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The repertoire features of T cell receptor β-chain of different age and gender groups in healthy Chinese individuals

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ABSTRACT

T cell immunity is dependent on T cell receptor (TCR) recognition of large numbers of antigenic peptides presented in the context of major histocompatibility complex (MHC) class I and II molecules. To explore whether the TCR β-chain repertoire changes with different ages and genders in healthy Chinese individuals, we analyzed the TCR β-chain repertoire in 154 healthy Chinese individuals by High-throughput sequencing (HTS) with age (age range: 6–70) and each age group contains about 20 individuals (male and female). Here we report that the extent of TCR diversity was not dependent on gender but it was significantly different between age groups. We found that the rearrangement of the V with J genes in T cell receptor β-chain was highest at the age of 21–30. Moreover, we found that the combination of V6-1 and J2-1 gene had the highest expression over a human lifespan. We further identified J2-1 and V7-2 gene expressed higher at the age of 6–10 and 11–20 than other age groups. However, D gene in TCR β-chain was not significantly diverse in different age groups. In addition, the T cell receptor β complementarity-determining region 3 (CDR3) amino acid sequence expressed the highest between the age of 21–30. These results showed different features of TCR β-chain repertoire in different groups of healthy Chinese individuals. In conclusion, it was expected that these TCR repertoires could serve as a useful tool for investigating the role of immune profiling in healthy Chinese individuals. These results stress the importance of considering age as a factor for immune response.

1. Introduction

T cell repertoire describes lymphocytes characterized by T cell receptor (TCR) expression, which plays a critical role in antigen recognition. Long read sequencing of TCR genes includes V, D, J and C regions. Importantly, the TCR diversity and the combination of V with J genes reflects T cell function and human immune status. The analysis of complementarity-determining region 3 (CDR3) is based on V-D-J gene rearrangement that has been used to evaluate T cell clonality and diversity [1,2]. Structural diversity of TCR repertoire has been directly examined by deep sequencing of the TCRβ CDR3 region [3]. Advances in high-throughput sequencing (HTS) technologies have rapidly progressed and enabled large-scale analysis of sequence data [4,5]. Furthermore, a number of advancements in the field have been described to determine the specificity of TCR repertoire by this technology [6]. Also, a healthy adult has several million TCRβ sequences in circulating T cells analysed by HTS [7]. A better understanding of the TCR diversity of different age stages will facilitate the development of novel strategies of immunotherapy.

It has become clear that the TCR repertoires is related to the

Abbreviations: TCR, T cell receptor; MHC, major histocompatibility complex; HTS, High-throughput sequencing; TRBD, D gene in TCR β-chain; TRBJ, J gene in TCR β-chain; TRBV, V gene in TCR β-chain; CDR3, complementarity-determining region 3; NGS, next-generation sequencing; TIL, tumor-infiltrating T

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Table 1
Summary of human samples and CDR3 sequences.

Cell type	Gender	Mean age at sample collection	Age group	Sample number	CDR3 nt sequences	Total CDR3 sequencing reads	CDR3 V-J combination
PBMC	male	7.3	6-10	13	12556	510922	554
PBMC	female	9.0	6-10	10	10884	553828	530
PBMC	male	16.2	11-20	12	8610	500786	518
PBMC	female	16.9	11-20	10	7866	215692	526
PBMC	male	26.3	21-30	12	33070	1618343	690
PBMC	female	25.4	21-30	15	32364	1396672	636
PBMC	male	34.9	31-40	10	12328	1062148	523
PBMC	female	38.3	31-40	10	11143	1297068	528
PBMC	male	45.7	41-50	11	7437	580696	510
PBMC	female	45.4	41-50	10	7599	479785	455
PBMC	male	55.5	51-60	10	9642	338726	521
PBMC	female	54.5	51-60	11	12960	665106	556
PBMC	male	63.1	61-70	10	6919	517767	521
PBMC	female	63.9	61-70	10	6820	181657	487
				Total:154			

occurrence and development of many diseases. HTS longitudinally investigated the change of the peripheral T-cell repertoire in two renal transplant patients [8]. A study found that the Vβ22 clone may have expanded or accumulated in both spleen and pancreas of the patients with type I diabetes [9]. Also, the changes of TCR repertoire sequencing have also been reported in various tumors. A report demonstrated that a "signature" set of TCRs exhibited lower divergences in glioma tumor-infiltrating T than that in peripheral blood [10]. The TCR repertoires of multiple primary esophageal squamous cell carcinoma samples were detected and researchers found that there are spatial heterogeneity [11]. A study showed that HTS of TCR can be applicated in diagnosing and monitoring therapy response in cutaneous T-cell lymphoma [12]. Eighty-eight cases with acute myeloid leukemia and thirty-eight healthy individuals were analyzed in order to further identify the potential mechanism of persistent immunodeficiency in leukemia patients [13].

Additionally, TCRβ repertoires have been analyzed in six healthy adults using a deep sequencing longitudinally over approximately 20 years, with ages ranging from 23 to 50 years [14]. The in-depth characterization of ten epitope-specific TCR repertoires of $CD8^+$ T cells from mice and humans demonstrated that each epitope-specific repertoire contains a cluster group of receptors that share core sequence similarities [15]. Although these studies have substantially increased our understanding of TCR diversity, the analysis of relationship between human T-cell repertoire with age was limited. Furthermore, the frequency, characteristic, and significance of CDR3 TCRβ chain are not well-understood in healthy Chinese individuals either.

In our study, PBMC (Peripheral Blood Mononuclear Cell) samples from 154 healthy individuals were collected (age range: 6–70 years old) with each age group consisting of 20 individuals (with a male: female = 1:1). The analysis of TCR repertoire was performed based on NGS. The results indicated that the extent of TCR diversity was not dependent on gender. However it was significantly different between age groups. Furthermore, compared with other age groups, TRBJ2-1 and TRBV7-2 expresses higher at the age of 6–10 and 11–20. However, D gene in TCRβ-chain (TRBD) was not significantly diverse in different age groups. In addition, we found that the combination of V with J genes was the highest at the age of 21–30. These different rearrangement proportions could be used to investigate the mechanism and function for some diseases. This study may enable us to reveal the diversity of TCR repertoire in healthy Chinese individuals with different age.

2. Materials and methods

2.1. Isolation of PBMC and sample preparation

Human peripheral blood samples were obtained from 154 healthy volunteers (age range: 6–70 years old). Peripheral blood samples were collected from healthy volunteers who tested negative for anti-Hepatitis B surface antigen antibodies and anti-HIV antibodies and exhibited no clinical or laboratory signs of other infectious diseases or any immunological disorders. Each age group includes about ten men and women, respectively. The 10 mL of whole blood was collected into heparinized tubes. PBMCs (3–10 × 10^6 cells per sample) were isolated using gradient separation with lymphoprep. Detailed sample information was provided in Table 1. This study was approved by the Institutional Ethical Committee of the First Affiliated Hospital of Zhengzhou University, China.

2.2. High-throughput sequencing of CDR3

Genomic DNA was isolated from PBMCs using the modified cetyltrimethylammonium bromide method of Brookman and Nicholson [16], combined with bead beating. The purified gDNA was used as template for the PCR amplification, which was performed to generate the library of TCRβ. Each tube (PCR mixture, volume 50 μL in total) contained 10 μl of 5 × HF buffer, 2.5 μL of DMSO, 1 μL of dNTP (10 mM), 0.5 μL of TCR-F, 0.5 μL the primer of TCR-R1 (or TCR-R2 in the other tube), 0.5 μL of enzyme, 200 ng of DNA and some ddH_2O (supplement Table 1 is the primers was used). The PCR amplification protocol was as follows: 94 °C for 3 min; 94 °C for 30 s, 60 °C for 30 s; and 72 °C for 60 s for 35 cycles; 72 °C for 7 min. The PCR products were purified, and the barcodes were confirmed. The region of these primers anneal is the CDR3 region by Multiplex PCR Amplifies. The TCRβ CDR3 regions were sequenced on the platform of Illumina X (MyGenostics, Beijing, China). According to the sequencing depth, the test covers about 90% of the TCR. The TCRβ CDR3 regions were discerned based on the definition established by the International ImMunoGeneTics (IMGT) collaboration, and the V, D, J segments contributing to each CDR3 region were identified by a standard algorithm [17].

2.3. CDR3 sequencing analysis

To evaluate the clonality of TCRβ repertoire in each sample, we defined clonality (clonality was calculated as 1-(Shannon's entropy)/$\log_2$ (number of productive unique sequences). To assess the similarity of TCRβ repertoire between samples, overlap metric of TCRβ repertoire was defined. TCR β repertoire overlap between two samples was defined as the total number of sequencing reads from shared TCR β sequences divided by the sum of sequencing reads detected in both samples, and ranged from 0 to 1:

$$\frac{\sum_{i=1}^{n} C_i(x) + C_i(y)}{\sum C(X) + \sum C(y)}$$

To evaluate the diversity of TCRβ repertoire between samples, a

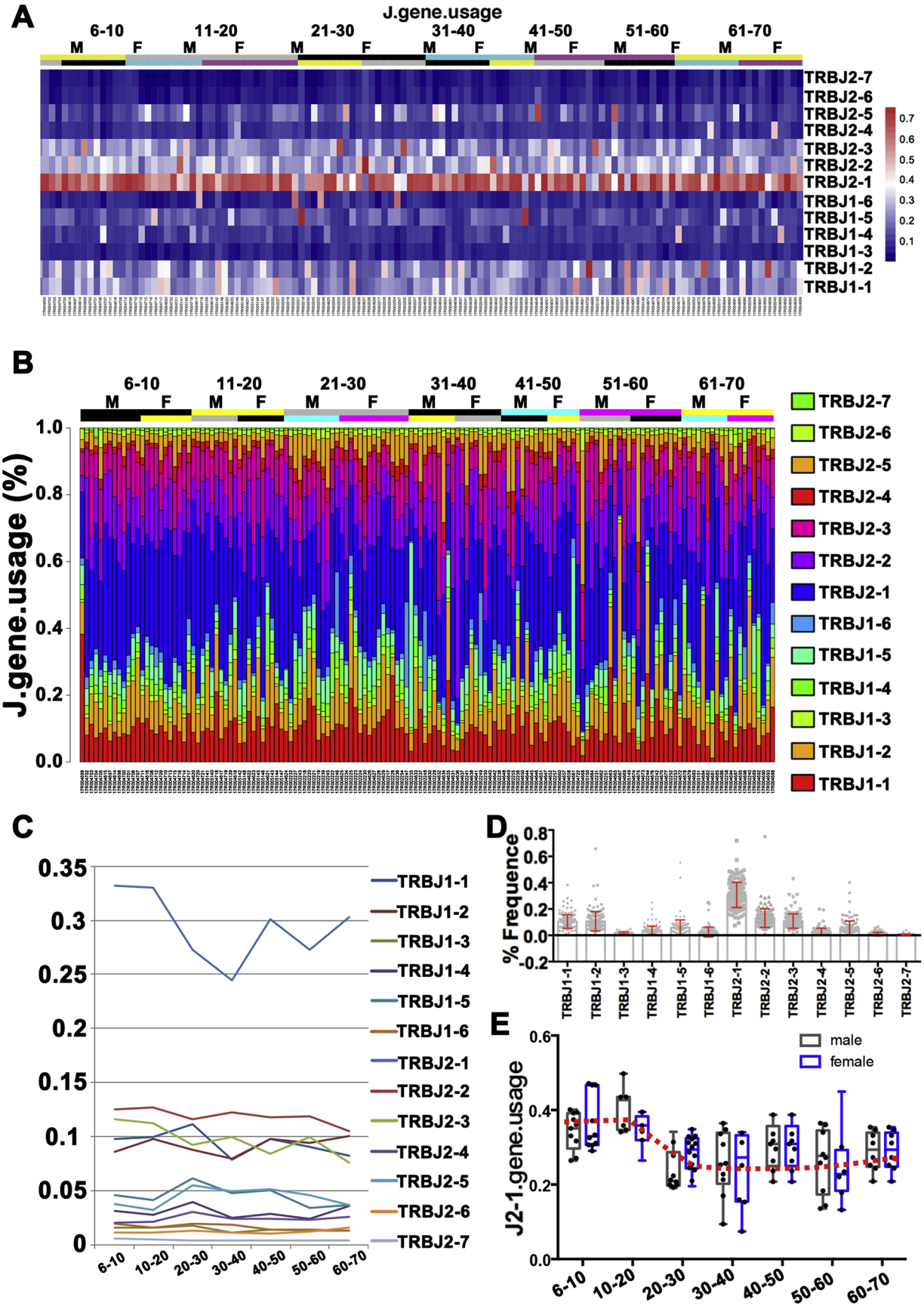

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Fig. 1. TRBJ gene segment usage in different age and gender groups. A, TRBJ gene segment usage landscapes are illustrated using heat map at different ages and genders. Each panel represents the differences expression of each TRBJ gene at different ages and gender groups. **B**, TRBJ gene segment usage landscapes are illustrated using heatmap at different ages and genders. Each panel represents the expression of each TRBJ gene at different groups. Each color represents a kind of TRBJ genes. **C**, The expression of each TRBJ gene with a line chart. Each color represents a kind of TRBJ genes and the line represents the average of the different groups. **D**, The expression of each TRBJ gene is shown as a scatter plot. Each dot indicates the percentage frequency of TRBJ in each individual. Bars in red indicate the mean values of individuals. **E**, J2-1 gene expression in different age groups and genders with a scatter plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

distance metric was defined. To convert the overlap metric into a distance metric, we computed (1/TCR overlap)-1. This distance metric was higher for less related TCR β repertoires and ranged from 0 to infinity. The calculations of overlap metric and distance metric were referenced to Chen et al [11]. We compared clonality and overlap of TCRβ repertoire between samples using an unpaired *t*-test and a one-way ANOVA adjusted for multiple comparison by Bonferroni correction, respectively (Table 1).

2.4. Statistical analysis

Statistical significances were tested using GraphPah Prism v6.0 (GraphPad Software) was used for graphing and statistical analysis.

3. Results

3.1. Usage of TRBV/J/D repertoires in healthy individuals

We amplified and sequenced TCRβ CDR3 regions of T cells from 154 healthy Chinese individuals. To reveal TRBV/J/D usage in whole TCR transcripts, the occurrence frequency of these samples was respectively calculated. Usage of TRBJ gene in the distribution of different age and gender groups was showed (Fig. 1). TRBJ gene expression was analyzed in different groups (Fig. 1A and B). Moreover, the usage of TRBJ gene was showed by a line chart at different age groups (Fig. 1C). The expression of TRBJ gene in whole healthy individuals was expressed with a scatter plot (Fig. 1D). As a whole, these results showed that TRBJ2-1 expression in whole healthy individuals with high level. In addition, TRBJ2-1 was expressed more highly at the age of 6–10 and 11–20 years than other age groups while no gender differences were observed in all groups (Fig. 1E). Furthermore, the expression of TRBD gene in the distribution of different age and gender groups was showed by a heat map (Fig.S1 A and S1B). However, the expression of TRBD gene was not different at ages and gender.

In addition, the distribution and usage of the TRBV genes in different groups were evaluated as well (Fig. 2A and B). The expression of each TRBV gene in whole healthy individuals was displayed with a scatter plot (Fig. 2C). We found that TRBV6-1 and TRBV27 were highly expressed throughout the whole healthy individuals. These two TRBVs may play a vital role in human growth or survival. And the two genes TRBV6-1 and TRBV27 may be expressed by sustained stimulation of one antigen, for example, influenza virus or other antigen, so there is no difference in different ages. Additionally, TRBV7-2 was expressed higher at the age of 6–10 and 11–20 years than other age groups while no gender differences in all groups (Fig. 2D). Also, the expression of the others TRBV genes in the distribution of different groups was analyzed (Fig.S2). Moreover, TRBV gene expression in different age and gender groups was showed (Fig.S2 A). Interestingly, TRBV6-4 gene expression was higher at the age of 11–20 and 21–30 years (Fig.S2B), and TRBV4-1 gene expression was higher at the age of 21–30 and 31–40 years (Fig.S2C). The expression of TRBV7-6 gene at the age of 21–30 and 31–40 years groups was higher (Fig.S2D). The usage of TRBV7-6 gene at the age of 11–20 years was higher (Fig.S2E). The expression of TRBV10-1 gene at the age of 6–10 and 11–20 years was higher (Fig.S2F). In total, we summarize that the usage of the TRBV/D/J gene throughout healthy individuals. Overall, this implied that the usage of TRBV/D/J genes is diverse in different age groups in healthy Chinese individuals.

3.2. Recombination of TRAV and TRAJ

We amplified and sequenced TCRβ CDR3 regions of T cells from 154 healthy Chinese individuals. To validate the usage of TCRs bearing a combination of TRBV with TRBJ genes, we analyzed the combination of TRBV with TRBJ genes at different age and gender groups (Fig. 3). Thus, we produced a heat map which represented the gene recombination of TRBV with TRAJ from 154 healthy individuals (Fig. 3A). The frequency of each recombination depended on the usage of TRBV or TRBJ. To visualize the usage of TCRs bearing a combination of TRBV with TRBJ genes, three-dimensional (3D) pictures of the TCR repertoires was used (Fig. 3B). The advantage of 3D images showed that the recombination of TRBV6-1/TRBJ2-1 and TRBV27/TRBJ2-1 was higher in whole individuals. In addition, we analyzed the number of gene recombination of TRBV with TRBJ at different age groups. Intriguingly, we found that the combination of TRBV with TRBJ genes was highest at the age of 21–30 years (Fig. 3C). In addition, the frequency of each gene recombination was analyzed and the results showed that the recombination of TRBV7-2 with TRBJ2-1 was higher at the age of 6–10 and 11–20 years (Fig. 3D). The occurrence tendency of each recombination was visualized by pie chart presentation (Fig.S3 A). Also, we found that TRBV7-6 with TRBJ2-1 was highly expressed at the age of 6–10 and 11–20 years (Fig.S3B).

3.3. The TCRB CDR3 amino acid sequence and CDR3 length distribution assay

The analysis of the TCRB CDR3 amino acid sequences and CDR3 length distribution has been effectively used to estimate the diversity of the TCR repertoire. In our study, the number of the TCRB CDR3 amino acid sequences was larger at the age of 21–30 years (Fig. 4A and 4B) and without gender differences. From this analysis, the occurrence ratio of the TCRB CDR3 amino acid sequences and the number of total TCRB CDR3 sequencing reads increased at the ages of 21–30 years (Fig. 4C), but without gender differences. In addition, the CDR3 length distribution of the TRB was showed (Fig.S4). These strongly suggest that the diversity of TCR has a strong relationship with age.

4. Discussion

It has become clear that TCR diversity is crucial for human immunity and the detection of preferential usage of TRBV/D/J would be benefit for investigating immune response induced by antigen-specific T cells, as different TCR clonotypes could recognize certain peptide [18]. Therefore, these investigating approaches could provide accurate results of TCR repertoires.

Using HTS, the results from four pediatric donors demonstrated that thymic repertoire is extremely diverse [19]. Previous studies of the V-J rearrangement of TCRβ chain in CDR3 repertoires showed that TCR diversity is different [20]. A deep sequencing of T cell repertoire from twins' cord blood showed that the difference slowly decreases with age [21]. The diversities of CDR3 polymorphism and length were demonstrated different in $CD4^+$ and $CD8^+$ T cells [22]. In addition, the TCR repertoire might be a significant determinant in tumor microenvironment. Large-scale sequencing of TCRβ CDR3 regions in colorectal

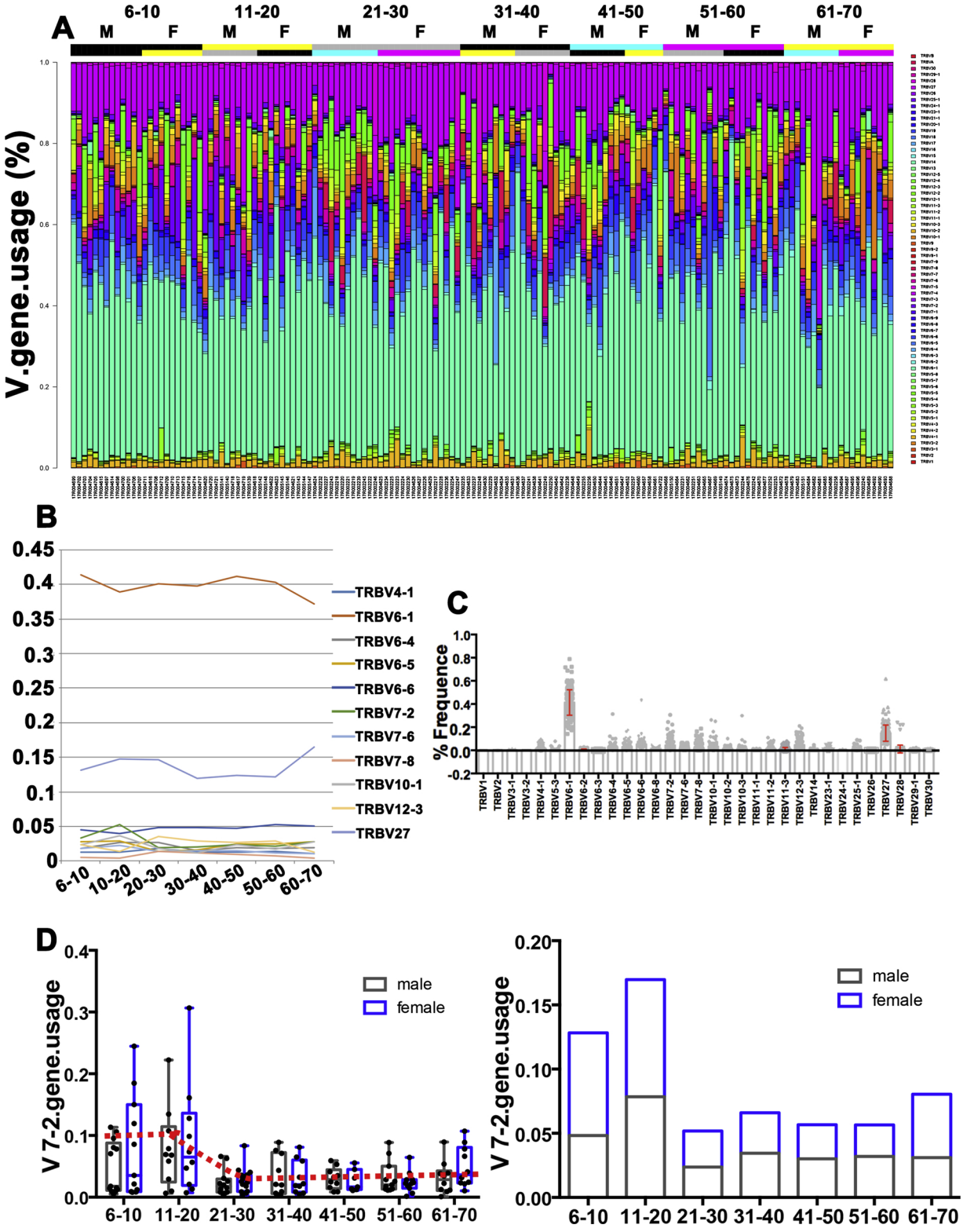

Fig. 2. TRBV gene segment usage in different age and gender groups. A, TRBV gene segment usage landscapes are illustrated using heat map at different ages and genders. Each panel represents the expression of each TRBV gene at different groups. Each color represents a kind of TRBV genes. **B**, The expression of each TRBV gene with a line chart. Each color represents a kind of V genes. **C**, The expression of each TRBV gene is shown as a scatter plot. Each dot indicates the percentage frequency of TRBV in each individual. Bars in red indicate the mean values of individuals. **D**, V7-2 gene expression in different age groups and genders with a scatter plot and histogram. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cancer tissue samples demonstrated that J- and V-regions are similar in PBLs and TILs [23]. TCR DNA sequencing had been used to evaluate TCR repertoire in breast cancer [24,25]. In addition, a study suggested that TCR repertoire might be a significant determinant in tumor microenvironment [26]. Analysis of TCR repertoire in germinal centres indicated that T follicular helper cells express antigen-responsive TCRs

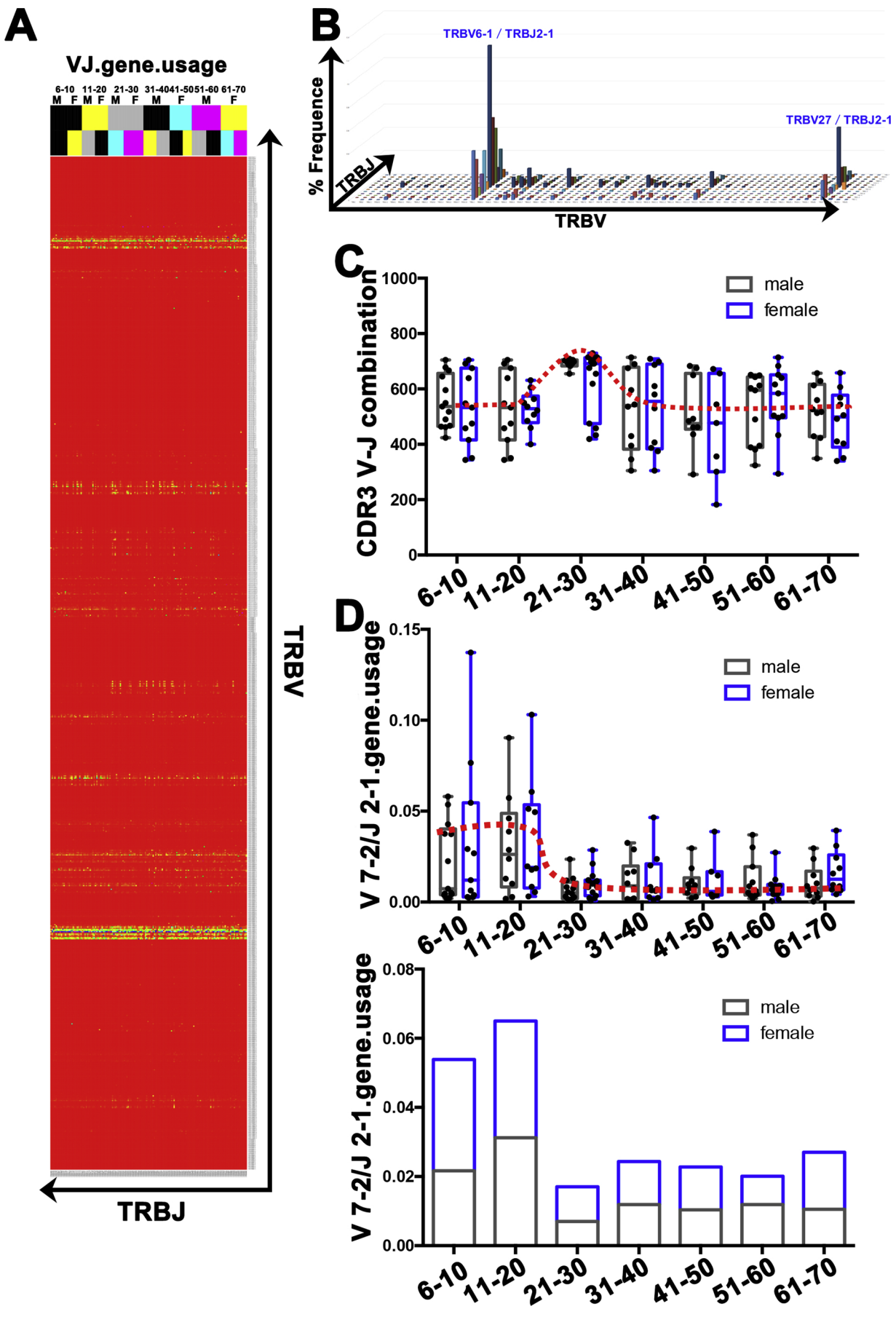

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Fig. 3. The Statistics of TRBV with TRBJ Recombination. **A**, Heat map representation of gene recombination of TRBV with TRAJ from 154 healthy individuals. The occurrence tendency of recombination is visualized by heat map presentation of the number of each recombination. Color in each pixel indicates the number of each recombination. **B,** 3D image of TRB repertoires. The numbers of TCR sequence reads bearing a given gene recombination of TRBV with TRBJ were counted. X-axis and Y-axis indicate TRBV and TRBJ, respectively. **C**, The number of gene recombination of TRBV with TRBJ at different ages groups. **D**, Recombination of TRBV7-2 with TRBJ2-1 was expressed with a scatter plot and histogram at different ages groups.

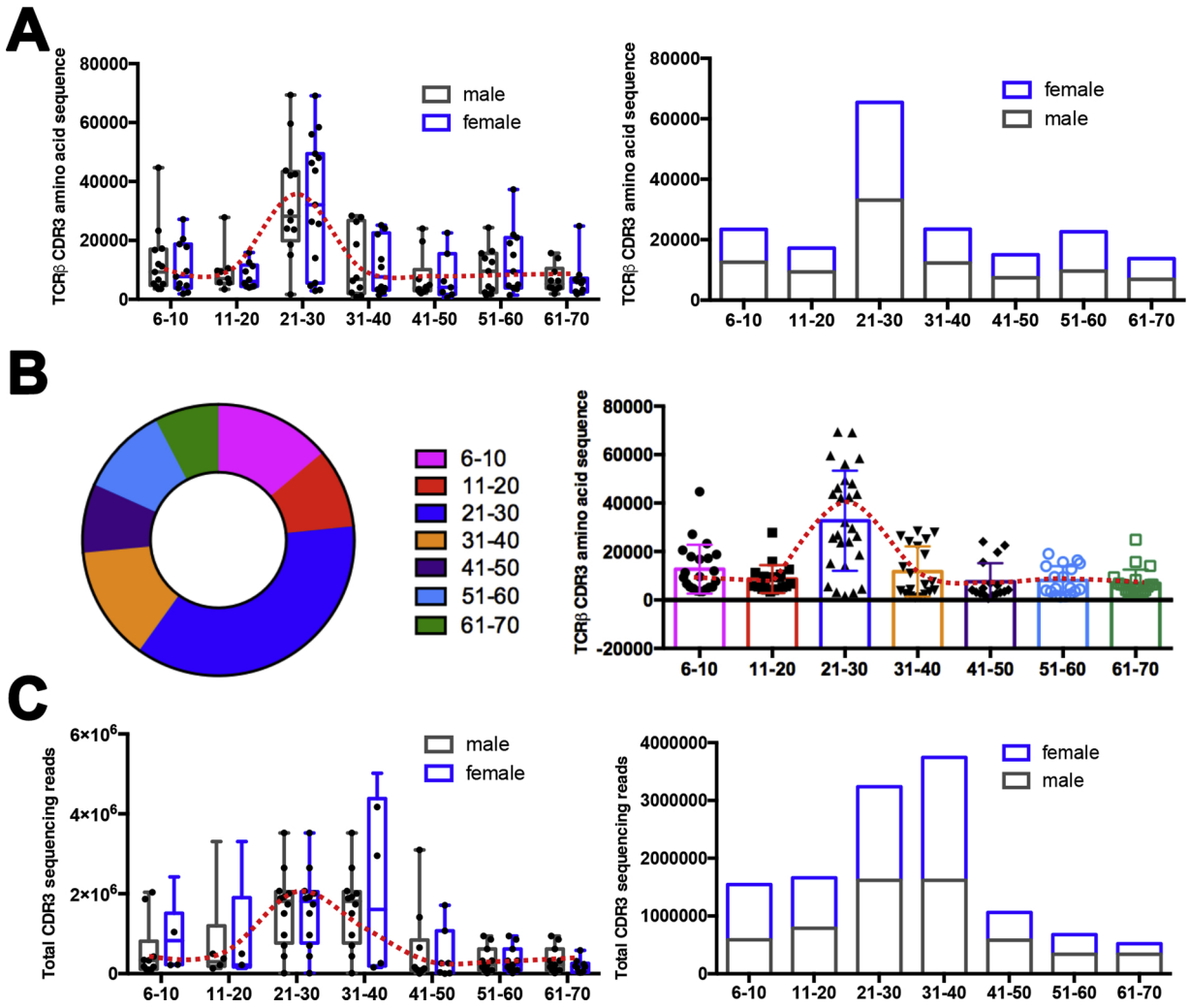

Fig. 4. Analysis of the TCRB CDR3 amino acid sequences. **A,** The number of the TCRB CDR3 amino acid sequences are showed with a scatter plot and histogram at different groups. **B**, The occurrence ratio of the TCRB CDR3 amino acid sequences are showed with pie chart and a scatter plot. Color indicates the ratio of each age groups. **C**, The number of the total TCRB CDR3 amino acid sequences are showed with a scatter plot and histogram at different groups.

to promote antibody responses [27]. Further studies suggested that TCRαβ chains are crucial for reactivity towards disease-associated autoantigens [28]. While, in Rasmussen encephalitis patients, it was found that treatment with rituximab/natalizumab/basiliximab do not change TCR diversity [29]. Taken together, current studies support that various TCR repertoires can be analyzed by HTS. However, analysis of T cell repertoire in healthy Chinese individuals of different age was limited in these studies.

Here, using HTS techniques, we further discovered that it is important to clarify the distribution of TCR repertoires of healthy Chinese individuals at different age and gender. In our study, we comprehensively examined TCR repertoires of a large number of individuals (n = 154) at different age and gender groups. Importantly, a large number amount of sequence data was evaluated. Thus, our study precisely revealed the normal range of gene expression level as well the extent of diversity and similarity of TCR repertoires in healthy Chinese individuals. Regarding TRBV, TRBD and TRBJ repertoires, we observed preferentially increased gene expression using TRBV, TRBD and TRBJ in peripheral blood from healthy individuals of different age. As expected, our study clearly demonstrated that there is a significant correlation between TCR diversity and age. Although we cannot definitively determine the association between immune response and diversity of TCR repertoires, it was certain that TCR repertoires could serve as a useful tool for investigating the role of immune profiling in healthy Chinese individuals. This study inspires us to further explore the development and function of immune system in healthy Chinese individuals of different age, as this could provide a theoretical basis for treatment of age-related diseases.

5. Conclusions

Our study showed that there are different features of TCRβ-chain repertoire in healthy Chinese individuals of different age. In the future, these TCR repertoires could serve as a useful tool for investigating the role of immune profiling and bring some guidance to the treatment of age-related diseases.

Compliance and ethics

The author(s) declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imlet.2019.03.007.

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