Received: 19 February 2022 | Accepted: 23 September 2022

DOI: 10.1111/ene.15582

european journal of neurology
the official journal of the european academy of neurology

ORIGINAL ARTICLE

Multiple sclerosis risk variants influence the peripheral B-cell compartment early in life in the general population

Casper L. de Mol[1,2] | Marvin M. van Luijn[3] | Karim L. Kreft[1] | Kirsten I. M. Looman[2,4] | Menno C. van Zelm[5] | Tonya White[6,7] | Henriette A. Moll[2,3] | Joost Smolders[1,3] | Rinze F. Neuteboom[1]

[1]Department of Neurology, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands

[2]Generation R Study Group, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands

[3]Department of Immunology, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands

[4]Department of Pediatrics, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands

[5]Department of Immunology and Pathology, Central Clinical School, Monash University and Alfred Hospital, Melbourne, Victoria, Australia

[6]Department of Child and Adolescent Psychiatry, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands

[7]Department of Radiology and Nuclear Medicine, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands

Correspondence
Rinze F. Neuteboom, Erasmus MC-Sophia, Room SK-1210, PO Box 2060, 3015 GD Rotterdam, the Netherlands.
Email: r.neuteboom@erasmusmc.nl

Funding information
Stichting MS Research, Grant/Award Number: 17-985 MS; ZonMw

Abstract

Background and purpose: Multiple sclerosis (MS) is associated with abnormal B-cell function, and MS genetic risk alleles affect multiple genes that are expressed in B cells. However, how these genetic variants impact the B-cell compartment in early childhood is unclear. In the current study, we aim to assess whether polygenic risk scores (PRSs) for MS are associated with changes in the blood B-cell compartment in children from the general population.

Methods: Six-year-old children from the population-based Generation R Study were included. Genotype data were used to calculate MS-PRSs and B-cell subset-enriched MS-PRSs, established by designating risk loci based on expression and function. Analyses of variance were performed to examine the effect of MS-PRSs on total B-cell numbers ($n = 1261$) as well as naive and memory subsets ($n = 675$).

Results: After correction for multiple testing, no significant associations were observed between MS-PRSs and total B-cell numbers and frequencies of subsets therein. A naive B-cell-MS-PRS ($n = 26$ variants) was significantly associated with lower relative, but not absolute, naive B-cell numbers ($p = 1.03 \times 10^{-4}$ and $p = 0.82$, respectively), and higher frequencies and absolute numbers of $CD27^{+}$ memory B cells ($p = 8.83 \times 10^{-4}$ and $p = 4.89 \times 10^{-3}$, respectively). These associations remained significant after adjustment for Epstein–Barr virus seropositivity and the *HLA-DRB1*15:01* genotype.

Conclusions: The composition of the blood B-cell compartment is associated with specific naive B-cell-associated MS risk variants during childhood, possibly contributing to MS pathophysiology later in life. Cell subset-specific PRSs may offer a more sensitive tool to define the impact of genetic risk on the immune system in diseases such as MS.

KEYWORDS
B-lymphocytes, genetic association studies, multiple sclerosis

INTRODUCTION

The pathophysiology of multiple sclerosis (MS) remains unclear, but is influenced by a complex interplay between genetic and environmental risk factors [1]. The International Multiple Sclerosis Genetics Consortium (IMSGC) has identified more than 200 autosomal single nucleotide polymorphisms (SNPs) that contribute to the disease process of MS [2].

MS patients show defects in peripheral B-cell tolerance checkpoints, with increased activation of potentially disease-inducing naive B cells [3]. In addition, increased presence of $CD27^+$ memory B cells was found in the cerebrospinal fluid (CSF) and brain of MS patients [4]. After their interaction with $CD4^+$ T helper cells, memory B cells migrate into the central nervous system (CNS) to mediate local pathology, under the influence of genetic control as evidenced by earlier studies [5–7]. Therapies that target the peripheral B-cell compartment (e.g., anti-CD20 antibody) reduce MS relapses and disability progression [8]. Altogether, this supports a combined role of genetics and B cells in MS, but the underlying early pathophysiology is not yet investigated. It remains unclear how genetic risk for MS affects B-cell development early in life, before a possible diagnosis of MS.

Recently, we have shown that children from a population-based study with a high polygenic risk for MS show alterations in the white matter microstructure of the brain and in the T-cell compartment of the immune system, which hints at early susceptibility factors for the disease process emerging in childhood [9, 10]. The importance of B cells and the interaction between B and T cells in MS pathophysiology warrant additional investigation of the influence of genetic risk for MS on the B-cell compartment and how this influences the distribution of naive and memory B cells during childhood [11].

In the current study, we investigate the effect of polygenic risk scores (PRSs) and functionally annotated risk variants for MS on the peripheral B-cell compartment in children from the general population. We hypothesize that children with high genetic risk for MS show alterations in the B-cell distribution of the immune system at an early age.

MATERIALS AND METHODS

Study participants

The study was performed in the context of the Generation R Study, a large population birth cohort ($n = 9749$) in the Netherlands [12]. Participants were selected on the basis of availability of genotyping data followed by detailed immunophenotyping at 6 years. Total B-cell numbers were measured in 3465 participants, and detailed phenotyping of B-cell subsets was conducted in 1079 subjects [13]. Parental MS was recorded at time of birth through questionnaires.

Standard protocol approvals, registrations, and patient consents

The Generation R Study has been performed according to the Declaration of Helsinki, and the study protocol has been approved by the Medical Ethical Committee of the Erasmus Medical Center. Written informed consent was given by the parents or legal representatives of all children.

B-cell immunophenotyping

Total B-cell numbers ($CD19^+$) were measured using a routine diagnostic lyse-no-wash protocol [13]. In addition, detailed phenotyping of B-cell populations was performed using whole blood and standardized six-color flow cytometry [14, 15]. Flow cytometry data were collected on a three-laser LSRII instrument (BD Biosciences) with standardized measurement settings, and analyzed using FACSDiva software version 6.2 [16].

Naive B cells ($CD27^-IgD^+$) and six memory B-cell subsets, including Ig-switched (IgG^+CD27^+, IgG^+CD27^-, IgA^+CD27^+, IgA^+CD27^-) and nonswitched natural effector ($CD27^+IgM^+IgD^+$) and IgM-only ($CD27^+IgM^+IgD^-$) B cells [14, 15]. Finally, $CD21^{low}$ B cells ($CD38^{low}CD21^{low}$) were defined [17]. The aforementioned subsets were used in our analyses based on their importance in MS pathophysiology and availability within the Generation R Study.

Genotyping

Genetic data extraction was performed on Illumina 610K and 660K SNP arrays on DNA collected at birth, from cord blood, or through venipuncture at a visit to the research center. A detailed description of the sample collection has been described earlier [18]. Participants of European ancestry were selected based on the HapMap Phase II data [19]. In addition, we imputed the genetic data using data from 1000 Genomes (Phase I, version 3) [20]. Quality control measures performed on the genetic data, a detailed description of the imputation method, and the calculation of principal components (PCs) have been published previously [9].

Polygenic risk scores

Weighted PRSs were calculated on the imputed genotype data originally generated by the IMSGC ($N = 41{,}505$; 14,802 cases/26,703 controls) [2]. PRSice 2 was used to calculate p-value thresholded and clumped PRSs in PLINK [21, 22]. To include the effect of suggestive MS risk variants [2], we calculated PRSs at several p-value thresholds ($p_T < 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1$). In addition, we calculated a PRS including only SNPs that reached genome-wide

significance ($p_T < 5 \times 10^{-8}$) in the final meta-analysis from the IMSGC ($N = 115{,}803$; 47,429 cases/68,374 controls) [2].

Due to the importance of the major histocompatibility complex (MHC) in MS pathophysiology [2], we calculated three sets of PRSs: one with all risk variants included, one without SNPs from the MHC region, and one with only SNPs from the MHC. The SNP rs3135388 was used as a tag variant for the *HLA-DRB1*15:01* haplotype [23]. Due to the low minor allele frequency of rs3135388, carriership was dichotomized into two groups: homozygous nonrisk and at-risk participants.

B-cell PRSs

Various genetic expression datasets were utilized to construct PRSs within the complete MS genome-wide risk variant sets that associated with particular B-cell subsets. B-cell-associated PRSs were constructed by assessing whether expression of a locus implicated by an SNP [2] was increased in a specific subset, defined by mRNA expression levels in the upper quartile of the concerning B-cell compartment [24–26]. In addition, expression quantitative trait loci were assessed for all MS risk SNPs and assigned to specific B-cell subsets [24]. Finally, gene ontology analyses were performed on MS risk SNPs to assign loci to functional pathways in the aforementioned lymphocyte subsets [27–29]. Identification of an association between an MS risk variant and B-cell subset in one of the analyses led to the inclusion of the SNP in the subset-associated PRS (Figure S1).

Statistical analyses

All statistical analyses were performed using the R statistical software package (version 3.5.1) [30]. First, we divided the MS-PRSs into quartiles. Using analyses of variance, we analyzed the effect of the different MS-PRSs' quartiles, using all *p*-value thresholds, on absolute B-cell counts. Second, the PRS with the strongest effect on absolute B-cell counts was used in further analyses with the different B-cell subsets of interest. To investigate the effects of the *HLA-DRB1*15:01* haplotype, we used multiple linear regression with rs3135388 as categorical determinant. All analyses were adjusted for age at B-cell measurement, sex, and the first 10 genetic PCs. In an additional sensitivity analysis, we adjusted for Epstein–Barr virus (EBV) seropositivity, *HLA-DRB1*15:01* positivity, and the $CD4^+/CD8^+$ ratio [10]. The measurement of EBV capsid antigen in the Generation R Study has been described previously [13], where EBV seropositivity was defined by a sample-threshold ratio above 0.8. When PRS-specific effects were observed, investigation of individual SNPs comprising the PRS was performed using multiple linear regression adjusted for age at B-cell measurement, sex, and the first 10 genetic PCs.

All B-cell populations were natural-log-transformed to establish normally distributed residuals in our regression models. False discovery rate adjustment was used to correct for multiple testing [31].

RESULTS

Population characteristics

Of the 3465 participants with absolute B-cell data, 2286 had genotype data available. One thousand two hundred sixty-one of these participants were eligible for total B-cell number analyses after selection on the quality of genotype data, relatedness, and European ancestry (Figure S2). Sex was evenly distributed in the population (50.8% male), and the median age was 6.0 years (interquartile range = 5.9–6.2; Table 1). We observed no significant difference in mean MS-PRS compared to participants who had no total B-cell counts available using a two-sided *t*-test ($n = 1569$, $p = 0.62$).

One thousand seventy-nine participants had detailed B-cell phenotyping data available. Of these participants, 675 remained eligible for genetic analyses after selection on the quality of genotype data, relatedness, and European ancestry (Figure S2). No significant differences were found between the characteristics of these participants and the participants who had total B-cell numbers available. No significant difference was found in the MS-PRS compared to the participants who had no detailed B-cell phenotyping data available ($n = 2155$, $p = 0.42$).

Total B-cell numbers

We found no significant association between the MS-PRSs' quartiles, concerning all thresholds, and absolute B-cell numbers (Table S1). We observed the strongest association with absolute

TABLE 1 Demographics and laboratory measurements in study participants

Characteristic	Absolute B-cell counts, $n = 1261$	B-cell phenotyping, $n = 675$
Age, median years (IQR)	6.0 (5.9–6.2)	6.0 (5.9–6.2)
Male, *n* (%)	641 (50.8)	340 (50.4)
EBV seropositivity, *n* (%)	512/1225 (41.8)	270/649 (41.6)
Reported presence of paternal MS, *n* (%)	2/1036 (0.2)	0/572 (0.0)
Reported presence of maternal MS, *n* (%)	2/1144 (0.2)	0/611 (0.0)
*HLA-DRB1*15:01* carriership, *n* at risk (%)	332/1261 (26.3)	175/675 (25.9)

Abbreviations: EBV, Epstein–Barr virus; IQR, interquartile range; MS, multiple sclerosis.

B-cell numbers when using a threshold of $p_T < 0.005$ to include SNPs in our PRS ($p = 0.07$). This threshold was used in subsequent detailed B-cell phenotyping analyses, which are described below.

Naive and memory B-cell subpopulations

The suggestive MS-PRS ($p_T < 0.005$) showed no significant associations with the relative frequencies of different B-cell subsets, including naive and memory B-cell subsets within the total B-cell population (Table S2).

Exclusion of the MHC region from the PRS did not significantly change these results (Table S3). The MS-PRS that only included variants from the MHC region showed significant associations with relative naive and memory B-cell frequencies, but these findings did not survive multiple testing correction (Table S4). Investigation of the effects of *HLA-DRB1*15:01* tag SNP rs3135388 showed comparable results to the MHC-PRS, a negative association with naive B-cell frequencies, and a positive association with memory B-cell frequencies, which were not significant after multiple testing correction (Table S5). The MS-PRS only including genome-wide significant ($p_T < 5 \times 10^{-8}$) risk variants from the MS genome-wide association studies (GWASs) showed similar significant associations with the B-cell subset frequencies of interest (Table S6). These findings were not significant after multiple testing correction.

Additional adjustment for EBV status did not significantly alter our observed results for the MS-PRSs (Tables S7 and S8). In conclusion, these results indicate no correlation between the suggestive and genome-wide significant MS-PRSs and proportions of subsets within the B-cell population.

B-cell PRSs

For a more targeted B-cell analysis, we grouped the autosomal genome-wide significant risk variants from IMSGC GWASs into B-cell subset-associated PRSs (Figure S1 and Table S9). We observed a significant association between the naive B-cell PRS ($n = 26$ variants) and naive B-cell frequencies ($p = 1.03 \times 10^{-4}$; Table S10). A post hoc Tukey test revealed significant negative associations between the different quartiles of the naive B-cell PRS (β first-fourth quartile = −0.034) and naive B-cell frequencies (Figure 1). None of the other B-cell subset-restricted PRSs showed significant associations (Tables S10 and S11).

When investigating the effects of the naive B-cell PRS on the other B-cell subpopulations of interest, we observed several additional significant associations (Table 2, Figure 1). We found a positive association with total memory B-cell frequencies ($p = 2.69 \times 10^{-4}$, β first-fourth quartile = 0.14) and relative CD27$^+$ memory B cells (Figure 1), resulting in a negative association with a naive/memory B-cell ratio. We observed no associations with CD21^{low} B-cell frequencies. The associations remained significant after adjustment for EBV seropositivity and *HLA-DRB1*15:01* status (Tables S12–S14). Similarly, following adjustment for the CD4$^+$/CD8$^+$ ratio,

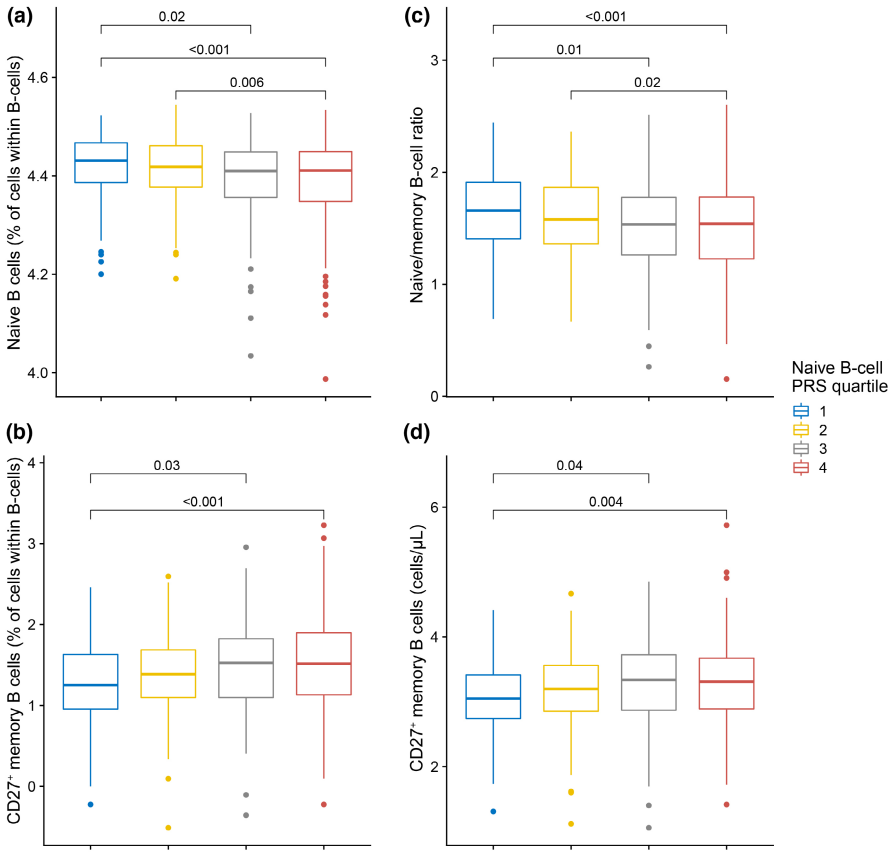

FIGURE 1 Effects of the naive B-cell polygenic risk score (PRS) quartiles on relative naive B cells and CD27$^+$ memory B-cell frequencies and absolute numbers. (a) Association between the naive B-cell multiple sclerosis (MS)-PRS and frequencies of naive B cells. (b) Association between the naive B-cell MS-PRS and frequencies of CD27$^+$ memory B cells. (c) Association between the naive B-cell MS-PRS and the naive/memory B-cell ratio. (d) Association between the naive B-cell MS-PRS and absolute values of CD27$^+$ memory B cells. Included were $n = 675$ children. Data are corrected for age, sex, and 10 genetic principal components. The PRS was divided into quartiles, and analyses of variance were used to investigate the effects of the PRS on B-cell subsets

TABLE 2 Analysis of variance results of the specific naive B-cell multiple sclerosis polygenic risk score and relative values of various B-cell subsets of interest

Immunological subset	Immunophenotype	Unadjusted *p*	FDR-adjusted *p*
Naive B cells	$CD27^{-}IgD^{+}CD19^{+}$	1.03×10^{-4}	1.17×10^{-3}[a]
Total memory B cells	$CD27^{+}IgD^{+}CD19^{+}$ + $CD27^{-}IgD^{-}CD19^{+}$ + $CD27^{+}IgD^{-}CD19^{+}$	2.69×10^{-4}	1.17×10^{-3}[a]
$CD27^{-}$ memory B cells	$CD27^{-}IgD^{-}CD19^{+}$	0.07	0.16
$CD27^{+}$ memory B cells	$CD27^{+}IgD^{-}CD19^{+}$	8.83×10^{-4}	2.87×10^{-3}[a]
IgM^{+} memory B cells	$CD27^{+}IgM^{+}CD19^{+}$	0.12	0.22
Natural effector B cells	$CD27^{+}IgM^{+}IgD^{+}CD19^{+}$	0.19	0.28
IgM-only B cells	$CD27^{+}IgM^{+}IgD^{-}CD19^{+}$	0.57	0.67
$CD27^{-}IgA^{+}$ B cells	$CD27^{-}IgA^{+}CD19^{+}$	0.16	0.26
$CD27^{+}IgA^{+}$ B cells	$CD27^{+}IgA^{+}CD19^{+}$	0.45	0.59
$CD27^{-}IgG^{+}$ B cells	$CD27^{-}IgG^{+}CD19^{+}$	0.72	0.75
$CD27^{+}IgG^{+}$ B cells	$CD27^{+}IgG^{+}CD19^{+}$	0.07	0.16
$CD21^{low}$ B cells	$CD38^{-/low}CD21^{low}CD19^{+}$	0.18	0.75
Naive B cells/total memory B cells		2.08×10^{-4}	1.17×10^{-3}[a]

Note: Included: $n = 675$ children. Data are corrected for age, sex, and 10 genetic principal components.

Abbreviation: FDR, false discovery rate.

[a]$p < 0.05$, significant values after FDR multiple testing correction.

the associations of the naive B-cell MS-PRS remained significant ($p = 5.73\times10^{-4}$; Table S15). Investigation of individual SNPs in the naive B-cell PRS showed significant associations of rs969625 (*BCL6*) and rs701006 (*OS9*) with naive B-cell frequencies (Tables S16 and S17). Gene ontology analysis of the SNPs comprising the naive B-cell PRS showed significant enrichment in various pathways, including B-cell activation and regulation of B-cell proliferation ($p = 6.05\times10^{-5}$ and $p = 8.45\times10^{-5}$, respectively; Table 3).

Absolute numbers of B-cell subsets

In addition to relative frequencies within total B cells, the absolute numbers of $CD27^{+}$ memory B cells were significantly associated with the naive B-cell MS-PRS. The association between the naive B-cell MS-PRS and absolute naive B-cell numbers was found to be non-significant, whereas the association with the ratio of naive/memory B-cell numbers remained significant (Table 4, Figure 1). The associations considering the $CD27^{+}$ memory B cells and naive/memory B-cell ratio persisted in significance after additional adjustment for EBV seropositivity and *HLA-DRB1*15:01* status (Tables S18–20). Thus, the naive B-cell PRS is superficially associated with an absolute (and relative) increase in memory B cells, which subsequently resulted in the observed relative decrease in naive B cells.

DISCUSSION

In this study, we reveal that naive B-cell-associated genetic risk variants for MS are associated with altered B-cell compartments in 6-year-old children from the general population. A selected set of 26 naive B-cell MS risk variants were associated with higher numbers of $CD27^{+}$ memory B cells in the blood. This was independent of EBV seropositivity, *HLA-DRB1*15:01* status, and the $CD4^{+}/CD8^{+}$ ratio. *HLA-DRB1*15:01* status, after multiple testing correction, was not associated with the distribution of B-cell subsets in children at an early age. These findings suggest that the presence of MS-risk SNPs involved in naive B cells has a specific impact on B-cell memory early in life, and can possibly contribute to the development of pathogenic B cells driving MS.

MS patients show defects in peripheral B-cell tolerance checkpoints, resulting in increased activation of mature naive B cells, which seem to be more sensitive to inflammatory stimuli such as interferon-γ and Toll-like receptor 9 ligands [3, 6, 32]. This could possibly predispose to their differentiation into memory B cells that are able to infiltrate the MS brain [6]. Because we did not find an association with absolute numbers of naive B cells, the observed increase in relative total memory B-cell numbers and relative and absolute $CD27^{+}$ memory B cells could be caused by elevated proliferation and activation of naive B cells expressing high levels of specific MS risk variants. Subsequently, this could predispose to an increased survival and clonal expansion of CNS-infiltrating B cells later in life in MS patients [6, 7].

We identified two autosomal genome-wide significant MS risk alleles that significantly altered the distribution of relative naive and memory B-cell subsets in pediatric blood. An intergenic variant near *BCL6* (rs969625) was associated with high MS risk and decreased naive/memory ratios, whereas an intronic variant in *OS9* (rs701006) corresponded to low MS risk and increased ratios [2]. Both *OS9* and *BCL6* are highly expressed in naive B cells [33] and although little is known about the role of *OS9* in B-cell maturation, *BCL6* is of importance in several pathways of the germinal center reaction, including

TABLE 3 Gene ontology analysis results of naive B-cell SNPs

GO biological process complete	Fold enrichment	Unadjusted *p*	FDR-adjusted *p*
Negative regulation of B-cell apoptotic process (GO:0002903)	>100	1.18×10^{-4}	0.03[a]
Negative regulation of immunoglobulin production (GO:0002638)	>100	1.59×10^{-4}	0.03[a]
Interleukin-15-mediated signaling pathway (GO:0035723)	>100	1.59×10^{-4}	0.03[a]
Regulation of B-cell proliferation (GO:0030888)	36.56	8.45×10^{-5}	0.02[a]
Regulation of antigen receptor-mediated signaling pathway (GO:0050854)	36.01	8.82×10^{-5}	0.02[a]
Positive regulation of angiogenesis (GO:0045766)	19.80	5.03×10^{-5}	0.02[a]
B-cell activation (GO:0042113)	18.86	6.05×10^{-5}	0.02[a]
Lymphocyte differentiation (GO:0030098)	18.71	7.07×10^{-7}	2.79×10^{-3}[a]
Regulation of lymphocyte differentiation (GO:0045619)	17.90	7.38×10^{-5}	0.02[a]
Regulation of cysteine-type endopeptidase activity involved in apoptotic process (GO:0043281)	14.53	1.62×10^{-4}	0.03[a]
Positive regulation of T-cell activation (GO:0050870)	14.40	1.68×10^{-4}	0.03[a]
Defense response (GO:0006952)	5.55	4.91×10^{-6}	7.06×10^{-3}[a]
Response to other organism (GO:0051707)	5.00	3.92×10^{-5}	0.02[a]
Negative regulation of signal transduction (GO:0009968)	4.86	1.47×10^{-4}	0.03[a]
Positive regulation of signal transduction (GO:0009967)	4.50	8.93×10^{-5}	0.02[a]
Intracellular signal transduction (GO:0035556)	4.25	1.39×10^{-4}	0.03[a]
Immune response (GO:0006955)	3.82	1.27×10^{-4}	0.03[a]
Positive regulation of cellular metabolic process (GO:0031325)	3.35	1.10×10^{-5}	0.01[a]
Positive regulation of nitrogen compound metabolic process (GO:0051173)	3.30	3.34×10^{-5}	0.01[a]
Positive regulation of macromolecule metabolic process (GO:0010604)	3.15	2.26×10^{-5}	0.01[a]

Note: Naive B-cell multiple sclerosis risk SNPs assigned to functional pathways using GO analysis [27–29].Abbreviations: FDR, false discovery rate; GO, gene ontology; SNP, single nucleotide polymorphism.

[a]$p<0.05$, significant enrichment values after multiple testing correction.

the regulation of apoptosis and activation, proliferation, and differentiation of B cells, resulting in the formation of plasmablasts/plasma cells and memory B cells [34]. Gene ontology analyses of all the genetic variants comprising the naive B-cell MS-PRS revealed significant enrichment in a number of functional pathways related to the germinal center reaction as well, including B-cell activation and regulation of B-cell proliferation. Additional negative regulation of B-cell apoptosis may potentiate a relative increase in autoreactive, possibly brain-homing, memory B-cell subsets in childhood, potentially contributing to clonal CSF expansion of memory B cells and the MS disease process later in life, which should be further studied in the near future [2, 6, 7].

Analyses involving the suggestive MS-PRS ($p_T<0.005$), MS-PRS only involving variants from the MHC complex, and the individual *HLA-DRB1*15:01* haplotype showed similar directions of effect on relative naive and memory B cells as the MS-PRS involving only naive B-cell risk variants. The *HLA-DRB1*15:01* haplotype is of importance in the interaction between B and T cells in adult MS patients, contributing to the ability of memory B cells to induce brain-homing $CD4^+$ T cells [10, 11, 35]. In an earlier study, we observed that higher genetic risk for MS and the *HLA-DRB1*15:01* haplotype increased the $CD4^+/CD8^+$ T-cell ratio in children from the general population [10]. The increased maturation of naive B cells observed in the current study in children with higher naive B-cell MS-PRS, independent of the $CD4^+/CD8^+$ T-cell ratio, was evident from higher frequencies and absolute numbers of $CD27^+$ memory B cells at a young age. The independent effects of genetic MS risk on B and T cells from children imply that certain risk variants have an impact on both compartments already at an early age, thereby affecting their interaction as one of the driving pathophysiological mechanisms behind MS.

The main remark that needs to be made on our current study, which often applies to PRS studies making use of disease-based genetic scoring, is on the subject of interpreting our findings and translating this to disease risk. Despite ongoing efforts to unravel the genetic architecture of MS, the SNP heritability for MS has been estimated at 19.2%, which limits the predictive ability of our PRS [2, 36]. Combined with the low population prevalence of MS and other factors contributing to MS risk not incorporated in this study (such as environmental risk factors and the interaction of these factors with genetic risk variants), it is unclear how our findings can be translated into an MS diagnosis later in life, because it remains elusive how many of the children in our sample will be diagnosed with MS in their life course. Because of this, our findings should be

TABLE 4 Analysis of variance results of the specific naive B-cell multiple sclerosis polygenic risk score and absolute values of various B-cell subsets of interest

Immunological subset	Immunophenotype	Unadjusted *p*	FDR-adjusted *p*
Naive B cells	$CD27^{-}IgD^{+}CD19^{+}$	0.82	0.86
Total memory B cells	$CD27^{+}IgD^{+}CD19^{+}$ + $CD27^{-}IgD^{-}CD19^{+}$ + $CD27^{+}IgD^{-}CD19^{+}$	0.02	0.09
$CD27^{-}$ memory B cells	$CD27^{-}IgD^{-}CD19^{+}$	0.22	0.40
$CD27^{+}$ memory B cells	$CD27^{+}IgD^{-}CD19^{+}$	4.89×10^{-3}	0.03[a]
IgM^{+} memory B cells	$CD27^{+}IgM^{+}CD19^{+}$	0.15	0.38
Natural effector B cells	$CD27^{+}IgM^{+}IgD^{+}CD19^{+}$	0.31	0.50
IgM-only B cells	$CD27^{+}IgM^{+}IgD^{-}CD19^{+}$	0.21	0.40
$CD27^{-}IgA^{+}$ B cells	$CD27^{-}IgA^{+}CD19^{+}$	0.36	0.50
$CD27^{+}IgA^{+}$ B cells	$CD27^{+}IgA^{+}CD19^{+}$	0.39	0.50
$CD27^{-}IgG^{+}$ B cells	$CD27^{-}IgG^{+}CD19^{+}$	0.79	0.86
$CD27^{+}IgG^{+}$ B cells	$CD27^{+}IgG^{+}CD19^{+}$	0.10	0.32
$CD21^{low}$ B cells	$CD38^{-/low}CD21^{low}CD19^{+}$	0.86	0.86
Naive B cells/total memory B cells		2.29×10^{-4}	2.97×10^{-3}[a]

Note: Included: $n = 673$ children, data are corrected for age, sex, and 10 genetic principal components.

Abbreviation: FDR, false discovery rate.

[a]$p < 0.05$, significant values after FDR multiple testing correction.

interpreted with caution, but we do believe they give important insights into the genetic pathophysiology of MS, which is necessary to unravel the mechanisms behind MS. In addition, on the topic of interpretability of our findings, MS GWASs are based largely on individuals with European ancestry [2]. Because of this, we included only participants of European ancestry in our analyses, but this limits the generalization of our results to individuals of non-European ancestry [36]. Because the genetic architecture of MS is found to be different in non-European ancestries [36, 37], additional steps need to be made in the inclusivity of genetic MS studies to allow broader application of results to populations of different ancestries.

Another limitation to our study involves the nonsignificance of the analyses considering the *HLA-DRB1*15:01* haplotype and MHC complex, which might suggest that these risk variants have no influence on the peripheral B-cell compartment in childhood. However, the lack of findings could also be due to power, although we did have a reasonable sample size for pediatric population-based studies. Suggestive genetic risk variants for MS and *HLA-DRB1*15:01* status could still alter this compartment at a young age, warranting population studies with large sample sizes to capture their polygenic effect. Additionally, our study is unable to analyze the functional properties of the B-cell subpopulations used in this study.

In addition to limitations, our study has several strengths. Despite being potentially underpowered for the detection of subtle polygenic effects, we included the largest number of children from the general population to date to explore the effects of MS genetics on the peripheral B-cell compartment in childhood. Furthermore, we constructed specific B-cell PRSs, based on a systems biology approach combining various databases, to investigate the influences of certain groups of MS risk variants, providing additional insights into early MS pathophysiology. Our study also demonstrates the importance of analyzing absolute values of immunological subsets in addition to relative frequencies to allow the correct interpretation of results.

Additional studies are warranted to investigate the longitudinal dynamics between MS genetics and the B-cell compartment, with serial sampling points of the immunological outcomes of interest. At the young age of our participants, the immune system is still in development, where the percentage of naive B cells may still vary in children throughout adolescence [38]. Longitudinal sampling would therefore increase the robustness of discovered associations. Further incorporation of environmental risk factors for MS in future studies such as EBV infection are needed to account for the epigenetic effects on B-cell-associated risk variants and fully characterize the early disease pathophysiology of MS.

To summarize, we report associations between B-cell-associated MS risk variants and circulating naive and memory B cells in children from the general population. We show that MS genetics correlate with the composition of the B-cell compartment early in life, possibly laying the foundation for MS disease later in life.

ACKNOWLEDGMENTS

We are thankful for the data issued by the International Multiple Sclerosis Genetics Consortium that were used in calculating the genetic risk scores for this study.

We are indebted to the children and parents, general practitioners, hospitals, midwives, and pharmacies in Rotterdam for their commitment to the Generation R Study.

FUNDING INFORMATION

This study was sponsored by the Dutch MS Research Foundation. The Generation R Study is made possible by continuous support from the Erasmus MC, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the NWO, and the Ministry of Health, Welfare, and Sport.

CONFLICT OF INTEREST

R.F.N. participates in trials with Sanofi Genzyme and Novartis. None of the other authors has any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

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)

ORCID

Casper L. de Mol https://orcid.org/0000-0002-3733-1706
Joost Smolders https://orcid.org/0000-0001-9766-8661

REFERENCES

1. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. *Neuron*. 2006;52(1):61-76. doi:10.1016/j.neuron.2006.09.011
2. Consortium IMSG. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. *Science*. 2019;365(6460):eaav7188.
3. Kinnunen T, Chamberlain N, Morbach H, et al. Specific peripheral B cell tolerance defects in patients with multiple sclerosis. *J Clin Invest*. 2013;123:2737-2741.
4. DiSano KD, Gilli F, Pachner AR. Memory B cells in multiple sclerosis: emerging players in disease pathogenesis. *Front Immunol*. 2021;12:676686. doi:10.3389/fimmu.2021.676686
5. Fransen NL, de Jong BA, Heß K, et al. Absence of B cells in brainstem and White matter lesions associates with less severe disease and absence of oligoclonal bands in MS. *Neurol Neuroimmunol Neuroinflamm*. 2021;8(2):e955.
6. van Langelaar J, Rijvers L, Janssen M, et al. Induction of brain-infiltrating T-bet–expressing B cells in multiple sclerosis. *Ann Neurol*. 2019;86(2):264-278.
7. Beltrán E, Gerdes LA, Hansen J, et al. Early adaptive immune activation detected in monozygotic twins with prodromal multiple sclerosis. *J Clin Invest*. 2019;129(11):4758-4768.
8. Hauser SL, Waubant E, Arnold DL, et al. B-cell depletion with rituximab in relapsing–remitting multiple sclerosis. *N Engl J Med*. 2008;358(7):676-688.
9. de Mol CL, Jansen PR, Muetzel RL, et al. Polygenic multiple sclerosis risk and population-based childhood brain imaging. *Ann Neurol*. 2020;87(5):774-787.
10. de Mol CL, Looman KIM, van Luijn MM, et al. T cell composition and polygenic multiple sclerosis risk: a population-based study in children. *Eur J Neurol*. 2021;28:3731-3741.
11. Jelcic I, Al Nimer F, Wang J, et al. Memory B cells activate brain-homing, autoreactive CD4+ T cells in multiple sclerosis. *Cell*. 2018;175:85-100.e23.
12. Kooijman MN, Kruithof CJ, van Duijn CM, et al. The generation R study: design and cohort update 2017. *Eur J Epidemiol*. 2016;31(12):1243-1264.
13. Van Den Heuvel D, Jansen MAE, Dik WA, et al. Cytomegalovirus- and epstein-barr virus-induced T-cell expansions in young children do not impair naive T-cell populations or vaccination responses: the generation R study. *J Infect Dis*. 2016;213(2):233-242.
14. van den Heuvel D, Jansen MAE, Nasserinejad K, et al. Effects of non-genetic factors on immune cell dynamics in early childhood: the generation R study. *J Allergy Clin Immunol*. 2017;139(6):1923-1934.e17.
15. van den Heuvel D, Jansen MAE, Bell AI, et al. Transient reduction in IgA + and IgG + memory B cell numbers in young EBV-seropositive children: the generation R study. *J Leukoc Biol*. 2017;101(4):949-956.
16. Kalina T, Flores-Montero J, Van Der Velden VHJ, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia*. 2012;26(9):1986-2010.
17. Driessen GJ, Van Zelm MC, Van Hagen PM, et al. B-cell replication history and somatic hypermutation status identify distinct pathophysiologic backgrounds in common variable immunodeficiency. *Blood*. 2011;118(26):6814-6823.
18. Medina-Gomez C, Felix JF, Estrada K, Peters MJ, Herrera L, Kruithof CJ, et al. Challenges in conducting genome-wide association studies in highly admixed multi-ethnic populations: the generation R study. *Eur J Epidemiol* 2015 30(4):317–30. Available from: https://pubmed.ncbi.nlm.nih.gov/25762173
19. The International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426(6968):789-796. doi:10.1038/nature02168
20. University of Michigan. 1000G Phase I Integrated Release Version 3 Haplotypes [Internet]. 2012. Available from: http://csg.sph.umich.edu/abecasis/MACH/download/1000G.2012-03-14.html
21. Euesden J, Lewis CM, O'Reilly PF. PRSice: polygenic risk score software. *Bioinformatics*. 2015;31(9):1466-1468.
22. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559-575.
23. Živković M, Stanković A, Dinčić E, et al. The tag SNP for HLA-DRB1$_*$1501, rs3135388, is significantly associated with multiple sclerosis susceptibility: cost-effective high-throughput detection by real-time PCR. *Clin Chim Acta*. 2009;406(1–2):27-30. doi:10.1016/j.cca.2009.05.004
24. Schmiedel BJ, Singh D, Madrigal A, et al. Impact of genetic polymorphisms on human immune cell gene expression. *Cell*. 2018;175(6):1701-1715.e16.
25. Chandra V, Bhattacharyya S, Schmiedel BJ, et al. Promoter-interacting expression quantitative trait loci are enriched for functional genetic variants. *Nat Genet*. 2021;53(1):110-119.
26. Wu C, Jin X, Tsueng G, Afrasiabi C, Su AI. BioGPS: building your own mash-up of gene annotations and expression profiles. *Nucleic Acids Res*. 2016;44:D313-D316.
27. Mi H, Muruganujan A, Thomas PD. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. *Nucleic Acids Res*. 2013;41(D1):D377-D386.
28. Healy J, Thomas EE, Schwartz JT, Wigler M. Annotating large genomes with exact word matches. *Genome Res*. 2003;13(10):2306-2315.
29. Huerta-Cepas J, Szklarczyk D, Heller D, et al. EggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res*. 2019;47(D1):D309-D314.
30. 3.5.1. RDCT. A language and environment for statistical computing. R foundation for R foundation for statistical Computing 2018.
31. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B*. 1995;57(1):289-300. doi:10.1111/j.2517-6161.1995.tb02031.x
32. Bar-Or A, Fawaz L, Fan B, et al. Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? *Ann Neurol*. 2010;67:452-461.
33. Berkowska MA, Schickel J-N, Grosserichter-Wagener C, et al. Circulating human CD27 − IgA + memory B cells recognize bacteria with polyreactive Igs. *J Immunol*. 2015;195:1417-1426.

34. Basso K, Dalla-Favera R. Roles of BCL6 in normal and transformed germinal center B cells. *Immunol Rev*. 2012;247(1):172-183.
35. Kisielow P, Teh HS, Blüthmann H, Von Boehmer H. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature*. 1988;335(6192):730-733.
36. Hone L, Giovannoni G, Dobson R, Jacobs BM. Predicting multiple sclerosis: challenges and opportunities. *Front Neurol*. 2022;12:761973.
37. Beecham AH, Amezcua L, Chinea A, et al. The genetic diversity of multiple sclerosis risk among Hispanic and African American populations living in the United States. *Mult Scler J*. 2020;26:1329-1339.
38. Blanco E, Pérez-Andrés M, Arriba-Méndez S, et al. Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. *J Allergy Clin Immunol*. 2018;141:2208-2219.e16.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: de Mol CL, van Luijn MM, Kreft KL, et al. Multiple sclerosis risk variants influence the peripheral B-cell compartment early in life in the general population. *Eur J Neurol*. 2022;00:1-9. doi: 10.1111/ene.15582

MANAGE-PD

Tool for Making Informed Decisions to **Aid Timely Management of Parkinson's Disease**

MANAGE-PD allows you to:

- Identify PD patients inadequately controlled on oral medications
- Determine which patients with PD may be adequately controlled on their current treatment regimen or may require changes to their treatment regimen

Scan the QR code to access to the web

Click here to access to the web

MANAGE-PD is an AbbVie Inc. registered Medical Device. It is a collaborative research and development effort between AbbVie Medical Affairs and Health Economics and Outcomes, the Parkinson's Foundation and an international panel of Movement Disorder Specialists.

PD: Parkinson´s Disease

Parkinson's Foundation

Veeva Code: ABBV-AA-00335-FM Approved: January 2022

abbvie