Short Report

# Genetic basis for relapse rate in multiple sclerosis: association with *LRP2* genetic variation

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Number of words in abstract: 101

Number of words in main text: 1053

Number of references: 10

Number of tables/figures: 1

**Abstract**

**Background:** In contrast to successes for MS susceptibility, the genetic basis for clinical heterogeneity remains largely unresolved.

**Objectives:** We investigate the first reported genetic association with relapse rate.

**Methods:** We genotyped variant rs12988804 in *LRP2* in a homogeneous study population of 527 Belgian MS patients with 970 documented relapses.

**Results:** The rs12988804*T allele is associated with a 1.16-fold increased hazard rate for a relapse occurring (P=0.0078), and a higher baseline relapse rate prior to immunomodulatory treatment (P=0.044).

**Conclusion:** Variant rs12988804 in *LRP2*, the first example of a genome-wide significant association with relapse rate in MS, is replicated in an independent study.

Genome-wide association studies (GWAS) in multiple sclerosis (MS) have led to the identification of 11 Human Leukocyte Antigen (HLA) and 110 non-HLA genetic risk variants[1-3]. From a clinical perspective, one of the most important aspects is prognosis, with implications for long-term outcome and disability and treatment. For example, some MS patients will experience frequent relapses necessitating the early start of aggressive therapy whereas others will remain relapse-free for longer periods without treatment. In contrast to the successes for genetic susceptibility, the basis for clinical heterogeneity between patients remains largely unresolved[4]. Addressing this key challenge has been hampered by the collection of detailed phenotypical information in sufficiently large sample sizes. Recently, Zhou et al.[5] undertook a GWAS for relapse risk in a total of 449 MS patients from three cohorts from Australia and the US, and demonstrated association with variant rs12988804 in the *LRP2* gene. As replication is essential in genetic studies, we investigated this finding in a single homogeneous cohort of 527 Belgian MS patients.

**Study Population and Methods**

*Patients*

A study population of N=527 bout-onset MS patients fulfilling McDonald diagnostic criteria 2010 were included at the Department of Neurology of the University Hospitals Leuven upon providing written informed consent and approval by the Ethics Committee of the University Hospitals Leuven (ML4733). Clinical data including relapse and treatment history were collected retrospectively during clinical follow-up by the same treating clinician (B.D.). Relapse was defined according to the 2010 McDonald criteria as the patient-reported symptoms or objectively observed signs typical of an acute inflammatory demyelinating event in the central nervous system with duration of at least 24 hours, in the absence of fever or infection.

*Genotyping*

SNP rs12988804 was genotyped with a Taqman assay (C_11829317_20) on a 7300 system (Life Technologies). Genotyping success rate was 99.05% and the minor allele frequency (T-allele) was 30%.

*Statistical analysis*

We applied two different methods for analysis of relapse history with genotype. The first method[5] is a Cox proportional hazards model calculated with the coxph function in the 'survival' package in R 3.3.2. This analysis includes all recorded relapses and considers as censors the start of immunomodulatory treatment or – for untreated patients – the end of follow-up. The second method[6] estimates a baseline yearly relapse rate before start of immunomodulatory treatment in the subset of patients (N=503) with a minimum baseline duration of 90 days. As baseline yearly relapse rate was not normally distributed, it was inverse-rank normalized and analysed with linear regression in R 3.3.2. We additionally evaluated whether covariates gender and age at onset influenced the analyses.

**Results**

Our study population consists of 527 bout onset MS patients, with 71% female and a median age at onset of 31 years. A detailed description of the study cohort is provided in table 1. The median baseline duration between onset and start of any immunomodulatory treatment or end of follow-up is 3.95 years. During this time, a total of N=970 new relapses after onset are documented, with an average of $1.84 \pm 1.66$ per patient. In a survival analysis using the Cox proportional hazards model and including all relapses, the T-allele is associated with a higher hazard rate of a relapse occurring [hazard ratio (HR) = 1.16 (95% CI = 1.01 - 1.33) compared to reference, P=0.0078]. A sensitivity analysis, limited to patients with at least two relapses

prior to treatment (N = 420) leads to similar results [HR = 1.16 (95% CI = 1.01 − 1.33), P = 0.0079]. In a secondary analysis as described previously[6], the rs12988804*T allele is associated with an increased yearly relapse rate at baseline, i.e. prior to the start of any treatment (P=0.044, beta = 0.15 ± 0.07). Including covariates gender and age at onset did not alter the results (P = 0.042).

**Discussion**

In a large dataset of N=527 Belgian MS patients with N=970 documented relapses upon onset, we observe a significant association of rs12988804 located in the *LRP2* gene with relapse rate in MS. The rs12988804*T allele is associated with a higher baseline yearly relapse rate before the start of any immunomodulatory treatment and with a 1.16-fold increased hazard rate for a relapse occurring. Our data replicate the identification by Zhou et al.[5] of the first genetic variant with relapse rate in MS. The magnitude of effect in our study (HR=1.16) is smaller than previously reported (HR between 1.74 and 2.75 across three cohorts). This may be due to the expected winner's curse in genetic studies as well as differences in study population. The GWAS of 449 MS patients by Zhou et al.[5] was based on three cohorts of MS patients from Australia and the US, each with their own recruitment strategies and characteristics of age, time of study entry, length of follow-up etc. We investigated a single, homogeneous dataset of N=527 Belgian patients for whom recruitment and data collection was performed by one expert MS clinician, thereby strengthening the validity of the observation. An important difference with Zhou et al.[5] is that our study considers relapse rate at baseline, i.e. prior to any immunomodulatory treatment, instead of including patients on a range of immunomodulatory treatments. This indicates that the *LRP2* variant exerts its influence on relapse rate as part of the natural course of disease.

The current finding is the first indication for a genetic basis for prognosis in MS. It is likely that additional GWAS studies will uncover novel genetic loci. Similarly, GWAS for other

autoimmune diseases such as inflammatory bowel disease[7] have led to the identification of multiple genetic variants influencing prognosis.

The *LRP2* variant associated with relapse rate has not been reported as associated with susceptibility so far. This mimics the case of variants associated with cerebrospinal fluid antibody responses in MS[8, 9]. Reversely, known genetic susceptibility factors – either alone[2] or combined in a genetic burden[6, 10] – appear to influence at most modestly clinical heterogeneity, with the exception of HLA and age at onset[3]. Together, this underscores that major genetic factors driving inter-patient heterogeneity are largely different from those for susceptibility and disease initiation. This concept is not unique to MS, but is an emerging theme shared with other autoimmune diseases[7]. Understanding the biology of prognosis has important clinical implications for treatment strategies and personalized medicine.

In summary, our data together with those from Zhou et al.[5] establish the first genetic variant associated with relapse rate in MS.

**Author contributions**

KH and KM processed samples and KH obtained genotype data. KH, MV and AG performed statistical analysis and data interpretation. IS and BD collected and handled clinical data. AG and BD designed and supervised the experiment. AG wrote the first draft of the manuscript, and all authors critically revised the manuscript.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

**References**

1.      The International Multiple Sclerosis Genetics Consortium. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet*. 2013; 45: 1353-60.

2.      The International Multiple Sclerosis Genetics Consortium and The Wellcome Trust Case Control Consortium 2. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011; 476: 214-9.

3.      Moutsianas L, Jostins L, Beecham AH, et al. Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nat Genet*. 2015; 47: 1107-13.

4.      Longbrake EE and Hafler DA. Linking Genotype to Clinical Phenotype in Multiple Sclerosis: In Search of the Holy Grail. *JAMA neurology*. 2016; 73: 777-8.

5.      Zhou Y, Graves JS, Simpson S, Jr., et al. Genetic variation in the gene LRP2 increases relapse risk in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2017.

6.      Hilven K, Patsopoulos NA, Dubois B and Goris A. Burden of risk variants correlates with phenotype of multiple sclerosis. *Mult Scler*. 2015; 21: 1670-80.

7.      Lee JC, Biasci D, Roberts R, et al. Genome-wide association study identifies distinct genetic contributions to prognosis and susceptibility in Crohn's disease. *Nat Genet*. 2017; 49: 262-8.

8.      Buck D, Albrecht E, Aslam M, et al. Genetic variants in the immunoglobulin heavy chain locus are associated with the IgG index in multiple sclerosis. *Ann Neurol*. 2012; 73: 86-94.

9.      Goris A, Pauwels I, Gustavsen MW, et al. Genetic variants are major determinants of CSF antibody levels in multiple sclerosis. *Brain*. 2015; 138: 632-43.

10.     Sondergaard HB, Petersen ER, Magyari M, Sellebjerg F and Oturai AB. Genetic burden of MS risk variants distinguish patients from healthy individuals but are not associated with disease activity. *Mult Scler Relat Disord*. 2017; 13: 25-7.

Table 1. Clinical description of study cohort

| Clinical data | MS patients | N |
|---|---|---|
| Male:Female, % | 29:71 | 527 |
| AAO, median (IQR), y | 31 (24-39) | 527 |
| Total relapses* | 970 | 527 |
| Relapse rate, median (IQR)*$^\phi$ | 0.62 (0.24-1.34) | 503 |
| Baseline duration, median (IQR), y | 3.95 (1.41-12.08) | 527 |
| - Untreated | 17.26 (9.11-27.92) | 147 |
| - Treated | 2.23 (0.94-5.83) | 380 |
| - First-line treatment | 2.05 (0.89-5.72) | 347 |
| - Second-line treatment | 3.65 (2.26-6.65) | 18 |
| - Other | 4.77 (2.96-8.45) | 15 |

MS: multiple sclerosis; N: number of individuals; AAO: age at onset; IQR: interquartile range;

*Baseline = onset till start of first treatment or end of follow up for untreated patients

$\phi$ > 90 days baseline