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Relevance of IL7R genotype and mRNA expression in Dutch patients with multiple sclerosis

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Abstract

Background: The interleukin 7 receptor (IL7R) has been recognized as a susceptibility gene for Multiple Sclerosis (MS). Analysis of rs6897932 (the most strongly MS-associated single nucleotide polymorphism (SNP)), showed effects of genotype on the relative expression of membrane-bound to total amount of IL7R mRNA.

Objective: We assessed the relevance of IL7R on MS phenotype (including clinical and magnetic resonance imaging (MRI) parameters) at DNA and mRNA level in Dutch patients with MS.

Methods: The genotype of rs6897932 was analyzed in 697 patients with MS and 174 healthy controls. The relevance of genotype and carriership of the C allele on MS phenotype (disease activity and severity, using clinical and MRI parameters) was assessed. In addition, relative gene expression of membrane-bound to total IL7R mRNA was analyzed with respect to disease phenotype in a subgroup of 95 patients with early relapsing MS.

Results: In particular, homozygosity for the risk allele is a risk factor for MS in our population ($OR_{CC\ vs\ CT\ and\ TT} = 1.65$ (95% CI: 1.18–2.30), two-sided $p = 0.004$). However, no effect of genotype or the relative expression of membrane-bound IL7R (presence of exon 6–7) to total amount of IL7R mRNA (presence of exon 4–5) was found on MS phenotype.

Discussion: Homozygosity for the IL7R exon 6 rs6897932 C allele is associated with a higher risk for MS in our Dutch population. No effect was found of genotype or mRNA expression on disease phenotype.

Keywords

genetics, genotype, IL7R, MRI, mRNA, multiple sclerosis, phenotype

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Introduction

The demyelinating neurodegenerative disease multiple sclerosis (MS) is the most common cause of chronic neurological disease in young adults.[1] Evidence indicates a complex interplay of genetic and environmental factors in the predisposition to this disease.[2–4] The strongest and most consistent genetic component is the major histocompatibility complex (MHC) HLA-DRB1*1501-DQB1*0602 haplotype.[5] More recently, several non-MHC single nucleotide polymorphisms (SNPs) were found, showing a more modest effect on susceptibility.[6–10] One of the SNPs identified and confirmed in several cohorts is rs6897932, located in exon 6 of the interleukin 7 receptor (IL7R) gene on

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chromosome 5p13.[8–15] In linkage studies the chromosome 5p12-14 region was related to MS susceptibility. A role for the IL7R gene in MS susceptibility was furthermore suggested because of its function in survival and proliferation of T and B cells.[16] For its action, the cytokine IL-7 depends on the expression of its corresponding receptor on the cell surface (IL-7R).[17,18] Two isoforms of the IL-7R receptor exist, a membrane-bound and a soluble isoform.

The SNP with the strongest association within the IL7R gene, identified by whole genome association studies and haplotype analysis, was rs6897932 (a non-synonymous SNP, leading to a coding change (T244I)).[9] In vitro analysis shows that the C allele augments an exonic silencer resulting in an approximately two-fold increase in the skipping of exon 6 when compared with the T allele, leading to increased production of the soluble form of IL-7R.[8]

In the family of cytokines and cytokine receptors, alternative splicing of pre-mRNA is a widespread regulatory mechanism that has been demonstrated to be involved in the control of gene expression, influencing both cell development and cell activation.[17,19] Due to the functional consequences of this polymorphism, it can be postulated that this SNP not only has an influence on disease susceptibility, but may also affect the disease course: patients carrying the risk allele may have a more active disease. To our knowledge no other studies have addressed the possible effect of IL7R polymorphisms and relative expression levels combined in one study on MS disease course.

In this study, our aim is to assess the importance of this IL7R SNP (rs6897932) in MS susceptibility in a Dutch population. Secondly, we study the relevance of this SNP (at DNA and mRNA level) on disease course (measured by clinical and imaging outcome parameters).

Material and methods

Subjects

We sampled 697 patients from MS natural history studies at the MS Center of the VU University Medical Center (VUmC), Amsterdam, the Netherlands. Dutch Caucasian patients only were included based on availability of at least one clinical assessment of disability using the Expanded Disability Status Scale (EDSS) and the availability of DNA material. Patients had a diagnosis of MS according to McDonald criteria[20] or Poser criteria,[21] depending on the time of data acquisition. Patients who presented with a first relapse with symptoms suggestive for demyelination that lasted at least 24 h, that however do not fulfil the McDonald criteria at their most recent follow-up were also included (Clinically Isolated Syndrome (CIS)).[20] We included 174 unrelated Dutch Caucasian healthy controls in this study. This group was also incorporated (and described) in the study by Schrijver et al.[22]

This study was carried out with the approval of the Medical Ethical Committee of the VUmc and informed consent was obtained from all participants.

MS phenotype

For all patients, the following clinical data on disease severity were collected: age at disease onset, most recent EDSS[23] scores, Multiple Sclerosis Severity Scales (MSSS)[24] and time to reach EDSS = 6 (a clinically relevant time-point indicating when a patient requires help in walking). When available Multiple Sclerosis Functional Composite (MSFC) scores[25] were collected and used as secondary outcomes of disease severity. The MSFC is a composite score consisting of three separate tests, the Timed 25-Foot Walk (T25FW), the 9-hole Peg Test (9HPT) and the Paced Auditory Serial Addition Test (PASAT). Testing was performed under standardized conditions and tests were practised at least once before baseline assessment. In patients that were followed-up annually from disease onset up to at least 2 years ($n = 210$), the following disease activity measures were collected: occurrence of new relapses, use of disease-modifying therapy and the number of intravenously administered methylprednisolone treatment courses.

Patient selection sub-study on expression analysis

For this sub-study we selected a group of 95 relapse-onset patients from our total MS (DNA) cohort ($n = 697$). This selection was based on the availability of mRNA. These patients were all sampled from one of our ongoing prospective natural history studies in early MS patients. Patients eligible for this study presented with CIS or were diagnosed with MS within the previous 6 months. RNA was collected at baseline and a magnetic resonance imaging (MRI) scan of the brain was performed at baseline and after 2 years of follow-up. At annual follow-up visits the above-described measures of disease severity and disease activity were collected.

Genotyping

Genomic DNA was isolated from anticoagulated blood using DNAzol reagent (Molecular Research Center, Inc, Cincinnati, Ohio, USA). The SNP in exon 6 of the interleukin 7 receptor gene (IL7R) (dbSNP rs ID: rs6897932; HGVS nomenclature version 2.0: NG_009567.1:g.22585C > T) was analyzed using the

C___2025977_10 predesigned and validated Taqman Genotyping Assay on Demand from Applied Biosystems (Foster City, CA, USA).

Expression analysis

Blood samples were collected using PaxGene RNA tubes followed by standard processing according to the manufacturer's instructions. Automated RNA isolation was performed, within 3 months after freezing, on the BioRobot MDX (Qiagen). cDNA synthesis was performed with the SuperScript III First-Strand Synthesis System (Invitrogen) according to the manufacturer's conditions. The total RNA input per reaction was 100 ng using a reverse IL7R primer (5'-TTC TTG GTT TCT TAC AAA GAT GTT CC-3') complementary to exon 7. Quantitative PCR was performed in duplicate on the ABI7300 with Platinum Quantitative PCR supermix-UDG w/Rox (Invitrogen). Quantitative analysis was done with the ABI7500 software.

The amounts of total (membrane-bound plus soluble) IL7R and membrane-bound form of IL7R mRNA were measured by assaying Hs00233682_m1 for exons 4–5 enabling the detection of total IL7R cDNA, and via Hs00904814_m1 spanning exons 6–7 reflecting only membrane-bound IL7R. Results are normalized to the expression level of 'housekeeping gene' glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to correct for experimental variations. Gene expression results were analyzed using the 2-Δ ΔCt method for relative quantification.[26] The relative expression of membrane-bound IL7R to total amount of IL7R mRNA was expressed as a 'fold-change' value.

Magnetic resonance imaging

Per protocol MRI scans were performed at baseline and at year 2 for patients from our ongoing natural history study in early MS. Scans were acquired either on 1.0 Tesla or 1.5 Tesla (Siemens AG, Erlangen, Germany) scanners with standard head coils, using standard 2D conventional or fast spin-echo Proton Density (PD)- and T2-weighted images (TR: 2200–3000 ms, TE: 20–30 and 80–100 ms) with slice thicknesses of 3–5 mm, a maximum gap between slices of 0.5 mm, and an in-plane resolution of $1 \times 1\,mm^2$. The number and volume of T2-weighted lesions, T1 hypo-intense lesions and gadolinium-enhancing T1-weighted lesions on brain MRI were assessed and compared with baseline. The lesions were identified by an independent rater, blinded for IL7R expression results and clinical data.

Disease severity on MRI was assessed by measuring the T1 hypo-intense lesion volume and the T2 lesion volume at baseline and after 2 years of follow-up. We used the following *disease activity* MRI parameters: number of new T2 lesions at follow-up and volume of T1 gadolinium-enhancing lesions.

Baseline scans were performed on the day of blood sampling for patients included in the expression analysis.

Statistical analysis

The annualized relapse rate and rate of intravenous methylprednisolone treatment courses were calculated from the most recent available data by dividing the total number of relapses and, respectively, the number of methylprednisolone courses by the total follow-up duration. Clinically relevant changes in EDSS and MSFC were assessed according to generally accepted guidelines.[27,28]

Hardy–Weinberg equilibrium (HWE) was evaluated using Pearson's goodness-of-fit χ^2 test (degree of freedom = 1) for genotyping results. Genotype distribution and allele frequencies were compared between healthy controls and MS subjects using Pearson Chi-Square testing and Fisher's exact test respectively; odds ratios were calculated including 95% confidence intervals (CI) using the best-fitting genetic model (recessive, dominant or additive).

To test for significant differences in MS phenotype, the parameters of disease severity and disease activity (clinically and using MRI parameters) were compared for every genotype using the Kruskal–Wallis test; differences between carriers and non-carriers of the risk allele were assessed using the Mann–Whitney test. In addition, multivariate regression analysis was performed to correct the results for relevant clinical parameters (disease duration, gender, use of disease-modifying therapy, age and onset type).

The 2-Δ ΔCT method was used to calculate the relative change in gene expression of membrane-bound to total IL7R mRNA. We assessed whether there were differences in the relative expression of membrane-bound to total IL7R mRNA between genotypes using the Kruskal–Wallis test. Differences in membrane-bound to total IL7R expression between groups for baseline characteristics (clinically and using MRI parameters) were tested with Mann–Whitney, Kruskal–Wallis and Spearman test where appropriate. Moreover, multivariate regression analysis was performed to correct the results for relevant clinical parameters (disease duration, gender, age, use of disease-modifying therapy and onset type).

For the time to first relapse and time to reach EDSS = 6, we constructed Kaplan–Meier curves, and with log-rank we tested differences between dichotomized levels of membrane-bound to total

IL7R expression and between genotypes. All reported *p*-values are based on two-tailed significance tests. The threshold for significance was set at 0.01. For all statistical procedures SPSS 15.0 for Windows was used.

Results

Patients

A total of 697 unrelated Dutch Caucasian patients were included in the DNA analysis. The median disease duration was 10.8 years (interquartile range (IQR): 10.5 years). The median EDSS was 4.0 (IQR: 4.0) (see Table 1 for more patient characteristics). MSFC results were available for 441 patients (data not shown).

Clinical data on disease activity was prospectively collected for 210 relapse-onset patients from disease onset (median follow-up 6 years). Some 57.1% of patients used disease-modifying therapy during follow-up. The median relapse rate in this group was 0.47. The median number of treatment courses with methylprednisolone per year was 0.12 (for more details on this subgroup see Supplementary table 1 for patient characteristics).

The control subjects ($n = 174$) consisted of 97 females (55.7%) and had a mean age of 46.5 years.

Association of genotype of rs6897932 (IL7R) and susceptibility to MS

Genotype frequencies in the healthy controls and in the total MS patient group did not deviate from HWE ($p > 0.01$). Genotype frequencies in MS patients were distributed as follows: CC 58.4%, CT 35.0% and TT 6.6%. In our MS patient cohort the frequency of the risk allele C is 75.8%, and in our healthy control cohort 67.8%.

Genotype distribution was significantly different between healthy controls and MS patients (Pearson Chi-Square $p < 0.01$). The recessive model fitted best (CC vs. CT and TT) and conferred an increased risk of disease for homozygous carriers of the C allele (0.58 vs. 0.46, OR: 1.65 (95% confidence interval: 1.18–2.30), $p = 0.004$). Homozygosity for the risk allele is a risk factor for MS development in our population. Genotype frequencies, odds ratios (OR) and 95% confidence intervals were calculated (see Table 2). Stratification for disease subtype revealed that the association of this SNP with MS is predominantly present in the secondary progressive (SP) MS patient group (see Table 2). Carriership of the risk allele was significantly more frequent in SP MS patients, when compared with healthy controls. The pre-study power of this susceptibility study is approximately 20% to detect a OR of 1.2

Table 1. Patient characteristics

	All ($n = 697$)	RR ($n = 367$)	SP ($n = 192$)	PP ($n = 111$)	CIS ($n = 27$)
Gender (*n*; % Male)	250 (35.9%)	112 (30.5%)	79 (41.1%)	49 (44.1%)	10 (37.0%)
Median age at onset (IQR)	32.1 (13.6)	29.9 (11.4)	29.8 (11.2)	42.1 (13.6)	39.6 (11.0)
Median disease duration at most recent visit (IQR)	10.8 (10.5)	8.2 (7.8)	17.1 (10.8)	12.2 (10.3)	4.1 (3.3)
Median EDSS at most recent visit (IQR)	4.0 (4.0)	3.0 (2.0)	7.0 (1.5)	6.5 (3.0)	2.0 (2.0)
Number of patients that reached EDSS 6 (%)	249 (35.7%)	32 (8.7%)	151 (78.6%)	64 (57.7%)	2 (7.4%)
Median time to reach EDSS 6 in years (IQR)	8.5 (9.0)	8.8 (7.4)	9.4 (10.6)	6.6 (7.3)	0.3 (*)
Median T2 lesion volume at baseline-scan in mm^3 (IQR) ($n = 226$)	1649 (3380)	1861 (3523)	3027 (5344)	2180 (3330)	607 (1259)
Median T1 hypo-intense lesion volume at baseline scan in mm^3 (IQR) ($n = 226$)	101 (333)	94 (326)	140 (573)	183 (687)	86 (171)
Median number of new T2 lesions at follow-up two years after baseline scan (IQR) ($n = 182$)	4 (7)	4 (9)	2 (14)	2 (5)	1 (4)

*Only based on two patients (0 and 8 months, no interquartile range could be calculated).

CIS, clinically isolated syndrome; EDSS, Extended Disability Status Scale; IQR, Interquartile range; MSSS, Multiple Sclerosis Severity Scale at final clinical follow-up; PP, primary progressive; RR, relapsing-remitting; SD, standard deviation; SP, secondary progressive.

Table 2. Genotype frequencies and allele frequencies of rs6897932 within IL7R gene in MS patients vs. healthy controls. Significant associations are printed in bold

	Total number:	Frequency risk allele (C) (%)	CC genotype *n* (%)	CT genotype n (%)	TT genotype *n* (%)	OR CC vs. CT and TT (95% CI) compared with healthy controls (= recessive model)	2-sided *p*-value (Fisher's exact test) CC vs. CT and TT compared with healthy controls
MS patients total cohort	697	75.8%	407 (58.4%)	244 (35.0%)	46 (6.6%)	1.65 (1.18–2.30)	0.004
RR	367	74.5%	207 (56.4%)	133 (36.2%)	27 (7.4%)	1.52 (1.06–2.18)	0.027
SP	192	79.4%	121 (63%)	63 (32.8%)	8 (4.2%)	2.00 (1.32–3.04)	0.001
PP	111	75.9%	61 (55 %)	40 (36%)	10 (9%)	1.43 (0.89–2.31)	0.15
Healthy controls	174	67.8%	80 (46.0%)	76 (43.7%)	18 (10.3%)	NA	NA

CI, confidence interval; OR, odds ratio; PP, primary progressive; RR, relapsing–remitting; SP, secondary progressive.

when comparing carriers of the CC genotype with the CT and TT genotype.

Association of rs6897932 (IL7R) and MS phenotype

EDSS was obtained for all 697 patients. A brain MRI scan was available from 226 patients at baseline and from 182 (80%) patients at follow-up after 2 years (see Table 1). See Supplementary table 2 for patient characteristics of the subgroup of which an MRI was available. No association could be detected between genotype of rs6897932 and disease severity (clinically and using MRI parameters). There was also no association of genotype with disease activity measures (using clinical and MRI parameters). After correction for disease duration, gender and onset type no significant differences were found Table 3.

IL7R gene expression profiles and MS characteristics

For 95 patients mRNA was available and IL7R mRNA expression profiles (the relative expression of membrane-bound IL7R (presence of exon 6–7) to total amount of IL7R mRNA (presence of exon 4–5)) were determined. These early MS patients had a median disease duration at baseline of 7 months (IQR: 10.3), a median EDSS of 2.5 (IQR: 1.5) and 51.6% of patients used disease-modifying therapy during follow-up. Baseline MRI scans were available for 93 patients.

Two patients were lost to follow-up before the second year and an additional 15 patients did not have a follow-up MRI scan of the brain after 2 years.

In this sub-study, 61.1% of the MS patients were homozygous for the C risk allele, while 32.6% carried the CT genotype and 6.3% of our patients were homozygous for the T allele. This distribution did not deviate from the original cohort. No association of genotypes of rs6897932 and the relative expression of membrane-bound IL7R to total amount of IL7R mRNA was found.

There were no differences observed in relative expression of membrane-bound IL7R to total amount of IL7R mRNA between patients that received an intravenous treatment course with methylprednisolone or experienced a relapse in the 3 months preceding the blood withdrawal compared with patients that did not experience such an event in the previous 3 months. Furthermore, no association was found between MS phenotype (clinically and using MRI scans of the brain) at the time of blood withdrawal and relative gene expression of membrane-bound to total IL-7R mRNA. Also, the membrane-bound to total IL-7R expression ratio neither predicted disease activity, nor did it predict disease severity using clinical parameters (after a median follow-up duration of 4 years and

Table 3. Genotype-Phenotype comparisons for genotype of rs6897932 and clinical and MRI parameters of disease activity and severity

	CC	CT	TT
Clinical parameters of disease severity:			
Median EDSS	4.0	4.0	3.5
Median MSSS	5.60	5.42	5.56
Median Time to reach EDSS = 6	110 months	91 months	151 months
Number of patients that reached an EDSS of 6	151/407 (37.1%)	83/244 (34.0%)	15/46 (32.6%)
MSFC scores:			
Median Timed walk test (sec) ($n = 228$)	4.0	4.0	3.7
Median 9-HPT (dominant hand) (sec) ($n = 227$)	18.2	18.3	17.8
Median 9-HPT (non-dominant hand) (sec) ($n = 228$)	19.3	19.2	18.7
Median PASAT 3 (number of correct answers) ($n = 222$)	56	55	55.5
Clinical parameters of disease activity			
Median number of methyl-prednisolone treatments relapse rate per year of follow-up (IQR) ($n = 210$)	0.12 (0.31)	0.12 (0.22)	0.14 (0.28)
Median number of relapses per year of follow-up (IQR) ($n = 210$)	0.50 (0.49)	0.47 (0.45)	0.33 (0.40)
MRI markers of disease severity:			
Median volume of T2 lesions at baseline (IQR)	1578 (2998)	2226 (4403)	1631 (2287)
Median volume of hypo-intense T1 lesions (IQR)	93 (317)	120 (405)	111 (197)
MRI marker of disease activity:			
Number of new T2 lesion IQR	4 (7)	4 (7)	2 (6)

No significant associations were found between genotype and the above mentioned parameters. Also after correction for disease duration, gender, age, use of disease modifying therapy and onset type no significant differences were found.
EDSS, Expanded Disability Status Scale; IQR, Interquartile range; MRI, magnetic resonance imaging; MSFC, Multiple Sclerosis Functional Composite; MSSS, Multiple Sclerosis Severity Scale; PASAT, Paced Auditory Serial Addition Test; 9-HPT, 9-hole Peg Test.

4 months) and MRI parameters of the brain, also when controlled for use of disease-modifying therapy, gender, age, onset type and disease duration. The pre-study power of our study to detect a clinically relevant difference of 0.5 point on the MSSS is approximately 50%.

Discussion

We present here the results on the effect of IL7R SNP rs6897932 and expression of alternatively spliced IL7R mRNAs on clinical disease course in a large and well-documented Dutch MS patient group. We confirm the relevance of the genotype of the exon 6 SNP rs6897932 on MS susceptibility in our Dutch MS population. In particular, the homozygote C (risk allele) genotype is associated with MS susceptibility in our study, possibly indicating a dose-dependent effect. However, an effect of heterozygotes can not be excluded due to the small OR (~ 1.2) as calculated in previous studies.[8,9,13] Although the power of our susceptibility study is relatively low (20%), due to the low number of healthy controls, the allele frequencies of the risk C allele in our MS population and healthy controls are however similar to other MS studies.[9,14] Moreover, the genotype distribution in our healthy controls is similar to a study with Dutch controls ($n = 465$),[29] strengthening the robustness of our positive association of the CC genotype and MS susceptibility.

In accordance with a previous study,[11] the association of this SNP with MS susceptibility is predominantly driven by the genotype distribution in our patients with secondary progressive MS. Because of this overrepresentation of the risk allele in the generally more disabled MS patients, we postulated that the same C allele is not only involved in disease susceptibility, but may also be involved in MS disease progression and severity. However, we did not find a correlation between genotype and disease severity. Previous studies demonstrated no effect of this IL7R SNP (and other susceptibility SNPs) on markers of disease severity in large cohorts.[8,30] We confirm this observation in our study in Dutch MS patients, by using more extensive disease severity markers (including imaging parameters) in a well-documented MS population. In addition, we show in our relapse-onset cohort that genotype is not related to disease activity parameters in early MS. More and more evidence points towards different genetic influence on MS severity compared with MS susceptibility.[31] Genes involved in MS severity are often involved in different processes than genes found related to MS susceptibility. However, modest effects of some genes might be present in both MS susceptibility and MS severity.[32] HLA-DR15, for example, was associated with a lower age of onset in MS[33] and with female sex.[34] Furthermore, carriers of DRB1*1501 presented with higher numbers of focal brain lesions at the time of initial presentation in the Optic Neuritis Treatment Trial,[35] and were associated with disease severity inferred by HMR spectroscopy and MRI measures.[36]

As mRNA might be a more direct reflection of the time-dependent biological relevance of the IL-7R receptor, we hypothesized that the membrane-bound to total IL7R expression ratio would be associated with disease phenotype at the moment of blood withdrawal, or influence the future disease course. However, no association was found between the expression profiles and MS phenotype (disease severity and disease activity) in our study. Moreover, surprisingly, no association was found between different genotypes for SNP rs6897932 and the membrane-bound to total IL7R expression. These expression results contradict previous results to some extent, as the association of the C major allele with alternative splicing of exon 6, leading to more soluble compared with membrane-bound IL7R, was seen in a study of 94 healthy controls,[8] but not in a study of 24 subjects with MS.[11] This might be due to the fact that we used whole blood mRNA instead of peripheral blood mononuclear cells. Differences in mRNA profiles have been described within different blood types.[37] Although we excluded that a recent relapse or prednisolone treatment (common in early MS) would influence the relative expression of membrane-bound to total IL7R mRNA, we cannot exclude other possible confounding factors. Also, the effect of disease-modifying drugs on measures of disease severity has not been taken into account in any of the reported studies.

Although we showed no influence of mRNA expression on disease phenotype in our homogenous group of early MS patients, this may change later on in the disease (since analysis has shown evidence for a role in disease susceptibility in secondary progressive MS). Unfortunately no mRNA was collected from secondary progressive MS patients or healthy controls. It is possible that mRNA expression would serve more as an indicator of disease severity as opposed to a predictor for disease phenotype. Future studies comparing early patients with patients in a more advanced stage of MS would be interesting, and should preferably also incorporate studies on protein level. Since knowledge on disease progression might be very important in developing future treatment in order to prevent disability accumulation, more studies (including genetic and environmental factors) are warranted to elucidate the mechanisms involved in disease progression.

In conclusion, we confirm the relevance of rs6897932 in MS susceptibility. Considering this and previous publications,[8–15] IL7R is firmly established as a (modest) genetic contributor to MS susceptibility. No effect of this polymorphism was found on disease phenotype (disease severity and disease activity). Due to the described effect of this SNP on mRNA expression and the overrepresentation of the risk allele in the more severely disabled MS patients (secondary progressive MS), we studied the effect on disease severity and activity. However, no effect was found of the genotype and the membrane-bound to total IL7R expression ratio on MS phenotype.

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Conflict of interest statement

L van der Voort has received research support from Bayer Schering Pharma, Biogen Idec, Merck Serono, and Teva Pharmaceutical Industries Ltd. C Oudejans serves on the editorial boards of BMC Medical Genetics, the Journal of Pregnancy, and the Journal of Alzheimer's Disease. H Vrenken received consultancy fees from Novartis and Merck Serono. Dr Polman serves on scientific advisory boards for Actelion Pharmaceuticals Ltd, Biogen Idec,

Bayer Schering Pharma, Teva Pharmaceutical Industries Ltd., Merck Serono, GlaxoSmithKline, UCB, Roche, and Antisense Therapeutics Limited; serves on the editorial boards of Lancet Neurology and Multiple Sclerosis; has received speaker honoraria from Biogen Idec, Bayer Schering Pharma, Novartis, and Teva Pharmaceutical Industries Ltd.; and receives research support from Biogen Idec, Bayer Schering Pharma, GlaxoSmithKline, Novartis, UCB, Merck Serono, Teva Pharmaceutical Industries Ltd., the European Community (EEC), and from the Dutch Multiple Sclerosis Society. Dr Killestein serves on scientific advisory boards for Novartis and Merck Serono; serves as a consultant for Merck Serono; has received research support from Bayer Schering Pharma, Biogen Idec, Merck Serono, Teva Pharmaceutical Industries Ltd., Genzyme Corporation, and Novartis. All other authors declare no disclosures.

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Supplementary table 1: Characteristics of patients (n=210) included in substudy on relationship genotype of rs6897932 and MS disease activity.

MS subtype (n=210)	
CIS	27
RR	173
SP	10
Gender (% Male)	31.4%
Median disease duration in years at most recent visit (IQR)	5.9 (3.4)
Median EDSS at most recent visit (IQR)	2.5 (2.0)
% of patients ever used disease modifying treatment	57.1%
Most recent disease modifying treatment:	
Interferons	81 (38.5%)
Glatiramere acetate	19 (9%)
Natalizumab	15 (7.1%)
Other (Mitoxantrone, Fingolimod)	5 (2.5%)
Median annual number of relapses during follow-up (IQR)	0.47 (0.47)
Median annual number of intravenous methylprednisolone treatments during follow-up (IQR).	0.12 (0.31)
Genotype distribution	
% CC	57.1
% CT	35.2
% TT	7.6

Supplementary table 2: Characteristics of patients (n=226) with MRI variables available, included in study on relationship genotype of rs6897932 and MS disease activity and disease severity.

MS subtype (n=226)	
CIS	26
RR	165
SP	10
PP	25
Gender (% Male)	34.5%
Median disease duration in years at most recent visit (IQR)	6.0 (3.3)
Median EDSS at most recent visit (IQR)	2.5 (1.6)
% of patients ever used disease modifying treatment	52.2%
Most recent disease modifying treatment:	
Interferons	81 (35.8%)
Glatiramere acetate	18 (8.0%)
Natalizumab	14 (6.2%)
Other (Mitoxantrone, Fingolimod)	5 (2.2%)
Genotype distribution	
% CC	55.8%
% CT	36.7%
% TT	7.5%