ORIGINAL ARTICLE

# The Skewness of Alpha Beta T-cell Receptors in the Peripheral Blood of the Patients with Type 1 Diabetes

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## *ABSTRACT*

***Objective:*** *To detect the skewness of T cell receptor (TCR) Vα and TCR Vβ of patients with Type 1 diabetes.*

***Methods:*** *The heparinized venous blood was collected from ten patients with Type 1 diabetes. The peripheral blood lymphocytes (PBL) were isolated and used to extract mRNA. Reverse amplyfication was performed for cDNA synthesis. The skewness of TCR Vα and Vβ was detected with real-time florescence quantitative polymerase chain reaction (RQ-PCR) and analysed by DNA melting-curve analysis technique, respectively.*

***Results:*** *Among the TCR Vα genes, the skewness frequency rate (SFR) of Vα22 was 30%; both of Vα5 and Vα24 were 20%; the SFR of Vα28 was 10%, which was the only gene that showed restricted-clone. In all the Vβ genes, Vβ7 and Vβ17 were the the highest expression genes, and their SFRs were both 60%. Vβ11 was near them with the SFR of 40%; the restricted clonal genes were Vβ18 and Vβ20, and their SFRs were 10% and 20%, respectivley.*

***Conclusions:*** *There were skewed genes in TCR Vα and TCR Vβ, which were probably relative to the onset of Type 1 diabetes.*


**Keywords:** Peripheral blood, TCR Vα, TCR Vβ, skewness frequency rate, Type 1 diabetes

# La Asimetría de los Receptores de Células T alfa βeta en la Sangre Periférica de los Pacientes con Diabetes Tipo 1

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## *RESUMEN*

***Objetivo:*** *Detectar la asimetría de RCT Vα y RCT Vβ de pacientes con diabetes tipo 1 (DT1).*

***Métodos:*** *Muestras de sangre venosa heparinizada fueron tomadas de diez pacientes con DT1. Los linfocitos de la sangre periférica (LSP) fueron aislados y usados para extraer el ARNm. La amplificación inversa se realizó para la síntesis del ADNc. La asimetría de RCT Vα y Vβ fue detectada mediante reacción en cadena cuantitativa fluorescente de la polimerasa en tiempo real (QF-PCR) y analizada mediante la técnica de análisis de curva de fusión del DNA, respectivamente.*


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***Resultados:*** *Entre los genes RCT Vα, el índice de frecuencia de asimetría (SFR, en inglés) de Vα22 fue de 30%; tanto el de Vα5 como el de Vα24 fue 20%; y el SFR de Vα28 fue 10%, siendo el único gene que mostró clon restringido. De todos los genes Vβ, los de mayor expresión fueron Vβ7 y Vβ17, y sus índices SFR fueron de 60%. El gen Vβ11 estuvo cerca de estos valores, con un SFR de 40%; los genes clónicos restringidos fueron Vβ18 y Vβ20, y sus SFR fueron de 10% y 20%, respectivamente.*

***Conclusiones:*** *Hubo genes sesgados en RCT Vα y RCT Vβ, probablemente relacionados con el inicio de DT1.*

**Palabras clave:** Sangre periférica, RCT Vα, RCT Vβ, tasa de frecuencia de asimetría, diabetes tipo 1

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## INTRODUCTION

Type 1 diabetes is one of the most prevalent autoimmune diseases in the world. To date, although there have been many experimental and clinical research on it, the exact mechanisms still remain unclear (1). However, a common view that T cells play an important role in the development of the disease had been proved by many researchers (2–5). As we known, the effects of T cells depend on the identification of major histocompatibility complex (MHC) binding the peptides of auto-antigens, so that the auto-respons of T cells specific to this combination might make the T cell receptor (TCR) changed, especially the genes located in complementarity determining region 3 (CDR3) (6). Accordingly, it is possible to find out the immune mechanisms of Type 1 diabetes onset from the specific changes of TCR.

According to the different composition of the double chains, TCR is divided into two subpopulations: γδ TCR and αβ TCR. Because of the high percentage (95%), the research on the relationship between the skewness of TCR and immune diseases usually focus on αβ TCR. To our knowledge, there are some reports concerning TCR Vβ, and few resolved the skewness of TCR Vα (7). In this study, using real-time florescence quantitative polymerase chain reaction (RQ-PCR) and DNA melting curve analysis technique, we simultaneously detected the skewness of TCR Vα and Vβ in the peripheral blood (PB) from the Type 1 diabetes patients, and hope to present a clue or idea for the future study on the onset mechanisms of Type 1 diabetes.

## SUBJECT AND METHODS

### Patients

Ten Type 1 diabetes patients and ten healthy volunteers were recruited. They were not treated with immunomodulating drugs in the previous six months prior to the study, and were seronegative for the markers of hepatitis viruses, HIV and other pathogenic infections. The patients with tumours and immunological disorders were excluded. This study's protocol was approved by the Hospital Ethics Committee.

### Extraction of RNAs and synthesis of the first cDNA

The sense primer, anti-sense primer and specific primers for TCR Vα and TCR Vβ genes were previously described (8, 9) and synthesized by the Guangzhou Daangene Corporation of China; and 5 mL of heparinized venous blood were collected from each of the Type 1 diabetes patients, and the peripheral blood lymphocytes (PBL) were isolated by Ficoll-Hypaque density centrifugation. Using an Omega RNA extraction kit and according to the manufacturer's instructions, the total RNA was extracted, and 1 μg total RNA was reversed transcribed with 250 pm olig (dT), 200 U Moloney murine leukaemia virus (M-MuLV) reverse transcriptase, and 2 μl of 10 mM dNTP mix (cDNA Synthesis Kit; MBI-Fermentas), in a total volume of 20 μl (six reactions for every sample). The cDNA was stored at -80 ℃.

### Skewness detection with RQ-PCR and melting-curve analysis technique

The cDNA products were continuously amplified with RQ-PCR in a 20 μl volume with 10 μl 2×Real-time PCR Master Mix (TOYOBO, JAPAN), which contained Taq-polymerase, dNTPs, PCR buffers, and SYBR green I. The final concentration of each primer was 0.3 μM. Subsequently, 1 μl liquid containing 10~50 ng reverse-transcribed total RNA was added to the reaction mixture as the PCR template. Reactions were performed in MJ Opticon 2 DNA engine and analysed with Opticon Monitor 3.0 software (Bio-rad, USA). The reaction conditions were shown as follows: pre-incubation at 94 °C for 3 minutes, 94 °C melting for 20 seconds, primer annealing at 56 °C for 30 seconds and extension at 72 °C for 30 seconds.

The above procedure was iterated 40 cycles for the whole amplification. Finally, the data obtained from the RQ-PCR were further analysed with the melting-curve analysis technique.

**Calculations of skewness frequency rates of TCR Vα and Vβ gene families**

In order to evaluate the usage of every TCR gene, respectively, the skewness frequency rates (SFR) of TCR Vα and Vβ gene families were calculated separately with the following formula:

SFR = N1 / N0 × 100%.

N1, is the summary of a skewed gene family; N0, is the total of the corresponding genes which included the skewed and the non-skewed.

## RESULTS

All the Vα gene families of the healthy subjects exhibited multi-peaks. Most of the TCR Vα gene families of the Type 1 diabetes patients exhibited multi-peaks; many genes showed oligo-peaks, single-peaks or low-level peaks. The different types of peaks, respectively, represented polyclone, oligoclone, monoclone and restricted-clone. The gene families with oligoclone, monoclone and restricted-clone, were totally called skewed gene family. The genes exhibiting oligolone and monoclone were named the predominant skewness (10). The details of the skewed gene families are as shown in Table 1.

Table 1: All the skewed Vα gene families in PBL of 10 Type 1 diabetes patients

| Patients | Monoclone | Oligoclone | Restricted-clone |
|---|---|---|---|
| 1 | Vα24 | Vα14,Vα26 | — |
| 2 | Vα4.1 | Vα12, Vα20 | — |
| 3 | — | Vα15, Vα24 | — |
| 4 | Vα11 | Vα17 | Vα28 |
| 5 | Vα13 | Vα1.1, Vα22 | — |
| 6 | — | Vα2, Vα6 | — |
| 7 | Vα22 | Vα4.2, Vα18 | — |
| 8 | Vα6 | Vα19, Vα23, | — |
| 9 | Vα22 | Vα5, Vα10, Vα29 | — |
| 10 | Vα32 | Vα16, Vα27 | — |

Among the TCR Vα genes, the frequency of Vα22 was 30%; Vα5 and Vα24 were next to it with the SFRs of 20%. There was no skewness for some genes: Vα1.2, Vα3, Vα7, Vα8, Vα9, Vα21, Vα25, Vα30 and Vα31. The SFR of Vα28 was 10%, which was the only gene that showed restricted-clone (Table 2).

All the Vβ gene families of healthy and control groups, exhibited polyclone. Most of the TCR Vβ gene families of the Type 1 diabetes patients exhibited polyclone, and many genes showed oligoclone, monoclone or restricted-clone. Vβ7 and Vβ17 were the predominant usage genes with the highest SFRs, which were 60%; Vβ11 was next to them with the SFR of 40%; the SFRs of Vβ2, Vβ6, Vβ18, Vβ21 and Vβ24 were all 20%. However, there was no skewness for Vβ3, Vβ4, Vβ5.2, Vβ8, Vβ10, Vβ12, Vβ15 and Vβ16 in all the Type 1 diabetes patients. The restricted-clonal genes were Vβ18 and Vβ20; and their SFRs were 10% and 20%, respectivley (Table 2).

Table 2: The skewness frequency rate of each Vα and Vβ gene family in PBL of ten Type 1 diabetes patients

| TCR Vα | Times for each predominant gene | Skewness frequency rate (%) | TCRR Vβ | Times for each predominant gene | Skewness frequency rate (%) |
|---|---|---|---|---|---|
| 1.1 | 1 | 10.0 | 1 | 3 | 30.0 |
| 1.2 | 0 | 0 | 2 | 2 | 20.0 |
| 2 | 1 | 10.0 | 3 | 0 | 0 |
| 3 | 0 | 0 | 4 | 0 | 0 |
| 4.1 | 1 | 10.0 | 5.1 | 1 | 10.0 |
| 4.2 | 1 | 10.0 | 5.2 | 0 | 0 |
| 5 | 1 | 10.0 | 6 | 2 | 20.0 |
| 6 | 2 | 20.0 | 7 | 6 | 60.0 |
| 7 | 0 | 0 | 8 | 0 | 0 |
| 8 | 0 | 0 | 9 | 1 | 10.0 |
| 9 | 0 | 0 | 10 | 0 | 0 |
| 10 | 1 | 10.0 | 11 | 4 | 40.0 |
| 11 | 1 | 10.0 | 12 | 0 | 0 |
| 12 | 1 | 10.0 | 13.1 | 1 | 10.0 |
| 13 | 1 | 10.0 | 13.2 | 1 | 10.0 |
| 14 | 1 | 10.0 | 14 | 0 | 0 |
| 15 | 1 | 10.0 | 15 | 0 | 0 |
| 16 | 1 | 10.0 | 16 | 0 | 0 |
| 17 | 1 | 10.0 | 17 | 6 | 60.0 |
| 18 | 1 | 10.0 | 18 | 2 | 20.0 |
| 19 | 1 | 10.0 | 19 | 1 | 10.0 |
| 20 | 1 | 10.0 | 20 | 3 | 30.0 |
| 21 | 0 | 0 | 21 | 2 | 20.0 |
| 22 | 3 | 30.0 | 22 | 1 | 10.0 |
| 23 | 1 | 10.0 | 23 | 1 | 10.0 |
| 24 | 2 | 20.0 | 24 | 2 | 20.0 |
| 25 | 0 | 0 | | | |
| 26 | 1 | 10.0 | | | |
| 27 | 1 | 10.0 | | | |
| 28 | 1 | 10.0 | | | |
| 29 | 1 | 10.0 | | | |
| 30 | 0 | 0 | | | |
| 31 | 0 | 0 | | | |
| 32 | 1 | 10.0 | | | |

TRAV: T cell receptor alpha chain; TRBV: T cell receptor beta chain; PBMC: peripheral blood mononuclear cell.

Table 3: All the skewed Vβ gene families in PBL of ten Type 1 diabetes patients

| Patients | Monoclone | Oligoclone | Restricted-clone |
|---|---|---|---|
| 1 | Vβ6, Vβ7 | Vβ8, Vβ13.2, Vβ17, Vβ21, Vβ24 | Vβ18 |
| 2 | Vβ7 | Vβ11, Vβ17, Vβ21 | — |
| 3 | Vβ11 | Vβ9, Vβ17, Vβ19 | — |
| 4 | — | Vβ2, Vβ17 | — |
| 5 | Vβ11, Vβ17 | Vβ1, Vβ7, Vβ23 | — |
| 6 | Vβ7 | Vβ11 | Vβ20 |
| 7 | Vβ6 | Vβ18 | — |
| 8 | Vβ5.1 | Vβ1, Vβ1 3.1, Vβ24 | Vβ20 |
| 9 | — | Vβ1, Vβ7, Vβ22 | — |
| 10 | Vβ7 | Vβ2, Vβ17 | — |

According to the the SFR of each gene family of Vα and Vβ, we respectively drew the histograms to further comprehensively analyse the skewness of the TCR Vα and the TCR Vβ of the Type 1 diabetes patients. The two histograms were further put together back to back, and a figure like a big key formed (Figure). A and B sides represented the predominant SFRs of the TCR Vα and Vβ gene families, respectively. The prominent columns were like the kits of the key; the higher the SFR of the Vα or Vβ gene family was, the higher the kit of the key would be.

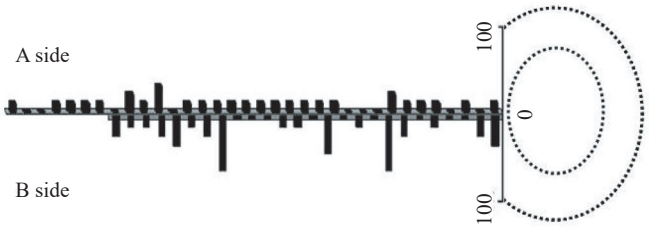


Figure: The key formed with the skewed TCR Vα and Vβ in P peripheral blood mononuclear cell BMC of ten Type 1 diabetes patients

According to different skewness frequency rates of every TCR Vα and Vβ genes, two histograms were drawn out, respectively. Moreover, both were put together back to back. It was easy to see that the merged gram looked like a big key, which was due to the different frequency of the each gene family ofTCR. A and B side respectively represents the skewed Vα and Vβ gene families. The prominent columns like the kits of the key, and the higher the frequency of the Vα and Vβ gene family was, the higher the kit of the key would be. To some extent, the key presented the total characterization of the skewness of Vα and Vβ of the ten Type 1 diabetes patients.

## DISCUSSION

In the past 30 years, more and more scholars had studied the associations between the TCR repertoires and tumour, infectious and autoimmune diseases. Among them, most of the reports focussed on the skewness of TCR Vβ, and few reports revolved around that of TCR Vα (11–13). Regarding Type 1 diabetes, such a situation looks more obvious (7). In an animal experiment, researchers found that TCR Vα5D-4 was the main gene in the mice model of Type 1 diabetes, and the invasion and clonal proliferation of T cells with TCR Vα5D-4 was observed in insulin. According to the evidence, the scholars thought that TCR Vα5D-4 probably was the stimulative factor for Type 1 diabetes in mice (14). In another basic test, Du *et al* (15) found that TCR Vα7 and Vα17 were the predominant expression genes. In this study, we directly detected the TCR Vα usage of peripheral blood (PB) of the Type 1 diabetes patients, and found that, compared with the sole character of polyclone for the healthy donors, the usage of TCR Vα showed variety: except the polyclone expression for most of the TCR Vα genes, there were numerous genes exhibiting oligoclone, monoclone and restricted-clone. In all the Type 1 diabetes patients, Vα22 was the gene of the highest SFR; Vα5 and Vα24 were next to it. Vα1.2, Vα3, Vα7, Vα8, Vα9, Vα21, Vα25, Vα30 and Vα31 were not predominantly used. These results were significantly different from those of the above animal experiments. This difference probably indicated that the onset mechanism for Type 1 diabetes was different between animal and human, and animal experiments could expose the mechanism of Type 1 diabetes onset, but the results could hardly truly reflect the skewed TCR repertoire of the Type 1 diabetes patients.

In the studies on the skewness of TCR Vβ of the animals with Type 1 diabetes, Liu *et al* (16) found that, in spite of $CD4^+$ or $CD8^+$ T cells, TCR-Vβ13S1A1 was identified as an allele of the TCR Vβ in the rat model with Type 1 diabetes. Further test showed that spontaneous diabetes could be prevented through the vaccination of the antibody specific to TCR-Vβ13S1A1. In another study, Codina-Busqueta *et al* simultaneously detected the skewness of TCR Vβ in peripheral blood mononuclear cells (PBMCs) of non-obese diabetic (NOD) mice and Type 1 diabetes patients, and found the monoclonaly expaned Vβ22 in both of them. These suggested that Vβ22 clone might have expanded or accumulated *in situ* by an autoantigen present in both NOD mice and Type 1 diabetes patients (6). Interestingly, in the simultaneous

study on NOD mice and paediatric patients with Type 1 diabetes, Marrero *et al* (17) found the common skewed TCR Vβ genes: TRBV1 (Vβ2), TRBV13-3 (Vβ8.1), and TRBV19 (Vβ6). Unfortunatly, this argument has not yet been proven by others, including our studies on Type 1 diabetes patients. In the previous study (18), we found that TCR Vβ7 was the common predominant usage gene in two Type 1 diabetes patients; and moreover, the two Vβ7 genes shared the same amino acid sequences. In the present study, TCR Vβ7 was also identified as the most predominant usage gene in all the ten Type 1 diabetes patients. Besides, Vβ17 was identified as another skewed gene while SFR was equal to Vβ7. These results were consistent with the report of Luppi (19), in which they found that the frequency of circulating TCR Vβ7 and Vβ17 T cells in PBMCs from the Type 1 diabetes patients increased. Similarly, an increase of Vβ7 expression was also reported in the PBMCs of the Type 1 diabetes patients in another study (20). In a study on two children with Type 1 diabetes, a marked over-representation of mRNA encoding TCR Vβ7 chain was observed (21). Based on the studies on Type 1 diabetes patients, it was possible to draw a conclusion that Vβ7 was the most predominant usage gene in Type 1 diabetes patients. However, this was denied by the Tzifis' study, in which they found that Vβ4 was the most predominant gene family in the paediatric patients with Type 1 diabetes (22). This difference probably depends on the different ages of the objects, or the different techniques used to assay the skewness of TCR Vβ genes. Additionally, besides the common characters of TCR Vβ usage existing in the Type 1 diabetes patients, there were possible individualized properties between the different individuals with Type 1 diabetes (23, 24).

According to Figure 1, we found that the merged histogram looked like a big key. The columns like the kits of the key, and the higher the SFR for each of the Vα and Vβ gene family was, the higher the kit of the key would be. To some extent, the key represented the total characteristic of the Vα and Vβ skewness of the ten Type 1 diabetes patients. As we know, the skewness of TCR (including TCR Vα and TCR Vβ) was specific to the associated antigen of Type 1 diabetes, so the key formed with the skewness frequency of each gene could be taken as the summary clonal changes of TCR Vα and Vβ. In other words, it should be a specific key to Type 1 diabetes. There is a Chinese proverb that says that, 'Open different locks with different keys'. In our opinions, an accurate 'key' specific to Type 1 diabetes will be drawn out through more studies in the future, and the door hiding the secrets for the disease onset will be opened to a greater extent.

## CONCLUSION

In this study, we found that Vα22, Vβ7 and Vβ17 were the predominant usage genes, while Vα28, Vβ18 and Vβ20 were the restricted clonal genes in the Type 1 diabetes patients. However, the function of the skewness of TCR Vα and Vβ genes on the onset of Type 1 diabetes needs further study in the future.

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