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Original research

# Environmental multiple sclerosis (MS) risk factors, genetic MS risk, and brain development in a general paediatric population

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## ABSTRACT

**Background** Neuroaxonal loss occurs in the early stages of multiple sclerosis (MS), but whether it results from early inflammatory brain damage or an ongoing neurodegenerative process remains unclear. We hypothesise that genetic and childhood environmental risk factors for MS may already have an impact on neurodevelopment before the typical age of onset for MS in the general population.

**Methods** We examined associations and interactions of genetic and environmental risk factors for MS with brain MRI outcomes, including volumetric (n=5350) and diffusion data (n=5649), at ages 9 and 13 years in a large, population-based childhood cohort without MS diagnoses. Polygenic risk scores (PRSs) were used to assess genetic burden, with rs10191329 as a marker of MS severity. Environmental factors at age 5 included Epstein-Barr virus (EBV) serology, vitamin D status, body mass index, duration of outdoor activities, and household parental smoking.

**Results** Genetic data were available for 2817 and 2970 participants with volumetric and diffusion data, respectively. The MS-PRS was positively associated with EBV viral capsid antigen titres among EBV-positive children ( $\beta=0.15$ ,  $p=2.98 \times 10^{-6}$ ). A negative association was observed between the MS-PRS and subcortical grey matter volume ( $\beta=-0.03$ ,  $p=0.014$ ). Interaction between the MS-PRS and household parental smoking was negatively linked to total brain ( $\beta=-0.21$ ,  $p=0.025$ ) and thalamic volumes ( $\beta=-0.22$ ,  $p=0.003$ ), where a higher MS-PRS and household smoking were associated with lower volumes. No associations were observed for rs10191329 with brain outcomes.

**Conclusions** Genetic and environmental risk factors for MS interact to influence brain volumes in childhood, suggesting a potential window for preventing MS in genetically susceptible individuals by reducing exposure to household smoking.

## INTRODUCTION

Multiple sclerosis (MS) is characterised by inflammatory demyelination and neuroaxonal loss within the central nervous system (CNS).<sup>1</sup> Diagnosed typically between the ages of 20 and 40 years, it remains unclear whether MS neurodegeneration stems from early inflammatory brain damage or a smouldering neurodegenerative process with superimposed inflammation. The exact age of onset remains uncertain, but brain volume loss at clinical

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Several environmental and genetic risk factors have been identified that contribute to multiple sclerosis (MS) susceptibility. How they interact with each other to add to MS disease risk early in life is largely unknown.

## WHAT THIS STUDY ADDS

⇒ This study shows that genetic risk for MS affects the immune response to Epstein-Barr virus infection in children from the general population and interacts with household parental smoking, resulting in lower total brain, subcortical grey matter, and thalamic volumes.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Early-life environmental and genetic factors for MS influence brain development at an early age in the general population, highlighting the possibility for early interventions targeting modifiable risk factors to mitigate future MS development.

onset and decreased premorbid cognitive performance suggests that disease pathophysiology begins earlier, before typical diagnosis.<sup>2–4</sup>

Migration studies indicate environmental factors critically influence MS pathophysiology before the age of 15 years.<sup>5</sup> Key risk factors include Epstein-Barr virus (EBV), 25-hydroxyvitamin-D (25(OH)D) levels, tobacco exposure, body mass index (BMI), and sun exposure, but how these interact early in life is unclear and remains to be elucidated.

Genetic risk factors are also crucial in MS pathophysiology. Genome-wide association studies (GWAS) discovered 233 genome-wide significant genetic variants and several suggestive variants contributing to MS diagnosis. Recently, a genetic variant linked to disease severity—rs10191329—was found.<sup>6,7</sup> Prior work showed polygenic risk for MS influences brain white matter (WM) in 9-year-old children in the general population, but multi-age associations were not assessed.<sup>8</sup> It remains unexplored how genetic risk for MS interacts with environmental risk factors in early life in the general population, which could be vital for understanding MS pathophysiology.

## Multiple sclerosis

This study uses brain MRI data from the Generation R study to analyse environmental MS risk factors and MS phenotypes on brain MRI, combined with MS polygenic risk scores (PRSs), to study the impact of MS risk factors cross-sectionally and across multiple ages on paediatric brain development in the general population. We hypothesise that environmental MS risk factors combined with genetic MS risk affect brain MRI volumetric regions and WM early on.

### METHODS

#### Population characteristics

Data from the Generation R study, a population birth cohort in the Netherlands, were utilised.<sup>9</sup> Participants included had good-quality genotype and/or environmental data on EBV serostatus, 25(OH)D levels, and measures of BMI, parental tobacco exposure, outdoor activity at age 5, and good-to-excellent quality neuroimaging at the 9-year or 13-year visits.

The Generation R study adhered to the Declaration of Helsinki, with ethics approval from the Erasmus Medical Centre. Written informed consent was obtained from parents/legal representatives and assent for children 12 years of age and older.

#### Genotype data

Genetic phenotyping was performed with the Illumina 610K and 660K single nucleotide polymorphism (SNP) arrays (Illumina, San Diego, CA, USA) on DNA from cord blood at birth or venipuncture at ages 5, 9, or 13 years.

Data were imputed with the 1000 Genomes Phase III version 5 reference panel.<sup>10</sup> European ancestry was defined by <4 standard deviation (SD) mahalanobis distance to the CEU subset of the 1000 Genomes Phase III version 5.<sup>11</sup> Quality control included imputation  $R^2 > 0.3$ , genotype missingness per individual <5%, genotype missingness per SNP <5%, SNP minor allele frequency >1%, Hardy-Weinberg Equilibrium  $p > 0.0001$ , and relatedness (identity by descent <0.185). Only overlapping SNPs between the two SNP arrays were selected for PRSs calculation (total SNPs=4 155 131).

#### Polygenic scoring

PRSs were calculated using PRSice2 at several suggestive thresholds ( $P_T < 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1$ ), based on the largest discovery MS susceptibility GWAS ( $n=41\,505; 14\,802$  cases and 26 703 controls).<sup>7 8 12 13</sup> An MS-PRS with genome-wide significant variants ( $P_T < 5 \times 10^{-8}$ ) was also calculated using GWAS meta-analysis data ( $n=115\,803$ ). SNP rs3135391 was used to characterise the *HLA-DRB1\*15:01* phenotype, dichotomised into 'no risk' and 'at risk' groups.

The presence of SNP rs10191329 was included in separate analyses to assess possible associations with brain outcomes.<sup>6</sup>

#### Environmental factors

**Epstein-Barr virus.** EBV serostatus was determined by detecting immunoglobulin G antibodies against EBV viral capsid antigen (VCA) using enzyme-linked immunoassays.<sup>14</sup> EBV-VCA titres are represented as a ratio, compared with a manufacturer-provided reference threshold sample, where a sample-threshold ratio >0.8 defined seropositivity.

**Vitamin D status measurement.** Blood sera at age 6 were measured using liquid chromatography/tandem mass spectrometry for both 25(OH)D2 and 25(OH)D3, to estimate vitamin D status. Serum 25(OH)D levels were residualised for sampling season using natural splines to account for non-linear seasonal effects.

**Body mass index assessment.** BMI calculation methods have been detailed previously.<sup>15</sup> SD scores adjusted for age and sex were computed using Dutch reference growth charts.<sup>16</sup>

**Household parental smoking and outdoor exposure.** At age 6, household parental smoking and outdoor exposure (ie, playing outside) were assessed through questionnaires. Smoking exposure was categorised as no exposure versus parental smoke exposure. Maternal smoking during pregnancy was used in a sensitivity analysis, which was categorised as: never smoked in pregnancy, smoked until pregnancy was known, and continued smoking during pregnancy. Outdoor exposure was assessed by average weekdays spent playing outside.

#### Imaging data

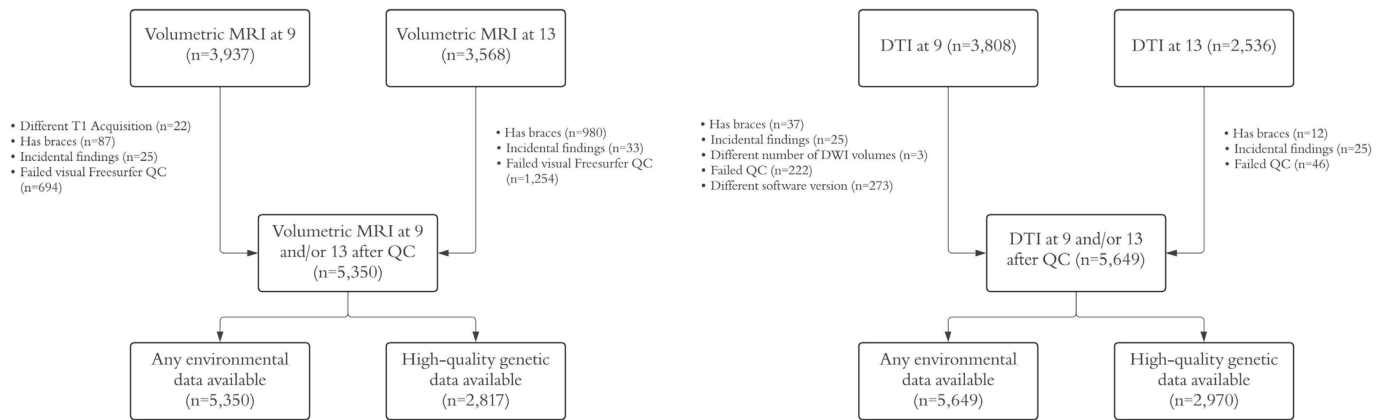
Brain imaging at age 9 and 13 used a 3T MR750w Discovery scanner (General Electric, Milwaukee, WI, USA). Volumetric and diffusion tensor imaging (DTI) measures were utilised, and the protocol and data processing have been described extensively.<sup>17 18</sup> Participants with incidental findings and braces were excluded, and images underwent extensive quality control to ensure validity. FreeSurfer v6.0 was used on T1-weighted images to obtain volumes for regions of interest (ROIs), including the hippocampus, thalamus, subcortical grey matter (GM) volume, total GM volume, and total WM volume.<sup>19</sup> For DTI outcomes, Functional MRI of the Brain Software Library (FMRIB FSL) and Camino Diffusion MRI Toolkit were used for data preprocessing.<sup>20 21</sup> Subsequently, AutoPtx probabilistic tractography plugin identified 12 WM tracts for average fractional anisotropy (FA) quantification.<sup>22</sup> Confirmatory factor analysis (CFA) on these WM tracts at the ages 9 and 13 modelled a single latent factor for global FA (online supplemental table 1).<sup>17</sup>

#### Statistical analyses

All analyses were conducted with R v4.3.1.<sup>23</sup> To compare descriptive statistics between the included participants in our study and the non-included participants from the Generation R study, analysis of variance (ANOVA) and  $\chi^2$  tests were used for numerical and categorical variables, respectively. In our first analysis, we used linear regression models to explore how MS genetics relate to EBV-VCA titres (in EBV-seropositive participants), 25(OH)D levels, and BMI, using environmental data from the 5-year visit. Next, we explored how the genetic and environmental factors related to pooled brain measures, for which we used linear mixed regression models with the participant as random effect to account for participants scanned during both the 9-year and 13-year visits.

Global associations tested different MS-PRS thresholds and environmental factors with total brain volume (TBV) and global FA. Next, to be consistent with our earlier work and limit our amount of statistical tests, the MS-PRS threshold with the strongest global association and the environmental factors were regressed onto subcortical volumes and individual WM tracts.<sup>8</sup> Additionally, to explore risk factor interactions in their association with the outcomes, we created a new multivariable model combining significant environmental factors and the most significant MS-PRS to test interaction effects. Finally, to investigate possible underlying age effects, since MRI data from 9 and 13 years of age were used, we tested for interaction effects between age and our determinants on volumetric outcomes and FA.

The analyses between the PRS and environmental factors were adjusted for age at measurement, sex, and the first 10 genetic principal components. All imaging analyses were adjusted for age at scanning, sex, and maternal education level. Imaging analyses



**Figure 1** Flowchart describing the selection process of the study. DTI, diffusion tensor imaging; DWI, diffusion weighted imaging; QC, quality control.

involving the MS-PRS were additionally adjusted for the first 10 genetic principal components. Analyses involving subcortical volumes were moreover adjusted for intracranial volume.

In all our analyses continuous determinants and outcomes were standardised to provide standardised  $\beta$  coefficients and allow the interpretability of effect sizes. Here, an increase of 1 SD in the determinant is associated with a  $\beta$ -sized increase or decrease in the SD of the outcome.

To account for multiple testing, we corrected the false discovery rate (FDR) using the Benjamini-Hochberg procedure.<sup>24</sup> In our first analysis examining the effects of the MS-PRS on environmental factors, this was applied across all environmental outcomes. In our imaging analyses, we applied the correction for the MS-PRS and environmental factors separately and divided between the global and subcortical outcomes.

## RESULTS

### Demographics and sample description

Selection of high-quality MRI data yielded 4121 unique participants with volumetric scans and 4283 participants with DTI scans. As participants underwent multiple sequences, 5350  $T_1$ -weighted scans were available for our imaging analyses and 5649 DTI scans (figure 1). Genetic data were available for 2817 participants with volumetric data and 2970 participants with DTI data after quality control (figure 1, table 1). Combining participants

with  $T_1$ -weighted scans and any available environmental data, we included 5350 participants for our environmental analyses, while 5649 participants with DTI scans had environmental data available (figure 1, table 1). Compared with participants not included, maternal education was relatively higher in our sample ( $p < 0.001$ ), but no significant difference was found in the height of the MS-PRS.

Age was normally distributed within all the study samples (table 1), and sex was evenly distributed across all groups. In all groups, the majority of participants consisted of children with high maternal education (table 1), particularly among participants with both genetic and imaging data available.

### Environmental MS risk factors

In EBV-positive children from the general population, a higher MS-PRS was associated with higher anti-EBV-VCA levels (eg, threshold 0.005:  $\beta = 0.150$ , standard error (SE) = 0.031,  $\Delta R^2 = 0.019$ ,  $p = 2.98 \times 10^{-6}$ ) (table 2). The results attenuated but remained statistically significant after excluding the major histocompatibility complex (MHC) region from the MS-PRS (online supplemental table 2). When investigating the effect of *HLA-DRB1\*15:01*, we found a similar association with anti-EBV-VCA levels (table 2). No statistically significant associations were observed between the MS-PRS thresholds and vitamin D status or BMI (online supplemental tables 3 and 4).

**Table 1** Descriptive characteristics of the study population

	Volumetric MRI+genetic data (n=2817)	DTI+genetic data (n=2970)	Volumetric MRI+environmental data (n=5350)	DTI+environmental data (n=5649)
Age (years) at MRI, median (IQR)	10.6 (9.9–13.7)	10.9 (9.9–13.7)	10.7 (9.9–13.7)	11.1 (9.9–13.7)
Female sex, n (%)	1407 (50.0)	1470 (49.5)	2761 (51.6)	2881 (51.0)
Level of maternal education, n (%)				
Low	32 (1.1)	35 (1.2)	332 (6.2)	365 (6.5)
Middle	889 (31.6)	929 (31.3)	2012 (37.6)	2132 (37.7)
High	1810 (64.3)	1915 (64.5)	2581 (48.2)	2699 (47.8)
Missing	86 (3.0)	91 (3.0)	425 (7.9)	453 (8.0)
EBV-VCA data available, n (%)	1936 (68.7)	2053 (69.1)	3449 (64.5)	3664 (64.9)
Vitamin D status available, n (%)	1831 (65.0)	1937 (65.2)	3264 (61.0)	3466 (61.4)
BMI available, n (%)	2681 (95.2)	2822 (95.0)	5015 (93.7)	5295 (93.7)
Data on parental smoking, n (%)	2402 (85.3)	2529 (85.2)	3956 (73.9)	4173 (73.9)
Data on outdoor activity, n (%)	2614 (92.8)	2754 (92.7)	4580 (85.6)	4832 (85.5)

BMI, body mass index; DTI, diffusion tensor imaging; EBV-VCA, Epstein Barr virus viral capsid antigen; n, number of participants.

**Table 2** Association between MS-PRSs and EBV-VCA titres in EBV-positive children. Table rows indicate different values of MS-PRS p value thresholds

MS-PRS p value threshold	$\beta$	SE	$\Delta R^2$	P value
$P_T < 5 \times 10^{-8}$	0.12	0.032	0.014	<b><math>2.03 \times 10^{-4}</math></b>
$P_T < 0.001$	0.14	0.032	0.018	<b><math>2.10 \times 10^{-5}</math></b>
$P_T < 0.005$	0.15	0.032	0.022	<b><math>2.98 \times 10^{-6}</math></b>
$P_T < 0.01$	0.14	0.032	0.019	<b><math>1.62 \times 10^{-5}</math></b>
$P_T < 0.05$	0.11	0.032	0.012	<b><math>5.03 \times 10^{-4}</math></b>
$P_T < 0.1$	0.08	0.032	0.007	<b>0.01</b>
$P_T < 0.5$	0.07	0.032	0.005	0.02
$P_T < 1$	0.08	0.032	0.006	<b><math>1.51 \times 10^{-2}</math></b>
rs3135391 (A)	0.16	0.032	0.026	<b><math>4.95 \times 10^{-7}</math></b>

Included: n=642 EBV-positive children. Analyses were corrected for age, sex, and 10 genetic principal components. EBV-VCA titres were represented as a ratio compared with a manufacturer-provided reference threshold sample. rs3135391 was dichotomised in the analyses into 'at risk' and 'no risk' groups.

Significant values after Benjamini-Hochberg multiple testing correction for the FDR are highlighted in bold.

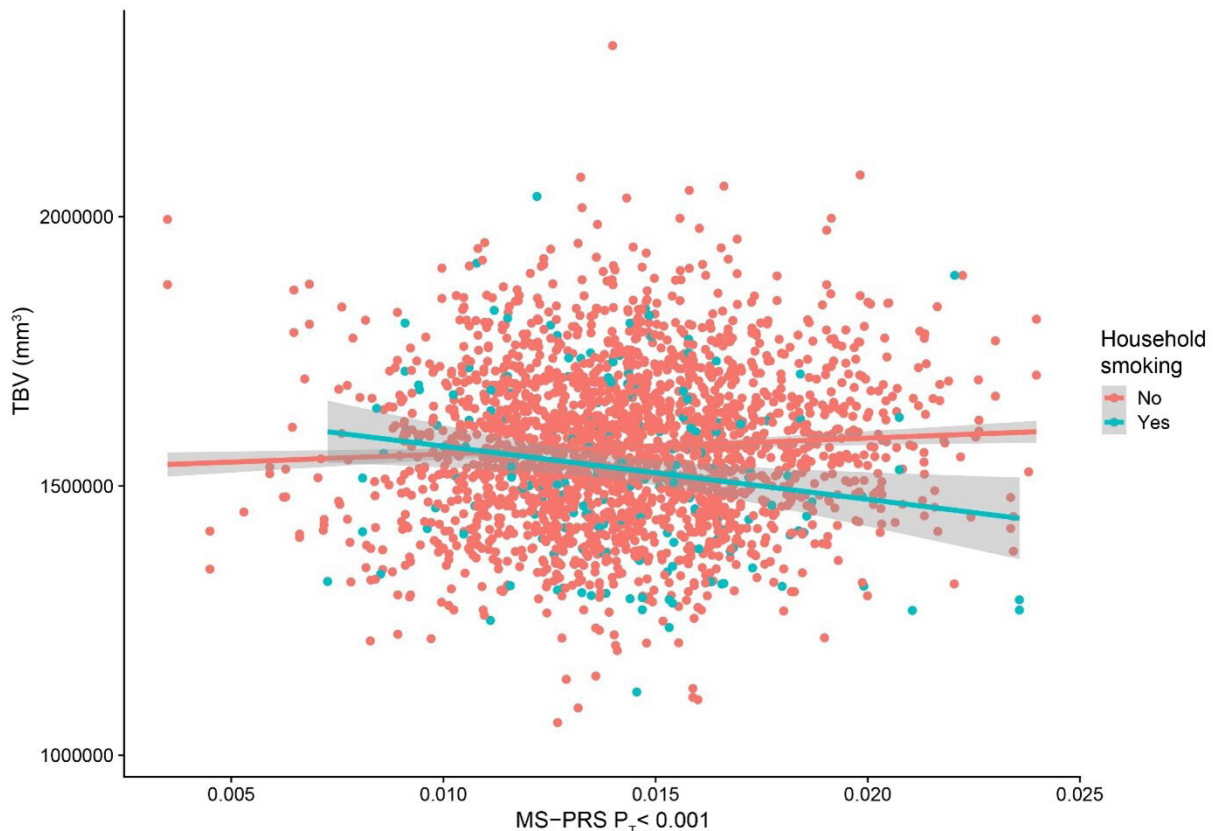
EBV-VCA, Epstein-Barr virus viral capsid antigen; FDR, false discovery rate; MS-PRS, multiple sclerosis polygenic risk score;  $P_T$ , polygenic threshold;  $\beta$ , standardised  $\beta$ ;  $\Delta R^2$ ,  $R^2$  difference.

**Structural MRI outcomes**

Higher MS-PRSs were associated with a higher TBV with statistical significance (online supplemental eTable 5). Vitamin D status and BMI associated positively with TBV, whereas parental smoking showed a negative association (online supplemental eTable 6). No significant association was found between outdoor exposure and TBV. In a combined model containing the MS-PRS and significant environmental factors, the MS-PRS association with TBV was not statistically significant (online supplemental eTable 7). We investigated interaction effects of the MS-PRS with BMI and parental

smoking, which revealed a significant negative interaction of the MS-PRS with parental smoking on TBV (figure 2, table 3).

Higher genetic MS burden was associated with lower total GM volume of subcortical regions ( $\beta = -0.033$ ,  $SE = 0.013$ ,  $p = 0.014$ ) (online supplemental eTable 8). For the environmental risk factors, we found several significant associations for vitamin D status and BMI on brain ROIs (online supplemental eTable 9). No significant associations were found between EBV, household parental smoking, and outdoor activity on the ROIs. When combining MS-PRS and BMI into a combined model



**Figure 2** Effect of MS-PRS on TBV based on parental smoking. MS-PRS, multiple sclerosis polygenic risk score;  $P_T$ , polygenic threshold; TBV, total brain volume.

**Table 3** Interaction effects between MS-PRS and parental smoking on brain volumes

Determinant	Outcome	$\beta$	SE	P value
$P_T < 0.001$ *	TBV	-0.21	0.095	<b>0.02</b>
Parental smoking				
$P_T < 0.001$ *	Subcortical GM volume	-0.17	0.070	<b>0.01</b>
Parental smoking				
$P_T < 0.001$ *	Thalamus volume	-0.22	0.075	<b><math>3.19 \times 10^{-3}</math></b>
Parental smoking				

Included: n=1501 children. Analyses were corrected for age, sex, level of maternal education, EBV serostatus, vitamin D levels, outdoor activity, standardised BMI, and 10 genetic principal components.  
Significant values after Benjamini-Hochberg multiple testing correction for the FDR are highlighted in bold.  
BMI, body mass index; EBV, Epstein-Barr virus; FDR, false discovery rate; GM, grey matter; MS-PRS, multiple sclerosis polygenic risk score;  $P_T$ , polygenic threshold; TBV, total brain volume;  $\beta$ , standardised  $\beta$ .

for subcortical GM volume, the MS-PRS association remained statistically significant (online supplemental table 10).

In an exploratory analysis we investigated the interaction effects of parental smoking and MS-PRS on subcortical volumes. Table 3 shows significant negative interactions between MS-PRS and parental smoking on subcortical GM volume and thalamic volume. In an additional analysis we tested whether this interaction was possibly biased due to an underlying effect of maternal smoking during pregnancy; however, no interaction effects between smoking during pregnancy and MS-PRS were observed (online supplemental table 11). Subsequent investigation of *HLA-DRB1\*15:01* status showed similar interaction effects (online supplemental table 12). When excluding the MHC region from our PRS, the associations were no longer significant (online supplemental table 13). However, the effect sizes remained in the same direction.

Next, we investigated the interaction effects between our independent variables and age, and found significant interactions for BMI and age on TBV and several ROIs (online supplemental table 14). No significant interactions effects were found between MS-PRS and age.

The MS-severity risk variant rs10191329 was not associated with any volumetric outcomes and did not show any significant age interactions (online supplemental table 15).

### DTI results

The MS-PRS showed positive associations with global FA, but no association survived correction for multiple testing. Similar results were obtained when the MS-PRS  $P_T < 0.01$  was regressed on specific WM tracts, with no statistically significant associations (online supplemental table 16 and 17).

The environmental risk factors did not show significant associations with global FA and no interaction effects were observed with age for the MS-PRS or environmental risk factors on FA (online supplemental table 18 and 19).

When investigating rs10191329, no significant associations were observed with DTI outcomes as well as any age interactions (online supplemental table 20).

### DISCUSSION

We found that genetic and environmental risk factors for MS interact upon certain aspects of brain structure during childhood and early adolescence. We found that EBV-positive children without MS, but with high polygenic risk for MS, had elevated anti-EBV-VCA titres. Furthermore, MS genetic risk was

associated with lower subcortical GM volumes. We also observed a negative interaction between MS-PRS and household smoking at the age of 5 years, associated with a lower TBV, subcortical GM volume, and thalamic volume.

The higher anti-EBV-VCA titres align with findings in MS patients, where elevated EBV titres are associated with genetic risk for MS.<sup>25</sup> In our results, *HLA-DRB1\*15:01* status was the strongest contributor to higher anti-EBV-VCA titres. Infection by EBV can result in latent infection of memory B cells. This could produce autoreactive B cells, which possibly bypass control of the immune system.<sup>26</sup> In addition, MS genetic risk variants have been shown to alter the transcriptomes of T and B cells.<sup>27 28</sup> Combined with the results of our earlier studies,<sup>29</sup> where the memory B cell compartment was increased because of higher genetic burden for MS, the higher titres found in our study may reflect a defective immune control of EBV due to genetic risk for MS, mainly driven by *HLA-DRB1\*15:01* status, possibly promoting the risk of MS in a genetically at-risk population.

Considering our volumetric results, lower subcortical volumes have been reported extensively in MS due to inflammation and degeneration of the CNS.<sup>30</sup> Lower GM volumes and cortical changes have been associated with genetic MS susceptibility risk in adults from the general population, but remain to be replicated.<sup>31 32</sup> Here, we show that in children from the general population, an association exists between polygenic risk for MS susceptibility and brain outcomes, resulting in lower subcortical GM volumes, and not for the MS severity risk variant rs10191329. The mechanisms mediating this association are uncertain. Since MS susceptibility genetic risk has been implicated in lymphocyte and microglia function, both of importance in brain development and homeostasis, the impact of these genes on functioning of these cells in normal brain development could underlie these associations.<sup>7 33 34</sup> In addition, we initially reported a positive association between MS-PRSs and TBV. This, however, attenuated to non-significance when environmental risk factors were included, highlighting the importance of incorporating both genetic and environmental factors in analyses regarding MS pathophysiology.

We found a negative interaction between genetic risk for MS and household smoking on TBV, and in an exploratory analysis on subcortical GM volume and thalamic volume. This indicates that a higher genetic MS risk is associated with an increased vulnerability to the negative effects of household smoking on brain development. The main hypothesis that could account for the increased MS risk by tobacco smoke is chronic inflammation of the respiratory tract, increasing the pro-inflammatory status of the immune system.<sup>35</sup> Interactions between genetic risk factors for MS, especially the MHC region including *HLA-DRB1\*15:01* status, and smoking have been described before in adults, where smoking could change the adaptive immunity system, increase oxidative stress, and lead to CNS auto-reactivity.<sup>35-38</sup> This seems to be in line with our results, where exclusion of the MHC region from our MS-PRS attenuated the results. Our results importantly add another potential mechanism of tobacco smoke exposure in individuals with higher polygenic MS risk. The increased brain vulnerability to the effects of parental smoking may increase exposure of CNS antigens to the developing immune system, increasing the risk of a brain specific autoimmune disease.

Our volumetric results are in line with studies in MS patients, where volumetric and cognitive differences are found before clinical presentation of the disease.<sup>2-4</sup> The results of our study add a potential mechanism for the observed prediagnostic cognitive and volumetric differences in MS patients, through the

presence of lower brain volumes in childhood because of genetic MS risk and the interaction with household parental smoking.

We found no interaction effect between age and genetic MS risk on volumetric or DTI outcomes. It is possible that genetic susceptibility for MS primarily influences disease pathophysiology by affecting the immune system rather than directly impacting brain development over time. Another possibility is that despite our large sample size we were still underpowered to investigate subtle interaction effects between age and genetic MS risk during childhood. When investigating the interaction of age with environmental risk factors, only significant interactions were observed for BMI considering volumetric MRI outcomes, underlining the role of body composition and nutrition on brain development, which has been studied extensively.<sup>15</sup>

Regarding our DTI results, we found no significant association between MS-PRS and global FA after multiple testing correction. The direction of the association was similar to our previous study in children of the Generation R study.<sup>8</sup> There are several explanations as to why we did not find a significant finding for these analyses. Due to the inclusion of an additional group of participants with genotype data available, the overall sample size and statistical power of our study increased, but fewer SNPs were included in our MS-PRS. In addition, through changes in the DTI quality control between our previous study and the current study, more participants could be included in the analyses of this paper. This consisted of more participants with lower education and more movement on DTI scans. The results of our previous study and current study are in need of replication in children from the same age, as the effect of MS genetic risk on FA in the general population may very well be specific to an early age.

Due to the unique study design of the Generation R study, which includes genetic data and information on environmental factors early in life, we were unable to replicate our study in an independent sample. Future studies in children before the age of onset for MS are needed to replicate our results, including environmental risk factors for MS, to study further the interaction with MS genetics. Results from studies in adults from the general population could differ significantly, as age-related brain atrophy may have already occurred and more importantly some participants have already been excluded due to an MS diagnosis.<sup>39</sup>

Our study has several strengths. First, we were able to study the effects of genetic MS risk both cross-sectionally and across multiple ages in a large sample of children from the general population. Second, our study incorporated multiple environmental MS risk factors to study their influence on brain development and their interplay with MS genetic risk. Finally, all participants were scanned in the same MRI scanner, allowing us to compare results without inter-scanner differences influencing our findings.

There were also limitations to our study. First, our sample size, while very large for paediatric imaging studies, may still have been too small to investigate subtle interaction effects of genetic MS risk and environmental risk factors with age on brain development. Second, only anti-EBV-VCA levels were available, as opposed to levels of the EBV nuclear antigen. Third, as mentioned before, we only included overlapping SNPs in our PRS. Finally, the relative underrepresentation of low maternal education in our study could have led to residual confounding in our results based on socioeconomic status.

In conclusion, we showed an interaction between genetic risk for MS and childhood environmental MS risk factors on paediatric brain volumes in the general population, and we found higher titres of anti-EBV antibodies in children with a higher genetic MS burden. Furthermore, our results point towards an

increased brain vulnerability to household smoking in children with a higher polygenic risk score for MS. How this increased vulnerability influences other MS risk factors may open a window for prevention of MS by limiting childhood exposure to household smoking or other toxic exposures associated with MS (ie, household chemicals),<sup>40</sup> and should be a focus for further studies.

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**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved by Ethics Committee Erasmus Medical Center (ID: 198.782/2001/31). Participants gave informed consent to participate in the study before taking part.

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**Data availability statement** Data are available upon reasonable request. The data used in this study are not publicly available due to legal and informed consent restrictions. Qualified researchers can request to access the data by contacting the Generation R study (datamanagementgenr@erasmusmc.nl).

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