Copy number variations across the blood–brain barrier in multiple sclerosis

Sahl Khalid Bedri1, Björn Evertsson1,2, Mohsen Khademi1, Faiez Al Nimer1,2, Tomas Olsson1,2, Jan Hillert1,2 & Anna Glaser1

1Department of Clinical Neuroscience and Centrum for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden
2Karolinska University Hospital, Tema Neuro, Stockholm, Sweden

Correspondence
Anna Glaser, Karolinska Institutet, Tomtebodavägen 18A Floor 5, 171 77 Stockholm, Sweden. Tel: +46 765557253; Fax: +46 8524 83049; E-mail: anna.glaser@ki.se

Funding Information
This project was supported by funds from Stiftelsen Goljes Minne, NEURO Sweden and The Nilsson-Ehle Endowments.

Annals of Clinical and Translational Neurology 2022; 9(7): 962–976
doi: 10.1002/acn3.51573

Abstract

Objective: Multiple sclerosis (MS) is a neuroinflammatory disease where immune cells cross the blood–brain barrier (BBB) into the central nervous system (CNS). What predisposes these immune cells to cross the BBB is still unknown. Here, we examine the possibility that genomic rearrangements could predispose specific immune cells in the peripheral blood to cross the BBB and form sub-populations of cells involved in the inflammatory process in the CNS.

Methods: We compared copy number variations in paired peripheral blood mononuclear cells (PBMCs) and cerebrospinal fluid (CSF) cells from MS patients. Thereafter, using next generation sequencing, we studied the T-cell receptor beta (TRB) locus rearrangements and profiled the T cell repertoire in peripheral CD4+ and CD8+ T cells and in the CSF.

Results: We identified deletions in the T-cell receptor alpha/delta (TRA/D), gamma (TRG), and TRB loci in CSF cells compared to PBMCs. Further characterization revealed diversity of the TRB locus which was used to describe the character and clonal expansion of T cells in the CNS. T-cell repertoire profiling from either side of the BBB concluded that the most frequent clones in the CSF samples are unique to an individual. Furthermore, we observed a difference in the proportion of expanded T-cell clones when comparing samples from MS patients in relapse and remission with opposite trends in CSF and peripheral blood.

Interpretation: This study provides a characterization of the T cells in the CSF and might indicate a role of expanded clones in MS pathogenicity.

Introduction

Multiple sclerosis (MS), is a chronic demyelinating inflammatory disease of the central nervous system (CNS) and a complex disease involving both genetic and environmental factors. Genetic analysis of MS has made significant progress in the past years as a result of large international research collaborations. These studies have focused on genome wide association studies (GWAS) of samples from several thousand MS patients and healthy controls and have resulted in the identification of more than 200 MS associated genetic variants, known as single nucleotide polymorphisms, which can be significantly associated with MS susceptibility.1-3 Currently the genetic variants associated with MS explain <1/3 of the total MS heritability.3 Hence, the issue of “missing heritability” in the field of MS genetics has been introduced4 and there have been several suggestions on how to reveal this missing heritability including analyzing genetic pathways, studying rare genetic variants, and applying more sophisticated analysis methods and whole genome or exome sequencing initiatives.5

There has also been attempts to study other types of genetic variants, such as copy number variations (CNVs), which include insertions, duplications, and deletions of a DNA segment ranging from a couple of thousands to a few million base-pairs. Baranzini et al. have compared CNVs between the genomes of peripheral blood mononuclear cells (PBMCs) and cerebrospinal fluid (CSF) cells from MS patients. Thereafter, using next generation sequencing, they studied the T-cell receptor beta (TRB) locus rearrangements and profiled the T cell repertoire in peripheral CD4+ and CD8+ T cells and in the CSF.
of MS before the age of 18 years, using comparative genomic hybridization arrays and finding de novo CNVs.  

In the present study, we hypothesize that genetic variants within a subpopulation of immune cells could make these cells more prone to invade the CNS with an impact on the inflammatory process and subsequent consequences for the MS process. This would represent a form of somatic mosaicism as this subpopulation of immune cells will be genetically different from the majority of cells in the peripheral circulation within the same individual. The possibility of somatic mosaicism in complex diseases has generally not been taken into consideration. It is however important for the understanding of the etiology of other diseases such as cancer development which is one of the classical examples, but somatic mosaicism has been also established in a number of monogenetic disorders such as hemophilia A and neurofibromatosis type 1. It has also attracted great interest in understanding the etiology of neuropsychiatric diseases such as schizophrenia. Furthermore, the interest in somatic mosaicism in autoimmune diseases is gaining momentum. To test our hypothesis, we aimed to identify CNVs between immune cells inside and outside the CNS in MS patients.

Materials and Methods

Samples collection

Paired peripheral blood (PB) and cerebrospinal fluid (CSF) samples were collected from MS patients with consent at the Neurology Clinic at Karolinska University Hospital, Sweden. Samples were also collected from patients with other neurological diseases (ONDs) and healthy controls (HC). These samples were collected as part of the Stockholm prospective assessment of MS (STOPMS) I (DNR 02–548, Stockholm) and II (DNR 2009/2107–31/2, Stockholm) projects. A total of 38 individuals (29 MS patients, six non-MS patients, and three HCs) were included in this study (Table 1). PB samples were collected in sodium citrate-containing cell preparation tubes (BD Vacutainer™ CPT™ Tube; BD Biosciences, Franklin Lakes, NJ, USA) and peripheral blood mononuclear cells (PBMCs) were isolated according to the manufacturer’s protocol. Isolated PBMCs were stained with brilliant Violet 421™ anti-human CD3 Antibody (BioLegend, San Diego, CA, USA) and anti-TCRγ/δ-PE (REA591; Miltenyi Biotec, Bergisch Gladbach, Germany) and cells were sorted on BD influx (BD Biosciences, Franklin Lakes, NJ, USA).

DNA extraction

DNA was extracted from paired PBMCs, CD4+, CD8+, γ/δ T, and CSF cells simultaneously using QIAamp DNA mini kit (Qiagen, Düsseldorf, Germany). Extracting enough DNA from the CSF cells was the bottleneck for including these samples in this study. The amount of DNA used for each analysis is mentioned in its respective methods sections.

CNV detection

The CytoScan HD Array (Affymetrix, Santa Clara, CA, USA) at the Array and Analysis Core Facility at Uppsala University was used for CNV comparison in the paired PBMCs and CSF cells samples. A minimum yield of 140 ng DNA was used. The CytoScan HD Array is specifically designed for CNV detection. It contains approximately 2.7 million markers covering all OMIM and RefSeq genes, with intragenic and intergenic markers spacing of 880 and 1737 base-pairs, respectively. The intensities from each probe was normalized to a reference panel using the chromosome analysis suite Software, calculating the log R ratio. CNVs between the PB and CSF were identified using the Nexus Copy Number software (BioDiscovery Inc, Hawthorne, CA, USA) and a threshold of a minimum five consecutive probes for calling a CNV was used.

Validation of the CNV

Further validation of the CNV regions identified by the array was performed using Taqman copy number assays on Quantstudio 7 flex real time PCR system (Applied Biosystems, Waltham, MA, USA). An approximate 2 ng of DNA per reaction was used. Taqman copy number assays for the human T-cell receptor (TCR) gamma (TRG) (Hs07530615_cn, Hs03646230_cn, and Hs04980855_cn), TCR beta (TRB) (Hs04330161_cn, Hs04329666_cn, Hs03643995_cn, and Hs07530853_cn), and TCR alpha (TRA) (Hs03308605_cn and Hs03094858_cn) regions were used. From the CT values of the real-time PCR run, the copy numbers of the target genes was calculated using PBMCs for each individual as a calibrator on the CopyCaller™ Software (Applied Biosystems, Waltham, MA, USA).
We investigated the clonality of the T cells by studying the TRB locus rearrangements using next generation sequencing. Library preparation was performed with the LymphoTrack®/C226 TRB assay-MiSeq kit (72250009; Invivoscribe, San Diego, CA, USA), where primers in the kit target the conserved Vβ and Jβ regions of the TRB locus. The amount of DNA used per library PCR reaction from the CD4+, CD8+, and CSF samples, was on average 47.9, 53.2, and 38.3 ng, respectively. Paired-end 2 × 250 sequencing was done using the MiSeq Reagent Kit v2 (MS-102–2003, Illumina, San Diego, CA, USA) on the Illumina MiSeq platform at the Bioinformatics and

| ID     | Sex | Age at sampling | Diagnosis                      | Treatment status | CSF-monouclear cells, × 10⁹/L | Method               |
|--------|-----|----------------|--------------------------------|-----------------|-------------------------------|----------------------|
| 14-036 | M   | 39             | RRMS, remission                | Not treated     | 10                            | CytoScan HD Array    |
| 14-087 | F   | 31             | RRMS, remission                | Not treated     | 22                            | CytoScan HD Array, MiSeq |
| 14-250 | F   | 22             | RRMS, relapse                  | Not treated     | 14                            | CytoScan HD Array, MiSeq |
| 14-265 | F   | 35             | RRMS, remission                | Not treated     | 8                             | CytoScan HD Array    |
| 15-138 | M   | 24             | HC                             | NA              | 4                             | CytoScan HD Array    |
| 15-237 | M   | 22             | HC                             | NA              | 2                             | CytoScan HD Array    |
| 14-131 | F   | 31             | RRMS                           | Not treated     | 8                             | CytoScan HD Array    |
| 14-155 | F   | 42             | RRMS, remission                | Not treated     | 8                             | CytoScan HD Array, MiSeq |
| 09-073 | M   | 36             | PPMS                           | Not treated     | 4                             | CytoScan HD Array    |
| 07-98  | M   | 33             | RRMS                           | Not treated     | 47                            | Taqman               |
| 07-381 | F   | 30             | RRMS                           | Not treated     | 5.8                           | Taqman               |
| 07-564 | M   | 34             | RRMS                           | Not treated     | 8.6                           | Taqman               |
| 08-454 | F   | 19             | RRMS                           | Not treated     | 12.8                          | Taqman               |
| 11-439 | F   | 32             | RRMS                           | Not treated     | 8.3                           | Taqman               |
| 12-447 | F   | 40             | RRMS                           | Not treated     | 9.8                           | Taqman               |
| 12-449 | F   | 26             | RRMS                           | Not treated     | 14.8                          | Taqman               |
| 14-111 | M   | 33             | RRMS                           | Not treated     | 2                             | Taqman               |
| 15-213 | M   | 21             | HC                             | NA              | 4                             | Taqman               |
| 17-8608| F   | 41             | RRMS, relapse                  | Not treated     | 2.9                           | Taqman               |
| 17-8805| F   | 38             | RRMS, remission                | Rituximab       | 6                             | Taqman               |
| 17-8813| M   | 43             | RIS                            | Not treated     | 2.8                           | Taqman               |
| 17-8823| F   | 40             | RRMS, relapse                  | Not treated     | 3                             | Taqman, MiSeq        |
| 17-8832| F   | 28             | RRMS, remission                | Fingolimod      | 5                             | Taqman               |
| 17-8838| F   | 44             | RRMS, remission                | Not treated     | 10                            | Taqman, MiSeq        |
| 17-8839| F   | 33             | Brain tumor + CIS, relapse     | Not treated     | 11                            | Taqman               |
| 17-8847| F   | 42             | OND¹                           | NA              | 2                             | Taqman               |
| 17-8848| F   | 55             | RRMS, relapse                  | Not treated     | 2                             | Taqman               |
| 14-003 | F   | 43             | OND²                           | NA              | 12                            | Taqman               |
| 14-205 | F   | 53             | OND³                           | NA              | 33                            | Taqman               |
| 14-272 | M   | 21             | OND⁴                           | NA              | 131                           | Taqman               |
| 17-8803| M   | 63             | PTSD (headache)                | NA              | 2                             | Taqman               |
| 17-8809| F   | 73             | OND⁵                           | NA              | 3                             | Taqman               |
| 17-8801| M   | 30             | RRMS, relapse                  | Not treated     | 44.7                          | MiSeq                |
| 18-8856| F   | 40             | RRMS, remission                | Not treated     | 4                             | MiSeq                |
| 17-465 | M   | 28             | RRMS, relapse                  | Not treated     | 42.2                          | MiSeq                |
| 14-137 | F   | 32             | RRMS, remission                | Not treated     | 2                             | MiSeq                |
| 16-098 | F   | 35             | RRMS, remission                | Not treated     | 8                             | MiSeq                |
| 16-223 | F   | 32             | RRMS, relapse                  | Not treated     | <1                            | MiSeq                |

CSF, cerebrospinal fluid; RRMS, relapsing remitting MS; PPMS, primary progressive MS; HC, healthy controls; RIS, radiologically isolated syndrome; CIS, clinically isolated syndrome; PTSD, post-traumatic stress disorder; OND, other neurological disease.
¹Tension headache.
²Demyelinating disease.
³SLE and aseptic meningitis.
⁴Idiopathic intrathecal hypertension.
⁵Herpes encephalitis.
Expression Analysis facility at Karolinska Institute. Eight samples were run per flow cell, including positive and negative controls. The generated FASTAQ data were processed using the MiXCR software to assemble the clonotypes and provide the highly variable CDR3 sequence. The software VDJtools was used for further analysis of the TCR repertoire.

### CDR3 sequences annotation

To identify the specificity of the detected T-cell clones we searched in the publicly available VDJdb database for matching TCR with previously known antigen specificity using the software VDJmatch version 1.3.1.

### Statistical analysis

Comparison of the copy number of the target genes between MS and non-MS patients was done using Wilcoxon rank-sum test. From the TRB locus sequencing, a unique CDR3 nucleotide sequence represents a unique T-cell clone. The frequency of a clone is defined as its sequence count compared to the total count of all sequences in a sample. Clones with a frequency ≥0.1% were considered as expanded and clones with a frequency <0.1% as non-expanded. Wilcoxon rank-sum test was also used to compare the proportions of expanded clones between independent groups. Statistical analysis and graphs were done using R software version 3.3.2.

### Results

#### Whole genome CNVs screening

In the initial screen we used the cytoscan HD array to search for CNV between paired CSF and PBMCs samples from six relapsing remitting MS (RRMS) patients, one primary progressive MS patient and two HC. We could detect CNVs in three regions on chromosomes 14, 7q, and 7p consistent with the TRA/D, TRG, and TRB loci. These CNVs were present in 8/9, 7/9, and 5/9 samples for TRA/D, TRG, and TRB, respectively and indicated deletions in all three regions when comparing CSF to PB (Fig. 1). The extent of the deleted regions for the different samples could be mapped using the array data. The deletions were larger in the TRA/D locus with a median length of ≈296 kb, while for TRG and TRB were ≈61 and 92 kb, respectively.

#### Validation of the identified CNVs

In order to confirm and further explore the CNVs across the TCR regions we analyzed a further 12 paired CSF and PBMCs DNA samples from eight RRMS, three OND patients, and one HC using TaqMan analysis with probes mapping across the TCR regions which had been identified in the previous screen (Table 2). The results from the TaqMan analysis confirmed the deletions in the CSF cells across the TRA/D and TRG regions where deletions could not be detected in the PB cells.

---

*Figure 1. Whole genome CNVs between PB and CSF cells from the seven MS patients (14-036, 14-087, 14-250, 14-265, 14-131, 14-155, and 09-073) and two HC (15-237 and 15-138). The upper panel shows the CNV frequency plot for all nine individuals with blue upward bars or red downward bars indicating more copy numbers in the PB or more copy numbers in the CSF cells respectively, with the chromosome numbers indicated at the top of the image. The panel below shows the annotation tracks for genes, exons, CNVs & miRNA according to a reference database. The lower panel shows the CNV between PB and CSF for each individual. CNV, copy number variation; PB, peripheral blood; CSF, cerebrospinal fluid; HC, heathy controls.*

© 2022 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals LLC on behalf of American Neurological Association.
Table 2. Taqman copy number assays used for the CNV validation.

| Taqman_assay id | Chromosome | Position | Gene      |
|-----------------|------------|----------|-----------|
| TRG             | chr7       | 38248115 | TCRG2     |
| Hs035646290_cn  | chr7       | 38278966 | TRGJ1P1   |
| Hs04980855_cn   | chr7       | 38369439 | TRG-AS1   |
| Hs043301611_cn  | chr7       | 142657429| TRBV12    |
| Hs04329666_cn   | chr7       | 142738548| TRBV24-1  |
| Hs03643995_cn   | chr7       | 142765864| PRSS2     |
| Hs07530853_cn   | chr7       | 142806573| TRBC2/IRBV30 |
| TRA/D           | chr14      | 22300377 | TRAV39    |
| Hs03308605_cn   | chr14      | 22300377 | TRAV39    |
| Hs03094858     | chr14      | 22409503 | TRAV39    |

CNV, copy number variation; TRG, T-cell receptor gamma; TRA/D, T-cell receptor alpha/delta; TRB, T-cell receptor beta.

be identified in 11/12 and 9/12 of the samples, respectively (Fig. 2A). The results for the TRB locus revealed a more complex structure where the extent of the deletion varied between samples. Even though the number of non-MS individuals was low, we compared the deletions in the TCR regions between the eight RRMS and four non-MS (three OND patients and one HC). Only deletions in the TRB locus of CSF cells were significantly different in RRMS than non-MS (p = 0.03), with RRMS having less copy numbers, that is, more deletions, in the TRB locus (Fig. 2B).

**CNVs in paired CD4+, CD8+ T, and CSF cells**

For the purpose of further study of TCR deletions in different types of T cells we compared CD4+ and CD8+ cells to CSF cell samples from six RRMS, one clinically isolated syndrome, one radiologically isolated syndrome, one SPMS, and two OND patients. The results from this analysis were in agreement with the previous observation that TRA/D and TRG deletions were consistent across the analyzed regions and that TRB displayed a more complex structure with variations in the extent of deleted regions within CSF samples from different individuals as well as from different types of T cells within one individual (Fig. 3).

**CNVs in paired γδ T and CSF cells**

Because the initial screen revealed less deletions of TRB compared to the TRA/D and TRG regions in CSF samples we wanted to examine the possibility of γδ T cells constituting a larger proportion of CSF cells as compared to PB. We therefore purified γδ T cells from PB and compared the CNV pattern in these cells with CSF samples and CD4+ and CD8+ cells. We did not detect any similarities between the CNV pattern of CSF and γδ T cells (Fig. 4). As expected, the γδ T cells demonstrated deletions in the TRG region. The position of our TRA probes did not allow specific detection of TRD deletions. However, we did detect deletions of TRB region in all the γδ T cells samples that we analyzed (Fig. 4). The extent of the TRB deletions varied between the γδ T cells samples.

**Clonality in MS patients**

Based on that the TRB locus displayed a more complex structure with inter-individual and intra-variations between CSF, and CD4+ and CD8+ T cells, we studied the TRB locus rearrangements and profiled the αβ T cell repertoire in peripheral CD4+ and CD8+ T cells and in the CSF. The average number of unique TRB sequences in the studied MS patients were 8206 (±1820), 6265 (±2810), and 6383 (±2021) in CD4+ T cells, CD8+ T cells and in CSF cells, respectively (Table 3). Each unique CDR3 or TRB nucleotide sequence, as a result of TRB locus rearrangements, is considered a unique clone, and when using a threshold for clonal expansion of 0.1% in all three compartments, most of their frequencies was under 0.1% (Fig. 5). CD4+ T cells were the most diverse compared to CD8+ T cells and CSF cells, which displayed comparable number of unique clones (Fig. 6).

**Intra- and interindividual overlap of expanded clones**

We wanted to study expanded clones in the CSF with a potential role in the neuroinflammatory process by comparing the occurrence of expanded clones in the CSF and the periphery. CSF samples had an average of 70.4 expanded clones, which could also be found (as expanded or non-expanded clones) in 39 and 43% of the paired CD4+ and CD8+ samples, respectively (Table 3).

Table 4 presents the five most frequent clones in the CSF and their frequency in the periphery if present in the CD4+ and CD8+ compartments.

When comparing the nucleotide sequence of the expanded clones between different individuals (interindividual comparison) there seems to be no overlap of the expanded clones. However, an interindividual comparison on the basis of the amino acid sequence shows an overlap of five clones between different pairs of individuals. CSF samples from patients 14–087 and 14–250 had two clones that were shared with another patient (Table 5).
Relapse and T-cell clonality

To investigate whether the clonality of the T cells in CSF, CD4\(^+\), and CD8\(^+\) cell compartments is related to the MS patient being under relapse or remission at the time of sampling, we compared the proportion of the expanded T-cell clones in each separate compartment in patients under relapse (for each CD4\(^+\) and CD8\(^+\) cell compartments \(n = 4\) and for CSF \(n = 5\)) and in remission (for each CD4\(^+\) and CD8\(^+\) cell compartments \(n = 4\) and for

Figure 2. Validation of CNVs in CSF cells using Taqman copy number assays targeting genes in the TRG, TRB, and TRA regions. CSF cells are compared to PBMCs and PBMCs in each individual was used as a calibrator in the CopyCaller™ Software (Applied Biosystems) to calculate the CN of the target genes, that is, for each target gene the CN in CSF cells is calculated in comparison to CN in the PBMCs. (A) Showing the individual variations in copy numbers of the target genes. (B) Comparing the copy numbers of the target genes of MS (\(n = 8\)) to non-MS (\(n = 4\)). Wilcoxon rank-sum test was used for the statistical testing and generating the \(p\) values presented in the figure. ns, not significant; CNVs, copy number variations; CSF, cerebrospinal fluid; CN, copy number; TRG, T-cell receptor gamma; TRA, T-cell receptor alpha; TRB, T-cell receptor beta; PBMCs, peripheral blood mononuclear cells; MS, multiple sclerosis.
The proportion of expanded T-cell clones was higher in patients under relapse than in remission in the CSF cells, while showing the opposite in the periphery in both CD8\(^+\) cells and CD4\(^+\) cells although the observed differences were not statistically significant (Fig. 7).

**T-cell clone specificity**

Taking advantage of publicly available databases for TCR sequences\(^{15}\) and their known targets, we performed an in silico investigation of the specificity of the T-cell clones. We used the CDR3 sequences of our identified T-cell clones provided by MIXCR and searched for matches in the VDJdb database. The CDR3 sequences and their target antigens matches with moderate and high confidence scores included clones specific for antigens presented by different viruses such as Epstein–Barr virus (EBV), cytomegalovirus, hepatitis C virus, yellow fever virus, influenza A, HIV, and Dengue virus. From 332 matching clones, nine were expanded, of which five clones were targeting three EBV antigens; EBNA3A, EBNA3B, and BMLF1. One of the clones targeting EBNA3A was expanded in all three compartments of patient 17-8838 and the same patient had another expanded clone targeting EBNA3B present in the CD8\(^+\) and CSF cells. In addition, another clone targeting a different epitope of EBNA3A antigen, RPPIFIRRL, was present in the CSF of patients 14-87 and 14-250 (Table S1).

**Discussion**

In the current study we wanted to explore the possibility of somatic mosaicism displayed as sub-populations of immune cells with genomic variation within the CNS in...
To explore this hypothesis, we performed a CNV comparison between CSF and PBMC samples from MS patients as well as OND patients and HC. The aim was to identify genomic regions which were over- or under-represented in the CSF samples which could be an indication of sub-groups of cells with specific genomic

Figure 4. CNVs in the TCR regions in paired γδ T and CSF cells. PBMCs in each individual was used as a calibrator in the CopyCaller™ Software (Applied Biosystems) to calculate the CN of the target genes, that is, for each target gene the CN in the CSF and γδ T cells is calculated in comparison to the CN in the PBMCs. CNVs, copy number variations; CSF, cerebrospinal fluid; TCR, T-cell receptor; CN, copy number; PBMCs, peripheral blood mononuclear cells.

Table 3. Showing the total number of clones and number of expanded clones with a frequency of ≥0.1% per sample.

| Patient id | Total no. of clones | No. of expanded clones | Total no. of clones | No. of expanded clones | Total no. of clones | No. of expanded clones | CD4-CSF | CSF/CD4 (%) | CD8-CSF | CSF/CD8 (%) |
|------------|---------------------|------------------------|---------------------|------------------------|---------------------|------------------------|--------|------------|--------|------------|
| 18-8856    | 9802                | 14                     | 7336                | 51                     | 5006                | 124                    | 54     | 44         | 43     | 35         |
| 17-465     | 9349                | 16                     | 8026                | 42                     | 7275                | 30                     | 10     | 33         | 13     | 43         |
| 17-8801    | 10,550              | 32                     | 4784                | 128                    | 6610                | 88                     | 43     | 49         | 43     | 49         |
| 17-8823    | 6653                | 30                     | 5379                | 65                     | 3930                | 130                    | 45     | 35         | 49     | 38         |
| 17-8838    | 8087                | 15                     | 5362                | 80                     | 10,595              | 24                     | 9      | 38         | 14     | 58         |
| 16-098     | 7560                | 27                     | 3306                | 68                     | 7702                | 58                     | 23     | 40         | 17     | 29         |
| 16-223     | 8714                | 3                      | 42,005              | 39                     | 7564                | 35                     | 8      | 23         | 16     | 46         |
| 14-127     | 491                 | 67                     | 3918                | 80                     | 6480                | 49                     | 26     | 53         | 23     | 47         |
| 14-155     | NA                  | NA                     | NA                  | NA                     | 4654                | 65                     | NA     | NA         | NA     | NA         |
| 14-250     | NA                  | NA                     | NA                  | NA                     | 3555                | 126                    | NA     | NA         | NA     | NA         |
| 14-87      | NA                  | NA                     | 6844                | 45                     | NA                  | NA                     | NA     | NA         | NA     | NA         |
| Average    | 8206                | 25.5                   | 6265                | 69.1                   | 6383                | 70.4                   | 27.25  | 39         | 27.25  | 43         |

Overlap between expanded CSF clones and CD4+ and CD8+ T cells clones. CSF, cerebrospinal fluid; NA, not available; CD4-CSF, number of expanded CSF clones overlapping with CD4+ T-cell clones; CSF/CD4, percentage of expanded CSF clones overlapping with CD4+ T-cell clones; CD8-CSF, number of expanded CSF clones overlapping with CD8+ T-cell clones; CSF/CD8, percentage of expanded CSF clones overlapping with CD8+ T-cell clones.

MS. In order to explore this hypothesis, we performed a CNV comparison between CSF and PBMC samples from MS patients as well as OND patients and HC. The aim was to identify genomic regions which were over- or under-represented in the CSF samples which could be an indication of sub-groups of cells with specific genomic
characteristics enabling a role in MS pathogenesis. The main results of this analysis revealed CNVs across the TCR regions. Although this is likely a general consequence of an over representation of the proportion of T cells in the CSF as compared to PB, this also enabled us to further describe the TCR rearrangement in T cells from CSF in order to search for potential signs of T-cell clonality in the CNS.

The main reason for choosing CNVs to study genomic variants is that CNVs are known to occur frequently in connection with mitosis and can hence provide the basis for somatic genomic rearrangements and the establishment of mosaicism. Our genomic comparison of CSF cells to PBMCs identified deletions in the TRA/D, TRG, and TRB loci in CSF cells, which were confirmed by qPCR. Comparison of sorted peripheral CD4+ and CD8+ T cells to CSF cells showed similar deletions in the TRA/D and TRG loci, while the TRB locus displayed a more complex structure with inter-individual and intra-variations between CD4+ and CD8+ T cells and CSF cells. The deletions in CSF cells were more specific to CD4+ and CD8+ than to γδ T cells, indicating that CSF cells seem to be predominantly T cells of the αβ and not the γδ type. Surprisingly though, we observed deletions in the TRB locus in peripheral γδ T cells. This would not be expected based on the sequential TCR rearrangement and we do not believe this is caused by contamination of the samples with αβ T cells as we would then have expected deletions in the TRA locus too, which we did not find. However, deletions in the TRB locus in γδ T cells have been observed before and attributed to the concurrent rearrangement of β, γ, and δ loci.

The TCR loci undergo rearrangement during the maturation of the T cells in the thymus and unselected genes are spliced out. Hence the deletions in the TCR loci, when comparing CSF cells to PBMCs, suggest that these T cells display the required diversity of adaptive immunity.

The presence of deletions in the TRG loci of CD4+ and CD8+ T cells could be explained by the successive model of TCR rearrangement, where TRG and TRD are first rearranged and if there is a γδ TCR product the cell commits to being a γδ T cell, if not, the next step is to rearrange the TRB and TRA loci and commit to being an αβ T cell. TCR rearrangement is a classic form of somatic variation and the detection of TCR regions in the initial CNV screening, as previously pointed out, is likely the result of the proportion of T cells in the CSF being higher than in the PBMCs, that is, T lymphocyte count bias. This allowed us to further characterize the TRB locus rearrangements using next generation sequencing and profile the αβ T-cell repertoire in paired CSF, CD4+, and CD8+ T cells.
CD8\(^+\) T cells. The results revealed intra- as well as interindividually diversity across the TRB locus.

We profiled the TCR repertoire in the periphery and the CSF using a combination of multiplex PCR and next generation sequencing which provided the frequencies and CDR3 sequences of the T-cell clones. Clones with a frequency ≥0.1% were considered clonally expanded. We examined the TCR repertoire in the CSF cells and compared to the peripheral CD4\(^+\) and CD8\(^+\) T cells and found no significant overlap between the TCR repertoire in the CSF and the periphery, which indicates a divergence in the TCR profile between the two compartments. Within the peripheral compartment we observed that the CD8\(^+\) T cells were less diverse than the CD4\(^+\) T cells,
Table 4. For each patient, the top five frequent clones in the CSF and their corresponding frequencies in the CD4<sup>+</sup> and CD8<sup>-</sup> T cells peripheral compartments when available.

| id     | CDR3aa     | V gene   | D gene   | J gene   | CSF     | CD4<sup>+</sup> | CD8<sup>-</sup> |
|--------|------------|----------|----------|----------|---------|----------------|----------------|
| 17-465 | CASSLTOQGGGETQYF | TRBV12-4 | TRBD2 | TRBJ2-5 | 0.0031 | 2.93E-06 | 0.0032 |
| 17-465 | CASRSGQ_GQYF | TRBV4-3 | TRBD1 | TRBJ2-7 | 0.0029 | 0.0001 | 0.0015 |
| 17-465 | CASSRQNPSLHF | TRBV25-1 | . | TRBJ1-6 | 0.0026 | 0 | 0.0007 |
| 17-465 | CASSQGSSGRLAGESYEYQF | TRBV3-1 | TRBD2 | TRBJ2-7 | 0.0026 | 2.93E-06 | 0.0007 |
| 17-465 | CASSQGSGVNNKLEFF | TRBV12-4 | TRBD1 | TRBJ1-4 | 0.0023 | 0 | 0.0059 |
| 17-8801 | CASSPAMNTAEFF | TRBV14 | . | TRBJ1-1 | 0.0327 | 3.77E-05 | 0.0085 |
| 17-8801 | CASSQVIIQOAAF | TRBV14 | . | TRBJ1-1 | 0.0127 | 0.0013 | 0.0300 |
| 17-8801 | CASSGTEAFF | TRBV4-1 | . | TRBJ1-1 | 0.0054 | 4.19E-06 | 0.0046 |
| 17-8801 | CASSLGQNGAEYGT | TRBV12-4 | TRBD1 | TRBJ1-2 | 0.0043 | 1.04E-06 | 0.0010 |
| 17-8801 | CASSQGSSTPYEQYF | TRBV5-1 | TRBD2 | TRBJ2-7 | 0.0036 | 4.19E-06 | 0.0021 |
| 17-8823 | CASSRGNVDEQOFF | TRBV18 | . | TRBD1-2 | 0.0155 | 0.0058 | 0.0008 |
| 17-8823 | CATSRGLQ_GFGANVLTF | TRBV15 | TRBD1 | TRBD1-2 | 0.0105 | 0 | 0.0028 |
| 17-8823 | CASSQDRTHDGNEQOFF | TRBV3-1 | TRBD1 | TRBD1-2 | 0.0092 | 0 | 0.0007 |
| 17-8823 | CASSQGMRITEAFF | TRBV10-2 | . | TRBJ1-1 | 0.0091 | 0.0002 | 0.0004 |
| 17-8823 | CASSV*LTTNTGELFF | TRBV18 | TRBD1-2 | TRBD2-2 | 0.0065 | 0.0004 | 0.0019 |
| 18-223 | CSASLASYGTF | TRBV5-1 | . | TRBJ1-5 | 0.0026 | 0 | 0.0150 |
| 18-223 | CASSLEDR_INQPQHF | TRBV7-4 | . | TRBJ1-5 | 0.0025 | 0.0004 | 0.0254 |
| 18-223 | CATSROLSGLRANGYTF | TRBV15 | . | TRBD1-2 | 0.0025 | 0 | 0 |
| 18-223 | CASSQVDRTHDGNEQOFF | TRBV18 | . | TRBD1-2 | 0.0023 | 0.0010 | 0.0402 |
| 18-8856 | CASSYVGDRTEAFF | TRBV6-5 | TRBD1 | TRBJ1-1 | 0.0082 | 0.0001 | 0.0009 |
| 18-8856 | CASSQAGRSYEQYF | TRBV14 | TRBD1-2 | TRBD2-2 | 0.0063 | 4.49E-06 | 0 |
| 18-8856 | CASSQVDRTHDGNEQOFF | TRBV18 | . | TRBD1-2 | 0.0057 | 0.0005 | 0.0004 |
| 18-8856 | CASSQDRTHDGNEQOFF | TRBV18 | TRBD1-2 | TRBD2-2 | 0.0054 | 0.0024 | 0.0063 |
| 18-8856 | CASSQDRTHDGNEQOFF | TRBV18 | TRBD1-2 | TRBD2-2 | 0.0044 | 0.0000 | 0.0007 |
| 16-098 | CAISEQQGEGYTF | TRBV10-3 | TRBD1-2 | TRBD2-2 | 0.0091 | 0.0042 | 0 |
| 16-098 | CASSLWFTNGELFF | TRBV7-8 | . | TRBD2-2 | 0.0050 | 0 | 0.0012 |
| 16-098 | CASSRGR_DTEAFF | TRBV14 | TRBD2 | TRBJ1-1 | 0.0049 | 0 | 0.0093 |
| 16-098 | CASSFGPSGTEAFF | TRBV27 | TRBD2 | TRBJ1-1 | 0.0049 | 0 | 0.0006 |
| 16-098 | CASSDESTETEAFF | TRBV10-1 | . | TRBJ1-1 | 0.0049 | 0.0008 | 0 |
| 14-137 | CASSQGSGCYEQYF | TRBV4-1 | TRBD2 | TRBJ2-7 | 0.0133 | 0.0015 | 0.0153 |
| 14-137 | CASSQSGNPOQHF | TRBV10-2 | TRBD1-5 | TRBJ1-5 | 0.0081 | 0.0029 | 0.0058 |
| 14-137 | CASSERGNSGQYTF | TRBV20-1 | TRBD1-2 | TRBD1-5 | 0.0065 | 0.0018 | 0.0147 |
| 14-137 | CASSERGNSGQYTF | TRBV20-1 | TRBD1-2 | TRBD1-5 | 0.0059 | 0.0145 | 0.0687 |
| 14-137 | CASRGLTQTYGTF | TRBV20-1 | TRBD1-2 | TRBD2-2 | 0.0047 | 0.0030 | 0.0008 |
| 14-137 | CASSWGGSNYGYTF | TRBV11-2 | TRBD1-2 | TRBD2-2 | 0.0574 | NA | NA |
| 14-137 | CASSQDRLTGQYTF | TRBV18 | . | TRBD1-2 | 0.0229 | NA | NA |
| 14-137 | CASSGLPSNQTFELFF | TRBV18 | TRBD1-2 | TRBD2-2 | 0.0226 | NA | NA |
| 14-137 | CASSQSGEYTF | TRBV18 | . | TRBD1-2 | 0.0100 | NA | NA |
| 14-137 | CASSLYATGAEFF | TRBV28 | . | TRBD1-1 | 0.0051 | NA | NA |
| 14-137 | CASSGQGSYEQYF | TRBV18 | TRBD2 | TRBJ2-7 | 0.0122 | NA | NA |
| 14-137 | CASR*RP_GVRRGQYTF | TRBV25-1 | TRBD1 | TRBJ2-2 | 0.0060 | NA | NA |
| 14-137 | CASSQRGPGAVKNEKLFF | TRBV4-1 | TRBD1 | TRBJ4-1 | 0.0058 | NA | NA |
| 14-137 | CASSLSRGQELFF | TRBV7-8 | TRBD1 | TRBD2-2 | 0.0055 | NA | NA |
| 14-137 | CASSRGLGQYTF | TRBV13 | TRBD2 | TRBD2-2 | 0.0041 | NA | NA |
| 14-137 | CASSQPRENYEQYF | TRBV14 | TRBD1 | TRBJ2-7 | 0.0150 | NA | NA |
| 14-137 | CARRPG_NTEAFF | TRBV12-3 | TRBD1 | TRBD1-1 | 0.0059 | NA | NA |
| 14-137 | CASSYVDRTEAFF | TRBV6-3 | . | TRBD1-1 | 0.0038 | NA | NA |
| 14-137 | CASSQAGPSYQYF | TRBV14 | TRBD2 | TRBD2-2 | 0.0038 | NA | NA |
| 14-137 | CASSQDRLTGGYTF | TRBV4-1 | TRBD1-2 | TRBD2-2 | 0.0030 | NA | NA |

Expanded clone frequencies are in bold. CSF, cerebrospinal fluid; NA, not available; Dot (.), sequencing reads not aligned to a D gene.
which was also reported in MS patients before undergoing autologous stem cell transplantation.\textsuperscript{22} The TCR repertoire analysis was performed in samples from RRMS patients before the initiation of treatment, and it would be interesting to follow up the TCR repertoire in these same patients after initiating treatment to track the frequencies of the clones that were abundant pretreatment.

Our results, indicate an increased proportion of expanded clones in samples from patients during relapse as compared to remission in CSF cells, while the proportion of expanded clones is higher in PB samples from patients during remission compared to relapse in both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells. Although the results were not statistically significant, this observation may propose a

| id  | Cell type | CDR3nt | CDR3aa   | V gene | D gene | J gene | Frequency |
|-----|-----------|--------|----------|--------|--------|--------|-----------|
| 16-223 | CD8\textsuperscript{+} | TGTGCCAGCAGTGAAGGTTATGGCTACACCTTC | CASSEGYGYTF | TRBV25-1 | . | TRBJ1-2 | 0.0037   |
| 17-465 | CD4\textsuperscript{+} | TGTGCCAGCAGTGAAGGCTATGGCTACACCTTC | CASSEGYGYTF | TRBV25-1 | . | TRBJ1-2 | 0.0014   |
| 14-137 | CSF      | TGTGCCAGCAGTATACAGGGGGCGGCGAATGGCTACACCTTC | CASQIQGANYGYTF | TRBV27 | TRBD1 | TRBJ1-2 | 0.0031   |
| 14-250 | CSF      | TGTGCCAGCAGTATCAGGGGGCGGCGAATGGCTACACCTTC | CASQIQGANYGYTF | TRBV27 | TRBD1 | TRBJ1-2 | 0.0012   |
| 14-87 | CSF      | TGGGCCAGCAGTATCGACACTAGAAACTGAACCTTC | CASSLAINEAFF | TRBV5-1 | . | TRBJ1-1 | 0.0012   |
| 17-8801 | CD8\textsuperscript{+} | TGGGCCAGCAGTATCGACTGAACCTGAACCTTC | CASSLAINEAFF | TRBV12-4 | . | TRBJ1-1 | 0.0015   |
| 14-250 | CSF      | TGGGCCAGCAGCAGCAAACAGCTGACGCCGCTACACCTTC | CASQQRLTGTYTF | TRBV4-1 | . | TRBJ1-2 | 0.0030   |
| 14-87 | CSF      | TGGGCCAGCAGCAGCAAACAGCTGACGCCGCTACACCTTC | CASQQRLTGTYTF | TRBV4-1 | . | TRBJ1-2 | 0.0229   |
| 14-137 | CD4\textsuperscript{+} | TGGGCCAGCAGCAGCAAACAGCTGACGCCGCTACACCTTC | CASQVAVGYGYTF | TRBV29-1 | TRBD2 | TRBJ1-2 | 0.0011   |
| 17-8801 | CSF | TGGGCCAGCAGCAGCAAACAGCTGACGCCGCTACACCTTC | CASQVAVGYGYTF | TRBV29-1 | TRBD2 | TRBJ1-2 | 0.0016   |

CSF, cerebrospinal fluid; Dot (.), sequencing reads not aligned to a D gene.

Figure 7. Difference in the proportion of expanded clones between MS patients under relapse or remission in CD4\textsuperscript{+}, CD8\textsuperscript{+} T, and CSF cell compartments. Expanded clones are clones with a frequency of ≥0.1% and the proportion of expanded clones is calculated as the number of expanded clones compared to the total sum of clones per sample. The bars represent the median of the expanded clones per cell compartment. Differences of the proportion of expanded clones between relapse and remission were tested using Wilcoxon rank-sum test. ns, not significant; CSF, cerebrospinal fluid.
role for clonal expansion in MS disease as well as a possible shift of expanded clones from the periphery into the CNS during relapse.

The obvious question is if the observed TCR rearrangements in the CSF cells are unique to MS. We only had access to a very limited number of non-MS samples but we did see less deletions in the TRB locus in these as compared to the MS samples. Although this may suggest differences in ηβ T cells ratio as well as levels of clonality between MS and non-MS samples, the number of available samples in the latter group was too small to determine such an effect. However, the sheer number of cells in CSF from the non-MS samples is surprising, although the samples were selected to have high numbers of cells.

Determining the antigen specificity of the expanded T-cell clones is a major step in deciphering the autoimmune response. Due to the environmental association of EBV infection with MS risk, there is an interest in studying T-cell response. Due to the environmental association of EBV, the samples were selected to have high numbers of cells.

In a GWAS, Sato et al. using DNA from PBMCs, reported CNVs that were also deletions in the TRG and TRA/D loci to be associated with MS and neuromyelitis. Highlighting the importance of sample selection and DNA source when studying somatic variations, they also attempted to correct for the DNA source and concentration when they sorted for different subsets of white bloods cells and the deletions were validated only in T cells. Interestingly they speculated that due to the large size of the deletions they are unlikely to be a result of rearrangement.

The original hypothesis of this study was the presence of sub-populations of cells within the CNS that are established as a result of genomic rearrangements and which may be involved in MS pathogenesis. By genomic comparison of cells from the CSF and PB we could detect an over representation of T cells in the CSF based on rearrangements of TCR. We further explored this finding by characterizing the genomic rearrangements of the TRA, TRB, and TRG regions and used the diversity of the TRB locus rearrangements to explore the character and clonal expansion of the T cells in the CNS. Although we did not detect any evidence of CNVs around the IGH, IGK, and IGL loci on chromosomes 14q32, 2p12, and 22q11 which may be an indication of an over representation of B cells in the CSF we can not guarantee that the available genetic markers from the CytoScan HD Array across the relevant genomic regions would allow such an observation. Nor did we see any other chromosomal regions indicating CNVs between CSF and PBMCs in multiple samples although the number of samples was limited.

In conclusion, in search of sub-populations of immune cells in the CNS, we have identified deletion type CNVs in the TCR loci of cells in the CSF consistent with specific TCR rearrangements of the T cells. We have further characterized these T cells and identified clonality which may advance the understanding of the neuro-inflammation process and eventually contribute to the development of therapeutics.

Acknowledgment

This project was supported by funds from Stiftelsen Goljes Minne, NEURO Sweden and The Nilsson-Ehle Endowments. The authors thank all MS patients, nurses, and neurologists for taking part in this study. The authors also acknowledge Merja Kanerva for handling the samples at the neurology clinic and Sabrina Ruhrmann for assisting with the cell sorting. Open Access funding enabled and organized by Projekt DEAL.
Conflict of Interest

No author has any conflict of interest related to this study to report.

Author Contributions

Conception and study design: A. G. and S.K.B. Data acquisition: all authors, Data analysis: S.K.B. and A.G. Data interpretation: S.K.B., A.G., and F.A.N. Drafting of the manuscript: S.K.B. and A.G. Critical revision of the manuscript: all authors.

References

1. Beecham AH, Patsopoulos NA, Xifara DK, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genetics. 2013;45 (11):1353-1360. doi:10.1038/ng.2770
2. Sawcer S, Hellenhal G, Pirinen M, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011;476(7359):214-219. doi:10.1038/nature10251
3. Baranzini SE, Oksenberg JR. The genetics of multiple sclerosis: from 0 to 200 in 50 years. Trends Genet. 2012;28(7):369-377. doi:10.1016/j.tig.2012.06.006
4. Lill CM. Recent advances and future challenges in the genetics of multiple sclerosis. Front Neurol. 2014;5:130. doi:10.3389/fneur.2014.00130
5. Womack JE, Jang HJ, Lee MO. Genomics of complex traits. Ann N Y Acad Sci. 2012;1271:33-36. doi:10.1111/j.1749-6632.2012.06733.x
6. Baranzini SE, Mudge J, van Velkinburgh JC, et al. Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. Nature. 2010;464(7293):1351-1356. doi:10.1038/nature08990
7. McElroy J, Krupp L, Johnson B, et al. Copy number variation in pediatric multiple sclerosis. 2013. doi:10.1016/j.jnra.2013.06.015
8. Youssoufian H, Pyeritz RE. Mechanisms and consequences of somatic mosaicism in humans. Nat Rev Genet. 2002;3 (10):748-758. doi:10.1038/nrg906
9. Kim J, Shin JY, Kim JJ, et al. Somatic deletions implicated in functional diversity of brain cells of individuals with schizophrenia and unaffected controls. Sci Rep. 2014;4:3807. doi:10.1038/srep03807
10. Van Horebeck L, Dubois B, Goris A. Somatic variants: new kids on the block in human Immunogenetics. Trends Genet. 2019;35(12):935-947. doi:10.1016/j.tig.2019.09.005
11. Van Horebeck L, Hilven K, Mallants K, et al. A robust pipeline with high replication rate for detection of somatic variants in the adaptive immune system as a source of common genetic variation in autoimmune disease. Hum Mol Genet. 2019;28(8):1369-1380. doi:10.1093/hmg/ddy425
12. Scinti F, Di Martino TM, Pensabene L, Bruni V, Concolino D. The Cytoscan HD Array in the diagnosis of neurodevelopmental disorders. High Throughput. 2018;7 (3). doi:10.3390/ht7030028
13. Bolotin DA, Poslavsky S, Mitrophanov I, et al. MiXCR: software for comprehensive adaptive immunity profiling. Nat Methods. 2015;12(5):380-381. doi:10.1038/nmeth.3364
14. Shugay M, Bagaev DV, Turchaninova MA, et al. VDJtools: unifying post-analysis of T cell receptor repertoires. PLoS Comput Biol. 2015;11(11):e1004503. doi:10.1371/journal. pcbi.1004503
15. Shugay M, Bagaev DV, Zvyagin IV, et al. VDJdb: a curated database of T-cell receptor sequences with known antigen specificity. Nucleic Acids Res. 2018;46(D1):D419-D427. doi:10.1093/nar/gkx760
16. VDJmatch: a software for database-guided prediction of T cell receptor antigen specificity. 2018. Accessed 04 Nov 2020. https://github.com/antigenomics/VDJmatch
17. R: A Language and Environment for Statistical Computing. 2016. Accessed 11 May 2018. https://www.R-project.org/
18. Dudley EC, Girardi M, Owen MJ, Hayday AC. Alpha beta and gamma delta T cells can share a late common precursor. Curr Biol. 1995;5(6):659-669. doi:10.1016/s0960-9822(95)00131-x
19. Margolis D, Yassai M, Hietko A, McOlash L, Gorski J. Concurrent or sequential delta and beta TCR gene rearrangement during thymocyte development: individual thymi follow distinct pathways. J Immunol. 1997;159 (2):529-533.
20. Abbas AK, Lichtman AH, Pillai S. Basic Immunology: Functions and Disorders of the Immune System. 4th ed. Elsevier/Saunders; 2014:ix,:320 pages.
21. Pardoll DM, Fowlkes BJ, Bluestone JA, et al. Differential expression of two distinct T-cell receptors during thymocyte development. Nature. 1987;332(6108):79-81. doi:10.1038/326079a0
22. Muraro PA, Robins H, Malhotra S, et al. T cell repertoire following autologous stem cell transplantation for multiple sclerosis. J Clin Invest. 2014;124(3):1168-1172. doi:10.1172/JCI71691
23. Alfredsson L, Olsson T. Lifestyle and environmental factors in multiple sclerosis. Cold Spring Harb Perspect Med. 2019;10(4). doi:10.1101/cshperspect.a028944
24. Lossius A, Johansen JN, Vartdal F, et al. High-throughput sequencing of TCR repertoires in multiple sclerosis reveals intrathecal enrichment of EBV-reactive CD8 T cells. Eur J Immunol. 2014;44(11):3439-3452. doi:10.1002/eji.201444662
25. Pender MP, Burrows SR. Epstein-Barr virus and multiple sclerosis: potential opportunities for immunotherapy. Clin Transl Immunology. 2014;3(10):e27. doi:10.1038/cit.2014.25
26. Venturi V, Kedzierska K, Price DA, et al. Sharing of T cell receptors in antigen-specific responses is driven by
convergent recombination. Proc Natl Acad Sci USA. 2006;103(49):18691-18696. doi:10.1073/pnas.0608907103
27. Venturi V, Chin HY, Asher TE, et al. TCR beta-chain sharing in human CD8+ T cell responses to cytomegalovirus and EBV. J Immunol. 2008;181(11):7853-7862. doi:10.4049/jimmunol.181.11.7853
28. Grut V, Biström M, Salzer J, et al. Cytomegalovirus seropositivity is associated with reduced risk of multiple sclerosis—a presymptomatic case-control study. Eur J Neurol. 2021;28(9):3072-3079. doi:10.1111/ene.14961
29. Sundqvist E, Bergström T, Daalhosein H, et al. Cytomegalovirus seropositivity is negatively associated with multiple sclerosis. Mult Scler. 2014;20(2):165-173. doi:10.1177/1352458513494489
30. Hayashi F, Isobe N, Glanville J, et al. A new clustering method identifies multiple sclerosis-specific T-cell receptors. Ann Clin Transl Neurol. 2021;8(1):163-176. doi:10.1002/acn3.51264
31. Sato S, Yamamoto K, Matsushita T, et al. Copy number variations in multiple sclerosis and neuromyelitis optica. Ann Neurol. 2015;78:762-774. doi:10.1002/ana.24511
32. Schwienbacher C, De Grandi A, Fuchsberger C, et al. Copy number variation and association over T-cell receptor genes—influence of DNA source. Immunogenetics. 2010;62(8):561-567. doi:10.1007/s00251-010-0459-7

Supporting Information

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

Table S1. The specificity of the identified T-cell clones when matching their CDR3 sequences with previously known targets in the VDJdb database.