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## Skewing of the antibody repertoire in cerebrospinal fluid B cells from healthy controls and patients with schizophrenia

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### Abstract

Autoantibodies play a role in the etiology of some neuropsychiatric disorders. To address the possibility that B cells and their antibodies may be involved in the pathophysiology of schizophrenia, we examined B cells in cerebrospinal fluid (CSF) and peripheral blood (PB) of 4 schizophrenic patients (SP) and 4 healthy control (HC) volunteers by analyzing immunoglobulin VH gene usage. All CSF samples contained measurable levels of B cells. We found for both SP and HC, CSF B cells represented a select subset of, and were not the same as, B cells in PB. Moreover, we found statistically significant differences in antibodies generated by CSF B cells in SP compared to CSF B cells in HC. Although binding characteristics of CSF SP-associated B cell antibodies is unknown, the study number is small, and pathophysiology has not been established, these results suggest the value of focusing further study on the distinctly separate population of CSF B cells in SP.

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CRedit authorship contribution statement

**SH-K** conceived the project, carried out experiments, analyzed data, and wrote the manuscript; **JL and DV** analyzed data, prepared figures and wrote the manuscript; **JAG and AKM** recruited study participants and obtained CSF and PB samples; **TLR** conceived the project, analyzed data, and wrote the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

## 1. Introduction

Schizophrenia is a chronic and severe mental disorder with a poorly understood etiology. A growing body of research suggests that abnormalities in the immune system contribute to the etiology and development of this debilitating disorder [1,2]. Much attention has focused on the possible role of B cell-derived autoantibodies in the pathogenesis of schizophrenia [3–8].

The presence of antibodies against brain tissue both in the serum and cerebrospinal fluid (CSF) was reported as early as 1939 [3]. These early findings, plus much subsequent work, suggest a general paradigm in which pathological antibodies in the serum might cross the blood-brain barrier and induce neuropsychiatric illness in susceptible individuals [9]. A role for B cells is further suggested by the finding that the B cell antigen CD19 is a schizophrenia-associated gene [10]. However, neither plasmapheresis nor hemodialysis produced benefit in patients with schizophrenia thereby reducing the likelihood that a serum antibody or other circulating factor is responsible [11,12], although the presence of serum antibodies against human brain tissue [13] in schizophrenia patients has been reported. Anti-NMDA receptor (NMDAR) encephalitis, a related encephalitis illness that can present with psychosis and can be associated with ovarian tumors, has been attributed to circulating antibodies against NMDAR and often responds to immune therapy [14,15]; however, this is considered a separate entity from schizophrenia.

An alternate paradigm suggests that disease-associated B cells and the pathological antibodies they produce are located within the central nervous system (and may not be present peripherally). This situation of B cell compartmentalization is observed in multiple sclerosis, where immunoglobulin oligoclonal bands are found in the CSF and lymphoid follicle-like aggregates are found in the meninges and other CNS sites [16,17]. Interestingly, B cells from the CSF of patients with MS appear to be part of the disease process in that certain heavy chain variable region gene (VH) usage is biased and antibodies recognize myelin basic protein [18–20]; successful therapy with B cell depletion is consistent with a pathogenic connection [21]. And although there are reports that MS B cells exchange between the CSF and the periphery [22–24], the degree to which, and the stage at which, this occurs remains uncertain.

We considered the possibility that pathological B cells may be CSF compartmentalized to a greater or lesser extent in schizophrenia, as they are in MS. This possibility is supported by the finding of an increased IgG ratio in patients with schizophrenia, suggesting intrathecal production of immunoglobulin [25]. However, not only is little known about CSF B cells in schizophrenia, little is known about CSF B cells in healthy controls. Other systematic reviews have also commented on the limited number of studies in schizophrenia based on CSF, while also highlighting that the control groups even in those studies typically consist of non-healthy subjects. As antibodies are synthesized exclusively by B cells, we studied the B cell population in healthy control volunteers (HC) and patients with schizophrenia (SP) to determine if there is a difference in the B cell repertoire in the CSF from SP as compared to HC. We compared paired samples of B cells from CSF and peripheral blood (PB) obtained from HC and SP and considered 3 specific issues; we asked: 1. Are B cells present in CSF from HC; 2. Are CSF B cells a random assortment of PB B cells or do they represent a select

subset; and, 3. Do CSF B cells from SP differ from CSF B cells from HC? Individual B cells generate only a single immunoglobulin molecule, and each immunoglobulin molecule contains only a single VH gene segment from among many possibilities [26]. Thus, we characterized individual B cells from CSF and PB, to determine similarities and differences, by analyzing the particular VH region utilized by each B cell's immunoglobulin.

## 2. Materials and methods

### 2.1. Subjects

CSF was collected from 12 HC and 13 SP by lumbar puncture. PB was additionally obtained from 4 out of 12 HC, and from 4 out of 13 SP. Paired samples of CSF and PB were simultaneously obtained from the same aforementioned 4 HC and 4 SP. Healthy controls were individuals who donated CSF and PB for no reason other than volunteerism. In particular, healthy control CSF was not obtained as part of a clinical evaluation for any disorder. Patients were recruited from the inpatient or outpatient departments at The Zucker Hillside Hospital. Schizophrenia was confirmed using the Structured Clinical Interview for DSM-IV (SCID). All patients were diagnosed with chronic schizophrenia and were stable on treatment with antipsychotic medications at the time of sample collection. Medication information is available for 12 of the 13 patients and treatment consisted of one individual each treated with aripiprazole, olanzapine, and risperidone; no patients were treated with clozapine.

Approval for the study was obtained from the North Shore Long Island Jewish-Feinstein Institute for Medical Research Institutional Review Board prior to initiation, and all participants provided written informed consent to a protocol approved by the same Institutional Review Board.

### 2.2. Sample processing

About 10–15 cc of CSF was collected by lumbar puncture and 8–10 cc of PB was obtained by venipuncture just prior to the lumbar puncture for each subject. All CSF samples were spun down at 400 g for 10 min at room temperature. After centrifugation, the supernatant was divided into aliquots, flash frozen with liquid nitrogen and stored at  $-80^{\circ}$  Celsius. PBMCs were isolated from the cell pellet after centrifugation using Ficoll-Paque density centrifugation and cryopreserved using standard protocols. CSF cells were spun down and frozen at  $-80^{\circ}$  C after suspending them in freezing media consisting of 90% FCS/10% DMSO.

### 2.3. Cell isolation

The cells from CSF and PB were stained with fluorescence-labeled antibodies to CD19, CD3, and CD7 (BD Biosciences). Cells were stained for 30 min and then washed; B cells (CD19<sup>+</sup>CD7<sup>-</sup>CD3<sup>-</sup>) were then isolated using the Influx cell sorter (BD Biosciences).

## 2.4. Next generation sequencing

Sequencing of sorted B cells was obtained from iRepertoire®. Samples were extracted and amplified using human B-cell receptor (BCR) heavy chain primers and pooled for sequencing on the Illumina MiSeq.

## 2.5. Data analysis

iRepertoire® provided raw data on V segment usage for B cell samples. Data analysis involved quantification of VH family and subfamily. Representation of VH region usage in CSF and PB for each subject was visually explored using bar charts. Wilcoxon rank-sum tests were used to test the difference in the proportion of VH region usage by B cells between patients with schizophrenia and healthy controls in both the CSF and PB. Two-sided p-values less than 0.05 were considered statistically significant unless otherwise specified. All analyses were conducted in R 3.6.1 (R Foundation for Statistical Computing; <http://www.r-project.org/>).

## 3. Results

### 3.1. Demographic and other details of the samples

Age of schizophrenia patients ranged from 20 to 49, while that of healthy controls ranges from 24 to 50 at the time of sample collection. There were two male subjects in the study, one in each group. Two out of 4 patients with schizophrenia were White, while one in 4 healthy controls was White (Table 1). Routine CSF analysis including quantification of total glucose and protein were also done, and were within normal range.

### 3.2. CSF samples from both healthy controls as well as patients with schizophrenia contain significant numbers of B cells

The presence of B cells among mononuclear cells in CSF fluid that populate a lymphocyte gate was evaluated by immunofluorescent staining that omitted cells expressing CD3 and CD7 and captured cells expressing CD19, as shown in Fig. 1. CD3-CD7-CD19+ B lymphocytes were selected by fluorescence activated cell sorting. We found B cells present in CSF samples obtained from self-reported healthy controls with no evidence of disease. The B cells isolated from CSF HC and from SP ranged from a cell count of 130–280, while 10,000 cells were isolated from the PB of each individual. There was no significant difference in the percentage of B cells among CSF lymphocytes obtained from HC and SP (data not shown). B cells were sort purified from PB samples obtained from the same schizophrenia patients and healthy controls.

### 3.3. The repertoire of CSF B cells differs from PB B cells in individual HC and SP

B cells located in the CSF might represent a random assortment, or, alternatively, a selected subset, of B cells in peripheral blood, and this might differ for SP vs HC CSF B cells. To address this issue, we evaluated the antibody repertoire of each CSF B cell sample in comparison with its corresponding PB B cell sample, making use of the fact that a single B cell produces a single antibody that expresses a single VH gene from among many in the genome. VH usage by individual antibodies was determined by deep sequencing of

sort-purified B cells. We evaluated the percent of total occurrences represented by each VH region for CSF B cells and corresponding PB B cells from the same subject. We found the CSF repertoire to be much more restricted than the PB repertoire in each individual. For example, among the 8 samples examined, the restricted CSF repertoire included 10 VH segments each of whose expression amounted to 25% or more of all VH segments expressed in a given sample, whereas the more broadly distributed PB repertoire did not include any VH segments whose expression reached the level of 25% or more of all VH segments expressed in any sample.

We evaluated the possibility that differences noted between CSF and PB repertoires resulted from comparison of few CSF B cells (hundreds) with many PB B cells (thousands) and thus could still represent random trans migratory events operating on the PB B cell pool, despite restricted expression of VH regions among CSF B cells. We assessed the significance of differences between unbalanced numbers of CSF and PB B cells for each subject by computing the expected CSF B cell VH usage on the basis of PB B cell VH usage and then comparing with observed VH usage by CSF B cells (Fig. 2). For all subjects, actual CSF B cell VH usage differed significantly from VH usage predicted by PB, which indicates that verifiable VH differences were found between CSF and PB B cells for each individual. Thus, to the extent that CSF B cells derive from the circulation, these results suggest operation of a non-random, selective process.

#### **3.4. The repertoire of SP CSF B cells differs from the repertoire of HC CSF B cells**

Inasmuch as CSF B cells appear to represent a select subset of PB B cells based on repertoire analysis, we questioned whether the CSF B cell populations in SP differ from the CSF B cell populations in HC. Fig. 3 shows the average (median) proportion of CSF and PB B cell VH usage from 4 SP and 4 HC samples. Wilcoxon rank-sum tests unadjusted for multiple testing revealed statistically significant differences in the proportion of CSF B cell VH usage between SP and HC subjects for two VH regions: VH1–8 and VH3–23. Visual inspection of Fig. 3 identified that VH regions such as VH1–2, VH3–30–3, and VH3–30 showed a big difference in median between SP and HC subjects. However, their difference was not statistically significant due to small sample size.

In contrast, VH1–8 and VH3–23 were utilized to a similar extent of PB B cells by SP and HC subjects, representing about 2% of all immunoglobulins for VH1–8 and about 11% of all immunoglobulins for VH3–23. Thus, the skewed presence of B cells expressing VH1–8 and VH3–23 in SP CSF (and not HC CSF) is not due to disproportionate expression in SP PB B cells as compared to HC PB B cells.

#### **3.5. The repertoire of SP PB B cells differs from the repertoire of HC PB B cells**

As we did for CSF B cells, we evaluated PB B cells by determining whether the PB B cell population differs between SP and HC subjects. We found statistically different utilization of PB B cells in two VH regions: VH3–53 and VH4–31 (Fig. 3). CSF B cell usage for VH 3–53 and VH4–31 were minimal with median of 0 for both SP and HC samples.

## 4. Discussion

A role for B cell-derived antibodies in the pathophysiology of some neuropsychiatric illnesses has been documented. However, in spite of studies showing the presence of peripherally circulating autoreactive antibodies [27,28], there is no evidence that such antibodies contribute to schizophrenia. We speculated, instead, that B cells within the central nervous system could be responsible for disease, somewhat akin to the current paradigm for the pathophysiology of multiple sclerosis. To begin to explore this issue, we looked at the repertoires of HC and SP CSF B cells in comparison with PB B cells from the same individuals.

We confirmed that B cells are present in the CSF of self-described healthy control volunteers, as well as schizophrenia patients. We determined VH usage by all B cells, in CSF and PB, by deep sequencing. Importantly, in both HC and SP, B cells in the CSF represented a different pool than B cells in the PB. For example, in 7 out of 8 HC and SP subjects, there was at least one VH gene segment that was vastly over utilized by B cells in CSF as compared to representation in PB. Because the number of CSF B cells analyzed (numbering between 130 and 282) was much less than the number of PB B cells analyzed (numbering in each case 10,000), the resultant data presented a variation of the urn problem. The question raised by the unbalanced number of samples is how confident one can be that, given the PB distribution of VH segment usage, the more limited and skewed distribution of VH segments among CSF B cells did not arise by random selection of a small number of CSF B cells from among a larger number of PB B cells. Careful analysis by Chi-square goodness of fit indicates that it did not, and thus B cells in the CSF represent a select subset of B cells in the periphery, although the means by which B cells are selected for CSF residence remains unknown. While it is known that some antipsychotic medications can affect B cells, the conclusion that CSF B cells and PB B cells represent different pools in both SP and HC is, in essence, medication-independent, because in this analysis the key element is whether differences in repertoire exist between B cell populations from 2 sites in the same individual, not what the repertoire might be in any given individual (unlike comparisons of B cells in SP versus HC). It is notable in connection with these results that B cell selection by the blood brain barrier has not been reported.

Beyond differences between CSF and PB B cell pools, a key question is whether the repertoire of CSF B cells in SP differs from the repertoire of CSF B cells in HC. Across 4 SP and 4 HC CSF samples, 2 VH regions were significantly different by resampling-based permutation tests, VH1–8 and VH3–23. In 2 of the 4 SP samples, VH1–8 was markedly overexpressed in CSF as compared to PB, suggesting that, as with MS, B cells expressing this VH region exist in the CSF separately from the PB. In contrast, in all 4 SP samples, VH3–23 is represented in CSF at a level that approximates the level in PB, suggesting that these B cells may be shared by, or may shuttle between, PB and CSF. Although VH3–23 is the most commonly used variable gene segment in PB of HC, it has also been reported to be associated with autoreactivity [29–31]. Thus, more than one mechanism may be at play in determining the repertoire of B cells in the CSF of SP.

The potential role of B cells in schizophrenia is receiving increasing attention [32]. In particular, B cell generated antibodies have been examined in PB and CSF. Beyond anti-NMDAR antibodies, little evidence exists to indicate a role for circulating or CNS antibodies as pathogenic effector agents for schizophrenia [33–35]. It is important to point out that our results do not pertain to antibodies but only to B cells. That is, our work describes the B cell repertoire, not the antibody repertoire. Still, the differences we have found between CSF and PB repertoires indicate CNS selectivity, and the differences we have found between CSF repertoires in SP and HC suggest the possibility of SP-specificity in B cell selection.

It is notable that the binding specificity of antibodies in the CSF B cell repertoire is as yet unknown and that, combined with the small number of SP samples involved in this study, makes general conclusions about schizophrenia pathogenesis difficult. And B cells have repertoire-based effector functions beyond antibody production, such as antigen presentation, which could influence neuropsychiatric illness. However, it is important to point out that the results presented herein indicate for the first time that the repertoires of CSF B cells differ significantly from the corresponding repertoires of PB B cells across multiple individuals, and this includes comparison of CSF and PB B cell repertoires from true healthy control volunteers (rather than patients with other neurological issues), for which no previous published information is available, to our knowledge. These results strongly suggest the value of future studies that focus more intently on the identity and function of CSF-specific B cells in SP and HC.

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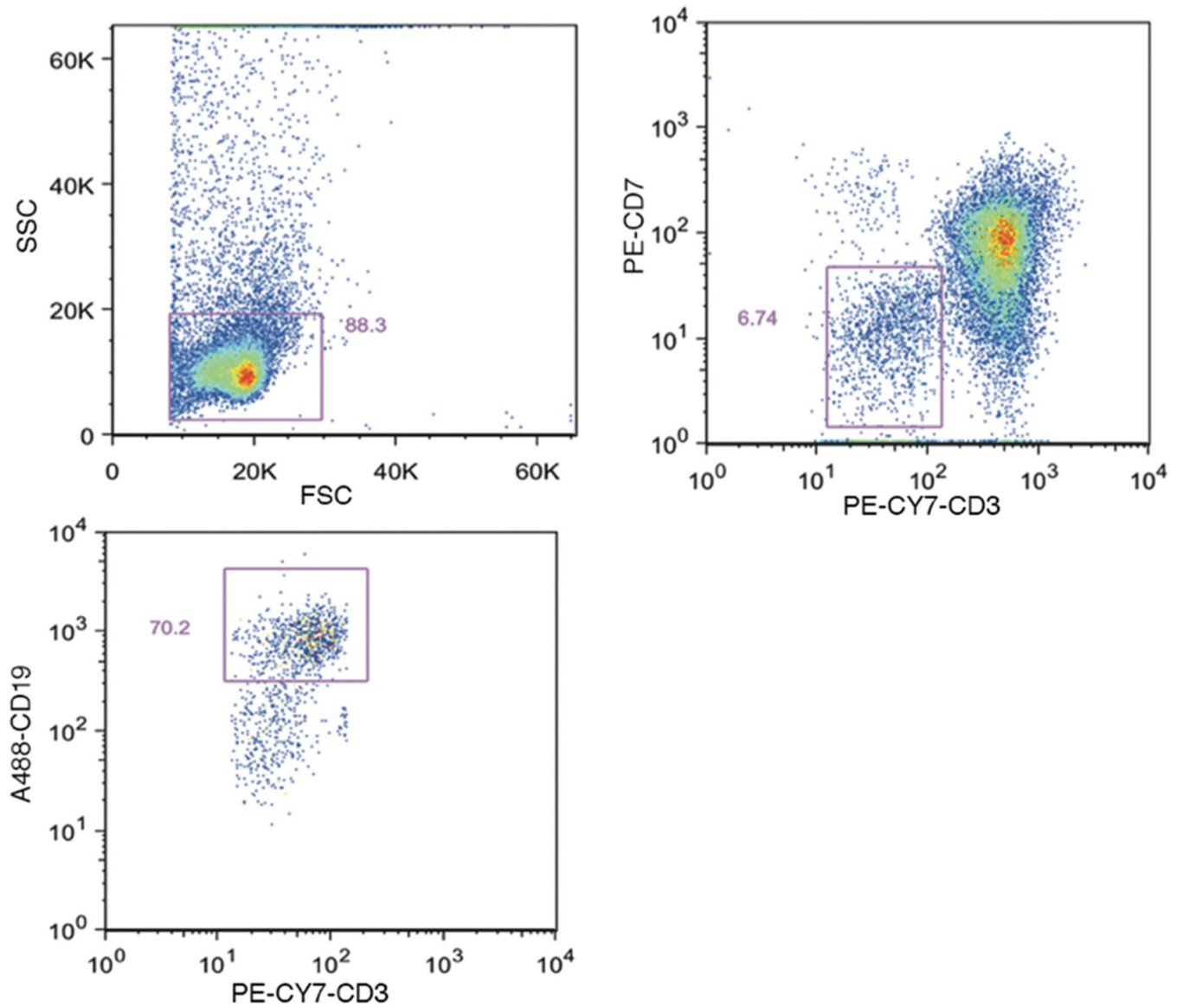
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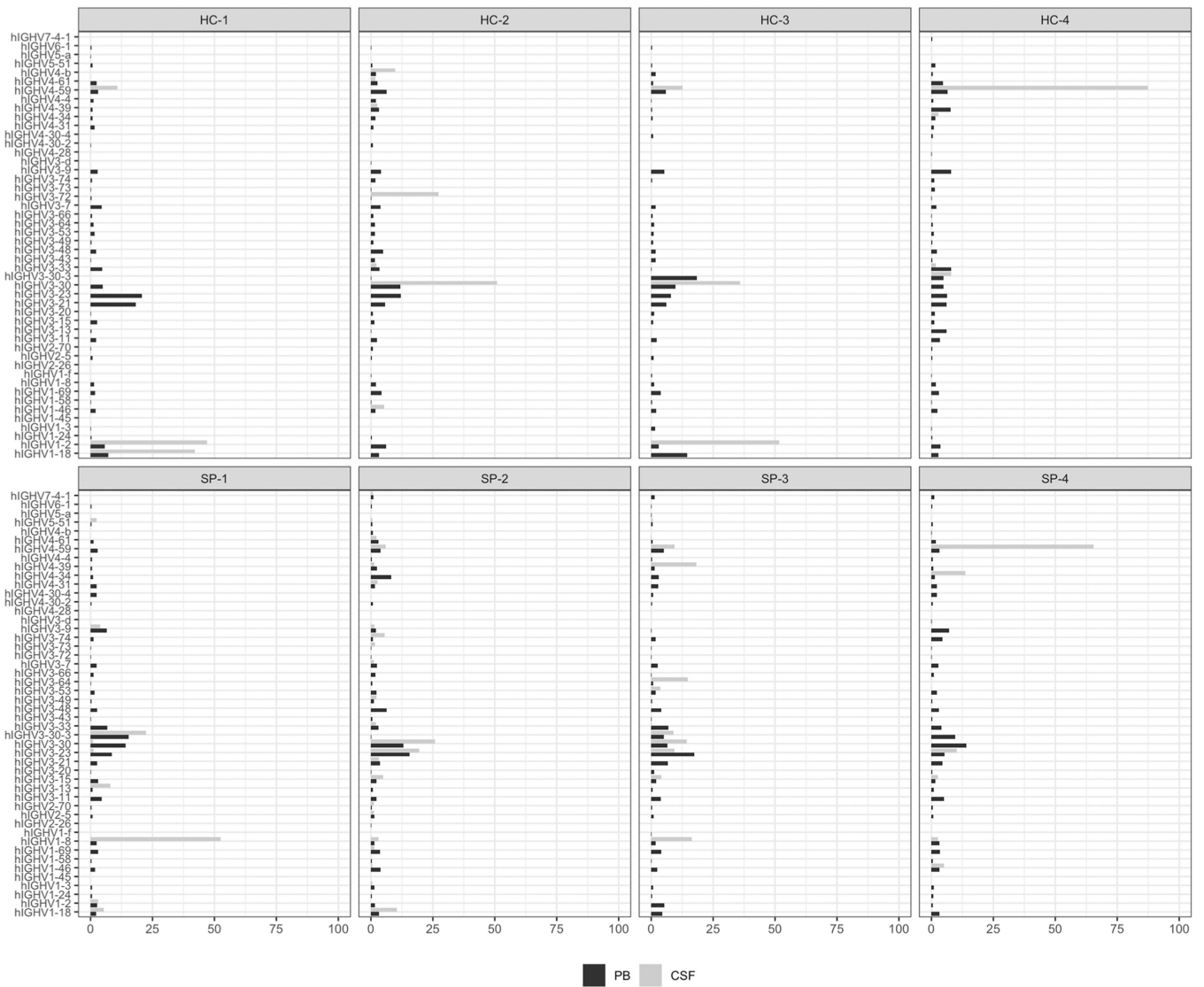
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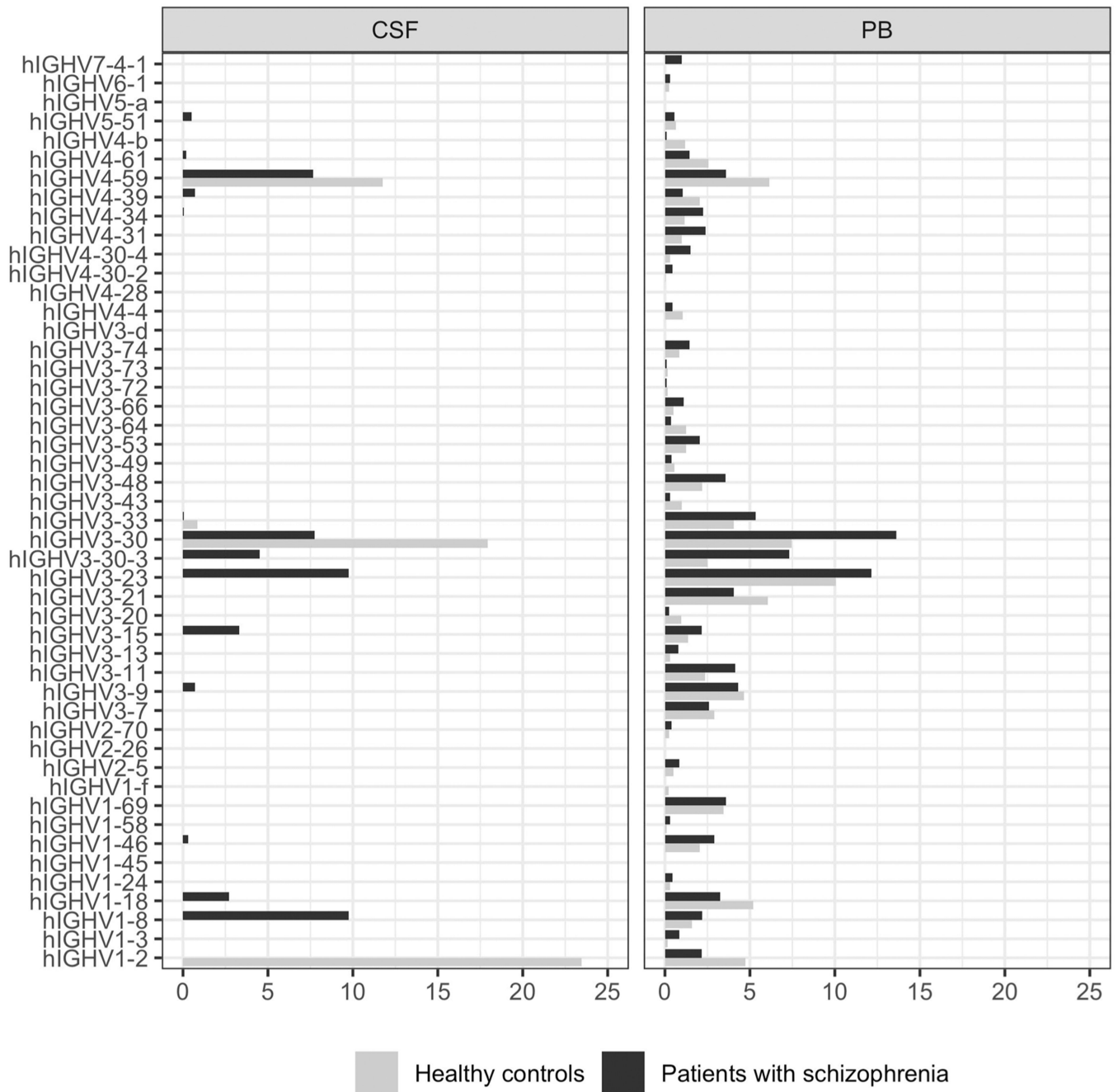
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**Fig. 1.** B cell isolation: Cells isolated from CSF were stained with immunofluorescent antibodies and isolated using an Influx cell sorter. The plot displays CD3<sup>-</sup>CD7<sup>-</sup>CD19<sup>+</sup>B cells. The plot shown is representative of 8 separate CSF samples.



**Fig. 2.** VH subfamily usage: Bar graphs representing VH subfamily usage as a percent of total immunoglobulin VH in matched CSF and PB samples are shown for 4 HC and 4 SP subjects.



**Fig. 3.** CSF and PB VH subfamily usage: The average percent VH subfamily usage of total CSF and PB immunoglobulin VH usage from 4 SP and 4 HC for whom paired CSF and PB samples were obtained is shown.

**Table 1**

Subject Demographics and other measures.

	<b>LP ID</b>	<b>Age</b>	<b>Sex</b>	<b>Race</b>	<b>Protein CSF</b>	<b>Glucose CSF</b>
Schizophrenia	SP-1	20	Male	White	42	33
	SP-2	49	Male	White	28	66
	SP-3	33	Female	African American	20	56
	SP-4	20	Male	Asian	16	60
Healthy Controls	HC-1	24	Male	African American	25	61
	HC-2	50	Female	African American	19	59
	HC-3	49	Male	White	24	61
	HC-4	33	Male	African American	40	61

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