# Increased Disability Progression in rs10191329$^{AA}$ Carriers with Multiple Sclerosis Is Preceded by Neurofilament Light Chain Elevations

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**Objective:** We examined the impact of the rs10191329 genetic risk variant on neuroaxonal damage as measured by serum neurofilament light chain (sNfL) levels, and disability progression in people with multiple sclerosis (pwMS).
**Methods:** In a cohort of pwMS (n = 740), 658 participants were prospectively monitored every 2 years for less than a decade while 82 of 740 pwMS were monitored retrospectively for up to 40 years. We investigated associations between rs10191329 variants and clinical outcome, including Expanded Disability Status Scale (EDSS), disability accrual (defined by EDSS-increase of at least 1.5 for patients starting at EDSS 0, at least 1.0 EDSS-points for patients with an initial EDSS between 1 and 4.5 and at least 0.5 points for patients starting with an EDSS equal or greater than 5) and progression to secondary progressive MS (SPMS). Clinical outcomes were analyzed using Kaplan–Meier and Cox proportional hazards analyses. Disability accumulation over time was depicted using a generalized mixed-effect model. Single-molecule array was used to assess sNfL levels.
**Results:** Homozygous, heterozygous, and non-carriers of the rs10191329 risk variant displayed comparable sNfL levels indicating similar neuroaxonal damage at the time of diagnosis. Importantly, in homozygous carriers we found highest sNfL levels in follow-up visits preceding elevated disease progression later in the disease course, a steeper increase in overall disability measures and higher probability of SPMS development.
**Interpretation:** These findings highlight how genetic variants may serve as new biomarkers for disease progression and can be used for personalized medicine and risk assessment in MS.

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In multiple sclerosis (MS), chronic disability accumulation is driven by an interplay of pathophysiological processes in the peripheral immune system and central nervous system (CNS) tissue.[1] Indeed, with the advent of high-efficacy treatments leading to partial or complete clinical relapse suppression, disability accrual has come

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more into the focus, even completely independent of relapse activity.[2–5] Unravelling the underlying pathophysiology and devising treatments that address the neurodegenerative facets of MS necessitate the identification of markers for the long-term disease course.[6] Despite a number of genetic risk loci associated with MS susceptibility, clinical data and magnetic resonance imaging have traditionally guided physicians in stratifying individual disease progression and determining the appropriate disease-modifying treatment (DMT) for people with MS (pwMS). Recently, the International Multiple Sclerosis Genetics Consortium (IMSGC) linked the potential first genetic variations with MS progression. Specifically, we, as part of the IMSGC, identified a significant association of rs10191329 in the DYSF-ZNF638 locus—encoding dysferlin—with a shortened median time to requiring a walking aid in homozygous carriers (rs10191329$^{AA}$) and increased brainstem and cortical pathology in brain tissue.[7] Furthermore, Gasperi et al. demonstrated a yearly 28% increase in brain atrophy per rs10191329$^{A}$ allele.[8] Although these findings are promising for elucidating the genetic role in MS progression and may be suggestive for new monitoring tools in the clinical routine, recent studies by Kreft et al. and Campagna et al. failed to establish any association between homozygous carriers (rs10191329$^{AA}$) and MS severity.[9,10] However, it is important to note that these studies lacked sufficient power; thus, their findings should not be interpreted as definitive evidence of no association.[11]

Leveraging this existing controversy, we aim to investigate whether the already reported disability accrual in homozygous (rs10191329$^{AA}$) patients is preceded by increased serum neurofilament light chain (sNfL).

## Methods

### Participants

The study was performed in compliance with the Declaration of Helsinki. The protocol for this study received prior approval from the local ethics committee (Ethics Committee of the Landesärztekammer Rhineland-Palatinate, number 837.019.10). All participants gave written informed consent. A genetic analysis was conducted in 2 distinct cohorts. The first cohort consisted of 119 asymptomatic first-degree relatives (parent, sibling, or child) of individuals with MS, for whom recent studies have shown an odds ratio of 7.0 for developing the disease (<10% will develop MS).[12] These participants were part of the Genes and Environment in MS (GEMS) study,[13] a longitudinal study recruiting individuals under 50 years of age. The second cohort (Supplement Figure 1) comprised 740 pwMS who met the revised McDonald criteria from 2017 for clinically isolated syndrome or MS, recruited from the MS outpatient clinics of the University Medical Centers of Mainz and Düsseldorf between 2008 and 2018. The MS cohort consisted of 2 subgroups: pwMS monitored prospectively (n = 658, monitored for less than a decade due to later inclusion) and pwMS analyzed clinically in a retrospective design (n = 82, monitored for more than a decade). The first subgroup was systematically monitored from diagnosis (MS × 0) on with MRI scans, clinical evaluations, a DMT synopsis every 2 years and biosampling. Subsequent visits were categorized as follows: MS*1 (median: 4.2 [IQR: 4.1–4.3] years from diagnosis) and MS*2 = total follow-up duration (median: 6.2 [IQR: 6.1–6.3] years from diagnosis). For the retrospective subgroup, all available visits from the MS outpatient clinic were collected and analyzed. The median follow-up duration for the retrospective cohort was 12.1 [IQR: 9–17.8] years and the median number of visits was 4 with an IQR of 3–6. The monitoring period regarding EDSS was categorized into 3 phases: 0–5 years, 5–10 years, and over 10 years after diagnosis, to capture the varying disease phases. The observed parameters included self-reported sex, age at diagnosis and at first symptom manifestation, DMT synopsis, EDSS scores, and disease course. Non-carriers (rs10191329$^{CC}$), homozygous (rs10191329$^{AA}$), and heterozygous carriers (rs10191329$^{AC}$) in the second prospective cohort were comparable in age, sex, DMT, and disease course. 22.5% of our participants were included in the IMSGC study.

### Genotyping

The single nucleotide polymorphism (SNP) rs10191329 was analyzed in pwMS using an allelic discrimination assay based on Taqman chemistry according to the manufacturer's protocol (Applied Biosystems). Genotyping was performed on 96-well plates with approximately 5% controls run in duplicates across plates. Genotyping efficiency was ≥99.5% for all SNPs. Deviation of the genotypes from Hardy–Weinberg equilibrium (HWE) as a potential marker for genotyping quality was assessed using Pearson's chi-squared test. The genotype distribution of rs10191329 did not deviate from HWE.

### Assessment of Clinical Data

To assess the predictive significance of rs10191329, we analyzed the longitudinal development of the EDSS score and the time taken to reach EDSS ≥4.5, indicating the ability to walk without assistance for ≥300 m but <500 m. Disability accrual was defined as a clinically meaningful increase in neurological disability measured by an EDSS worsening at MS*2 compared to the previous assessment at MS*1 by ≥1.5 points for patients starting at EDSS

0, at least 1.0 EDSS point for patients with an MS*1 EDSS between 1 and 4.5 and at least 0.5 point increase for patients starting with an MS*1 EDSS equal or greater than 5. Additionally, we converted the EDSS to the global Age-Related Multiple Sclerosis Severity score (gARMSS).[14] The main test results of our findings, using gARMSS instead of EDSS, are shown in Supplement Table 1 for comparison with previous publications. We also investigated whether patients underwent a transition to secondary progressive MS (SPMS). Subsequently, we classified DMT into 3 categories: (1) DMT-naïve, (2) basic/moderate (including azathioprine, daclizumab, dimethyl fumarate, glatiramer acetate, interferons, methotrexate, secukinumab, S1P-modulators, and teriflunomide), and (3) high efficacy (HE) DMT (comprising alemtuzumab, mitoxantrone, natalizumab, ocrelizumab, ofatumumab, and rituximab).

### sNfL Measurements

For the generation of the sNfL data, we selected a subset of GEMS participants who self-reported being asymptomatic at the time of sampling. Participants are distributed throughout the United States. Blood samples were collected remotely by a local phlebotomist and shipped in room temperature packs overnight to a central laboratory where serum was prepared and cryopreserved. Sample collection and sNfL measurement was performed in the pwMS cohort as previously described in detail.[15] In brief, venous blood was spun at 1,300 g at room temperature for 15 minutes, a maximum 2 hours after sampling, and stored locally at −80°C. sNfL levels in the GEMS and pwMS serum samples were measured in duplicate in a blinded manner according to the manufacturer's instructions using the NF-Light Advantage Kit (Quanterix) with a single molecule array (SiMoA) at the Amsterdam University Medical Center (HD-X, Quanterix) and the University Medical Center Mainz (HD-1, Quanterix), respectively. The intra- and inter-assay coefficient of variation were both below 10%.

### Statistical Analyses

Normality of variables was assessed through the Shapiro–Wilk test. Group comparisons were assessed using the one-way analysis of variance (ANOVA) for normally distributed variables, and the Mann–Whitney *U* or Kruskal–Wallis test for non-normally distributed variables with subsequent post-hoc tests and adjustments for multiple comparisons. Categorical variables were examined using the Chi-square test, with the Fisher-exact test and post-hoc multiple *z*-tests of two proportions employed when appropriate.

We employed analysis of covariance (ANCOVA) to account for potential additional confounding variables beyond genotype, with age- and BMI-adjusted sNfL percentiles[16] at MS*1 as dependent variable. We included sex, relapses, DMT group at MS*1, time spent in a specific DMT group (categorized as basic/moderate or HE; calculated as the ratio of days on DMT to total days in the study), type of DMT regimen (escalation vs induction), number of DMT switches, symptom duration, and EDSS as covariates. Because studies indicate a temporal relationship of sNfL upregulation with clinical relapses as well as recently also Gd lesions,[17] we included relapse occurrence or presence of Gd lesions 3 months prior to or after sample collection as covariates. Treatment regimens for patients who initiated therapy directly with a HE DMT were classified as induction therapy. In contrast, patients who had previously received a drug from the basic/moderate efficacy group were classified as undergoing escalation therapy.

To investigate the effect of the genetic progression marker on the rate of disability accrual in our retrospective cohort, we developed a generalized linear mixed-effects model (LMM) with serial EDSS as the dependent variable. The primary predictor was the interaction between genotype and time since first symptoms. Individuals were included as random effects, and further covariates, beyond genotype and time since disease onset, included sex, age at onset, and time spent in a specific DMT group.

The incidence of SPMS conversion on the one hand and attainment of EDSS ≥4.5 on the other hand were analyzed with the help of two different Kaplan–Meier curves with log-rank tests and post-hoc rank tests with Bonferroni correction. Finally, to model the time-dependent occurrence of these two mentioned outcomes while controlling for confounders, we performed two separate Cox proportional hazard analyses. Each model included the covariates of sex, age at symptom onset, and type of DMT. Given that a primary progressive MS (PPMS) course is known to cause higher EDSS, we included it along with the other covariates in the EDSS outcome. For the SPMS outcome, PPMS patients were excluded from analyses. DMT groups were included in both models as time-dependent covariates (coxph function in the survival package version 3.7–0 in R). The proportional hazards assumption was examined by inspection of scaled Schoenfeld residuals. Post-hoc tests were Bonferroni-adjusted to account for multiple comparisons.

The lower and upper hinges of the box plots represent the first and third quartiles, respectively. The upper whisker extends from the hinge to the largest value within 1.5 times the interquartile range (IQR) from the hinge. Similarly, the lower whisker extends from the hinge to the

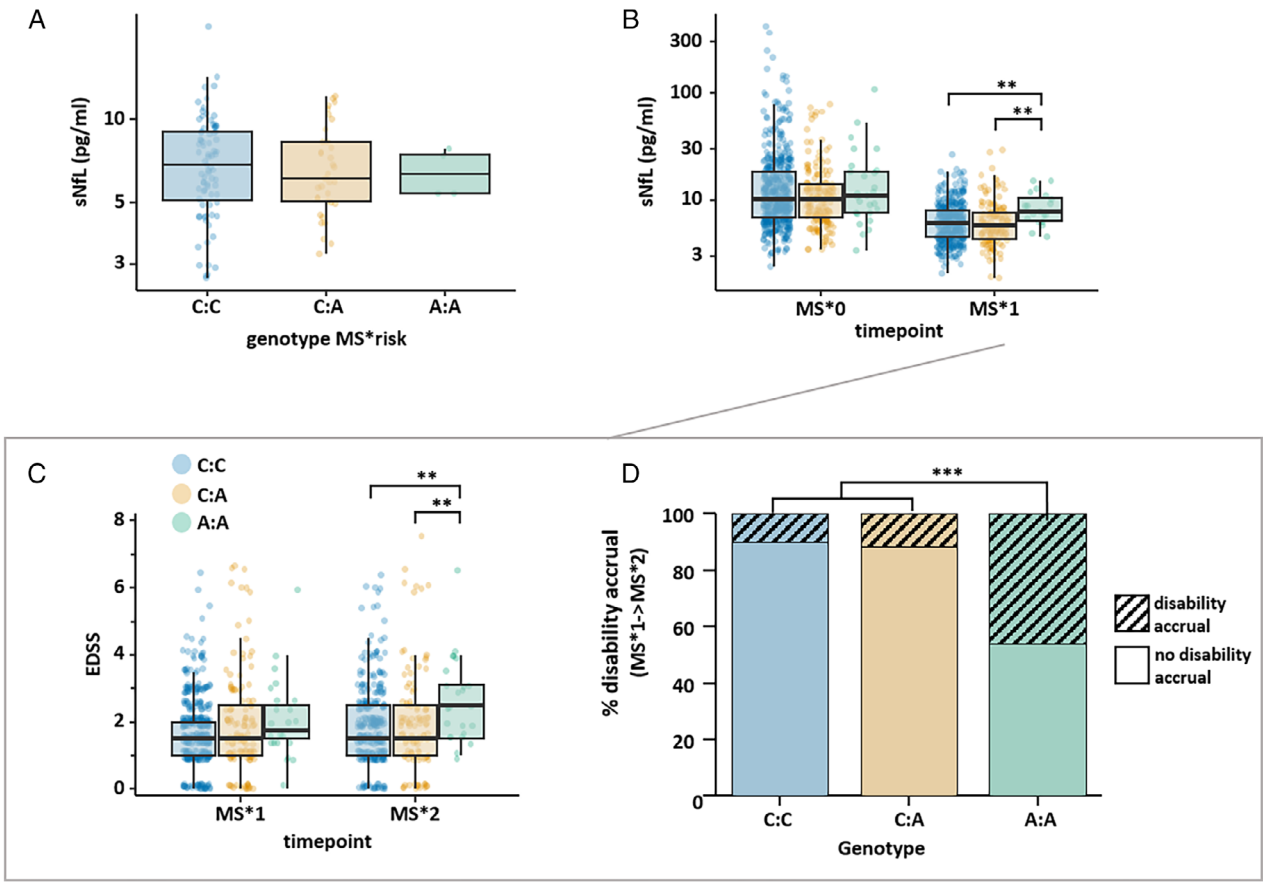


**FIGURE 1: Despite no difference in people at risk for MS and at diagnosis, pwMS homogyzgous carriers of the risk allele exhibit increased neurofilament already early in the disease course, followed by increased disease severity. Box plots presenting sNfL levels in (A) asymptomatic first-degree relatives of persons with MS (no significant differences) and (B) in pwMS at MS*0 and MS*1, with Kruskal–Wallis tests for each time point and post-hoc tests with Dunn's correction for multiple comparisons. At MS*0 we observed no significant differences ($p = 0.227$). At MS*1 homozygous carriers had significantly increased sNfL levels compared to non-carriers ($p = 0.005$) and heterozygous carriers ($p = 0.005$). Further ANCOVAs were performed accounting for confounders such as age, BMI, EDSS, or Gd-enhancing lesions (for details see Table 2). (C) Box plot illustrates the course of EDSS in the early cohort with significantly increased values in homozygous carriers at MS*2 ($p = 0.005$, compared with non-carrier; $p = 0.009$, compared with heterozygous carrier; Kruskal–Wallis tests with Dunn's multiple comparisons correction). A jitter was applied to the data points for visualization purposes. (D) A bar chart is shown with the respective percentages of patients with and without disability accrual at MS*2 relative to MS*1 ($p < 0.001$, Fishers exact test two-sided; significant differences between homozygous carrier and both non-carrier and heterozygous carrier confirmed by multiple z-tests of two proportions). [Color figure can be viewed at www.annalsofneurology.org]**

smallest value within 1.5 times the IQR from the hinge. Statistical significance was established at a threshold of $p < 0.05$.

### *Software*

For data analyses, we used IBM SPSS Statistics version 23 (IBM, USA), Graphpad Prism version 9.1.0 (GraphPad Software, USA), RStudio version 2024.04.2 (Posit PBC) with R version 4.4.0 (https://www.r-project.org/) and the following software packages: data.table version 1.15.4, dplyr version 1.1.4, ggplot2 version 3.5.1, lme4 version 1.1-35.3, lmerTest version 3.1-3, MASS version 7.3-61, ms.sev version 1.0.4, multcomp version 1.4-25, nlme version 3.1-165, officer version 0.6.6, rvg version 0.3.3, stats version 4.4.0, survminer version 0.4.9, survival version 3.2-11, and tidyverse version 2.0.0.

## Results

### *Higher sNfL in rs10191329$^{AA}$-pwMS Is Followed by Subsequent Increased Disability*

When investigating sNfL in a cohort of asymptomatic first-degree relatives of pwMS who are at increased risk of MS and thus represent a potential pre-stage of MS (n = 119, 75.6% female, median age: 35 years, IQR: 29–42.5 years), homozygous (rs10191329$^{AA}$), heterozygous (rs1091329$^{AC}$), and non-rs10191329 carriers (rs10191329$^{CC}$) displayed comparable sNfL levels at baseline (rs10191329$^{AA}$ median: 6.4 pg/ml, IQR: 5.4–7.7; rs10191329$^{AC}$ median: 6.1 pg/ml,

## Table 1. Demographic and Clinical Data

**Prospective cohort**

| | Total (n = 658) | rs10191329$^{CC}$ (n = 462) | rs10191329$^{AC}$ (n = 169) | rs10191329$^{AA}$ (n = 27) | *p*-Value |
|---|---|---|---|---|---|
| Female | 450 (68.4%) | 315 (68.2%) | 114 (67.5%) | 21 (77.8%) | 0.569[a] |
| Disease course | | | | | |
| CIS | 110 (16.7%) | 79 (17.1%) | 25 (14.8%) | 6 (22.2%) | 0.570[a] |
| RRMS | 548 (83.3%) | 383 (82.9%) | 144 (85.2%) | 21 (77.8%) | |
| Median age at diagnosis [IQR] | 31 [25–40] | 31 [25–40] | 30 [24–40] | 32 [23–44] | 0.878[b] |
| Median age at onset [IQR] | 30 [25–39] | 31 [25–39] | 30 [24–39] | 32 [22–44] | 0.888[b] |
| Number of relapses between MS*0 and MS*1 (mean ± SD) | 1.0 ± 1.3 | 0.9 ± 1.2 | 1.1 ± 1.5 | 1.2 ± 2.0 | 0.928[b] |
| Number of DMT switches between MS*0 and MS*1 (mean ± SD) | 1.4 ± 0.9 | 1.4 ± 0.9 | 1.5 ± 0.9 | 1.7 ± 1.2 | 0.606[b] |
| DMT | | | | | |
| Naive | 146 (22.2%) | 100 (21.6%) | 39 (23.1%) | 7 (25.9%) | 0.423[a] |
| Basic/moderate | 445 (67.6%) | 313 (67.8%) | 117 (69.2%) | 15 (55.6%) | |
| High | 67 (10.2%) | 49 (10.6%) | 13 (7.7%) | 5 (18.5%) | |
| Time spent in DMT group | | | | | |
| Mild/moderate | 0.71 ± 0.37 | 0.71 ± 0.38 | 0.72 ± 0.36 | 0.65 ± 0.37 | 0.672[b] |
| High | 0.08 ± 0.24 | 0.08 ± 0.24 | 0.06 ± 0.21 | 0.16 ± 0.30 | 0.016[b] |
| EDSS at MS*1 | 1.7 ± 1.3 | 1.6 ± 1.1 | 1.7 ± 1.6 | 2.0 ± 1.3 | 0.261[b] |
| Years between onset and MS*1 | 4.3 [4.2–4.7] | 4.3 [4.2–4.7] | 4.3 [4.2–4.7] | 4.3 [4.2–4.6] | 1.000[b] |
| Years between diagnosis and MS*1 | 4.2 [4.1–4.3] | 4.2 [4.1–3.4] | 4.2 [4.1–4.3] | 4.2 [4.1–4.3] | 0.067[b] |

**Retrospective cohort**

| | Total (n = 82) | rs10191329$^{CC}$ (n = 31) | rs10191329$^{AC}$ (n = 27) | rs10191329$^{AA}$ (n = 24) | *p* |
|---|---|---|---|---|---|
| Sex (female) | 52 (63.4%) | 17 (54.8%) | 21 (77.8%) | 14 (58.3%) | 0.161[a] |
| RRMS | 68 (82.9%) | 26 (83.9%) | 23 (85.2%) | 19 (79.2% | 0.837[a] |
| PPMS | 14 (17.1%) | 5 (16.1%) | 4 (14.8%) | 5 (20.8%) | 0.837[a] |
| Age at diagnosis (years ± SD) | 36.7 ± 11.7 | 34.4 ± 12.0 | 38.6 ± 10.4 | 37.7 ± 12.7 | 0.358[c] |
| Age at onset (years ± SD) | 34.6 ± 11.8 | 32.8 ± 10.4 | 35.5 ± 10.4 | 35.9 ± 13.3 | 0.554[c] |
| DMT* | | | | | |
| Naive | 7 (8.5%) | 3 (9.7%) | 3 (11.1%) | 1 (4.2%) | 0.648[a] |
| Basic/moderate | 40 (48.8%) | 15 (48.4%) | 14 (51.9%) | 11 (45.8%) | 0.911[a] |
| High | 35 (42.7%) | 13 (41.9%) | 10 (37.0%) | 12 (50%) | 0.643[a] |
| SPMS Conversion (n = 71) | 16 (22.5%) | 2 (7.7%) | 6 (25%) | 8 (38.1%) | 0.043[a] |

[a]Chi-squared test.
[b]Kruskal-Wallis test.
[c]Ordinary one-way ANOVA.
CIS = clinically isolated syndrome; DMT = disease modifying therapy (basic/moderate efficacy category: azathioprine, daclizumab, dimethyl fumarate, glatiramer acetate, interferon, teriflunomide, methotrexate, S1P-modulators; high efficacy: alemtuzumab, ocrelizumab, ofatumumab, rituximab, natalizumab, mitoxantrone); EDSS = Expanded Disability Status Scale; IQR = interquartile range; PPMS = primary progressive multiple sclerosis; RRMS = relapsing remitting multiple sclerosis; SD = standard deviation; SPMS = secondary progressive multiple sclerosis.

IQR: 5.0–8.3 pg/ml; rs10191329$^{CC}$ median: 6.8 pg/ml, IQR: 5.0–9.0 pg/ml, Kruskal–Wallis test $p = 0.830$; Figure 1A). Our prospective pwMS cohort (n = 658 pwMS) was composed of 462 non-carriers (rs10191329$^{CC}$; 70.2%), 169 heterozygous (rs10191329$^{AC}$; 25.7%), and 27 homozygous carriers (rs10191329$^{AA}$; 4.1%). We found no significant difference between sex, disease course, age at onset, number of relapses between MS*0 and MS*1 or category of DMT at MS*1 (Table 1). At the time of diagnosis in this cohort of pwMS, our analysis also revealed no significant difference in sNfL concentration between non-carriers (rs10191329$^{CC}$; median: 10.2 pg/ml, IQR: 6.9–18.4 pg/ml) and homozygous carriers (rs10191329$^{AA}$; median: 10.9 pg/ml, IQR: 7.5–18.7 pg/ml, Kruskal–Wallis test $p = 0.227$; Figure 1B). Importantly, at visit MS*1, approximately 4 (median: 4.3, IQR: 4.2–4.7) years after diagnosis, homozygous carriers (rs10191329$^{AA}$) presented with significantly higher sNfL levels (median: 7.8 pg/ml, IQR: 6.2–11.0 pg/ml) compared with non-carriers (rs10191329$^{CC}$; median: 6.1 pg/ml, IQR: 4.5–8.0 pg/ml, $p = 0.005$) and heterozygous carriers (rs10191329$^{AC}$; median: 5.8 pg/ml, IQR: 4.3–7.8 pg/ml, $p = 0.005$, both Dunn's-adjusted post-hoc tests; Figure 1B). sNfL levels are influenced by several parameters such as age, BMI, gadolinium-enhancing (Gd) lesions or DMT.[18] We validated our results using an ANCOVA model with age- and BMI-adjusted sNfL percentiles[16] at MS*1 as dependent variable and incorporation of sex, DMT group at MS*1, type of DMT regimen (escalation vs induction), number of DMT switches, presence of Gd lesions or relapses in a 6-month window around blood collection (±3 months), symptom duration, and EDSS as covariates. Besides the DMT group and presence of Gd lesions at MS*1, rs10191329 genotype status was a significantly independent factor ($p = 0.039$). Post-hoc analyses revealed significantly elevated sNfL levels at MS*1 between homozygous carriers (rs10191329$^{AA}$) compared with non-carriers (rs10191329$^{CC}$; $p = 0.035$) and heterozygous carriers (rs10191329$^{AC}$; $p = 0.042$, both Bonferroni-adjusted, Table 2). We conducted a sensitivity analysis excluding participants with Gd lesions on MRI scans or clinical relapses both in a 6-month window around MS*1 (3 months prior to and 3 months after blood collection; n excluded: 26 non-carriers, 9 heterozygotes, 1 homozygote). The rs10191329 carrier status was still a significantly independent predictor for sNfL levels at MS*1 ($p = 0.038$) and the post-hoc tests again resulted in significantly elevated sNfL levels in homozygous carriers (rs10191329$^{AA}$) compared with non-carriers (rs10191329$^{CC}$; $p = 0.034$) and heterozygous carriers (rs10191329$^{AC}$; $p = 0.043$, both Bonferroni-adjusted). Our finding therefore demonstrates the impact of the progression risk allele on the early sign of neuroaxonal damage,

**Table 2. sNfL at MS*1 (ANCOVA)**

| Effect | Percentile sNfL at MS*2 (Age- and BMI-Adjusted) *F* | *p*-Value |
|---|---|---|
| Time since onset | 0.365 | 0.546 |
| Sex | 1.086 | 0.298 |
| DMT category at MS*1 | 6.335 | **0.012** |
| Escalation vs. induction treatment | 0.283 | 0.595 |
| Number of DMT switches between MS*0 and MS*2 | 0.134 | 0.715 |
| Occurrence of relapses at MS*1 | 0.075 | 0.784 |
| Presence of Gd-enhancing lesions at MS*1 | 12.932 | **<0.001** |
| EDSS at MS*1 | 3.838 | 0.051 |
| rs10191329 status | 3.279 | **0.039** |

Analysis of covariance with age- and BMI-adjusted sNfL percentiles at MS*1 as dependent variable and rs10191329 status as grouping factor. Included covariates are listed in column 1. Post-hoc tests with Bonferroni correction revealed significant differences between homozygous carrier and non-carrier ($p = 0.035$) and homozygous carrier and heterozygous carrier status ($p = 0.042$).

namely sNfL, but not yet clinical disability. However, in the following years (MS*2) we observed a significant increase in EDSS of homozygous carriers (rs10191329$^{AA}$; $p = 0.005$, compared with non-carriers; $p = 0.009$, compared with heterozygous carriers (rs10191329$^{AC}$); Kruskal–Wallis tests with Dunn's multiple comparisons correction, Figure 1C; for test results with gARMSS instead of EDSS see Supplement Table 1) and occurrence of disability accrual compared with heterozygous (rs10191329$^{AC}$) and non-carriers (rs10191329$^{CC}$; $p < 0.001$; Fishers exact test 2-sided and multiple *z*-tests of 2 proportions; Figure 1D).

### *Increased Long-Term Disability of rs10191329$^{AA}$-pwMS*

Consistent with the prospective cohort, no significant differences in EDSS were detected at the early disease stage in the retrospective cohort. However, at later stages of the disease, the homozygous (rs10191329$^{AA}$) group exhibited significantly higher EDSS scores compared with non-carriers (rs10191329$^{CC}$) at 5 to 10 years after diagnosis ($3.9 \pm 2.3$ vs $1.4 \pm 1.7$, $p = 0.04$; Kruskal–Wallis tests with Dunn's multiple comparisons correction) and more than 10 years after diagnosis ($6.6 \pm 2.4$ vs $2.1 \pm 2.1$,

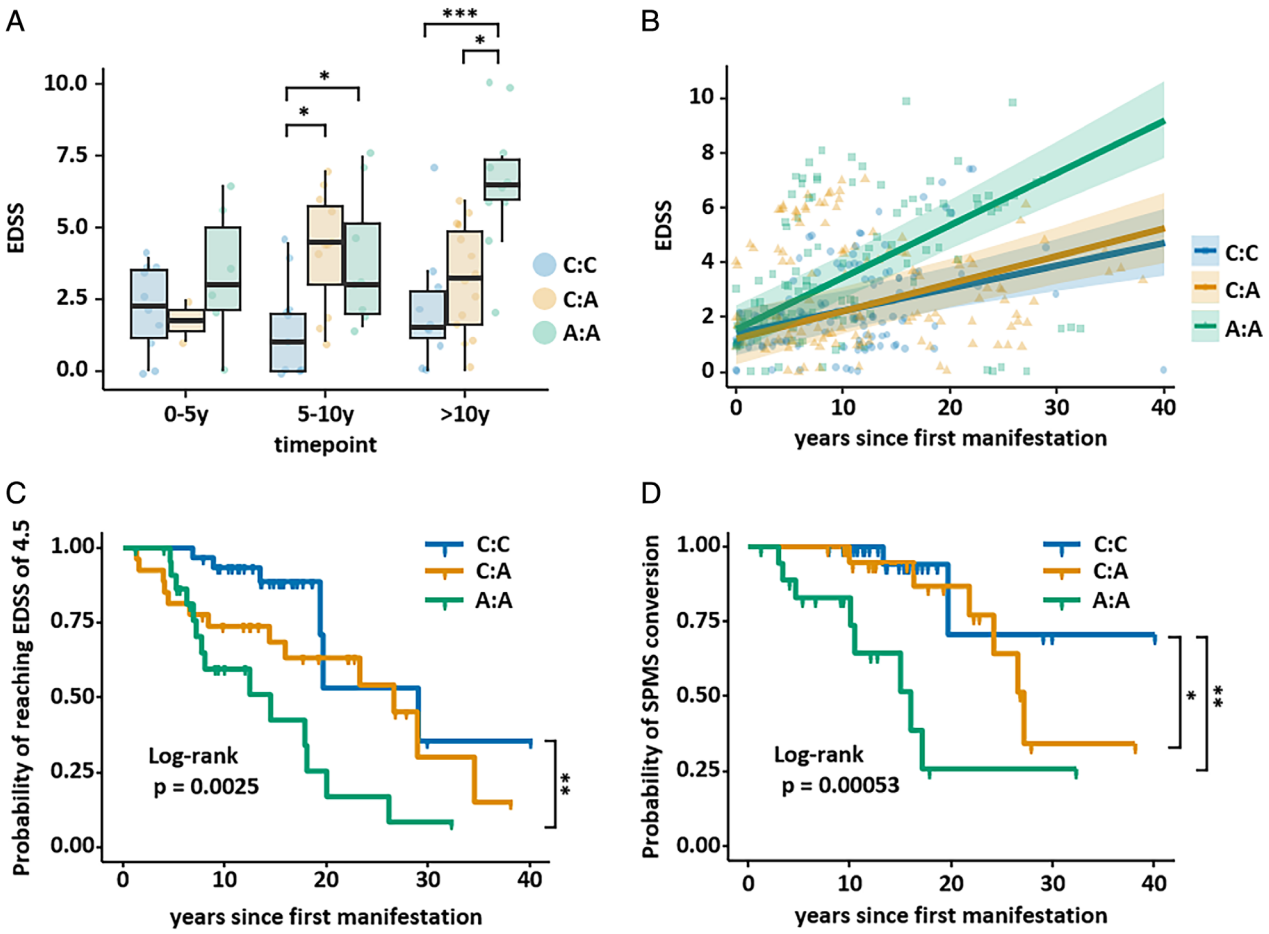


**Figure 2: Evidence for increased long-term disability in carriers of the severity risk allele. (A) A box plot illustrates the trajectory of EDSS. Notably, EDSS exhibited a statistically significant increase between years 5 and 10 in homozygous carriers compared with non-carriers ($p = 0.047$), and beyond the 10-year mark compared with both non-carriers ($p < 0.001$) and heterozygous carriers ($p = 0.012$, Kruskal–Wallis tests with Dunn's multiple comparisons correction). A jitter was applied to the data points for visualization purposes. (B) Evolution of group-specific EDSS scores over the duration of the symptoms (time since first manifestation). Individually observed EDSS scores are denoted by squares, triangles, and circles, while regression lines and the corresponding standard error of the means, represented as surrounding areas, are derived from a linear mixed-effects model demonstrating a significant interaction term between disease duration and homozygous genotype ($\beta = 0.11$ [0.06–0.16], $p < 0.001$). Disease duration, genotype, age at onset, time spent in a specific DMT group, and sex were incorporated as covariates (Table 3). Genotype groups are delineated by different colors (*blue*: non-carrier; *orange*: heterozygous; *green*: homozygous). A jitter was applied to the data points for visualization purposes. (C, D) Kaplan–Meier analysis revealed (C) differences in the survival proportion (log-rank $p = 0.003$), which was due to an elevated risk of attaining an EDSS score of ≥4.5 in rs10191329$^{AA}$ ($p = 0.002$ compared with rs10191329$^{CC}$; Bonferroni-adjusted); additionally, there were (D) differences regarding the survival-proportion (log-rank $p < 0.001$) due to a heightened risk of transitioning to SPMS in homozygous carriers ($p = 0.002$ compared with rs10191329$^{CC}$; $p = 0.033$ compared with rs10191329$^{CA}$; Bonferroni-adjusted). These analyses were further validated using Cox proportional hazard analyses after inclusion of several covariates (Tables 4 and 5). [Color figure can be viewed at www.annalsofneurology.org]**

$p < 0.001$; and $6.6 \pm 2.4$ vs $3.2 \pm 1.9$ in the heterozygous (rs10191329$^{AC}$) group, $p = 0.012$; Kruskal–Wallis tests with Dunn's multiple comparisons correction, Figure 2A). Furthermore, there were two cases of death due to MS in the rs10191329$^{AA}$ group. Regarding the other variables, especially the use of HE DMT, no significant differences were found.

To investigate the impact of the genetic progression marker on the rate of disability accrual in our retrospective cohort we developed a generalized LMM. The primary predictor in this model was the interaction between genotype and time since the first symptoms. The model was adjusted for repeated measurements and included covariates such as genotype, time since disease onset, sex, age at onset, and time spent in a specific DMT group. We found a significant impact of allele dosage on disability accrual over time, with homozygous carriers (rs10191329$^{AA}$) accumulating disability faster ($\beta = 0.11$ [0.06–0.16], $p < 0.001$) than non-carriers (rs10191329$^{CC}$) or heterozygous carriers (rs10191329$^{AC}$) (Table 3). Individual EDSS courses and regression lines with standard errors are shown in Figure 2B.

**Table 3. EDSS Progression over Time (Generalized Linear Mixed-Effects Model)**

| | EDSS | | |
|---|---|---|---|
| **Predictors** | **Estimates** | **CI** | ***p*-Value** |
| (Intercept) | −0.44 | −2.12–1.24 | 0.606 |
| Time (since onset) | 0.08 | 0.05–0.12 | **<0.001** |
| C:A | −0.17 | −1.25–0.91 | 0.754 |
| A:A | 0.16 | −0.89–1.22 | 0.763 |
| Female | −0.04 | −0.79–0.72 | 0.921 |
| Age at onset | 0.07 | 0.03–0.10 | **<0.001** |
| Time spent on basic/moderate DMT | −1.46 | −2.59–−0.34 | **0.011** |
| Time spent on high DMT | 2.12 | 0.33–3.92 | **0.021** |
| Time * C:A | 0.02 | −0.03–0.07 | 0.493 |
| Time * A:A | 0.11 | 0.06–0.16 | **<0.001** |

A:A = homozygous; C:A = heterozygous.
We developed a generalized linear mixed-effects model (LMM) with serial EDSS as the dependent variable. The primary predictor was the interaction between genotype and time since onset. Individuals were included as random effects, and covariates included genotype, time since onset, sex, age at onset, and time spent in a specific DMT group.

**Table 4. EDSS 4.5 Hallmark (Cox Proportional Hazards Analyses with DMT as Time-Varying Covariate)**

| | Reaching EDSS 4.5 | | |
|---|---|---|---|
| **Predictors** | **HR** | **CI** | ***p*-Value** |
| C:A | 2.32 | 0.73–7.39 | 0.153 |
| A:A | 5.43 | 1.88–15.63 | **0.002** |
| DMT group basic/moderate (time varying) | 2.57 | 0.77–8.56 | 0.125 |
| DMT group high (time varying) | 6.87 | 2.17–21.82 | **0.001** |
| Age at onset | 1.05 | 1.01–1.08 | **0.006** |
| Female | 0.96 | 0.38–2.43 | 0.925 |
| PPMS course | 4.29 | 1.67–10.99 | **0.002** |

Cox proportional hazards analyses with reaching EDSS hallmark of at least 4.5 as the dependent variable. Group of disease modifying therapy (DMT, basic/moderate, and high) were incorporated as time-varying covariate. rs10191329 status, sex, age at onset, and whether the disease course was primary progressive multiple sclerosis (PPMS) were added as further covariates. The proportional hazards assumption was examined by inspection of scaled Schoenfeld residuals. Multiple comparison resulted in significant differences between A:A and C:C (adjusted $p = 0.005$).
CI = 95% confidence interval; HR = hazard ratio; $p$ = corresponding $p$-value.

### *rs10191329$^{AA}$ Is Associated with Higher Probability of SPMS Conversion*

To unravel whether homozygous (rs10191329$^{AA}$)-pwMS have a higher risk to reach the EDSS hallmark 4.5, a Kaplan–Meier risk analysis was performed revealing significant differences ($p = 0.003$) in the survival distribution between the three genotype groups (Figure 2C). Subsequent log-rank tests confirmed significant differences between homozygous carriers (rs10191329$^{AA}$) and non-carriers (rs10191329$^{CC}$) (Bonferroni adjusted $p = 0.002$). Next, we strengthened these results by using Cox proportional hazard analyses to add covariates to the model. We examined the likelihood of reaching EDSS ≥4.5, incorporating rs10191329 status, sex, age at first manifestation, time since onset, DMT (time-varying) and PPMS status as covariates. Significant factors included rs10191329$^{AA}$ (hazard ratio [HR]: 5.43, 95% CI: [1.88–15.63], $p = 0.002$), age at onset (HR: 1.05, 95% CI: [1.01–1.08], $p = 0.006$), PPMS disease course (HR: 4.29, 95% CI: [1.67–10.99], $p = 0.002$) and HE DMT (HR: 6.87, 95% CI: [2.17–21.82], $p = 0.001$, Table 4).

In our cohort, 14 of 33 patients with an EDSS of at least 4.5 were classified as SPMS. Therefore, as these relevant clinical outcomes are not necessarily equivalent, we performed a separate analysis for SPMS conversion. Patients with PPMS were excluded for SPMS conversion analysis. Kaplan–Meier analyses indicated significant overall differences ($p = 0.001$, Figure 2D). Post-hoc tests revealed these differences between homozygous carriers (rs10191329$^{AA}$) and non-carrier (rs10191329$^{CC}$; Bonferroni-adjusted $p = 0.002$) as well as between homozygous (rs10191329$^{AA}$) and heterozygous (rs10191329$^{AC}$; adjusted $p = 0.033$). Cox proportional hazard analyses, incorporating the same covariates as previously used in the EDSS outcome except for PPMS, confirmed this conclusion. The analyses identified rs10191329$^{AA}$ (HR: 19.32, 95% CI: [3.28–113.71], $p = <0.001$), age at onset (HR: 1.10, 95% CI: [1.04–1.17], $p = 0.0016$), and HE DMT (HR: 11.19, 95% CI: [1.68–74.64], $p = 0.013$) as independent predictors for SPMS progression (Table 5).

**Table 5. SPMS Conversion (Cox Proportional Hazards Analyses with DMT as Time-Varying Covariate)**

| | SPMS conversion | | |
|---|---|---|---|
| **Predictors** | **HR** | **CI** | ***p*-Value** |
| C:A | 2.28 | 0.41–12.82 | 0.348 |
| A:A | 19.32 | 3.28–113.71 | **0.001** |
| DMT group basic/moderate (time varying) | 1.40 | 0.26–7.45 | 0.694 |
| DMT group high (time varying) | 11.19 | 1.68–74.64 | **0.013** |
| Female | 0.59 | 0.14–2.40 | 0.460 |
| Age at onset | 1.10 | 1.04–1.17 | **0.001** |

Cox proportional hazards analyses with SPMS conversion as the dependent variable. Category of disease-modifying therapy (DMT; basic/moderate or high) were incorporated as time-varying covariate. rs10191329 status, sex, and age at onset were added as further covariates. The proportional hazards assumption was examined by inspection of scaled Schoenfeld residuals. Multiple comparison resulted in significant differences between A:A and both C:C (adjusted $p = 0.003$) and C:A (adjusted $p = 0.005$).
CI = 95% confidence interval; HR = hazard ratio; $p$ = corresponding $p$-value.

## Discussion

We aimed to determine whether the discovery that the risk allele of the SNP rs10191329 is related to MS-severity[6] can be supported by sNfL biomarker measures.[16,19,20] In our cohorts, we observed no genotype-driven differences in sNfL levels in healthy people at risk of MS or at the time of MS diagnosis; furthermore, there were no clinical differences at this stage, underlining that the rs10191329 risk variant is not linked to MS susceptibility or peripheral inflammatory processes that are most prominent in the very early disease phases. However, homozygous carriers (rs10191329$^{AA}$) revealed increased levels of sNfL in the early stages of the disease when disability measures were still similar in all groups. Importantly, homozygous carriers showed higher rates of disability accrual thereafter and over time, a steeper association of EDSS, and in addition an increasingly higher EDSS beyond 5 years and a higher probability of SPMS development. rs10191329$^{AA}$ stood out in multiple variable regression as a significant predictor of reaching EDSS hallmarks and, notably, SPMS conversion. The latter was positively associated not only with rs10191329$^{AA}$ but also with rs10191329$^{AC}$. As expected, a higher EDSS score was also influenced by the primary progressive disease course. Two participants in the homozygous (rs10191329$^{AA}$) group died at the ages of 52 and 58 due to MS during our study, in contrast to no reported deaths in the other groups. This finding suggests that, even in the early stages of the disease, there is underlying axonal damage in individuals with the rs10191329$^{AA}$ genotype, which may not yet be clinically apparent.

sNfL serves as a predictor for disability accrual and CNS atrophy in MS.[21] Consequently, the rationale for diverging trajectories in EDSS levels[7] and brain atrophy,[8] particularly to the disadvantage of homozygous carriers (rs10191329$^{AA}$), is plausible. Kreft et al. and Campanga et al. recently reported a lack of association between rs10191329 and disease phenotype,[9,10] in contrast to our findings, as well as those reported by Gasperi et al. and the IMSGC.[8] Notably, our retrospective cohort exhibited a mean age at diagnosis/onset 4 years older than that described by Kreft et al. (37 years vs 33 years), and included a higher proportion of male participants (42% [10/24] male vs 32% [12/37] male in the homozygous group). Both older age at diagnosis and male sex are known predictors of poor outcomes in MS.[22] Importantly, in our study, the factors of sex and age were evenly distributed across our genotype groups and were included in our regression models, suggesting valid differences in our cohort. Another potential reason for the discrepancy lies in the differences regarding DMT. Although not statistically significant in either study, our long-term cohort appeared to be shifted toward high-efficacy treatments, while in the cohort reported by Kreft et al.,[9] 81% of homozygous carriers (rs10191329$^{AA}$) were treatment naive, indicating a higher proportion of potentially milder disease forms. To mitigate any bias, DMT was included in our multiple linear regression models. Furthermore, both studies by Kreft et al.[9] and Campanga et al.[10] were not powered to support a null result indicating that while an association was not seen, the possibility of an association has not been rejected.[11] At the same time, we note that our study is not without limitations due to the relatively small size of the retrospective cohort and the lack of disability confirmation. Future large studies including many centers may add to combining the MS severity risk genotype with the sNfL biomarker. Additional studies to estimate the potential impact of treatment on disability accrual based on clinical trial data using a propensity score for likelihood of therapeutic response are also warranted.

Ultimately, we bolstered the previously reported association of rs10191329$^{AA}$ with disability accrual through the inclusion of sNfL. The recognized limitations of the EDSS, including issues with inter- and intra-rater reliability[23] or sensitivity in detecting disability accrual compared to the Multiple Sclerosis Functional Score (MSFC),[24] are well-established. In

the era of precision medicine, there is a need for additional sensitive outcome measures. sNfL, which is capable of detecting neuroaxonal damage independent of disease drivers up to 6 years before MS onset,[25] holds promise for discriminating outcome differences and is integrated in current clinical studies.

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## Author Contributions

M.P., F.S., F.L., S.B., and F.Z. contributed to the conception and design of the study; all authors contributed to the acquisition and analysis of data; M.P., F.S., F.L., S.B., and F.Z. contributed to drafting the text or preparing the figures.

## Potential Conflicts of Interest

The authors declare no relevant conflicts of interest.

### *Data Availability*

The data supporting the findings of this study will be available upon reasonable request to the corresponding author of the study.

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