A Genetic Risk Variant for Multiple Sclerosis Severity is Associated with Brain Atrophy

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The minor allele of the genetic variant rs10191329 in the *DYSF-ZNF638* locus is associated with unfavorable long-term clinical outcomes in multiple sclerosis patients. We investigated if rs10191329 is associated with brain atrophy measured by magnetic resonance imaging in a discovery cohort of 748 and a replication cohort of 360 people with relapsing multiple sclerosis. We observed an association with 28% more brain atrophy per rs10191329*A allele. Our results encourage stratification for rs10191329 in clinical trials. Unraveling the underlying mechanisms may enhance our understanding of pathophysiology and identify treatment targets.

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The determinants of disease severity and long-term outcome of multiple sclerosis (MS) are largely unknown. In a recent genome-wide association study including more than 12,000 people with mostly longer MS disease duration (mean age and disease duration were 51.7 and 18.2 years, respectively), the minor allele A of the single-nucleotide polymorphism rs10191329 in the *DYSF-ZNF638* locus had a frequency of 17% and was associated with an unfavorable long-term disability outcome. Moreover, heritability enrichment analysis of MS severity pointed to central nervous system tissues, in contrast to the immune-related nature of MS susceptibility.[1] Against this backdrop, we tested the hypothesis that the minor allele of rs10191329 is associated with accelerated brain atrophy.

Methods

Participants

The study was performed in accordance with the Declaration of Helsinki and approved by the local ethics committees. We considered data of all individuals included in the in-house cohort studies. Inclusion criteria were age 18–70 years at baseline, relapsing MS or clinically isolated syndrome with fulfilled magnetic resonance imaging (MRI) criteria for dissemination in space, availability of data on disease-modifying therapy at baseline and follow-up, available genotyping data, availability of a pair of T1-weighted and fluid-attenuated inversion recovery sequences acquired with the identical protocol

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at the same scanner with an interval of at least 12 months, and successful visual quality check of raw and processed MRI.

MRI Acquisition and Processing

Details of MRI acquisitions are given in Table 1. The primary outcome variable, brain atrophy, was measured through percentage brain volume change per year (yPBVC) using the software SIENA (version 6.04).[2] Secondary outcome variables were white matter (WM) lesion volume at baseline and new WM lesion volume, as well as the brain volumes of the cerebral cortex, thalamus, putamen, and WM. Of these volumes, baseline values and percentage-wise change per year were determined by the software package SAMSEG (version 7.3.2; FreeSurfer, Charlestown, MA, USA).[3,4]

Genotyping and Imputation

DNA samples from the discovery cohort were genotyped on an Illumina Infinum Global Screening Array-24 (GSA), whereas samples from the replication cohort were genotyped either on a GSA or an Infinium Human Omni Express Bead Chip as part of larger cohorts. Before imputation, we performed quality control of the discovery cohort using PLINK (version 1.95, Boston, MA, USA),[5] removing variants out of the Hardy–Weinberg equilibrium ($p < 1e\text{-}6$), with a minor allele frequency <0.001 and missingness >0.02, and individuals with sample missingness >0.045 or excess heterozygosity, as well as duplicates and population outliers. The pre-imputation quality control of the replication cohort has been described in detail previously.[1] Phasing was performed using standard settings with SHAPEIT2 (version 2.r837, Oxford, UK)[6] for the discovery cohort and EAGLE (version 2.4.1, Boston, MA, USA)[7] for the replication cohort. Imputation was performed using the 1,000 Genomes Phase 3 reference panel and IMPUTE2 (version 2.3.2, Oxford, UK)[8] for the discovery cohort, and the Haplotype Reference Consortium panel and Minimac4 (version 1.0.2, Ann Arbor, MI, USA)[9] for the replication cohort. Per-cohort imputation quality scores for rs10191329 were high (discovery/OmniExpress replication/GSA replication: 0.934/0.969/0.973). We calculated dosages (2× the probability of being homozygous for the minor allele +1× the probability of being heterozygous). After imputation, we removed related individuals and further population outliers, reducing the dataset to the individuals included in the present study.

Statistical Analysis

In the primary analysis, we tested for an association of rs10191329 with yPBVC. We used the German cohort for discovery and the Swedish cohort for replication. We used a multivariate linear regression model, adjusting for age, sex, 8 ancestry components, the MRI scanner, and the genotyping array. Because of the directed nature of our hypothesis, a one-sided p value <0.05 was considered significant. We confirmed p values by nonparametric permutation testing (n = 10,000 permutations). In exploratory analyses, we tested for associations of rs10191329 with 10 other MRI metrics, as well as the Expanded Disability Status Scale (EDSS)[10] scores at baseline and the yearly absolute EDSS change for which we combined both cohorts' results using inverse variance-weighted fixed effects meta-analysis. The regression models for the yearly EDSS change were not adjusted for the MRI scanner. Because these secondary analyses were exploratory, we did not adjust for multiple testing.

Results

Study Participants

The main characteristics of the cohorts are summarized in Table 1.

Association of rs10191329 with Brain Atrophy, Other MRI Metrics and Yearly EDSS Change

We found that the minor allele of rs10191329 (rs10191329*A) was associated with higher rates of brain atrophy (estimate [est] = −0.109, standard error [SE] = 0.036, one-sided $p = 0.001$; Table 2). We found the same association in the replication cohort (est = −0.115, SE = 0.044, $p = 0.005$). We confirmed both p values by nonparametric permutation testing (not shown). The joint analysis ($p = 6.541 \times 10^{-5}$) showed that each rs10191329*A allele was associated with a decrease of the yPBVC by 0.11 (confidence interval 0.06–0.17) corresponding to 27.5% (confidence interval 15.0–42.5) of the mean yPBVC of 0.40. Figure shows the yPBVC distribution per imputed genotype and the association statistics of the primary analysis.

Exploratory analyses (Table 2) on additional MRI metrics yielded associations of rs10191329*A with higher baseline WM lesion volume (est = 1.258, SE = 0.441, $p = 0.004$) and higher yearly percentage volume changes of the thalamus (est = −0.134, SE = 0.049, $p = 0.007$) and putamen (est = −0.074, SE = 0.033, $p = 0.026$). To investigate if the association of rs10191329 with the yPBVC was independent of the association with the baseline WM lesion volume, we repeated the primary analysis adjusting for the baseline WM lesion volume. We found that rs10191329 was still significantly associated with the yPBVC (joint $p = 0.002$).

EDSS scores at baseline and follow-up were available for subsets of 731 (98%) patients from the German cohort and 281 (78%) patients from the Swedish cohort.

Table 1. Baseline Characteristics of the Cohorts and Magnetic Resonance Imaging Parameters

	Discovery cohort TU of Munich	Replication cohort KI Stockholm
n	748	360
Age (yr)	35 (28–44)	37 (30–45)
Sex (female %)	489 (65.4)	275 (76.4)
Type of multiple sclerosis (CIS/RRMS/SPMS)	42/706/0	0/336/24
EDSS	1 (0–2)	2 (1–3)
Interscan interval (yr)	3.0 (2.0–5.0)	2.9 (2.0–5.6)
rs10191329 minor allele frequency	16.1%	15.6%
Disease-modifying therapy at baseline and follow-up (%)	320 (42.8), 577 (77.1)	158 (43.9), 316 (87.8)
Overlap with recent IMSGC cohort[1] (%)	239 (32)	312 (87)
Volumes in ml at baseline (SAMSEG):		
White matter lesions	3.2 (1.2–7.0)	3.4 (1.2–8.1)
Cerebral cortex	500.6 (465.9–540.4)	501.5 (466.2–546.1)
Thalamus	12.4 (11.6–13.3)	12.0 (11.1–13.0)
Putamen	10.7 (9.9–11.5)	9.9 (9.1–10.7)
White matter	398.2 (369.5–434.0)	402.0 (375.1–440.6)
Intracranial	1491.2 (1406.0–1616.6)	1515.6 (1423.3–1620.2)
MRI parameters (n, type, field strength, TE, TI, TR, voxel dimensions in ml)		
Scanner 1	467, Philips dStream Achieva, 3 T. T1-w: 4 ms, N/A, 9 ms, 1.0 × 1.0 × 1.0; FLAIR: 140 ms, 2,750 ms, 10,000 ms, 1.5 × 1.0 × 1.0 mm	39, Siemens Aera, 1.5 T. T1-w: 3.02 ms, 1,100 ms, 1900 ms, 1.0 × 1.0 × 1.5 mm; FLAIR: 333 ms, 1800 ms, 5,000 ms, 1.5 × 1.0 × 1.0 mm
Scanner 2	281, Philips Ingenia, 3 T. T1-w: 4 ms, N/A, 9 ms, 0.75 × 0.75 × 0.75; FLAIR: 140 ms, 2,750 ms, 10,000 ms, 1.0 × 1.0 × 1.0 mm	220, Siemens Avanto, 1.5 T. T1-w: 3.55 ms, 1,100 ms, 1900 ms, 1.0 × 1.0 × 1.5 mm; FLAIR: 333 ms, 2,200 ms, 6,000 ms, 1.5 × 1.0 × 1.0 mm
Scanner 3	N/A	43, Siemens Trio, 3 T. T1-w: 3.39 ms, 900 ms, 1900 ms, 1.0 × 1.0 × 1.5 mm; FLAIR: 388 ms, 2,300 ms, 6,000 ms, 1.5 × 1.0 × 1.0 mm
Scanner 4	N/A	58, Siemens Vision Plus, 1.5 T. T1-w: 7.0 ms, 300 ms, 1,350 ms, 1.0 × 1.0 × 1.5 mm; FLAIR: 110 ms, 2,500 ms, 9,000 ms, 1.0 × 1.0 × 5 mm

Note: Values are given in n (%) and median (interquartile range). Apart from lesion volume and intracranial volume, volumes were corrected for head size by mean scaling (divided by scanner-wise mean intracranial volume and multiplied by individual intracranial volume).

Abbreviations: CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; IMSGC = International Multiple Sclerosis Genetics Consortium; KI = Karolinska Institute; MRI = magnetic resonance imaging; N/A = not applicable; RRMS = relapsing–remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; T = Tesla; T1-w = T1-weighted; TE = time to echo in milliseconds; TI = time to inversion in milliseconds; TR = time to repetition in milliseconds; TU = Technical University.

Table 2. Associations of rs10191329 With Magnetic Resonance Imaging Parameters and Expanded Disability Status Scale

	Discovery (n = 748)		Replication (n = 360)		Meta-analysis	
Outcome	**Estimate (SE)**	**1-sided *p***	**Estimate (SE)**	**1-sided *p***	**Estimate (SE)**	**1-sided *p***
Primary analysis						
Yearly PBVC	−0.109 (0.036)	**1.260×10^{-3}**	−0.115 (0.044)	**4.860×10^{-3}**	−0.111 (0.028)	**6.541×10^{-5}**
Exploratory secondary analyses (joint analysis)						
Baseline parameters						
WM lesion	1.100 (0.540)	**2.107×10^{-2}**	1.571 (0.761)	**1.994×10^{-2}**	1.258 (0.441)	**4.318×10^{-3}**
Cerebral cortex	−0.472 (3.095)	4.394×10^{-1}	−1.866 (4.717)	3.463×10^{-1}	−0.892 (2.588)	7.303×10^{-1}
Thalamus	−0.057 (0.083)	2.491×10^{-1}	−0.118 (0.137)	1.954×10^{-1}	−0.073 (0.071)	3.052×10^{-1}
Putamen	−0.043 (0.078)	2.908×10^{-1}	−0.132 (0.11)	1.153×10^{-1}	−0.073 (0.064)	2.519×10^{-1}
White matter	−1.976 (2.977)	2.535×10^{-1}	−0.248 (4.498)	4.781×10^{-1}	−1.449 (2.482)	5.593×10^{-1}
EDSS	0.105 (0.081)	9.556×10^{-2}	−0.037 (0.166)	4.116×10^{-1}	0.078 (0.072)	2.802×10^{-1}
Yearly change rates in %						
WM lesion[a] volume	0.000 (0.044)	4.972×10^{-1}	0.194 (0.077)	**6.373×10^{-3}**	0.047 (0.038)	2.157×10^{-1}
Cerebral cortex	−0.073 (0.051)	7.430×10^{-2}	−0.080 (0.095)	2.004×10^{-1}	−0.075 (0.045)	9.454×10^{-2}
Thalamus	−0.095 (0.058)	5.079×10^{-2}	−0.230 (0.093)	**6.725×10^{-3}**	−0.134 (0.049)	**6.730×10^{-3}**
Putamen	−0.048 (0.039)	1.102×10^{-1}	−0.136 (0.061)	**1.394×10^{-2}**	−0.074 (0.033)	**2.624×10^{-2}**
White matter	0.061 (0.051)	1.192×10^{-1}	−0.137 (0.087)	5.926×10^{-2}	0.010 (0.044)	8.213×10^{-1}
EDSS[b,c]	0.032 (0.028)	1.260×10^{-1}	0.072 (0.099)	2.314×10^{-1}	0.035 (0.027)	1.927×10^{-1}

Note: We performed multivariate linear regression analyses to investigate associations of rs10191329 with magnetic resonance imaging parameters. For all longitudinal parameters, we calculated the yearly change rates as percentages of the baseline volume. The *p* values <0.05 are marked in bold.
Abbreviations: EDSS = Expanded Disability Status Scale; n = number of analyzed individuals; *p* = *p*-value; PBVC = percentage brain volume change; SE = standard error; WM = white matter.
[a]The yearly absolute change rate of the while matter lesions volume (in ml/year) was investigated as the outcome parameter.
[b]EDSS scores at baseline and follow-up were available for subsets of 731 patients from the discovery cohort and 281 patients from the replication cohort.
[c]The yearly absolute EDSS change was investigated as an outcome parameter.

We could not observe any association of rs10191329 with the baseline EDSS scores or the yearly absolute EDSS change in this study (see Table 2).

Finally, we downloaded the summary statistics of several genome-wide association studies of longitudinal brain changes in >15,000 participants performed by the ENIGMA consortium.[11] We only found a nominally significant association of rs10191329 with the yearly change rate of the lateral ventricles ($p = 0.032$), but not with any of the other 14 investigated metrics, including yearly change rates of the total brain volume ($p = 0.690$), thalamus ($p = 0.953$), or putamen ($p = 0.613$).

Discussion

Our primary hypothesis was based on the results of a recent GWAS of MS severity.[1] We chose the measure of

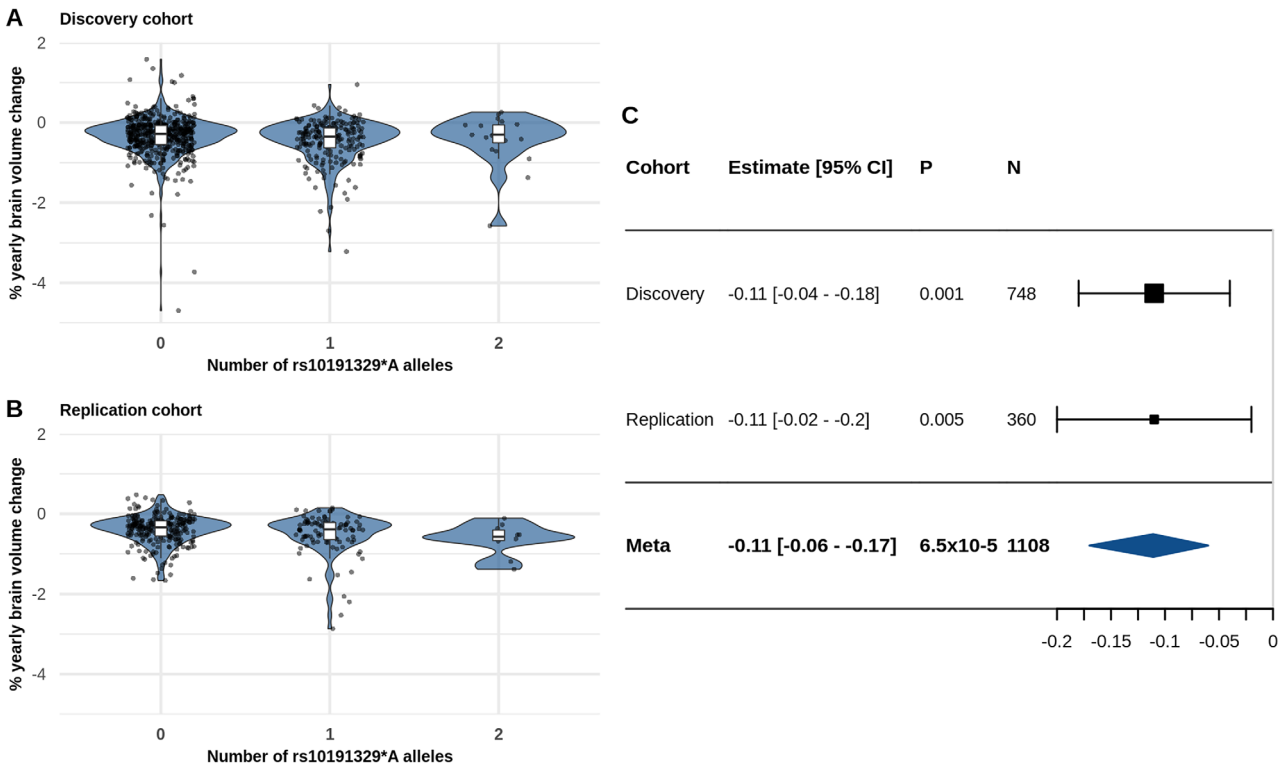

Figure: rs10191329*A is associated with higher brain atrophy rates in multiple sclerosis. The percentage of yearly brain volume change per genotype for rs10191329 is shown for (A) the discovery cohort and (B) the replication cohort. (C) Forest plot showing the association statistics for the discovery analysis, the replication, and the inverse variance-weighed fixed effects meta-analysis. For better visualization of plots A and B, all individuals with a rs10191329*A dosage <0.3 were considered to be noncarriers, individuals with dosage >0.7 and <1.3 were considered to be heterozygous, and individuals with dosage >1.7 were considered to be homozygous for rs10191329*A. CI = confidence interval; P, one-sided *p* value; N, number of analyzed individuals. [Color figure can be viewed at www.annalsofneurology.org]

yPBVC as the most robust in vivo marker of brain atrophy in MS.[12–14] In the discovery cohort, we found rs10191329*A to be associated with a more pronounced yPBVC in MS patients and could replicate this finding in an independent cohort. The biological effect, with an estimated 28% increase in brain atrophy per additional allele, is considerable. The fact that we demonstrated the association of rs10191329*A with brain atrophy in two cohorts based on MRI data from six scanners points toward a high degree of generalizability. However, our main result and particularly its effect size necessitate further confirmation and distinction.

We conducted exploratory joint analyses of both cohorts on additional MRI metrics. These analyses did not point to specific brain regions being primarily affected. Instead, we observed nominally significant associations of rs10191329 with baseline WM lesion volume and atrophy of the thalamus and putamen. Considering the lack of an association of rs10191329 with comparable longitudinal MRI metrics in controls,[11] we believe that carriers of the minor allele of rs10191329 are more prone to MS-related brain tissue damage already at the stage of WM lesion formation. Given the impact of rs10191329 on brain atrophy, it seems reasonable to consider stratification for this genotype in clinical trials involving brain atrophy measurements. At this point, however, we have only demonstrated an association of a noncoding genetic variant with brain atrophy in MS. The elucidation of the molecular and cellular mechanisms underlying this association may reveal mechanisms of disease progression and open new avenues for treatment.

Finally, the present results illustrate that it is possible to successfully relate genetic variants to the course of MS by using brain MRI as a proxy. Genetic variants exerting effects in the same order of magnitude as rs10191329 should be detectable in large-scale multicenter MRI studies—an ambitious, but feasible and hopefully fruitful, step to better understand the pathomechanisms leading to brain atrophy and disability progression in MS.

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Author Contributions

C.G., T.G., I.K., B.H., and M.M. contributed to the conception and design of the study; C.G., T.W., J.M., S.C., T.M., R.O., A.P., C.V., C.B., M.L., T.F.M.A., F.H., L.A., K.S., P.S., A.H., B.W., J.S.K., C.Z., A.Ba., F.P., A. Be., T.G., I.K., B.H., and M.M. contributed to the acquisition and analysis of data; C.G., B.H., and M.M. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

A.Ba. is an employee and shareholder of Regeneron Pharmaceuticals. The company is a biotechnology company that invents, develops, and commercializes medicines for people with serious diseases; it has a significant interest in MS genetics. The other authors have nothing to report.

Data Availability Statement

Raw data were generated at both contributing centers. Derived data are available from the corresponding authors on request. The data are not publicly available due to data privacy laws.

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