Original Article

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Flow Cytometric Analysis of T Cell Vβ Repertoire in Common Variable Immunodeficiency Patients with TACI Mutations

TACI Mutasyonu Bulunan Yaygın Değişken İmmün Yetmezlik Hastalarında T Hücre Vβ Repertuvarının Akan Hücre Sistemi ile Analizi

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Abstract

Objective: TCR (T Cell Receptor) which is expressed on T cells is responsible for recognizing antigens presented by HLA molecules of the APCs (Antigen Presenting Cells) and initiation of the immune response. It has been reported that TACI (Transmembrane activator and calcium modulating cyclophilin ligand interactor) mediates the interaction of B cells and dendritic cells which are both responsible for the processing of T cells.
Materials and Methods: In this study, 24 TCRVβ clones were analyzed by using multiparametric flow cytometry in seven patients with TACI mutation [two homozygous (c.310T> C) five heterozygous (c.310T> C, c.226G> C, c.260T> A)], four CVID (Common Variable Immunodeficiency) patients who had not TACI defect (non-TACI) and five healthy controls. In this study group, serum Ig levels and infection history, CD4+ and CD8+ cell percentages, and HLA profiles were investigated.
Results: Increased TCRVβ13.2 clone was observed in patients with TACI defects unlike control individuals and non-TACI-CVID patients (p=0.02). We found that there was a statistically significant decrease in TCRVβ8 clone (p=0.012) in TACI deficient CVID patients and non-TACI-CVID patients compared to control individuals. TCRVβ20 clones in non-TACI-CVID patients were decreased compared to TACI-CVID patients and control individuals (p=0.009). Decreased TCRVβ8 was also associated with TACI deficient CVID patients.
Conclusion: Further studies and large cohorts are needed to understand the relationship between TCRVβ8, TCRVβ13.2, and CVID with TACI mutations.

Keywords: TACI, CVID, primary immunodeficiency, TCRVβ repertory

Öz

Amaç: T hücrelerinde ifade edilen T Hücre Reseptörü (THR), Antijen Sunan Hücre (ASH) üzerindeki HLA molekülleri tarafından sunulan antijenleri tanımaktan ve immün cevabı başlatmaktan sorumludur. TACI'nin (Transmembrane activator and calcium modulating cyclophilin ligand interactor), T hücrelerine sunumdan sorumlu olan B hücreleri ile dendritik hücrelerdeki interaksiyonuna aracılık ettiği bildirilmiştir.
Gereç ve Yöntem: Bu çalışmada dört yaygın değişken immün yetmezlik (YDİY) hatası mutasyonu [iki homozigot (c.310T> C), beş heterozigot (c.310T> C, c.226G> C, c.260T> A)] bulunan yedi hasta, TACI dahil herhangi genetik mutasyonu bulunmayan dört hasta (TACI-olmayan) ve beş sağlıklı kontrol bireyde 24 TCRVβ klonu multiparametrik akan hücre sistemi ile incelendi. Bu çalışma grubunda, serum Ig seviyeleri, enfeksiyon öyküleri, CD4+ T ve CD8+ T hücre oranları ile HLA profilleri incelendi.
Bulgular: TACI defekti bulunan hastalarda, TACI-olmayan hastalardan farklı olarak artmış TCRVβ13.2 klonu gözlendi (p=0.02). TACI defekti olan ve olmayan hasta gruplarında kontrol bireyler ile kıyaslandığında TCRVβ8 klonunda istatistiksel olarak anlamlı bir düşüklük saptadık (p=0.012). TACI olmayan hasta grubunda, TACI-YDİY hastaları ile kıyaslandığında TCRVβ20 klonu azaldı (p=0.009). Artmış TCRVβ13.2 oranı TACI defektli YDİY hastaları ile ilişkili bulundu.
Sonuç: TCRVβ8, TCRVβ13.2 ile TACI mutasyonu bulunan YDİY hastaları arasındaki ilişkinin anlaşılması için daha ileri çalışmalar ile daha büyük hasta gruplarına ihtiyaç duyulmaktadır.

Anahtar kelimeler: TACI, YDIY, primer immün yetmezlik, TCRVβ repertuvarı

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Introduction

Transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), a member of tumor necrosis factor receptor superfamily (TNFRSF), has essential roles in various stages of B cell development by interacting with APRIL (a proliferation-inducing ligand) and BAFF.[1-3] After stimulation, TNFRSF transmits the signals to canonical and non-canonical NF-Kβ pathways via TRAFs (TNFR-associated factors) and regulates B cell survival and proliferation.[1-3] Heterozygous, homozygous, and compound heterozygous mutations of TNFRSF13B gene encoding TACI may be associated with a phenotype of CVID and selective IgA deficiency by leading to abnormal B cell immunity.[4-7] TACI deficiency is characterized by low levels of serum immunoglobulins (IgA, IgM, and IgA), recurrent infections including gastrointestinal and respiratory system infections, lymphoproliferation, and autoimmunity, such as other CVID patients.[4-7]

Although TACI is widely known as an important factor for B cell development and function, it has roles in T cell development.[8,9] Thereby, functions of TACI may be divided into three main topics: 1) T cell activation. TACI is expressed on T cells and following inhibition of TACI with TACI-Fc, T cell activation was shown to be blocked.[9] 2) B cell development and maturation. B cell maturation is dependent on functional BCR (B cell receptor) generation. Before entering the circulation, immature B cells egress from the spleen and transit T1 (Transitional 1) stage via BCR signaling to T2 (Transitional 2) stage by the interaction of TACI with its specific ligand.[10] 3) B cell functions via dendritic cells and T cells. Mature B cells replace the T cell zone and interact with T cells for acquiring the proper signals to produce immunoglobulin that has optimal affinities to antigens. FDCs (follicular dendritic cells) provide a suitable environment by producing chemoattractants such as CXCL13 and helps this interaction in the T-B border. TACI mediates B cell- dendritic cell interactions via BLyS (B Lymphocyte Stimulator) to prime naive CD8+ T cells. Antigens presented by B cells are recognized by specific TCR (T Cell Receptor) on T cells.[11]

TCR which is expressed on T cells is responsible for recognizing antigens presented by HLA molecules of the APCs (Antigen Presenting Cells) and initiation of the inflammatory response. In αβ T cells, TCR is a heterodimer of α and β chains and contains variable regions on both α and β chains (~ 90% of T cells consists of αβ heterodimers).[12-14] These variable regions differ from ~10^{15}-10^{20} and they are related to internal factors such as HLA diversity, TCR gene polymorphisms, and external factors such as viruses and superantigens.[15-17] TACI on B cells was shown to affect antigen presentation to CD8+ T lymphocytes.[11]

In light of these studies, we investigated TCRVβ diversity among CVID patients with or without TACI gene mutations and control individuals.

Material and Methods

Patients

Eleven patients with CVID who were followed up in our clinic at Hacettepe University and five healthy control individuals were included in the study after signing the informed consent forms. The study was approved by the Ethics Committee Review Board of Hacettepe University (No: TSA-2016-9087). All TACI-CVID (n=7) and non-TACI-CVID (n=4) patients were diagnosed as CVID according to ESID (European Society of Immunodeficiency) criteria.[33]

Mutation Analysis by Next Generation Sequencing and Sanger Sequencing

We extracted genomic DNA using standard procedures from whole blood. Eleven patients with CVID were analyzed with NGS-based gene panel (including 266 genes) screening and presence of possible variants was confirmed by Sanger sequencing. Two patients were analyzed with WES (Whole-exome sequencing) and verified by Sanger sequencing.[18]

Flow cytometric analysis of TCRVβ clones in samples of patients and controls

Peripheral blood samples were collected from patients and control individuals. Flow cytometry was performed by using the TCRVβ repertoire analysis kit obtained from Beckman Coulter according to the manufacturer's instructions (USA). Eight tubes were prepared for each patient and control individual. Peripheral blood samples (100 μl) were incubated with PERCP- Cy5.5 CD3 and two fluorescein-tagged antibody mix targeting specific TCRVβ clones. Following 1 hour incubation, flow cytometric analysis was performed by using BD FACS CANTO II. Cell percentages were evaluated for comparison of Vβ clones as seen in the representative flow cytometer image in Figure 1.

Calculation of Gini-Skewing Index

To show the distribution of TCRVβ usage in patients and healthy controls, Gini-Skewing Index was calculated according to a published study by Kornelis SM and et al.[19]

HLA Allele Determination

To determine HLA alleles to impute classical two-digit HLA alleles in HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ-B1, and HLA-DQ-A1 using Turkish population-specific reference panels, polymerase chain reaction-reverse sequence-specific oligonucleotide probe (PCR-rSSO) method was performed.[20]

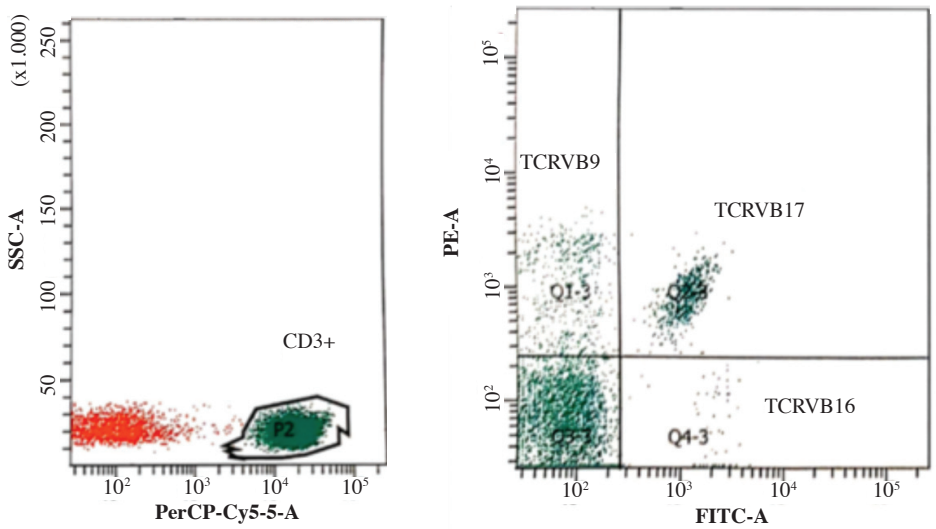

Figure 1. Representative image of TCRVβ in CD3+ T cells in healthy controls.

Table 1. Lymphocyte counts and percentages of CD4+ T and CD8+ Tcells in the patients.

	P1	P2	P3	P4	P5	P6	P7
Lymphocyte ($x10^3$ cell/μl)	1,3 (1,3-3,5)	0,8* (1,3-3,5)	1,2* (1,3-3,5)	1,9 (1,3-3,5)	1,7 (1,3-3,5)	1,0* (1,3-3,5)	0,9* (1,3-3,5)
CD4+ T%	22* (29,0-59,0)	27* (29,0-59,0)	32 (29,0-59,0)	38 (29,0-59,0)	33 (29,0-59,0)	33 (29,0-59,0)	33 (29,0-59,0)
CD8+ T%	42** (19,0-29,0)	5* (19,0-29,0)	37** (19,0-29,0)	21,8 (19,0-29,0)	14.2* (19,0-29,0)	36,2** (19,0-29,0)	36** (19,0-29,0)

**Describes low levels of CD4+ T and CD8+ T lymphocyte levels compared to reference values*
***Describes high levels of CD4+ T and CD8+ T lymphocyte levels compared to reference values*

Statistical Methods

The numbers are given as median (interquartile range, IQR). Furthermore, odds ratios were calculated. For statistical evaluation, Kruskal-Wallis and Mann-Whitney U tests were performed using Graphpad-Prism 8.2.1. and SPSS version 14. p<0,05 was accepted as statistically significant.

Results

Clinical and laboratory findings of the patients

Seven TACI-CVID, four non-TACI-CVID patients, and five normal control individuals were evaluated. The median ages of the patients with TACI-CVID, non-TACI-CVID, and control individuals were 29, 26, and 32 years, respectively. Seventy-one percent of TACI-CVID patients were male and 29 % were female. The female/male ratio was 1:1 among non-TACI-CVID patients. The female ratio was 80 % among control individuals. Two siblings (P1 and P2) among TACI-CVID patients and one patient among non-TACI-CVID (NT-P3) patients had a history of consanguinity. Seventy-one percent of TACI-CVID patients (P1, P4-P7) had autoinflammatory disorders. None of the patients had autoinflammatory disorders among non-TACI-CVID patients. P4 and P5 had HSV, and P7 had CMV infection. Herpes labialis was observed in P2 and Giardia lamblia in P1 (Table 2). According to IUIS criteria, all patients had a marked decrease in IgG levels except for P2 and a marked decrease in IgM or IgA levels. P1 and P2 had a low level of CD4+ T cell ratio compared to reference values. Four patients (P1, P3, P6, and P7) had an increased level of CD8+ T cell ratio in contrast to P2 and P5 who had a decreased ratio of the CD8+ T cells (Table 1). The patients showed also a poor response to vaccines and had history of recurrent infections.

All TACI-CVID patients had pneumonia and Herpes simplex infections. Four patients showed autoimmune manifestations such as ITP (Idiopathic Thrombocytopenic Purpura) and AIHA (Autoimmune Hemolytic Anemia). Malignity was not observed in TACI and non- TACI-CVID patients (Table 2).

The TCRVβ profiles and HLA alleles were evaluated

Table 2. Comparison of clinical findings between TACI-CVID and non-TACI-CVID groups.

	Patient Number	Gender	Age (year)	Consanguinity	Autoinflamatory Disorder	Infection	Allergy	Malignity
TACI-CVID	P1	F	39	+	ITP	Gairdia lamblia	ND	-
	P2	M	44	+	ND	Herpes labialis	ND	+
	P3	M	38	-	ND	ND	ND	-
	P4	F	29	-	ND	HSV, Saccharomyces cerevisiae	ND	-
	P5	M	4	-	HA	HSV	ND	-
	P6	M	26	-	Anchilozan spondilite	ND	ND	-
	P7	M	31	-	ITP	CMV	ND	-
Non-TACI-CVID	NT-P1	F	28	-	ND	*E.coli*	ND	-
	NT-P2	M	26	-	ND	ND	ND	-
	NT-P3	F	11	+	ND	CMV, EBV	ND	-
	NT-P4	M	8	-	ND	ND	ND	-

ND; Not Determined, HA;Hemolytic Anemia, ITP; Idiopathic Thrombocytopenic Purpura, CMV; Cytomegalovirus, EBV; Epstein-Barr Virus

in three groups and compared. IgE and IgA levels were low in all patients. IgG levels were low in patients except for P2. IgM levels were also low in P3-P7. P1 and P2 showed normal IgM levels during follow-up.

Mutations in the patients

Patients with CVID were evaluated by the NGS immunodeficiency gene panel including 266 genes. Three different TNFSRF13B mutations were determined in seven TACI-CVID patients. Two patients with homozygous and two patients with heterozygous c.310T> C mutation which was autosomal dominantly inherited were associated with CVID (OMIM: *604907). In two patients with heterozygous c.226G> C mutation which was recorded as a pathogenic mutation in NCBI with the number of NM_002834.4(PTPN11) and associated with immune thrombocytopenia. Non-TACI-CVID patients had no known mutations including TNSFR13B related to CVID according to NGS panel screening. Control individuals had no mutation and/or variant for TNSFR13B.

Distribution of TCRVβ clones in the patients and the healthy controls

After evaluation of TCRVβ clones by the conventional method, we observed that TCRVβ8 clones were significantly decreased in TACI deficient CVID patients and non-TACI-CVID patients compared to control individuals (p=0,012). Median values for TCRVβ8 were 8.5 (5,6-12,5), 3.7 (2,8-NA:not applicable) and 11.6 (13,5-20,45) in TACI-CVID, non-TACI-CVID and the healthy control samples, respectively. Increased percentage of TCRVβ13.2 clones were only observed in CVID patients with TACI defect whereas in healthy controls and non-TACI-CVID patients TCRVβ13.2 percentages were decreased (p=0,048). Relevant median values for TCRVβ13.2 percentages were 17.35 (19,25-25,25), 4.8 (3,2-NA), and 6 (3,95-13,7) in TACI-CVID, non-TACI-CVID, and the healthy controls, respectively. TCRVβ20 expression in non-TACI-CVID (median; 5,7 (3,9-NA) patients was statistically significantly decreased compared to TACI-CVID (median; 16 (12,6-18,1) patients and control individuals (median; 20,8 (18,05-21,8) (p=0,009) (Figure 2, and 4). The frequency of TCRVβ20 clone was associated with the decreased disease occurrence (OR=0,77). In contrast, decreased TCRVβ8 clone percentage was not related to TACI-CVID and non-TACI CVID (OR=1,285). Increased TCRVβ13.2 ratio was associated with TACI-CVID (OR=0,77).

Evaluation of Gini-Skewing Index

Control samples showed unequal distribution compared to patient samples although they were age-matched control individuals. Lower Gini-index in TCRVβ8 (p=0.012), TCRVβ13.2 (p=0,02), and TCRVβ20 (p=0.009) showed that equal distribution of the clones was observed among TACI deficient patients compared to non-TACI CVID patients. Non-TACI CVID patients demonstrated unequal distribution for these specific clones. In contrast to the conventional evaluation of decreased TCRVβ8 in all CVID patients, Gini-Skewing Index showed that this distribution was unequal in non-TACI CVID patients (Figure 3).

HLA allele analysis in patients

In our study, we analyzed HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ-B1, and HLA-DQ-A1 alleles. There was no correlation between HLA alleles and TCRVβ8 and TCRVβ13.2 clones.

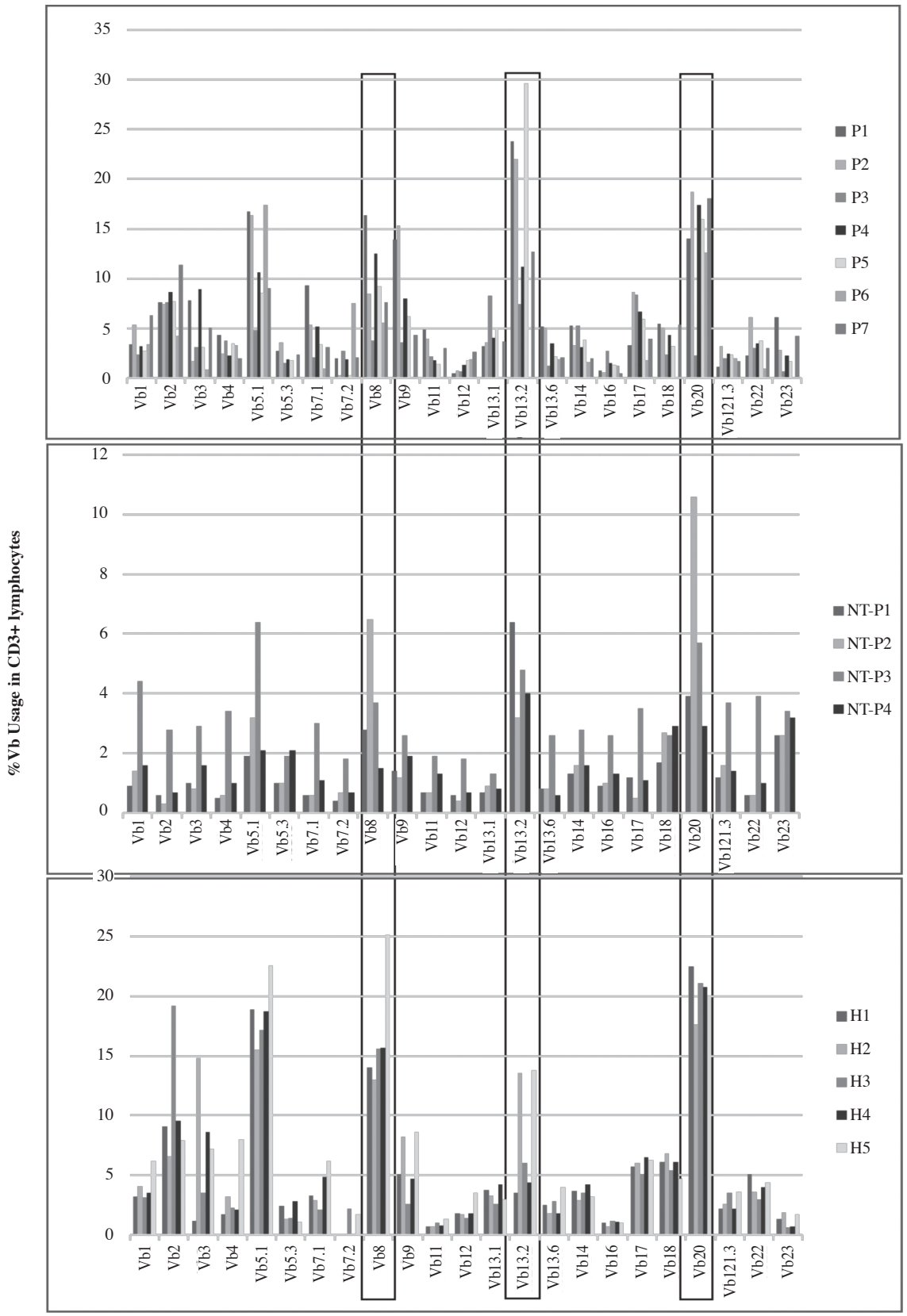

Figure 2. The percentage of 24 TCRVβ clones expressed in CD3+ T lymphocytes in patients with TACI - CVID (middle) and healthy controls (below). CVID; Common Variable Immunodeficiency

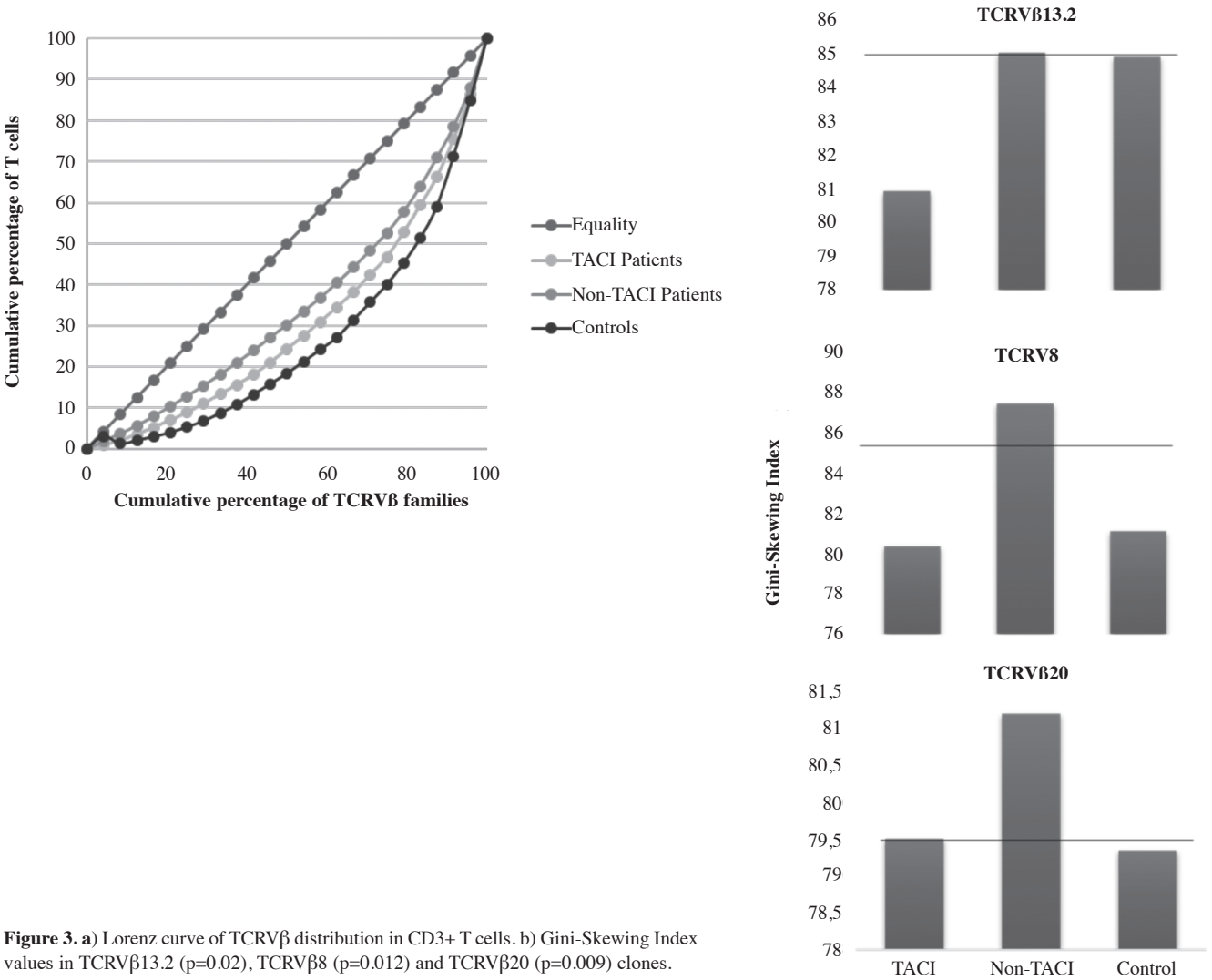

Figure 3. a) Lorenz curve of TCRVβ distribution in CD3+ T cells. b) Gini-Skewing Index values in TCRVβ13.2 (p=0.02), TCRVβ8 (p=0.012) and TCRVβ20 (p=0.009) clones.

Discussion

T cell receptor repertoire diversity is hypothetically considered to be between 10^{15}-10^{20} and associated with intrinsic factors such as HLA diversity and TCR gene polymorphisms and extrinsic factors such as viruses and superantigens. Deep TCR repertoire analysis showed that TCR diversity is thought to be about 10^6 in the laboratory conditions.[13]

TACI is a molecule that is responsible for B cell development and survival, and its deficiency may be associated with CVID. Furthermore, TACI mediates the interaction of B cells and dendritic cells and is responsible for the processing of T cells.[4-7] Due to its function, in patients with TACI gene defects, B cell function is impaired. Hypogammaglobulinemia and selective IgA deficiency are observed in most of the patients with TACI deficiency.[30-31] Therefore, it is inevitable to show its contribution to TCR diversity. Patients with CVID presenting reduced TCRVβ diversity in their CD8+ T cells are reported to have more severe complications.[11] Also, decreased Complementary Determining Region 3 (CDR3) regions and the length of N nucleotides were shown after thymic involution with aging.[22] Thus, control individuals were selected in the appropriate age range for patients with TACI defect, but there was no significant decrease in TCRVβ clones even in P6 with the youngest age among our patients compared to the other patients. We did not group our patients and control individuals according to gender because there was no study investigating the association of TCRVβ with gender in patients and/or healthy individuals.

In Crohn's disease, despite increased TCRVβ8 expressing CD8+ T cell counts, decreased cytotoxicity was observed.[23] In our study, we found that the percentage of CD8+ cells increased in three patients and decreased in two patients. There was no correlation between viral infection

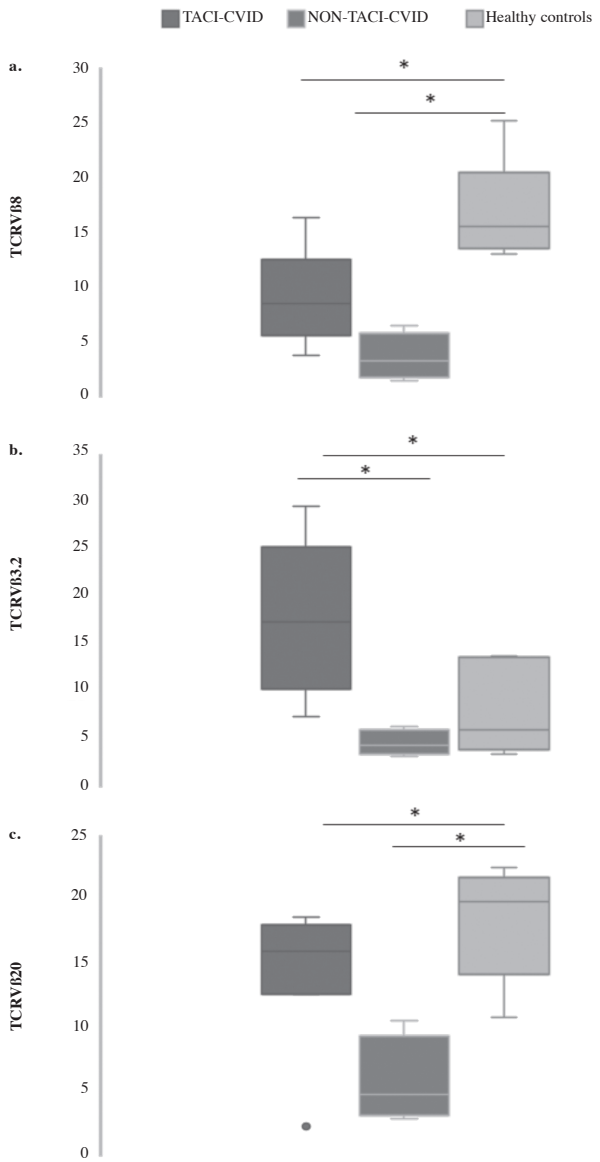

Figure 4. Box diagram showing the difference. **a)** TCRVβ8 **b)** TCRVβ13.2 **c)** TCRVβ20 among TACI-CVID patients, Non-TACI CVID patients and healthy controls. Mann –Whitney U test was performed for pairwise comparison of the TCRVβ usage.

and autoimmunity findings in CD8+ cell percentage. TCRVβ8 was associated with a high level of IgE levels in mice.[24] IgE levels in our patients were low. This decrease may be related to decreased levels of TCRVβ8 in our patients compared to the control group. It has been reported that TCRVβ8+ T cells protect against demyelinating disease in the viral model of multiple sclerosis so that a limited number of T cell diversity is responsible for clearing TMEV infection in mice.[25] Viral infections were observed in four and parasitic infection in one patient in the TACI-CVID group. Lowest TCRVβ8 expression was found in two patients (P3 and P6) without history of infection.

Preferential usage of TCRVβ20 is associated with *Staphylococcus aureus* infection and cutaneous lymphoma.[26-28] Among TACI-CVID and non-TACI-CVID patients only 1 patients had a malignant disease. None of the patients had a Staphylococcus aureus infection. Although TCRVβ 13.2 increase was observed in our patients, there was no correlation between TCRVβ 13.2 and laboratory findings. We did not observe a correlation between mutation type and preferential usage of TCRVβ clones. Likewise, we did not find a correlation between HLA alleles and expressions of TCRβ8/TCRVβ13.2 and TCRVβ20 in our patients. Although the relationship between HLA allele usage and TCRVβ clones is poorly known, one of the aims of a continuing project investigating the relationship between HLA and TCR interaction was reported in The 17th International HLA6 Immunogenetics Workshop that is to elucidate the changes in CDR1/CDR2 regions as a function of HLA types.[29] Understanding the link between HLA and TCRVβ clones will clarify preferential usage of TCRVβ clones and specific APCs involving in the disease progress and further studies are needed to shed light on these interactions.

This study showed the relevance of TCRVβ13.2, TCRVβ8, and TCRVβ20 in TACI-CVID and not-TACI CVID.

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Ethics Committee Approval: Hacettepe University (No: TSA-2016-9087).

Conflict of Interest: The authors declare that there is no conflict of interest.

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Informed Consent: The patients were involved the study after signing informed consent forms.

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