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ORIGINAL RESEARCH ARTICLE

Characteristics of T-cell and B-cell receptor repertoires in patients with autoimmune hepatitis

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Abstract

Introduction: Autoimmune hepatitis (AIH) is a chronic liver disease characterized by inflammation resulting from the body's immune system attacking its own liver cells. T-cell receptor (TCR) and B-cell receptor (BCR) repertoires are crucial mediators of autoimmune diseases, with the cause of B-lineage dysregulation remaining elusive.

Objective: This study aims to comprehensively describe the changes in TCR and BCR repertoires in the peripheral blood and liver of patients with AIH.

Methods: High-throughput sequencing and multiplex polymerase chain reaction amplification were applied to explore the TCR and BCR gene repertoire characteristics in 60 patients with AIH and control groups.

Results: The findings revealed a significant reduction in the diversity of BCR heavy-chain (BCR-H) repertoire and the length of BCR-H complementarity-determining region 3 (CDR3) in the peripheral blood of AIH patients. Interestingly, the pre-selection BCR-H CDR3s in AIH patients were abnormally short, suggesting irregularities in the early stages of B-cell development and repertoire formation in the bone marrow. Furthermore, compared with controls, peripheral blood samples from AIH patients showed reduced diversity in the TCR β-chain (TCR-β) repertoire, longer CDR3 lengths, biased TCR-β variable gene usage, and extensive TCR-β sharing. The analysis revealed a comparable distribution of clone diversity between liver tissue samples from AIH and control groups, potentially attributable to the architectural characteristics of AIH liver T and B cells, which are marked by the presence of multiple antigen-responsive T and B cells rather than a single reactive T and B lymphocyte clone.

Conclusion: This study reveals distinct alterations in the TCR and BCR repertoires among patients with AIH, potentially offering novel insights for its prevention and diagnosis.

Keywords: T cell receptor; Immunoglobulin heavy chain; Immune repertoire; High-throughput sequencing; Autoimmune hepatitis

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1. Introduction

Autoimmune hepatitis (AIH) is a chronic liver condition driven by the immune system, marked by a gradual loss of the body's ability to tolerate its own tissues, which can subsequently trigger persistent hepatic inflammation, acute liver failure, and ultimately cirrhosis if left untreated.[1] Despite decades of research, the precise mechanisms underlying AIH pathogenesis remain only partially understood. Increasing evidence suggests that a multifactorial etiology is involved, encompassing genetic susceptibility, environmental triggers, and immune dysregulation.[2] In particular, both T and B cells have emerged as central players in disease development, as indicated by the frequent detection of infiltrating $CD4^+$ T lymphocytes and plasma cells within affected liver tissues of patients with AIH.[3,4]

T and B lymphocytes identify antigens using highly specialized surface receptors, specifically the T-cell receptor (TCR) and B-cell receptor (BCR). The distinct antigenic specificity of each receptor is primarily determined by the hypervariable complementarity-determining region 3 (CDR3), which corresponds to the β chain in TCRs and the heavy chain in BCRs. Antigen receptors are generated through a random process called somatic recombination, which rearranges variable (V), diversity (D), and joining (J) gene segments from the germline repertoire. Additional sequence diversity arises from random nucleotide insertions and deletions occurring at the V(D)J recombination junctions. However, this inherently error-prone process frequently produces nonfunctional receptors due to frameshift mutations or the incorporation of premature stop codons.[2] To compensate for this, T and B cells attempt rearrangement of the second allele, and if successful, an in-frame rearrangement results in the coexistence of a functional and a nonfunctional receptor gene within the same cell. Functional receptors are subject to strict positive and negative selection to maintain self-tolerance and establish a functional repertoire for further investigation.[4-6] Interestingly, transcripts corresponding to nonfunctional TCRs and BCRs remain detectable within cells, and although they cannot be translated into functional receptor chains, their presence provides valuable insights into the pre-selection immune repertoire and the underlying mechanisms of immune receptor diversity.[7,8]

The diverse and dynamic nature of human TCR and BCR repertoires presents major challenges for systematic analysis and understanding. Recent advances in next-generation and high-throughput sequencing (HTS) technologies have enabled the detailed examination of millions of TCR β-chain (TCR-β) and BCR heavy-chain (BCR-H) sequences.[9]

Therefore, this study aims to quantitatively evaluate the immune repertoires of patients with AIH alongside multiple disease control groups, thereby determining the extent to which AIH specifically influences TCR and BCR diversity and clonal composition. This comprehensive immunogenomic approach not only enhances our mechanistic understanding of AIH pathogenesis but also aids in identifying potential diagnostic and prognostic biomarkers that could serve as valuable tools for disease monitoring, clinical management, and the development of novel therapeutic strategies.

2. Materials and methods

2.1. Biological materials

The TCR and BCR CDR3 sequences used in this study were retrieved from the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/view/PRJEB37143).[5] Immune repertoire sequencing using next-generation sequencing technology was employed to analyze peripheral blood mononuclear cells (PBMCs) from a well-characterized cohort consisting of 60 patients with AIH and 60 age- and sex-matched healthy donors (HDs). Additional control groups were included to confirm that changes in the immune repertoire were specific to AIH and not merely reflective of general inflammatory responses linked to chronic liver damage.

The study included PBMC samples from 30 patients diagnosed with primary biliary cirrhosis (PBC) and 30 patients with chronic hepatitis C virus (HCV) infection who had not received antiviral treatment. Moreover, liver tissue specimens were analyzed, including samples from 12 patients with AIH and 6 control liver samples obtained from diagnostic biopsies of individuals without AIH, comprising those with non-alcoholic fatty liver disease (n = 3), non-alcoholic steatohepatitis (n = 1), and hepatic hemangioma (n = 2).

All study protocols were reviewed and approved by the Ethics Committee of the Hamburg Medical Association (*Ethikkommission der Ärztekammer Hamburg*, Germany; project number PV4081). Written informed consent was obtained from all participants before the collection and research use of biological samples.

2.2. DNA extraction, library construction, and HTS

T and B lymphocytes were isolated from PBMCs using anti-human cluster of differentiation (CD)3 and CD19 magnetic beads, according to the manufacturer's protocol (555335 and 551520, Becton, Dickinson and Company, USA), yielding highly purified cell populations.[6,7] Genomic DNA was extracted from PBMCs and freshly frozen liver tissues using the GenElute Mammalian Genomic DNA Miniprep Kit (G1N350, Sigma-Aldrich, Germany) following the

manufacturer's protocol. The extracted DNA served as the template for TCR-β and BCR-H repertoire analysis using the immunoSEQ assay. Immunosequencing of CDR3 regions was performed following the methodology established in previous studies.[8-10] Briefly, a multiplex polymerase chain reaction amplification strategy with integrated bias control was employed to amplify V(D)J rearrangements of genomic DNA, after which HTS was conducted to obtain comprehensive profiles of TCR and BCR repertoires.

The initial processing of raw sequencing data from the immunoSEQ assay was conducted using the immunoSEQ Analyzer software (http://www.adaptivebiotech.com/immunoseq; Adaptive Biotechnologies, USA). This pipeline incorporates rigorous quality control measures to minimize amplification and sequencing bias. For the TCR-β repertoire, sequence annotation was conducted using the MiXCR library. In contrast, BCR-H repertoire sequences were aligned to the international ImMunoGeneTics reference database (version 3; http://www.imgt.org) for annotation.

To ensure the reliability of downstream analyses, nonproductive sequences and those supported by fewer than two reads were excluded. In addition, batch correction was applied to remove potential inter-assay variations across datasets. Each unique CDR3 nucleotide sequence was defined as an individual clone,[11] and the relative abundance of each clone was calculated after alignment. For each repertoire, the frequency distribution of distinct nucleotide sequences, corresponding amino acid sequences, and *V-J* gene combinations was computed. Comprehensive repertoire analyses were then conducted, including the assessment of clonal diversity, gene segment usage bias, and CDR3 length distribution. Diversity metrics were quantified using multiple indices—including Shannon's H index, Simpson index, inverse Simpson index, and D50 diversity index.[12,13]—to provide complementary measures of repertoire complexity. Clonal sharing between samples was assessed using the overlap coefficient, defined as:

$$overlap(X,Y)=\frac{|X\bigcap Y|}{min(|X|,|Y|)} \quad (I)$$

with amino acid sequences serving as the comparison unit and nucleotide sequences representing the species identity. These analyses collectively enabled the systematic evaluation of repertoire structure, clonality, and inter-individual similarity in both TCR and BCR repertoires, following previously established methodologies.[14,15]

2.3. Data analyses

PBMC samples were analyzed using a one-way Analysis of Variance to assess group-level differences, while liver tissue samples were evaluated using unpaired two-tailed *t*-tests to compare pairwise contrasts. Statistical significance was determined based on two-tailed $p<0.05$, and all analyses were performed using Statistical Package for the Social Sciences version 24.0 (IBM, United States) to ensure reproducibility and standardization of results. To further account for multiple comparisons, Holm-Bonferroni corrections were systematically applied across all diversity and repertoire-sharing metrics, thereby reducing the potential inflation of type I error and mitigating false discovery rate concerns in intergroup statistical evaluations.

3. Results

3.1. TCR-β and BCR-H CDR3 repertoire diversity analysis

To comprehensively quantify the overall diversity of the AIH cohort's in-frame and out-of-frame TCR-β and BCR-H CDR3 repertoires, we employed multiple statistical indices, including Shannon's H index, Simpson index, inverse Simpson index, Gini index, and D50 diversity index, all of which are widely applied to capture different aspects of repertoire complexity at the amino acid sequence level. These indices were used to provide a multidimensional assessment of sequence diversity, where lower Simpson's and Gini values, together with higher Shannon, inverse Simpson's, and D50 values, consistently indicated greater diversity within the immune repertoire.

As shown in Figure 1, the HD group displayed the highest degree of repertoire diversity across both TCR-β and BCR-H CDR3 regions. In contrast, patients with PBC exhibited significantly reduced in-frame TCR-β CDR3 diversity compared with both the HD and AIH groups ($p<0.05$) (Figure 1A-E). However, no significant differences were observed in the diversity of out-of-frame sequences (Figure S1A-E). Moreover, the diversity of BCR-H CDR3 in PBMC samples from AIH patients, for both in-frame and out-of-frame sequences, was significantly lower than that observed in the HCV groups ($p<0.05$) (Figure 1F-J; Figure S1F-J). In contrast, there were no statistically significant differences in TCR-β and BCR-H CDR3 repertoires between AIH and control liver tissue samples (Figure S2).

3.2. Clonal distribution of TCR-β and BCR-H CDR3 repertoires

The diversity of the TCR-β and BCR-H CDR3 repertoires was assessed by analyzing the distribution of various CDR3 clones to evaluate clonal architecture and repertoire constraints. Clonal expansion was assessed by calculating the cumulative proportion of the repertoire represented by the most frequent TCR-β and BCR-H amino acid clones, which were subsequently categorized into four groups: top 10, top 11–100, top 101–1000, and clones ranked beyond 1000.

As shown in Figure 2, the average overlap rate of the 10 most abundant TCR-β and BCR-H clones in PBMC samples from AIH patients was significantly lower than that observed in other liver diseases, suggesting a reduced dominance of common clones. Moreover, the average overlap rate of over 1,000 low-frequency TCR-β and BCR-H clones in PBMC samples from the three liver disease groups was lower than that in the

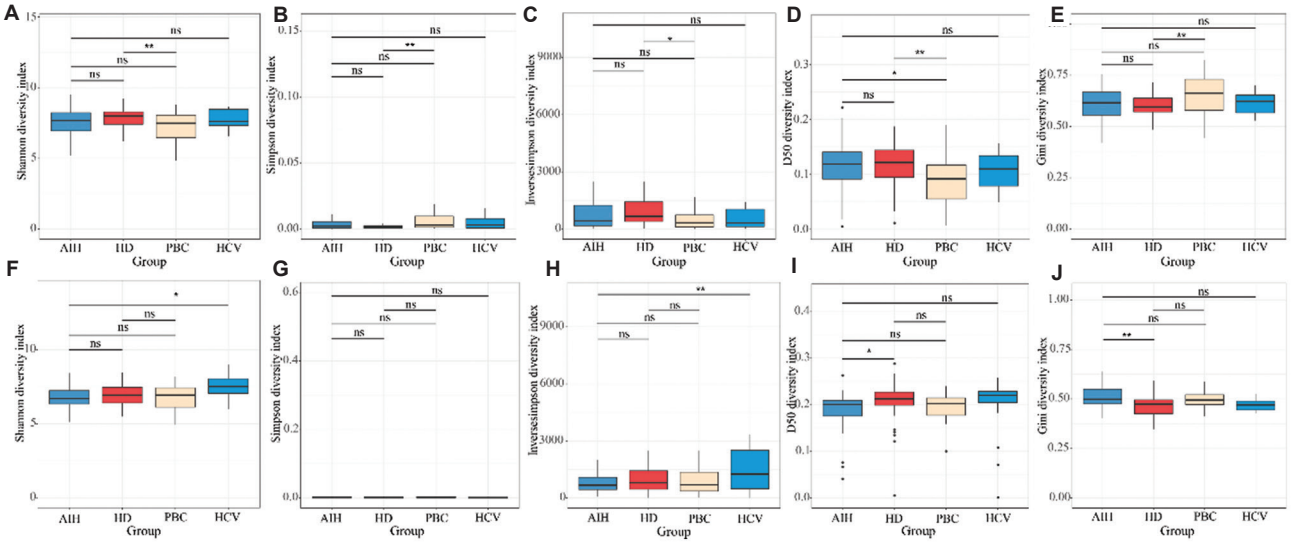


Figure 1. In-frame TCR and BCR repertoire diversity indices in the peripheral blood of AIH patients and control groups (HD, PBC, and HCV). The diversity of the TCR repertoire was assessed using (A) Shannon index, (B) Simpson index, (C) inverse Simpson index, (D) D50 diversity index, and (E) Gini diversity index. The BCR repertoire diversity was assessed using the Shannon index (F), Simpson index (G), inverse Simpson index (H), D50 diversity index (I), and Gini diversity index (J). Data were compared using an unpaired *t*-test, and multiple comparisons were adjusted using the Bonferroni-Holm correction.

Notes: Single asterisk (*) and double asterisk (**) indicate statistically significance at $p<0.05$ and $p<0.01$, respectively. "ns" denotes no statistical significance.

Abbreviations: AIH: Autoimmune hepatitis; BCR: B-cell receptor; HCV: Hepatitis C virus; HD: Healthy donor; PBC: Primary biliary cholangitis; TCR: T-cell receptor.

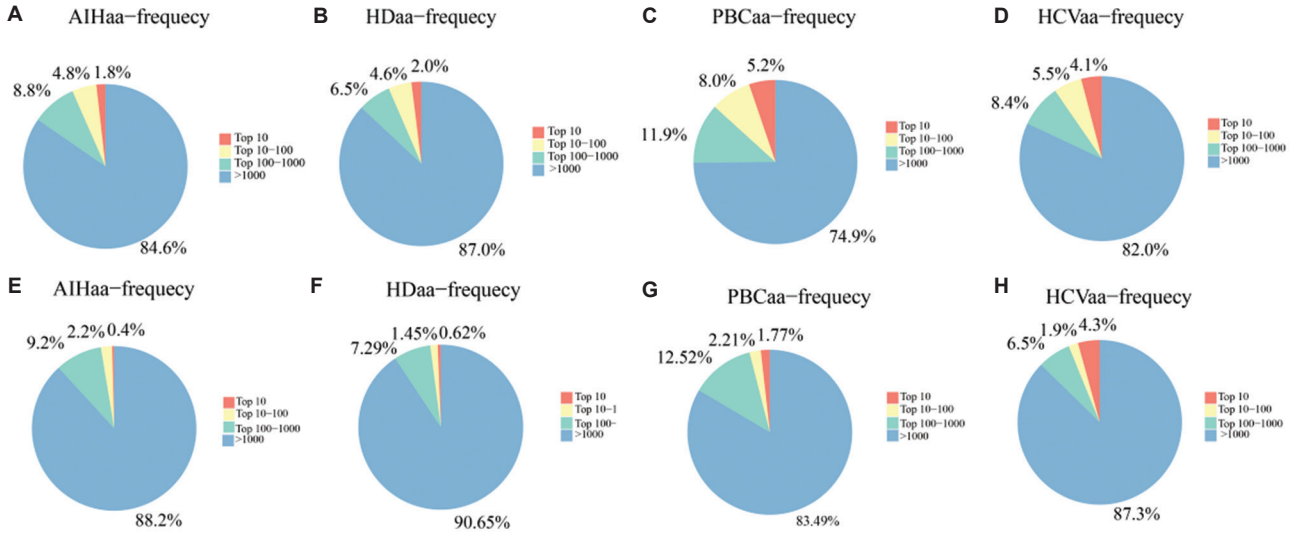


Figure 2. Analysis of TCR-β and BCR-H CDR3 repertoires in peripheral blood samples from AIH patients and control groups. Frequency distributions of (A-D) TCR-β CDR3 and (E-H) BCR-H CDR3 repertoires. Each pie chart displays the percentages of the most prevalent clones, classified into four categories: top 10, the top 11-100, the top 101-1000, and those over 1000.

Abbreviations: AIH: Autoimmune hepatitis; BCR-H: B-cell receptor heavy chains; CDR3: Complementarity-determining region 3; HCV: Hepatitis C virus; HD: Healthy donor; PBC: Primary biliary cholangitis; TCR-β: T-cell receptor β-chains.

HD cohort, although the difference was not statistically significant.

To further investigate tissue-specific distribution, we examined the frequency distribution of TCR-β and BCR-H CDR3 clones in liver tissue samples from AIH patients and control groups (Figure S3). Interestingly, clone abundance analysis revealed that the mean overlap rate of the 10 most common TCR-β clones in liver tissue samples from AIH patients was higher than that in the control group (8.7% vs. 7.1%), indicating the presence of dominant T-cell clones in AIH. In addition, the proportion of highly diverse clones (>1000 types) in AIH liver tissue samples was markedly increased compared with controls (42.7% vs. 32.4% for TCR-β, and 52.2% vs. 23.3% for BCR-H).

3.3. TCR-β/BCR-H CDR3 length distribution and recombination patterns

In the AIH group, each sequence's CDR3 region was sequenced, and the distributions of amino acid and nucleotide lengths were analyzed. The TCR-β CDR3 amino acid lengths ranged from 4 to 86 amino acids, with a peak at 14 amino acids, while the BCR-H CDR3 lengths ranged from 4 to 68 amino acids, peaking at 16 amino acids (Figure 3A and B). Patients with PBC had significantly shorter in-frame TCR-β CDR3 lengths compared with those with AIH. In addition, the out-of-frame TCR-β CDR3 length distribution in the HD group was significantly shorter than that observed in AIH patients (Figure 3C and D). Notably, this trend was reversed when examining the nucleotide length distribution of BCR-H CDR3. In the AIH-PBMC group, relative to the HD group, either in-frame or out-of-frame BCR-H CDR3 repertoires showed a shift toward shorter clonotypes at the nucleotide level, suggesting that the BCR-H repertoire was aberrant in AIH patients (Figure 3F and G).

To elucidate the molecular basis underlying these features, we analyzed recombination patterns, as variations in TCR-β and BCR-H CDR3 lengths may arise from atypical nucleotide insertions and/or deletions (Figure 3E and H). Nonetheless, no biases were detected in the nucleotide composition of the CDR3 sequences. Furthermore, statistical analysis indicated that the distribution of TCR-β and BCR-H CDR3 indel lengths, at both the nucleotide and amino acid levels, was comparable between AIH and control liver tissue samples (Figure S4).

Since recombination patterns alone could not fully explain the observed differences in CDR3 length distributions, we next examined whether clonal selection processes might contribute to these patterns. Interestingly, the preferential enrichment of specific CDR3 lengths coincided with a decreased frequency of public clonotypes shared among AIH individuals, implying that these length-skewed clones may recognize common antigenic determinants associated with disease pathogenesis. Moreover, when compared with the PBC and HCV groups, the repertoire of AIH patients exhibited distinct clonotype length signatures, further supporting the hypothesis that length variation reflects disease-specific immune responses.

3.4. TCR-β and BCR-H CDR3 repertoire complexity score and skewness analysis

To further evaluate repertoire abnormalities, complexity scores and skewness values were calculated as quantitative measures of TCR-β and BCR-H receptor diversity, thereby providing an improved means of identifying perturbations in CDR3 length distributions.[16,17] The complexity score was obtained by quantifying the number and relative contributions of dominant peaks in the CDR3 length spectrum, with dominant peaks defined as those whose amplitudes were equal to or >10% of the total peak height sum. This approach allowed assessment of the extent to which a small number of peaks dominated the overall distribution.

In parallel, skewness was calculated to evaluate the asymmetry of the CDR3 length distribution relative to the mean, serving as an indicator of preferential expansion at specific lengths. As illustrated in Figure S5A-D, both AIH and PBC cohorts exhibited reduced complexity scores in their TCR-β CDR3 length repertoires compared with those of the HD group, indicating a more restricted and less diverse distribution. Moreover, statistically significant differences were observed in both the complexity score and skewness values of TCR-β and BCR-H CDR3 profiles between AIH and HCV patients. Conversely, AIH liver tissue samples exhibited TCR-β and BCR-H CDR3 profiles comparable to those of control liver tissue samples, with no significant differences detected (Figure S6A-D).

3.5. Amino acid composition of TCR-β and BCR-H CDR3 repertoires

Recent research has shown that a 13-amino acid CDR3 sequence containing hydrophobic amino acids at the sixth and seventh positions promotes T-cell self-reactivity.[18,19] Our analysis revealed that the amino acid composition at positions 6 and 7 was highly conserved in the TCR-β CDR3 and BCR-H CDR3 repertoires among patients with AIH, as well as in both healthy and diseased control groups (Figures S5E-P and S6E-H).

3.6. Skewed usage of *TRβV* and *IGHV* genes and CDR3 sequences

To investigate whether pathogenic factors associated with AIH, PBC, and HCV influence gene usage preferences,

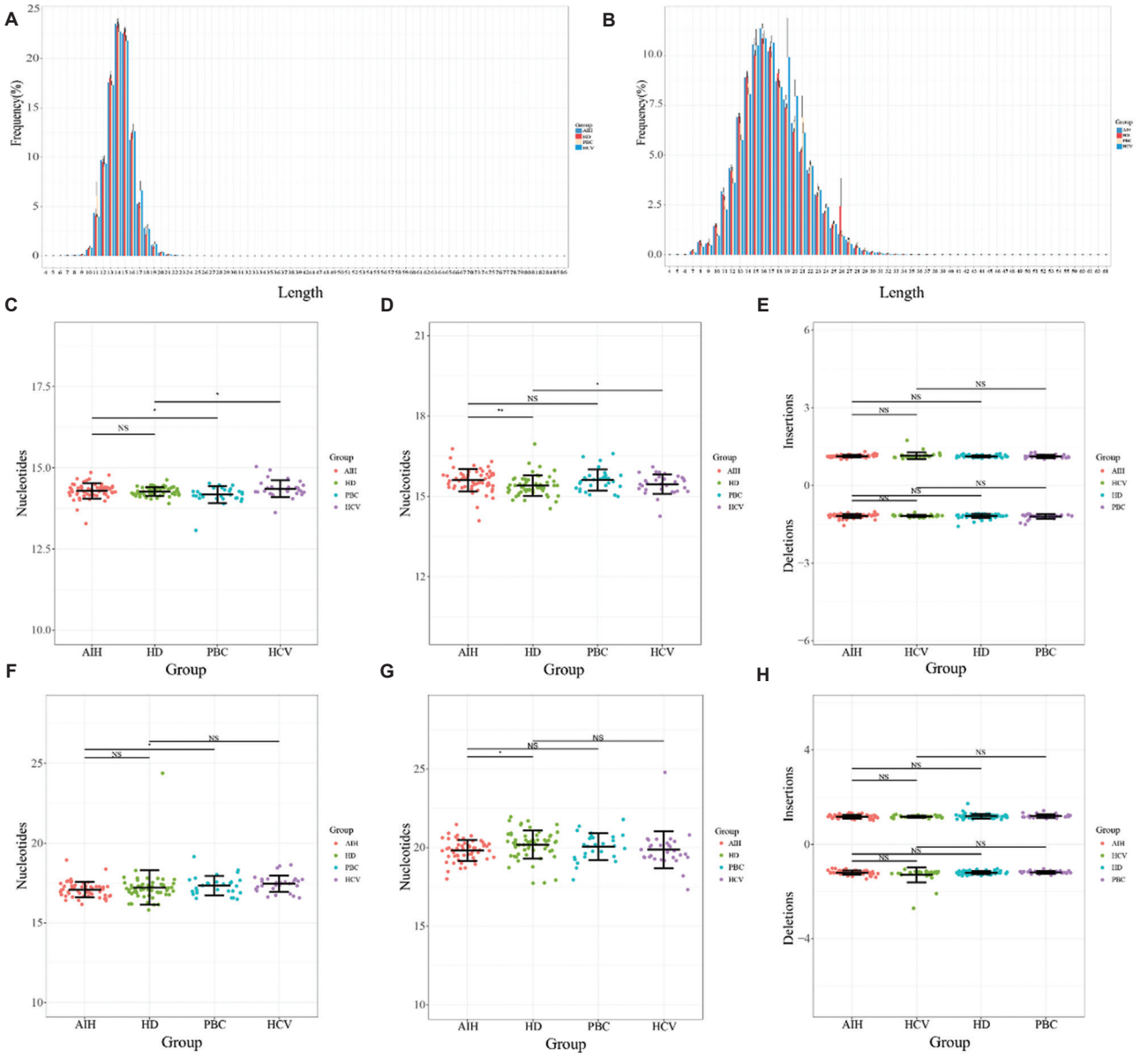


Figure 3. Distribution of lengths and recombination patterns of TCR-β and BCR-H CDR3 in peripheral blood samples from AIH patients. Amino acid lengths of (A) TCR-β CDR3 and (B) BCR-H CDR3 in AIH, HD, PBC, and HCV patients. Nucleotide lengths of the (C) out-of-frame and (D) in-frame TCR-β repertoires across groups, along with the (E) average length of nucleotide insertions or deletions in TCR-β CDR3. Similarly, nucleotide lengths of the (F) out-of-frame and (G) in-frame BCR-H repertoires in the AIH cohort, along with the (H) average length of nucleotide insertions or deletions in BCR-H CDR3. Data are presented as mean ± standard deviation and compared using an unpaired *t*-test.

Notes: Single asterisk (*) and double asterisk (**) indicate statistical significance at $p<0.05$ and $p<0.01$ (two-tailed), respectively. "NS" denotes no statistical significance.

Abbreviations: AIH: Autoimmune hepatitis; BCR-H: B-cell receptor heavy chain; CDR3: Complementarity-determining region 3; HCV: Hepatitis C virus; HD: Healthy donor; PBC: Primary biliary cholangitis; TCR-β: T-cell receptor β-chain.

we compared the utilization frequencies of variable gene segments in unique TCR-β (Figure 4A-F) and BCR-H (Figure 4G-L) repertoires derived from both PBMC and liver tissue samples. The analysis demonstrated that, relative to control liver tissue samples, several TCR-β variable (*TRBV*) genes—including *TRBV4*, *TRBV5*, *TRBV6*, *TRBV7*, *TRBV10*, *TRBV11*, *TRBV12*, *TRBV13*, *TRBV14*, *TRBV15*, *TRBV18*, *TRBV19*, *TRBV20*, *TRBV27*, *TRBV28*, *TRBV29*, and *TRBV30*—exhibited significantly lower utilization frequencies in AIH liver tissue samples (Figure 4A and B).

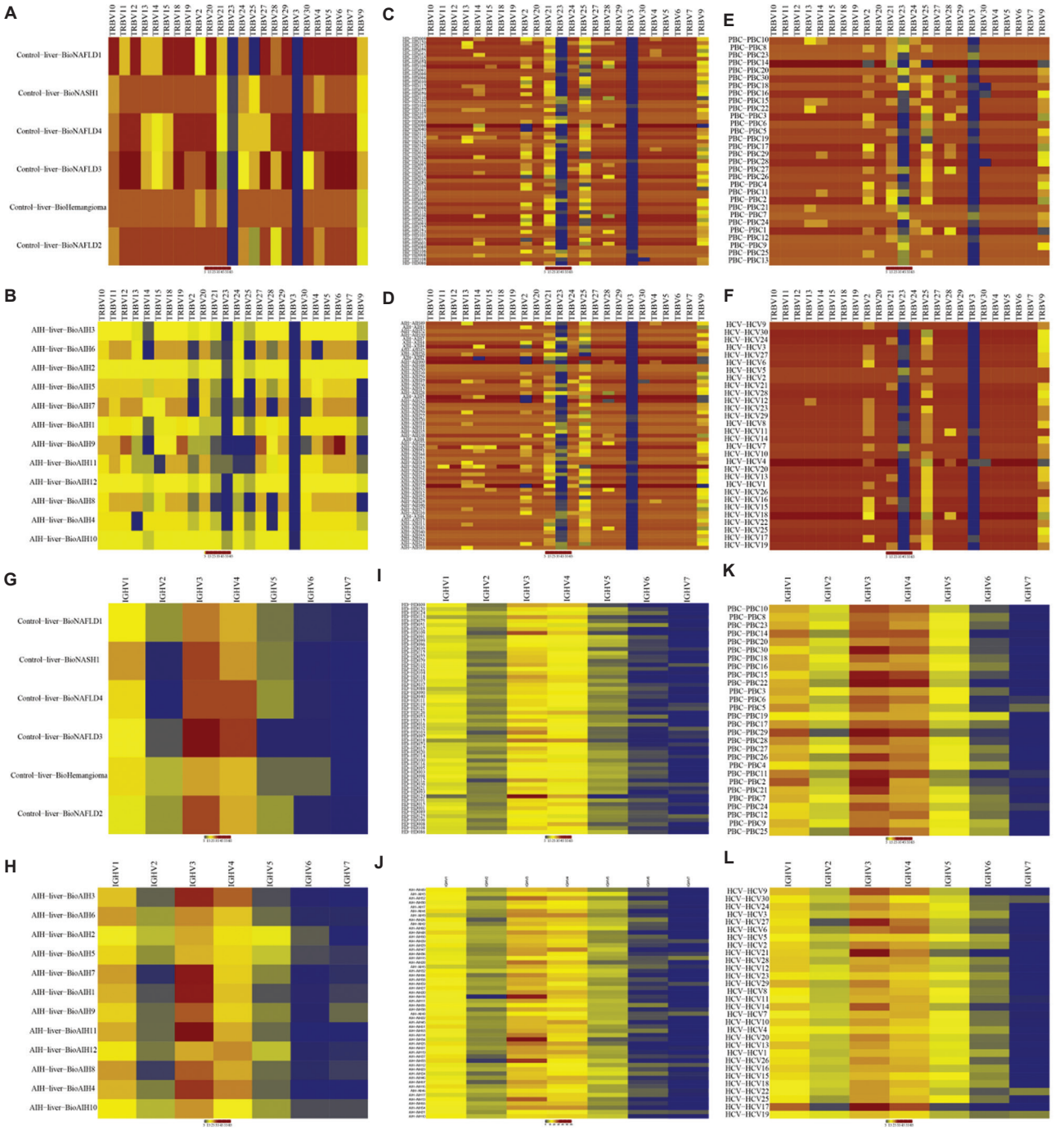


Figure 4. Heatmaps showing the frequency of unique *TRBV* and *IGHV* gene usage. (A-C, G-I) Liver tissue samples from (A and G) control, (B and H) AIH, and (C and I) HD groups. (D-F, J-L) PBMC samples from (D and J) AIH, (E and K) PBC, and (F and L) HCV groups.
Abbreviations: AIH: Autoimmune hepatitis; HCV: Hepatitis C virus; HD: Healthy donor; IGHV: Immunoglobulin heavy chain variable; PBC: Primary biliary cholangitis; PBMC: Peripheral blood mononuclear cell; TRBV: T-cell receptor β-variable.

In contrast, in PBMCs, the immunoglobulin heavy chain variable (IGHV) region (*IGHV3* and *IGHV4*) segments were consistently more frequently utilized across all three liver disease groups than in the HD group (Figure 4I-L).

Beyond gene usage, we also examined intergroup public clonotypes, as public clonality is regarded as an important marker of shared immune responses across individuals. By analyzing both PBMC and liver tissue samples from AIH

patients, we identified several TCR-β and BCR-H CDR3 sequences that underwent clonal expansion specifically within the AIH cohort. As illustrated in Figure 5, AIH patients displayed an increased frequency of shared TCR-β and BCR-H CDR3 public clones in PBMCs compared with all three control groups. However, the expansion of BCR-H clones appeared relatively modest in number. Furthermore, significant differences in the clonal abundance of both TCR-β and BCR-H CDR3 repertoires were observed between AIH and control groups.

Overall, these findings suggest that the unique repertoire biases observed in AIH are not merely a consequence of non-specific liver inflammation but may reflect disease-specific immune mechanisms. We hypothesize that the differential abundance of expanded CDR3 clonotypes across AIH, PBC, and HCV groups represents distinct pathogenic processes, with each condition driving characteristic TCR and BCR repertoire signatures.

3.7. Sequence sharing patterns of TCR-β and BCR-H CDR3 repertoires

The concept of sequence sharing within the immune repertoire was further investigated by distinguishing between "private sequences"—defined as TCR-β or BCR-H CDR3 sequences that occur exclusively in a single individual—and "public sequences," which are shared across two or more individuals within the same cohort. To evaluate repertoire variability in AIH, we quantified both the proportion and overlap of private and public sequences across groups.

As shown in Figure 6A and B, PBMC samples from the AIH and HD groups exhibited significantly greater TCR-β CDR3 overlap compared with other disease groups ($p<0.05$). In contrast, BCR-H CDR3 overlap was most pronounced in the HCV group, where shared clones were significantly more frequent than in the AIH, PBC, or HD groups. To

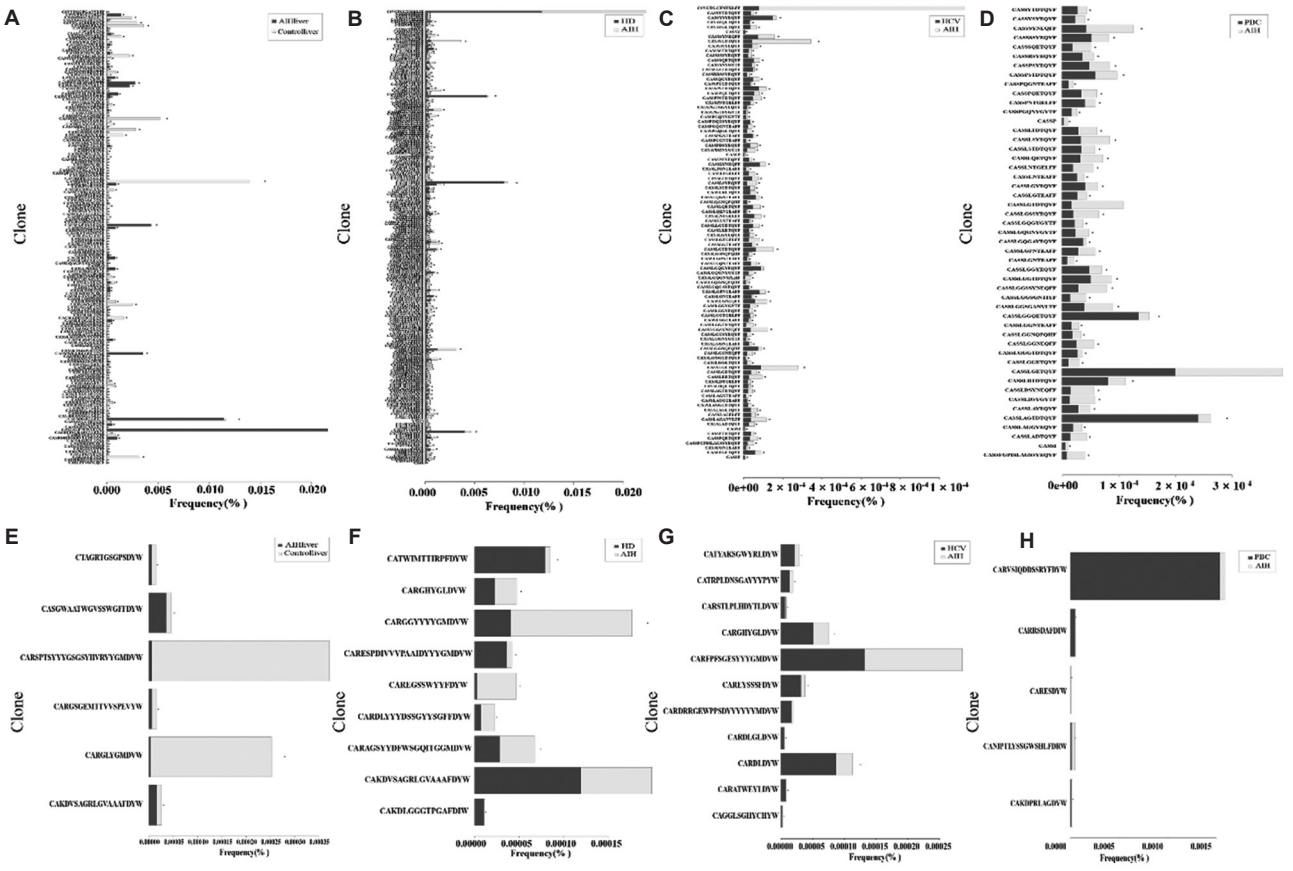


Figure 5. Frequent clones identified in the liver tissue and peripheral blood of AIH patients. Frequent (A) TCR-β CDR3 and (E) BCR-H CDR3 clones in liver tissue samples from AIH and control groups. Comparison of (B) TCR-β CDR3 and (F) BCR-H CDR3 clone abundance between PBMC samples from AIH and HD groups. Comparison of (C) TCR-β CDR3 and (G) BCR-H CDR3 clone abundance between PBMC samples from AIH and HCV groups. Comparison of (D) TCR-β CDR3 and (H) BCR-H CDR3 clone abundance between PBMC samples from AIH and PBC groups.
Abbreviations: AIH: Autoimmune hepatitis; BCR-H: B-cell receptor heavy chain; CDR3: Complementarity-determining region 3; HCV: Hepatitis C virus; HD: Healthy donor; PBC: Primary biliary cholangitis; PBMC: Peripheral blood mononuclear cell; TCR-β: T-cell receptor β-chain.

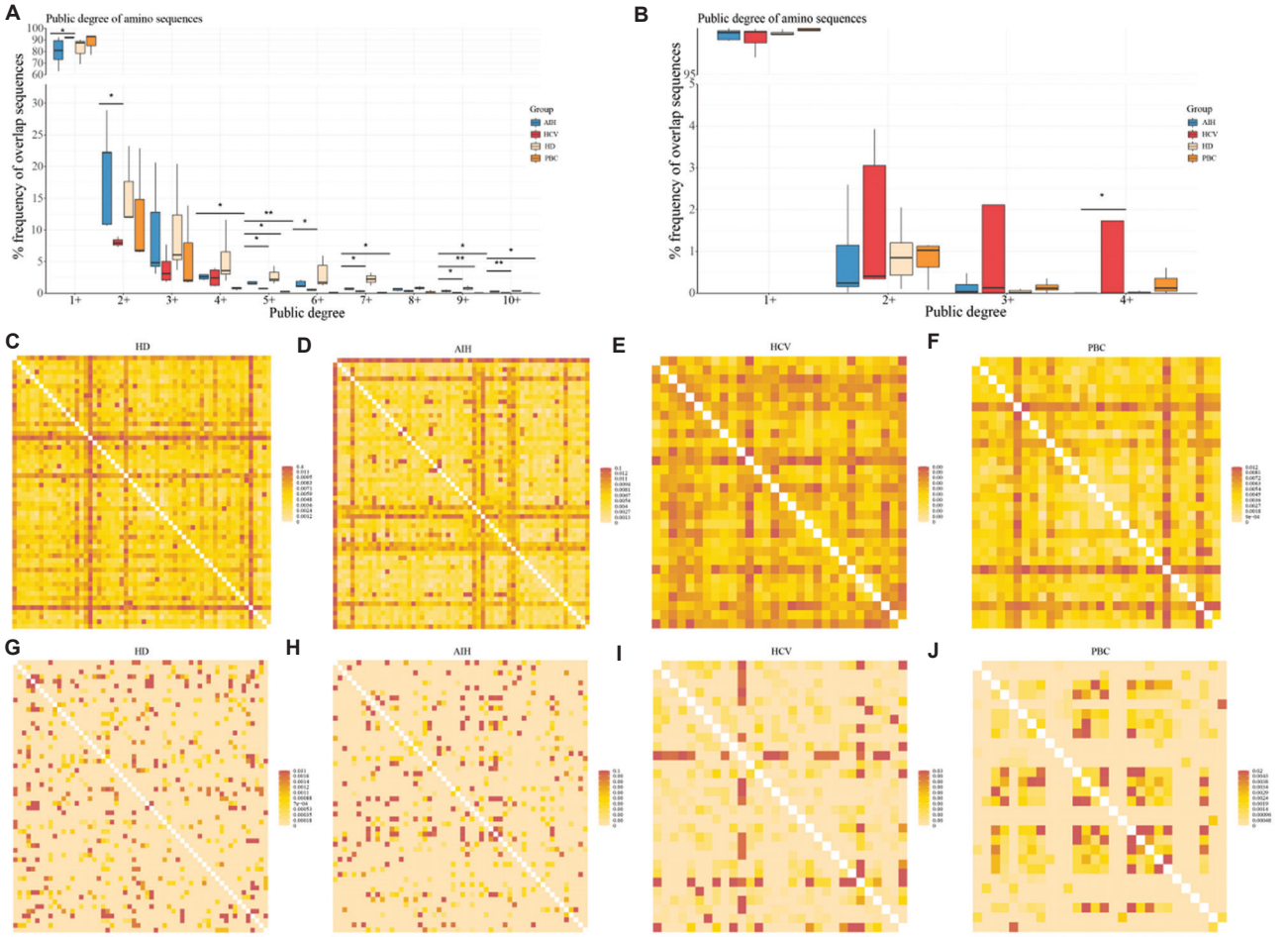


Figure 6. Clone sharing in PBMC samples within the AIH group. Overlap of (A) TCR-β CDR3 and (B) BCR-H CDR3 amino acid sequences among PBMC samples from AIH, HCV, PBC, and HD groups. (C-F) Heatmaps showing the percentage of shared TCR-β repertoires between any two individuals in the (C) HD, (D) AIH, (E) HCV, and (F) PBC groups. (G-J) Heatmaps showing the percentage of shared BCR-H repertoires between any two individuals in the (G) HC, (H) AIH, (I) HCV group, and (J) PBC groups. Data were compared using an unpaired *t*-test, with Bonferroni–Holm correction applied for multiple comparisons.

Notes: Single asterisk (*) and double asterisk (**) indicate statistical significance at $p<0.05$ and $p<0.01$, respectively.

Abbreviations: AIH: Autoimmune hepatitis; BCR-H: B-cell receptor heavy chain; CDR3: Complementarity-determining region 3; HCV: Hepatitis C virus; HD: Healthy donor; PBC: Primary biliary cholangitis; PBMC: Peripheral blood mononuclear cell; TCR-β: T-cell receptor β-chain.

provide a more comprehensive view of sequence-sharing patterns, we also employed heat map-based analysis to calculate and visualize the overlap index of TCR-β and BCR-H amino acid repertoires across all pairwise sample comparisons within each group (Figure 6C-J; Figures S6 and S7). This approach enabled the identification of both intra- and inter-individual sequence convergence, thereby highlighting group-specific signatures of immune receptor sharing.

4. Discussion

AIH is an idiopathic disease characterized by impaired immune regulatory mechanisms.[2] The presence of autoantibodies and elevated γ-globulin levels is diagnostic hallmark, and the involvement of T and B cells is central to disease pathogenesis.[5,20,21] This study aimed to explore the diversity and composition of TCR-β and BCR-H CDR3 repertoires in T and B cells derived from PBMCs and liver tissue from AIH patients using HTS. Compared with the control group, our findings revealed that all three liver disease cohorts showed significantly reduced diversity and increased clonality within the TCR-β and BCR-H repertoires.

Bao *et al.*[22] reported that patients with PBC demonstrate reduced diversity in peripheral $CD4^+$ TRβV CDR3 compared with healthy individuals. Furthermore, this reduction in diversity was more pronounced among patients with advanced stages of the disease. We postulated

that liver-derived repertoires, being the most functionally relevant components, could provide deeper insights. However, the liver repertoire metrics indicated a similar distribution of clones between AIH and control liver tissue samples, consistent with the findings of Schultheiß *et al*.[5] This similarity may be attributed to the architectural features of liver T cells in AIH, which consist of multiple antigen-reactive T cells rather than a single dominant clone.

Variations in the V(D)J rearrangement process can lead to alterations in the lengths of TCR-β and BCR-H CDR3 regions, thereby enhancing their capacity to recognize a diverse array of antigens. This, in turn, may induce modifications in T- and B-cell functionality, potentially contributing to immune dysregulation.[23] In this study, we observed that, compared with PBMC samples from the HD group, BCR-H CDR3 lengths were shifted toward shorter clonotypes in PBMC samples from the AIH group, indicating that AIH-related enrichment of shorter BCRs may enhance the potential for autoreactivity.[24] However, the transition of TCR CDR3 to longer clonotypes in PBMC samples from the AIH group is inconsistent with previous findings[24] and may require further investigation.

Notably, the pre-selected BCR-H CDR3 regions of AIH and PBC patients also showed abnormal shortening. This suggests that the initial stages of B-cell development and repertoire formation in the bone marrow of AIH and PBC patients are aberrant.[25] In addition, the average length of nucleotide deletions or insertions at each position among groups was evaluated, and the findings indicated that the overall length distribution was similar across all groups. This aligns with previous findings, which demonstrated that the average CDR3 nucleotide length was not significantly associated with the mean length of inserted nucleotides in either pre-selection or post-selection repertoires. However, a positive correlation was observed with the length of the original germline sequence.[25]

Moreover, one or more V(D)J subfamilies might undergo selective rearrangement and abnormal replication due to disease.[26] The *TRBV* (*IGHV*) gene usage map revealed skewness and restriction in the V-gene usage of AIH patients. Previous studies have confirmed restricted and skewed V(D)J segment usage in autoimmune diseases such as primary Sjogren's syndrome,[27] PBC,[28] and rheumatoid arthritis.[29] Information on the richness and complexity score of CDR3 length in the T- and B-cell repertoires of AIH patients remains limited. In this study, the findings demonstrated that AIH and HCV patients exhibited statistically significant differences in their TCR-β and BCR-H CDR3 repertoires, with HCV patients displaying higher complexity scores. This might be related to the emergence of viral escape mutations during long-term HCV infection[30] and the extent of T-cell infiltration in AIH patients,[31] with specific mechanisms requiring further study.

The notion of public TCR-β and BCR-H sequences was first proposed more than 5 years ago.[32-34] We define TCR-β or BCR-H sequences that are shared among all individuals in a population as public sequences.[35] In contrast, sequences detected exclusively in a single individual are defined as private sequences. Interestingly, we observed an atypical phenomenon in which unrelated individuals within the same group shared identical TCR-β or BCR-H amino acid sequences, and these sequences were consistently detected across multiple individuals.[36] In healthy individuals, V(D)J recombination of TCRs and BCRs occurs randomly, resulting in a polyclonal and multi-family repertoire of T and B cells. However, under persistent stimulation by disease-associated antigens, including both exogenous and self-antigens, antigen-specific T- and B cells undergo selective clonal expansion. Given the homeostatic nature of the immune system, the total number of immune cells in the body remains relatively stable. Consequently, the preferential expansion of certain T or B cell subfamilies leads to a relative reduction in others. At the molecular level, this process is reflected by an increased abundance of specific TCR or BCR sequences, accompanied by a relative decrease in other TCRs or BCRs, ultimately resulting in reduced repertoire diversity.

Notably, TCR or BCR diversity is strongly associated with health: higher diversity confers greater resistance to pathogens, whereas reduced diversity predisposes individuals to disease. Our findings indicated that the overlap of the TCR-β and BCR-H repertoires was more pronounced in patients with AIH compared with other groups. Shared clonotypes within the TCR-β repertoire are likely induced in response to identical antigenic epitopes and are essential for pathogen-specific immune responses and infection control.[37] In this study, we found that, compared with healthy controls, patients with AIH exhibited a higher degree of overlap in their TCR-β and BCR-H repertoires. Moreover, patients with all three types of liver disease showed a significant reduction in TCR-β and BCR-H repertoire diversity, accompanied by a marked increase in clonality. These findings suggest that reduced immune receptor diversity and clonal expansion may play key roles in the pathogenesis of AIH and other liver diseases. Specifically, in AIH patients, the excessive overlap and restricted diversity of TCR-β and BCR-H repertoires may limit the breadth of antigen recognition by the immune system, thereby contributing to disease progression.

5. Conclusion

In summary, through HTS, we conducted a comprehensive characterization of the TCR-β and BCR-H CDR3 repertoires in PBMC and liver tissue samples from patients with AIH. Our analysis revealed multiple repertoire alterations, including significantly reduced overall TCR and BCR diversity, a tendency toward shortened BCR-H and TCR-β CDR3 clonotypes, skewed and restricted usage of *TRBV* and *IGHV* gene segments, and abnormal V(D)J rearrangement patterns. Furthermore, we observed an unusually high degree of clonotype sharing among unrelated individuals, with prominent overlap between the TCR-β and BCR-H repertoires, suggesting antigen-driven clonal expansion. Collectively, these findings indicate that restricted receptor diversity, biased V(D)J usage, and increased clonal overlap may impair immune repertoire breadth, thereby contributing to the breakdown of self-tolerance and the pathogenesis of AIH.

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Conflict of interest

The authors declare no competing interests.

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Ethics approval and consent to participate

Written informed consent was obtained from all human participants for participation through ethics committee-approved consent forms.

Consent for publication

Written informed consent was obtained from all human participants for data.

Availability of data

All TCR and BCR CDR3 sequences used in the evaluation were downloaded from the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/view/PRJEB37143). The data underlying this article will be shared on reasonable request to the corresponding author.

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