The IGHG1 alleles determine the B cell repertoires in the cerebrospinal fluid of patients with multiple sclerosis

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Introduction

Clonally expanded B-cells accumulate in the brain and the cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients. These cells are characterized by somatic mutations in immunoglobulin heavy chain genes and have a biased usage of certain variable heavy-chain genes indicating antigen driven response.
Refinement of single-cell transcriptomics and high-throughput sequencing has made it possible to link the B-cell receptor heavy- and light-chain sequences to the complete immunoglobulin alleles and functional B-cell phenotypes.

Materials and methods

We recruit patients who undergo diagnostic lumbar puncture in connection with sample collection. We use flow cytometry to sort single cells from the CSF and then amplify all the genes expressed in each cell. Next different bioinformatic tools are applied to analyze the gene expression (transcriptome) and to reconstruct the Ig receptors on the single cell level. This gives us information about what kind of B cell is involved (phenotype), and the clonal relationship between the cells.

Conclusions

MS patients have a number of subgroups of B cells in the spinal fluid, and for the first time we can link information on phenotype and Ig on single cell level. We show that VH4 segment and kappa light chain dominance are not found in all MS patients, and that it is possibly dependent on IGHC alleles.

Results

❖ Numerous families of clonally related B-cell clones are found in all patients, (Fig.1).

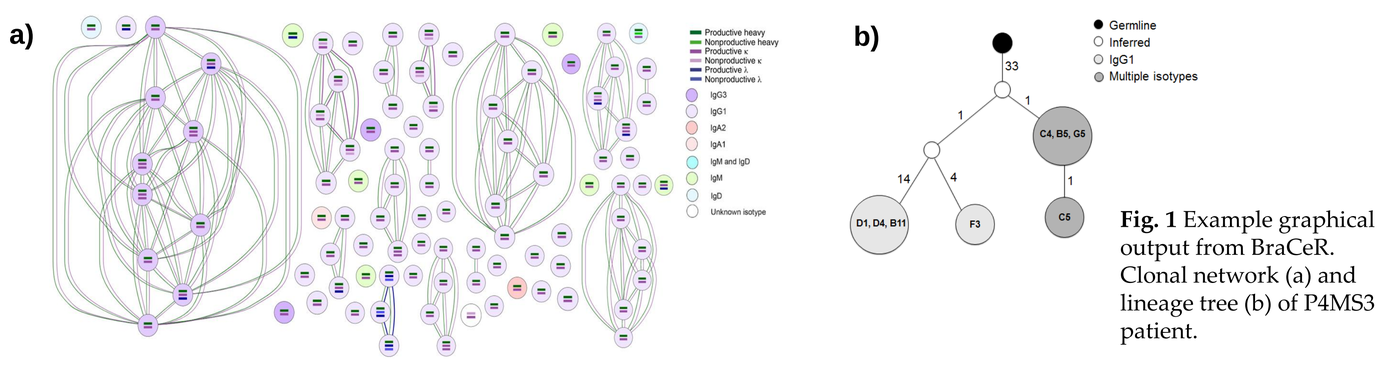

Fig. 1 Example graphical output from BraCeR. Clonal network (a) and lineage tree (b) of P4MS3 patient.

❖ The isotypes of the plasmablasts are dominated by IgG1 (median 92.2% [67-100%]). We also found clonally expanded IgA and IgM cells. The dominant light chain depends on the IGHG1 allele (Fig. 2).

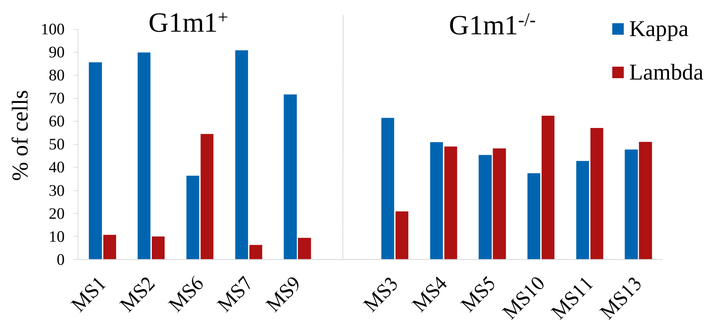

Fig.2 Productive light-chains usage in populations of PBs in each patient.

❖ A biased usage of VH4 gene segments in plasmablasts among patients carrying the IGHG1*01 allele (Fig. 3).

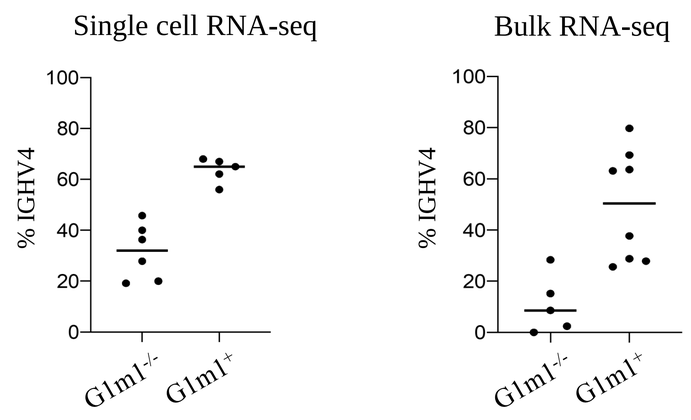

Fig.3 Percent of plasmablasts using IGHV4 gene from single cell RNA-seq (left) and previosuly published, reanalyzed bulk sequencing data (right panel).

❖ VH4 preferentially pairs with KV1 family genes, but only in patients with IGHG1*01 allele (Fig. 4).

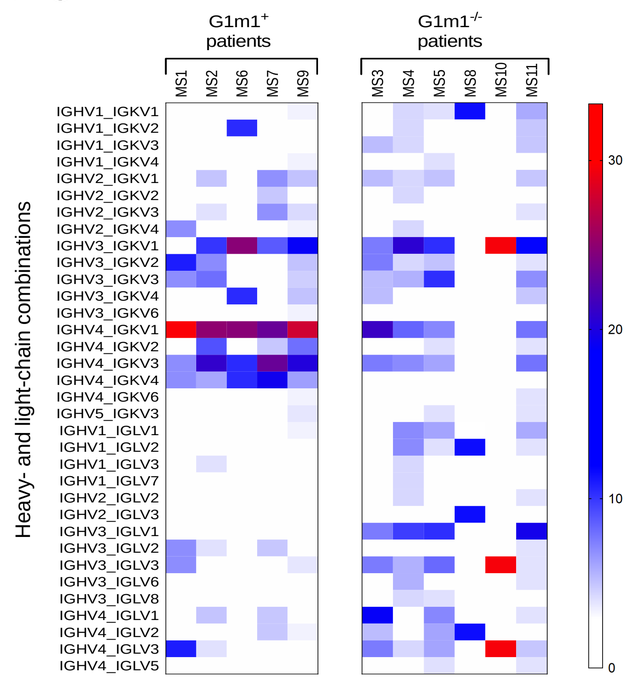

Fig.4 Pairing of variable segments of heavy and light chains in the B cell receptors of CSF plasmablasts.

Disclosure:

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