Progression of multiple sclerosis

The role of microglia and neurons

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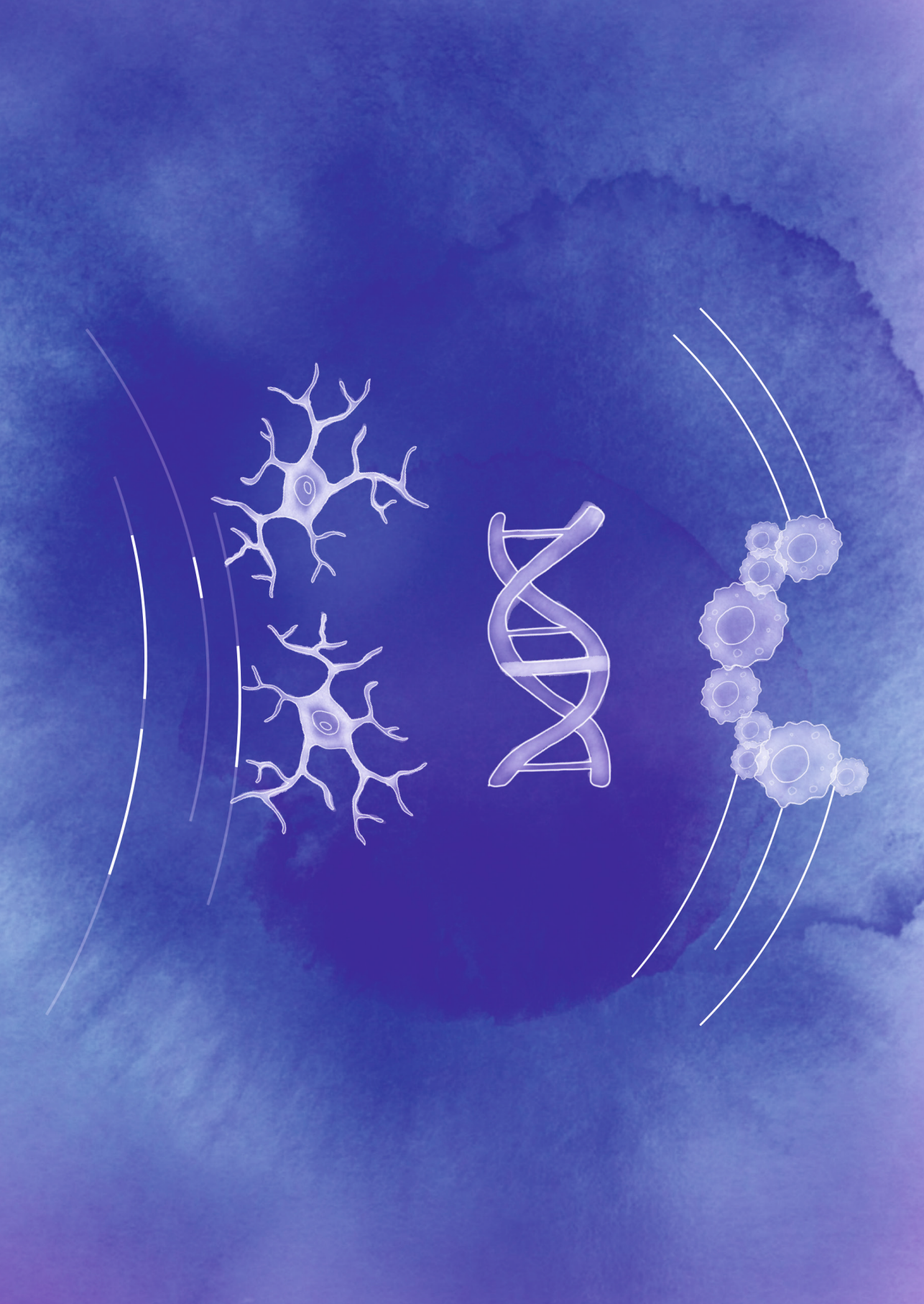

CHAPTER 8

Multiple sclerosis severity-associated genetic locus exacerbates neuropathology

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that results in significant neurodegeneration in the majority of those affected and is a common cause of chronic neurological disability in young adults. Here, to provide insight into the potential mechanisms involved in progression, we conducted a genome-wide association study of the age-related MS severity score in 12,584 cases and replicated our findings in a further 9,805 cases. We identified a significant association with rs10191329 in the *DYSF–ZNF638* locus, the risk allele of which is associated with a shortening in the median time to requiring a walking aid of a median of 3.7 years in homozygous carriers and with increased brainstem and cortical pathology in brain tissue. We also identified suggestive association with rs149097173 in the *DNM3–PIGC* locus and significant heritability enrichment in CNS tissues. Mendelian randomization analyses suggested a potential protective role for higher educational attainment. In contrast to immune-driven susceptibility, these findings suggest a key role for CNS resilience and potentially neurocognitive reserve in determining outcome in MS.

MS affects more than 2.8 million individuals worldwide, profoundly reducing quality of life for the majority of affected individuals [1,2]. Clinically, the disease is characterized by recurrent episodes of largely reversible neurological dysfunction, known as relapses, followed by a steady and unrelenting accumulation of chronic neurological disability, referred to as progression [1]. Case-control genome-wide association studies (GWAS) have identified more than 200 variants associated with susceptibility to the disease. Yet they do not associate with disease severity [3], which suggests that an independent genetic architecture determines the clinical course of the disease, as has been seen in other autoimmune [3] and neurological conditions [4].

To shed light on the genetic basis of MS progression, a large in-depth characterization of the genetic architecture underlying severity of MS was performed. This study combined cross-sectional and longitudinal analyses of MS-specific disability outcomes in 12,584 people of European ancestry with MS [5]. An association signal with a higher age-related MS-severity score at rs10191329 (DYSF–ZNF638 locus) reached genome-wide significance ($p = 9.7 \times 10^{-9}$), which was confirmed in the replication population and retained genome-wide significance in fixed-effects meta-analysis ($p = 3.6 \times 10^{-9}$). The direction of effect was consistent across all replication centers without evidence of heterogeneity (Q-statistic = 1.5, P = 0.99; I2 = 0%). In individuals who had been assessed longitudinally, analysis of serial EDSS across all visits revealed that the homozygous DYSF–ZNF638 risk allele carriers displayed faster disability progression ($p = 0.002$). Adjusted Cox proportional hazards analyses showed that the risk allele rs10191329A at the DYSF–ZNF638 locus was associated with faster 24-week confirmed disability worsening, (hazard ratio = 1.1 per unit increase in allele dosage, 95% confidence interval 1.02–1.18, $p = 7.9 \times 10^{-3}$), a metric used as the primary outcome in progressive MS therapeutic trials [6]. In homozygous carriers, the lead variant also conferred a 3.7-year shorter median time to using a walking aid (EDSS 6.0; hazard ratio = 1.22, 95% confidence interval 1.09–1.38, P = 9.3×10^{-4}), a clinically relevant MS disability milestone that typically tracks with the progressive phase of the disease and fixed neurological disability [7]. Furthermore, MS severity is associated with variation in genes that are preferentially expressed within the central nervous system (CNS). Prioritized MS severity genes (*DYSF, ZNF638*) even displayed cell type specificity for oligodendrocyte lineage cells. In conclusion, this recent study provided evidence for a role of genetic variation in MS progression, identifying a genetic locus associated with disability accrual in MS [5].

Here, we aim to further explore the association between the severity locus rs10191329 and MS pathology. We examined the variant's association with disease-relevant markers of tissue injury in an independent MS autopsy cohort

comprising 4,652 tissue blocks from 290 individuals. Following informed consent, brain donors with pathologically confirmed MS recruited to the Netherlands Brain Bank since 1990 were clinically and pathologically characterized (**Table 1**). Autopsy procedures were approved by the Ethical Committee of the VU University Medical Center in Amsterdam, the Netherlands. As previously described [8], blocks were dissected at standardized CNS locations (including the brainstem), with additional blocks targeted to MS lesions using macroscopic and post-mortem MRI assessment. Sections were double-labelled for proteolipid protein (PLP) and human leukocyte antigen (HLA) using immunohistochemistry and lesions were subsequently characterized in each dissected tissue block. For each individual, a brainstem lesion count was quantified using one section per standardized block. Areas of cortical grey matter demyelination were identified and classified by location (subpial, intracortical, leukocortical). These lesion locations were selected based on their recognized importance to MS pathophysiology [8,9]. DNA was extracted from whole blood or frozen cerebellar tissue, or when neither were available from formalin-fixed paraffin-embedded cerebellar tissue. Genotyping for rs10191329 was performed using the KASP genotyping platform (LGC Genomics). Pathological characterization was undertaken blind to genotype status. Differences in brainstem lesion load and rate of cortical lesions between genotype groups were examined using quasi-Poisson regression adjusted for sex, age at onset and initial disease course. To account for a variable number of supratentorial blocks sampled between individuals, cortical lesions were considered as a rate by adding the number of tissue blocks with visible cortex as an offset. Individuals with missing dependent variables or covariates were excluded. P values less than 0.025 were considered significant (adjusting for 2 pathological variables).

Consistent with estimates from the longitudinal analysis [5], homozygous risk allele carriers showed on average a four-year shorter median time to EDSS 6.0, although the differences were not significant in this smaller cohort (**Table 1**). Pathologically, homozygous carriers displayed a 1.83-fold higher number of lesions in the brainstem (95% confidence interval 1.09–3.06, P = 0.023; Methods), as well as a 1.76-fold higher rate of cortical lesions across sampled supratentorial tissue (95% confidence interval 1.15–2.69, P = 0.001; **Fig. 1**), confirming that the risk allele at the DYSF–ZNF638 locus is associated with more extensive injury at key brain locations. It is well established that focal lesions such as those in the brainstem result in axonal loss, and that cortical demyelination, which occurs independently of white matter lesions, is associated with selective neuronal loss [10]; both of these degenerative features are prominent determinants of progression [10,11].

Here, we demonstrate the association of rs10191329 with a more extensive MS-specific brainstem and cortical pathology which likely results in axonal and neuronal degeneration, and drive progression [10,11]. The specific pathways regulating this accelerated worsening still need to be uncovered. Our data provide a biological validation for a role of genetic variation in MS progression as suggested by its association with disability scores . MS has undergone a therapeutic revolution in the past few decades, with the emergence of ever more effective immune therapies that reduce and even halt relapses. Despite this, treatment of progression remains an unmet need. We have validated a genetic locus associated with faster disability progression in MS. A better understanding of the mechanisms underlying this association will provide new directions for functional characterization and drug development targeted on the neurodegenerative component of the disease.

Table 1: Donor demographics and clinical disease duration of MS donors the risk SNP rs10191329.

Variable	n	rs10191329 C/C	n	rs10191329 C/A	n	rs10191329 A/A	p-value[c]	SMD
Female, no. (%)	155	97 (62.6%)	68	43 (63.2%)	6	6 (100%)	0.188	0.728
Disease course, no. (%)	153		63		6		0.354	0.373
relapsing onset		103 (67.3%)		37 (58.7%)		5 (83.3%)		
primary progressive		50 (32.7%)		26 (42.3%)		1 (16.7%)		
Years to EDSS 6.0, median [IQR][a]	131	14.0 [7.0, 22.0]	52	13.0 [8.0, 20.3]	5	10.0 [4.0, 13.0]	0.450	0.499
Age at onset, mean (SD)	144	34.0 (11.1)	63	35.4 (11.0)	6	35.3 (7.9)	0.707	0.087
Age at death, mean (SD)	155	62.8 (12.8)	68	64.6 (13.6)	6	56.7 (12.0)	0.293	0.418
Disease duration, mean (SD)	155	31.2 (16.7)	68	31.9 (14.8)	6	21.3 (10.8)	0.304	0.521
Total tissue blocks, median [IQR][b]	204	18.0 [0.0, 26.0]	80	21.0 [1.8, 27.0]	6	40.0 [31.5, 41.8]	0.001	1.252
Targeted tissue blocks, median [IQR][b]	146	13.0 [8.0, 19.0]	65	14.0 [8.0, 19.0]	6	28.0 [20.3, 29.8]	0.006	1.120

[a]Information on time to EDSS 6.0 was only available for individuals who reached this milestone during the clinical observation period; [b]The number of tissue blocks was significantly different between genotype groups, driven by a higher number of non-standardized blocks targeted to MS lesions in homozygous risk allele carriers (post-hoc Dunn test adjusted P = 0.004), in support of a higher lesion burden in this group; [c]Fisher's exact test; one-way ANOVA (Kruskal-Wallis test for nonnormal continuous variables, reported as median [IQR]). P-values are two-sided. IQR, interquartile range; SD, standard deviation; SMD, standardized mean difference.

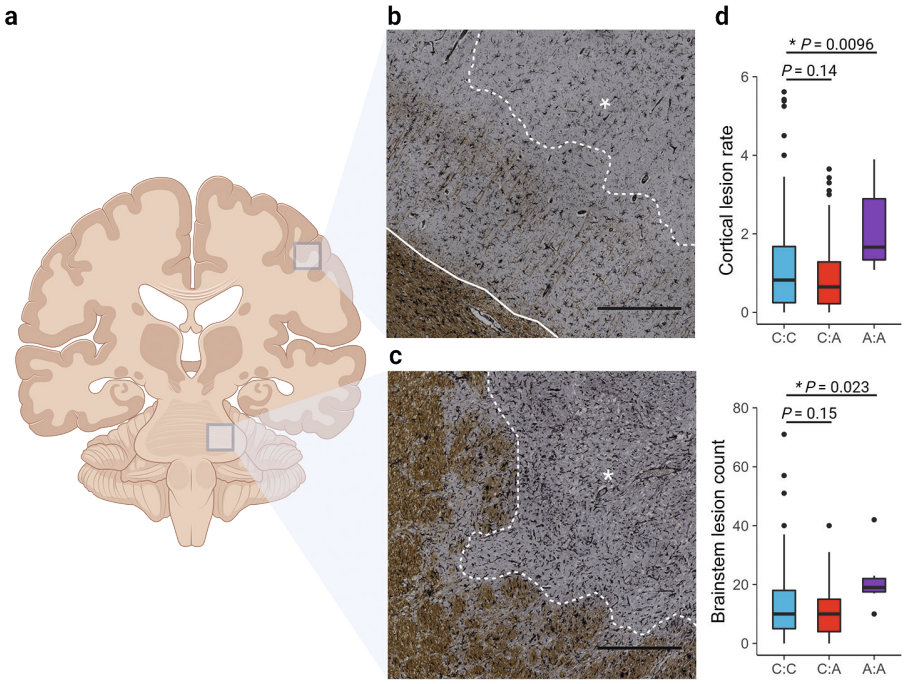

Figure 1: Cortical lesion rate and brainstem lesion count are higher in homozygous rs10191329 risk allele carriers. A) Schematic representation of tissue sampling locations. Demyelinating lesions were quantified on a brainstem section dissected in a consistent manner across individuals. Cortical lesions were identified on supratentorial tissue blocks targeted to macroscopic or MRI-visible MS lesions. B) Brain tissue section immunostained for the proteolipid protein marker of myelin (brown). A subpial cortical lesion characterized by the loss of myelin is marked by an asterisk and delineated by the dotted white line. The solid white line separates normal-appearing grey matter (sparse brown) from white matter (dense brown). C) A lesion spanning grey and white matter in the brainstem of the same donor, marked by an asterisk and delineated from normal-appearing tissue by the dotted white line. The donor was homozygous for the A allele of rs10191329. D) The displayed cortical lesion rate was calculated by dividing the number of lesions by the number of tissue blocks containing cortex. Box plots show median, first, and third quartiles; whiskers represent the smallest and largest values within 1.5-times the interquartile range; outliers are depicte das dots. Two-sided *P* values were obtained from generalized linear models comparing lesion count in the cortex (offset by the relevant number of tissue blocks: n = 174 donors) and brainstem (n = 181 donors) across genotype groups adjusting for covariates; significant differences are marked with an asterisk. Scale bars, 0.5mm. Image of brain in A created with BioRender.com. Figure from Harroud *et al.*, 2023, Nature, doi: 10.1038/s41586-023-06250-x

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Conflict of interest
Authors report no conflict of interest.

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