Research Square

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Evaluation of T Cell Repertoire in Primary Immunodeficiencies With Dna Repair Defects

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Abstract

Inborn errors of immunity include multiple genetic abnormalities affecting different components of the innate and adaptive immune systems. More than 450 genes have been described so far including DNA repair defects which may result in predisposition to infections, but also malignancies, neurologic abnormalities and growth retardation. The group of patients with DNA repair and methylation defects exhibit impaired adaptive immunity, which increases susceptibility to infections due to impaired repertoire diversity. In this context, we aimed to investigate the TCRvβ repertoire and its interaction with clinical entities in a group of IEI patients with DNA repair defects including ATM, DCLRE1C, DNA-PRKDC, DNA ligase-4, and BLM.

Thirty-nine patients with evidence of DNA repair defects and radiosensitivity and 15 age-matched healthy controls were included in this study. Peripheral lymphocyte subset and TCR-vβ repertoire analyses were performed by flow cytometry. To contrast TCR-repertoire in patients with DNA repair defects to healthy controls, we extracted data on lymphocyte phenotype, thymic function, immunoglobulins, and analysis of the TCRvβ repertoire from a prior study.

The entire TCR-vβ repertoire was detected in all patients. However, compared with the control group, 9 of 24 clones (37.5%) were statistically significantly lower, whereas only 3 clones had high levels ($p < 0.05$). In addition, 62.5% of all clones had lower values than the control group. Some unique vβ clones have been associated with some clinical entities. Clonotypes associated with infections, autoimmunity and lymphoid proliferation were detected in the patient group. Lower TCR-vβ-9 and TCR-vβ23, higher TCR-vβ7.2 were detected in the patients with pneumonia ($n = 13$) (respectively $p = 0.018$, $p = 0.044$ $p = 0.032$). In addition, AT patients with pneumonia ($n = 10$) had a lower TCR-vβ-9 clone than patients without pneumonia ($n = 25$) ($p = 0.008$).

In summary, we observed skewed clonal proliferation of most TCR-vβ clones in DNA repair defects, especially AT. In addition, our study demonstrated that some TCR-vβ clones might be predictive of some clinical entities. To further investigate the impact of the diversity of the TCR repertoire on the clinical phenotype, future studies should focus on the analysis of naïve and memory T cells, the detection of the source of oligoclonality, and the relationship between clonality and clinical entities.

Introduction

Inborn errors of immunity (IEI) include multiple genetic abnormalities affecting different components of the innate and adaptive immune systems[1]. IEI make affected individuals more susceptible to infections, autoimmune diseases, and malignancies[2]. The group of IEI patients with DNA repair and methylation defects exhibit impaired adaptive immunity, which increases susceptibility to infections due to impaired repertoire diversity[3].

T-cell receptors (TCRs) are one of the most important molecules of the adaptive immune system, located on the surface of T cells[4]. Their function is to recognize peptides bound to one of the cell surface products of major histocompatibility complex (MHC) genes[5]. TCRs are composed of two distinct chains: α and β or γ and δ. Each of these chains carries constant and variable regions. The diversity of the variable regions accounts for the specificity of TCRs[6].

Recombination of variable (V), diverse (D), and joining (J) gene segments accounts for the diversity of the antigen repertoire. In addition, diversity is further enhanced by junctional diversity, terminal deoxynucleotidyl transferase (TdT) and ARTEMIS activity[7]. These molecules provide non-templated (N) nucleotide additions at the V-D and D-J junctions and random deletion of nucleotides at the recombination edges[7]. In addition, selection of the TCR repertoire and interaction with antigens occurs in the thymus. The complex of recombination-activating genes 1 and 2 (RAG1 and RAG2) provides DNA double-strand breaks (DSBs) near the V-, D-, and J-coding gene segments and recombination signal sequences[8]. This process is known as V (D) J recombination. RAG1 and RAG2 gene mutations can cause severe combined immunodeficiency (SCID) [7].

Homologous recombination (HR) and non-homologous DNA end joining (NHEJ) are two important DNA repair pathways. In both physiological and pathological DSBs, the only DNA repair mechanism is NHEJ, which is also the most rapid[9]. Abnormal NHEJ leads to susceptibility to DNA damage and predisposition to immunodeficiency, which manifests in neurologic abnormalities, growth retardation, and infections[9]. Specific genes are part of this process; for example, ataxia-telangiectasia mutated kinase (ATM) plays a role in both pathways. Moreover, these specific gene defects cause combined immunodeficiency, namely RAG deficiency (TCRβ and B-cell receptor (BCR) [10], ataxia-telangiectasia (AT) (TCRα and BCR) [7], Cernunnos deficiency (TCRβ, TCRδ and BCR) 3, Wiskott-Aldrich syndrome (TCRβ) [11], and common variable immunodeficiency (CVID) (TCRβ, BCR) [12].

Ataxia-telangiectasia (AT) (OMIM #208900) is caused by mutation of the ATM gene, which encodes a protein belonging to a family of protein kinases with a phosphatidylinositol 3-kinase (Pi3K) domain[13]. The main function of the ATM protein is to regulate the DSB-induced DNA damage response, mediate the cellular antioxidant response, and activate the tumor suppressor p53 to regulate cell cycle arrest, apoptosis, senescence, and metabolism[14]. ATM is essential for the phosphorylation activity of DNA PKcs[15]. DNA PKcs is necessary for hyperphosphorylation of Artemis protein, and both play important roles in IR (ionizing radiation)-induced DSB repair[16]. ATM is also involved in telomere maintenance, carbon metabolism, and angiogenesis. ATM has a complementary role in V(D)J recombination. ATM -deficient patients have shorter complementarity determining region 3 (CDR3) lengths in the variable regions of the β-chain of the TCR (TCR V-Beta), resulting in more pathology-associated TCRs[7].

The clinical picture is characterized by progressive debilitating neurodegeneration, cerebellar ataxia, oculocutaneous telangiectasia, recurrent sino-pulmonary infections, hypersensitivity to ionizing radiation, insulin-resistant diabetes, and increased risk of malignancies[17,18]. In addition, growth failure, poor pubertal development, gonadal atrophy, skin abnormalities, and cardiovascular disorders have also been reported in A-T patients[17,18]. However, the clinical picture exhibits high variability that correlates with residual ATM kinase activity[19]. In 2006, a prospective study reported a median survival of 25 years[20]. Immunologic manifestations in AT patients include low immunoglobulin (Ig)A and IgG2 subclasses, B- and T-cell lymphopenia, and impaired diversity of the T-cell receptor (TCR) repertoire[21].

To measure TCR diversity and clonality, the first technique is to amplify CDR3 and visualize the length distribution ("spectra typing"). This method is routinely used in clinical practice in the evaluation of hematologic malignancies to demonstrate clonality. Results can be classified as clonal, oligoclonal, or polyclonal in a standardized international consensus for clinical testing (BIOMED2 consortium agreement)[22,23]. However, results are still largely qualitative and over- or under-represented due to polymerase chain reaction (PCR) bias.

The other technique, high-throughput sequencing (HTS), has allowed T-cell clonotypes to be defined more precisely, and the precise use of V, D, and J gene segments and junctional diversity of each clonotype has been revealed. However, these large data sets require sophisticated bioinformatics support[24]. As with the first technique, HTS-TCR can be compromised by PCR biases and errors[25].

Alternatively, Vβ-specific antibodies can assess the diversity of the TCR repertoire with flow cytometric analysis. Although this method does not cover all TCRvβ families and fewer vα genes, it assesses actual protein expression on the cell surface. Moreover, it is a simple method and can be performed in centers with flow cytometry[26].

We aimed to investigate the TCRvβ repertoire and its interaction with clinical entities in a group of IEI patients with DNA repair defects including ATM, DCLRE1C, DNA-PRKDC, DNA ligase-4, and BLM.

Material And Methods

Patients

All patients with genetic evidence of DNA repair defects underwent clinical exome (CES) or whole exome sequencing (WES). 39 IEI patients with evidence of DNA repair defects and radiosensitivity and 15 age-matched healthy controls were included in this study. To contrast TCR-repertoire in patients with DNA repair defects to healthy controls, we extracted data on lymphocyte phenotype, thymic function, immunoglobulins, and analysis of the TCRvβ repertoire from a prior study.[27, 28]

Approvals were obtained from the human research at Istanbul University-Cerrahpasa Faculty of Medicine, Ethics Committee (08.07.2022–431711). Informed consent for the performed tests was obtained from all patients and/or their parents. All patients were diagnosed with IEI based on the updated clinical diagnostic criteria of the European Society for Immunodeficiencies (ESID).[29]

Immunological Assessments

Peripheral lymphocyte subset and TCR-Vβ repertoire analyses were performed by flow cytometry according to previous studies[27]. Whole blood was incubated with monoclonal antibodies against surface markers in the dark at room temperature for 20 minutes. After red cell lysis, cells were washed and proceeded with flow acquisition[30,31]. Staining of the Beta Mark TCRVβ Repertoire kit was performed according to the manufacturer's instructions (Beckman Coulter, FR). Stained cells were captured using the Navios EX cytometer (Beckman Coulter). Cytobank software (Beckman Coulter) was used to analyze all available samples using the Navios Bechman Coulter cytometer.

Shannon entropy, species richness, and species evenness were calculated for each TCRβ repertoire using the R package Vegan. Shannon entropy was defined using the following equation:

where pi is the proportion of species i and S is the number of species in a repertoire. Species richness was defined as the number of species found in a population, and species evenness was defined as Shannon entropy divided by the logarithm of species richness. The Shannon index H measures the diversity of the repertoire, and the Gini coefficient indicates the homogeneity of the repertoire[32].

Statistical Analysis

PASW Statistics 18 program for Windows was used for data entry and statistical analysis. Descriptive statistics and graphs were used to present the results. The Kolmogorov-Smirnov distribution test was performed to determine the normal distribution of the analyzed data. Mann Whitney U test was used to compare two groups. Statistical significance was accepted as $p < 0.05$. In addition, some statistical analyzes and graphical plots were performed using GraphPad Prism 9 (GraphPad Software Inc, San Diego, CA) and SPSS 20 (IBM, Chicago, IL, USA).

Results

Demographic characteristics of patients

After performing WES or CES, 39 IEI patients with mutations in DNA repair and methylation genes were recruited for this study. According to molecular diagnosis, we divided our patients into two groups, including atypical SCID (n = 3; DCLRE1C- OMIM #602450) (n = 1), DNA-PRKDC-OMIM #615966 (n = 1), DNA ligase-4-OMIM #606593 (n = 1), and combined immunodeficiency with DNA repair defects with syndromic features (n = 36; Bloom syndrome-OMIM # 210900 (n = 1), AT-OMIM #208900 (n = 35)). (Fig. 1A, Table 1).

Table 1
Clinical and demographic features of the patients.

Patient	Subgroup	Genetic Defect	Gender, Age (mo)	Age at diagnosis (mo)	Age at onset	Infections	Other clinical findings	Treatment
P1	AT	ATM p.Vol1506* (c.4514delC)	F, 90	31	18	None	Ataxia, NDD, Short stature (+)	IgRT sc
P2	AT	ATM (c.6047A > G, P.Asp2016Gly)	F, 176	53	6	Rec RTI, Candida albicans (+)	NDD, Short stature	IgRT sc; Azithromycin TMP-SMZ px
P3	AT	ATM (c.6047A > G, P.Asp2016Gly)	F, 74	18	18	None	Lymphadenopathy, NDD, Short stature	IgRT sc, Azithromycin
P4	AT	ATM p.Phe2387(c.7159-7160insAGCC)	F, 120	24	64	Rec RTI, Rec Otitis media and sinusitis	Factor 11 deficiency, Bronchiolitis obliterans, Atopic dermatitis, NDD, Short stature	IgRT sc
P5	AT	ATM p.Phe2387(c.7159-7160insAGCC)	F, 39	6	13	None	Congenital hypothyroidism, NDD	IgRT sc
P6	AT	ATM p.A2622V (c.7865C > T)	M, 162	72	18	None	Febrile convulsion, NDD, Short stature	None
P7	AT	ATM p.K1192K (c.3576G > A)	M, 171	143	18	Rec RTI and diarrhea	Alopecia, Dystonia, Scoliosis, Enuresis, Encopresis, NDD, Short stature	IgRT IV
P8	AT	ATM p.Glu2014(c.6040G > T)	F, 58	24	18	Rec RTI (S. Pneumoniae +), Urinary tract infections, moniliasis	Autoimmune hemolytic anemia, Hepatosplenomegaly, Lymphadenopathy, Protein-losing enteropathy, NDD, Short stature, arterial aneurysm, Congenital hypothyroidism	IgRT sc
P9	AT	ATM c.B050 C > T (p. Gln2684*)	M,92	25	10	Rec RTI	NDD, Short stature	IgRT IV, Azithromycin TMP-SMZ px
P10	AT	ATM c.3576G > A (p.K1192)	M, 134	84	60	None	NDD	TMP-SMZ px
P11	AT	ATM c.6701T > c (p.L2234P) (novel)	M, 162	119	14	Rec Otitis media and sinusitis	NDD	IgRT IV; Azithromycin TMP-SMZ px
P12	AT	ATM p.Arg.35 (c.103C > T) heterozygous, (c.2251-4A > G) heterozygous	F, 225	72	48	Herpes Zoster	Non-Hodgkin's lymphoma	None
P13	AT	ATM c.6046G > p.Asp2016Tyr	M, 183	162	18	Rec Otitis media	Atopic dermatitis, Dysmorphic face	IgRT IV and TI SMZ px
P14	AT	ATM p.Gln852*(c.2554 > T)	F, 80	47	12	Rec Otitis media, Septic arthritis	Granuloma at skin, Dysmorphic face, Hepatosplenomegaly, Lymphadenopathy, NDD, Short stature	IgRT IV, TMP-S px, hydroxychloro

ATM: Ataxia telangiectasia mutated
RTI: respiratory tract infections
NDD: neurodevelopmental delay
IgRT: Immunoglobuline replacement therapy
Px: prophylaxis
TMP-SMZ: trimethoprim sulfamethoxazole
WPW: Wolf Parkinson White

Patient	Subgroup	Genetic Defect	Gender, Age (mo)	Age at diagnosis (mo)	Age at onset	Infections	Other clinical findings	Treatment
P15	AT	ATM c.4973delC	M, 63	25	13	Rec sinusitis, Atelectasis in thorax CT	Dysmorphic face, Hepatomegaly, T cell leukemia, Chronic diarrhea, NDD, Short stature	IgRT IV, Voriconazole a 6-MP
P16	AT	**ATM c.3576G > A, p.Lys1192=**	**F, 114**	**73**	**13**	**None**	**Atopic dermatitis, Dysmorphic face, NDD**	**None**
P17	AT	ATM c.6583_6584delCA (p.H2195X)	F, 111	54	11	Rec RTI, Urinary tract infections	Dysmorphic face, NDD, Short stature	Azithromycin
P18	AT	**ATM c.3576G > A, p.Lys1192=**	**F, 124**	**103**	**18**	**None**	**NDD**	**TMP-SMZ px**
P19	AT	ATM c.8122 G > A (p.Asp2708Asn) heterozygous, c.8024_8026delGAG (p.Gly2675del)heterozygous	F, 93	72	15	None	Epilepsy, Short stature	None
P20	AT	**ATM c.3802delG (p. Val1268Terfs)**	**M, 110**	**73**	**14**	**Rec RTI, sinusitis and diarrhea**	**Nephrolithiasis, Dismorphic face, Cleft lip and palate, NDD, Short stature**	**TMP-SMZ px**
P21	AT	ATM c.4973delC c.4973delC	M, 91	27	12	Rec RTI	Dismorphic face, Autoimmun thyroiditis, Short stature	**IgRT IV and TI SMZ px**
P22	AT	**ATM c.2921 + 1G > A**	**M, 62**	**30**	**15**	**Rec Otitis media and sinusitis**	**NDD**	**None**
P23	AT	ATM c.7788G > A (p.Glu2596=)	M, 101	18	12	None	NDD	None
P24	AT	**ATM c.3576G > A (p.Lys1192=)**	**F, 37**	**36**	**24**	**None**	**NDD, Vitiligo**	**None**
P25	AT	ATM c.3576G > A (p.Lys1192=)	**M, 97**	96	24	None	**NDD, Vitiligo**	None
P26	AT	**N/A**	**F, 42**	**40**	**21**	**None**	**None**	**IgRT IV**
P27	AT	ATM c.2284_2285delCT (p.Leu762ValfsTer2)	M, 57	52	12	Rec RTI	None	IgRT IV
P28	AT	**ATM c.756_757delTG(p.Cys252*) heterozygous/ c.7865C > T(p.Ala2622Val) heterozygous**	**M, 65**	**41**	**24**	**Rec RTI (**S. Pneumoniae, H. İnfluenza, **Rhinovirus,Parainfluenza virus)**	**Lymphadenopathy, Short stature**	**IgRT IV**
P29	AT	ATM c.756_757delTG(p.Cys252*) heterozygous/ c.7865C > T(p.Ala2622Val) heterozygous	F, 29	12	12	Rec RTI (*S. Pneumoniae, H. İnfluenza*, Rhinovirus,Parainfluenza virus)	None	**IgRT IV**
P30	AT	**ATM p.Glu2596=(c.7788G > A)**	**F, 137**	**91**	**60**	**Rec RTI, Rec Otitis media and sinusitis, Oral moniliasis, Persistent fever**	**NDD, Short stature, Corneal punctuate epitheliopathy, Febrile seizure**	**IgRT IV**

ATM: Ataxia telangiectasia mutated
RTI: respiratory tract infections
NDD: neurodevelopmental delay
IgRT: Immunoglobuline replacement therapy
Px: prophylaxis
TMP-SMZ: trimethoprim sulfamethoxazole
WPW: Wolf Parkinson White

Patient	Subgroup	Genetic Defect	Gender, Age (mo)	Age at diagnosis (mo)	Age at onset	Infections	Other clinical findings	Treatment
P31	AT	ATM p.Asp2016Gly (c.6047A > G)	F, 153	82	72	Persistent fever, Rec RTI	Lymphadenopathy, NDD, Short stature, Bronchiectasis	IgRT IV
P32	AT	N/A	F, 69	15	13	Rec RTI (S. Pneumoniae, H. İnfluenza, Adenovirus)	Short stature, Bronchiectasis	IgRT IV
P33	AT	ATM p.Glu2596Glu (c.7788G > A)	F, 115	63	24	None	Dismorphic face, NDD, Short stature, WPW Syndrome	None
P34	AT	ATM p.Glu2596Glu (c.7788G > A)	F, 80	54	12	None	NDD, Short stature	None
P35	AT	ATM p.Glu2596Glu (c.7788G > A)	F, 61	36	12	None	NDD, Short stature	None
P36	Bloom Syndrome	BLM chr15.91346925, c.3535del (p.Thr1179LeufsTer3)	M, 132	125	1	Rec RTI	Dismorphic face, Short stature, Hyperpigmented macules	IgRT IV
P37	DNA PRKDC Deficiency	PRKDC c.10423_10426dup (p.Glu3476Alafs*48) heterozygous, c.7783C > T p(Arg2595Cys) heterozygous	F, 53	46	37	Rec diarrhea and recurrent abscess, Oral moniliasis	Anal fistula, Inflammatory bowel disease like symptoms, Dysmorphic face, Lymphadenopathy, Sclerosing cholangitis, NDD, Short stature	IgRT IV
P38	Artemis Deficiency	DCLRE1C c.632G > T homozygous	M, 85	83	77	Rec RTI	Bronchiectasis	IgRT IV and TI SMZ px
P39	DNA Ligase 4 Deficiency	LIG4 c833G > A, p.Arg278His, c.724_725delTT, p.Leu242IlefsTer5 compound heterozygous	M, 221		18	Rec RTI and Otitis media, persistent fever	Atopic dermatitis, Dysmorphic face, NDD, Short Stature, Vitiligo, Epilepsy, Microcephaly	IgRT IV and TI SMZ px

ATM: Ataxia telangiectasia mutated

RTI: respiratory tract infections

NDD: neurodevelopmental delay

IgRT: Immunoglobuline replacement therapy

Px: prophylaxis

TMP-SMZ: trimethoprim sulfamethoxazole

WPW: Wolf Parkinson White

A total of 39 patients (n = 17 (43.5%) males and n = 22 (56.5%) females) with a median age of 93 months (IQR 25–75: 63–134 months) were included. Thirty-one (79.5%) of all patients were from a blood-related family. The median age of all patients at symptom onset was 18 months (IQR 25th-75th: 12–24 months), and the median age at diagnosis was 54 months (IQR 25th-75th: 30–82 months). The median duration of diagnostic delay was 31 months in all groups (IQR 25th-75th: 12–57 months), and it was shorter in AT patients (36.8 months) than in the others (88.5 months). However, these differences were not statistically significant ($p = 0.110$).

Clinical Findings and Immunological Results of Patients

Clinical findings of the patients are given in Table 1, Fig. 1B and Table S1.

Immunological work-up revealed absolute T-cell, B-cell and total CD4 + and CD8 + T cell counts which is consistent with previous studies, only T-cell and CD4 + T-cell counts were lower than in previous studies.[7,21,33] The immunological parameters are given in Table S2, Table S3 and Fig S1.

Oligoclonality of the TCR repertoire and correlation with clinical phenotype

In general, TCR repertoires are thought to be dynamically variable, with diversity decreasing with age[34]. Therefore, previous studies have included age-matched controls[7,21]. When analyzing the TCR-β repertoire in patients with different pathogenic backgrounds, we also included 15 age-matched healthy controls to compare the diversity and complexity of the repertoire.

Overall, the entire TCR-vβ repertoire was detected in all patients. However, compared with the control group, 9 of 24 clones (37.5%) were statistically significantly lower, whereas only 3 clones had high levels ($p < 0.05$). In addition, 62.5% of all clones had lower values than the control group (Figs. 2 and 3).

The statistically significantly lower clones were TCR-Vβ 7.1(*$p < 0,01$ $p = 0,006$*), Vβ3 (*$p = 0.01$*), Vβ9 (*$p < 0,01$ $p = 0,005$*), Vβ17 (*$p < 0,01$ $p = 0,009$*), Vβ2 (*$p = 0$*), Vβ22(*$p < 0,05$ $p = 0,021$*), Vβ21.3 (*$p < 0,01$ $p = 0,005$*), Vβ11(p *< 0,01 p = 0,004*), and Vβ4 (*$p = 0$*). And the statistically significant higher clones were TCR-Vβ-20 (*$p < 0,01$, $p = 0,004$*), Vβ12 (*$p < 0,01$ $p = 0,004$*), Vβ14 (*$p < 0,05$ $p = 0,037$*)(Fig. 2).

In other words, the TCR-vβ repertoire of AT patients showed limited diversity compared to healthy controls (HC). Richness is measured by the number of unique TCRs in a given sample. Evenness indicates how evenly distributed the frequencies of each unique TCR are in the estimated population. Shannon entropy considers both richness and evenness to measure diversity. In our study, restriction by low Shannon's H-index ($p < 0.05$ $p = 0,019$) and the evenness of clonality by higher Gini coefficient ($p < 0.05$ $p = 0.008$; Figs. 4A and 4B) confirmed. This situation predisposed to oligoclonality. These results were also true for all patient groups compared to HC (Shannon's H index ($p < 0,05$ $p = 0.012$) and Gini coefficient ($p < 0.01$ $p = 0.002$)). When comparing atypical SCID and healthy controls, repertoire diversity using the Shannon H index and evenness of clonality using the Gini coefficient were not statistically evaluated because only 3 patients had atypical SCID.

In addition, some unique Vβ clones have been associated with some clinical entities. Clonotypes associated with infections, autoimmunity and lymphoid proliferation were detected in the patient group. Lower TCR-vβ-9 and TCR-vβ23, higher TCR-vβ7.2 were detected in the patients with pneumonia ($n = 13$) (respectively $p = 0.018$, $p = 0.044$ $p = 0.032$, Fig. 5A1). In addition, AT patients with pneumonia ($n = 10$) had a lower TCR-vβ-9 clone than patients without pneumonia ($n = 25$) ($p = 0.008$; Fig. 5A2).

Moreover, some additional clones, including TCR-vβ23, TCR-vβ21.3, TCR-vβ22, and TCR-vβ13.2, were associated with chest imaging findings (TFI; consolidation, atelectasis, bronchiectasis, pulmonary nodules; $n = 10$) and were observed in lower frequency in patients with TFI (Fig. 5B1). As expected, the Gini coefficient indicated heterogeneity of the repertoire and was significantly higher compared to patients without findings in the chest radiograph ($p = 0.041$, Fig. 4C). When comparing TFI (+) AT patients ($n = 8$) and TFI (-) AT patients ($n = 27$), TCR-vβ11 and TCR-vβ5.2 were significantly lower in the AT group with TFI than when comparing HC and all patients ($p = 0.023$ and $p = 0.039$, respectively) (Fig. 5B2).

The number of patients with bronchiectasis was too small to compare statistically, but clones with similar statistical significance to patients with thorax findings in imaging were detected as possibly associated with pulmonary pathologies (Fig. 5C). The Gini coefficient was also higher than in patients without bronchiectasis ($p = 0.024$, fig. S3). In addition, sinusitis ($n = 6$) and otitis media (OM, $n = 7$) were also associated with some clones. However, their statistical significance was limited (Fig. 5D1). Moreover, the same TCR-vβ clone was significantly lower than in the patients with OM, when comparing AT patients with and without OM (Fig. 5D2). The oligoclonality could be attributed to recurrent or chronic infections causing selective expansion of some clones.

The patients with lymphoproliferation (lymphadenopathy and/or hepatosplenomegaly, $n = 9$) had some distribution abnormalities, including TCR-vβ2 higher than in the lymphoproliferative patient group ($p = 0.041$, Fig. 6). Expansions of TCR-vβ9, TCR-vβ5.1, TCR-vβ22, and TCR-vβ14 were detected in two patients affected by malignancy (T-cell leukemia, $n = 1$ and non-Hodgkin's lymphoma, $n = 1$, respectively $p < 0.001$, $p = 0.009$, $p = 0.009$, $p = 0.013$).

Distribution abnormalities, including clonal expansion of TCR-vβ4 and TCR-vβ2 and clonal decrease of TCR-vβ11 and TCR-vβ5.1, were shown in patients with autoimmunity ($n = 4$, respectively $p = 0.033$, $p = 0.026$, $p = 0.012$, $p = 0.044$, Fig. 5E1). TCR-vβ4, TCR-vβ11 and TCR-vβ5.1 showed the same pattern when comparing AT patients with ($n = 4$) and without autoimmunity ($n = 31$) (respectively $p = 0.038$, $p = 0.007$ $p = 0.028$; Fig. 5E2). Consequently, we suggest that the TCR-vβ repertoire in DNA repair defects, especially in AT patients, is biased by either underutilization or oligoclonal expansion of most vβ families.

Restriction of TCR repertoire and correlation with immunological phenotype

We found that some immunological parameters were significantly associated with certain Vβ clones. CD4 + T-cell lymphopenia was associated with decreased TCR-vβ5.3 and TCR-vβ11 ($p = 0.042$, $p = 0.012$, respectively, Fig. 7A). CD8 + T-cell lymphopenia showed an association with decreased TCR-vβ18 ($p = 0$, Fig. 7B). The same pattern was seen when compared within AT patients.

In addition, TCR-vβ11 levels showed a weak positive correlation with the number of CD4 + T cells (Pearson correlation analysis, $r = 0.347$), and TCR-vβ7.1 levels also showed a weak positive correlation with the number of CD8 + T cells (Pearson correlation analysis, $r = 0.386$), but not statistically significant with a group of CD8 + lymphopenic compared to a group of normal CD8 + T cells. This abnormal distribution may predict normal levels of CD4 + T cells and CD8 + T cells correlate with a normal TCR repertoire.

Discussion

We performed flow cytometry analysis of TCRvβ in 39 IEI patients with DNA repair defects. Different TCR repertoire characteristics in different disease groups and clinical phenotypes were detected in the cytometry data compared with healthy controls. The results provide evidence for clinical entities associated with specific TCR clones and indicate the function of ATM and other DNA repair genes during V(D)J recombination.

Successful VDJ recombination is very important for the adaptive immune system. The results of its failure have been shown in IEI such as TB-NK + SCID, in which components of the VDJ recombination mechanism are deficient or absent[35]. In addition, other immunodeficiencies such as Omenn syndrome, Wiskott-Aldrich syndrome (WAS), immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome (IPEX), and common variable immunodeficiency (CVID) have been studied in terms of antigen receptor diversity, particularly in the TCR repertoire[36–38].

ATM function is required for both mechanisms (NHEJ and HR) of DSB repair[9]. Other DSB repair defects (including DCLRE1C, DNA-PRKDC, DNA ligase-4, BLM) also affect DNA instability[7,15,39,40]. The diversity of the TCR repertoire is an important parameter for the immune response to antigenic challenge. Because the severity of T-cell deficiency determines the T-cell repertoire, the diversity of the TCR repertoire is more limited in patients with severe clinical phenotypes[41]. Our results confirm previous studies on DNA repair defects; in particular, AT patients were characterized by significantly reduced diversity of the TCR repertoire[7,21,42]. However, the results should be evaluated with caution because of the limited sample size of the atypical SCID group in this study.

From a clinical perspective, we consider the TCR repertoire as a comprehensive biomarker that should be included in the assessment of immune parameters. Because at present, the identification of complete T-cell epitopes seems to be far away to cover all clinical entities. In this study, it was found that underutilization or oligoclonal expansion of most vβ families causes bias in the TCRvβ repertoire in DNA repair defects, especially in AT patients. These results confirm and extend previous observations on the clonality of the TCRvβ repertoire of AT[7,21]. Giovannetti et al showed that diffuse oligoclonal expansions distort the TCRvβ repertoire of AT patients and also investigated that the restriction of the TCR repertoire at AT depends on TCR generation limitations. They observed expansion of TCRvβ genes in the CD4 subset in various clones, with the exception of TCR-vβ14 from our study[21]. Our study also did not examine vβ gene expression.

Fang et al illustrated TCRβ recombination in DNA repair and methylation defects[7]. As in our study, TCRβ rearrangements were detected in all patients. The TCRβ repertoire of patients from AT was severely restricted. This restriction concerned the diversity of the repertoire, as indicated by a low Shannon's H index, and also the evenness of clonality, as represented by a higher Gini coefficient compared with HC. Our study revealed a low Shannon's H index and a high Gini coefficient in all patients and AT patients compared with HC. Similarly, abnormalities in relative TCR-vβ utilization in healthy subjects were approximately 60–70% of T cells expressing TCR-vβ. In our study, this ratio was % 62.6, and TCR-vβ19 usage in AT and TCRvβ 7.3 usage in ICF2 were significantly reduced compared with HC. Our study did not include these two clones[7]. Our study showed expansion of three clones (TCR-vβ20, TCR-vβ12, and TCR-vβ14) and reduction of several clones.

In CVID patients, clonality of the TCRβ repertoire is clearly increased independent of clinical phenotype, age, and gender.[43] In our study, age or gender did not affect TCR diversity.

These expansions and oligoclonality likely originate from the relatively few cells that have functional TCR rearrangements[21]. Chronic infections, particularly the viral infections EBV and CMV, which are associated with detectable T-cell activation, induce clonal expansions in healthy individuals[44]. However, chronic infection is unlikely to cause these expansions. This is because CD8 + T cells are the predominant cells of antigenic stimulation[45] and oligoclonality predominates in CD4 + T cells. Accordingly, our patients had no proven infection with EBV or CMV.

Another study included 8 patients with MHC-II and showed some distribution abnormalities, including clonal expansion of different clones in different patients. However, relatively minor differences in the TCR repertoire of CD4 + T cells were described in that study[46]. Two patients with IPEX had an abnormal repertoire of CD4 + T cells that included expansions of TCR-vβ8 and TCR-vβ22[37]. Unfortunately, we did not observe the TCR repertoire of CD4 + or CD8 + T cells.

The reduction of naive CD4 + T cells contributes to the alteration of the TCR repertoire. In addition, expansion of CD8 + T cells affects the TCR repertoire. Ozturk et al discovered that some parameters of immune reconstitution were associated with specific vβ clones: TCR-vβ5.2 and TCR-vβ18 clones were higher in patients with CD4 + T-cell reconstitution than in patients with lower CD4 + T-cell counts[27]. In parallel with these parameters, some clones were associated with CD4 + T-cell lymphopenia and CD8 + T-cell lymphopenia in our study. However, the significance of these clones is speculative at present.

Given the reduced repertoire diversity, we predicted that there would be limited T-cell-dependent antibody response, leading to frequent infections. To assess this, we examined repertoire diversity against specific infections, such as pneumonia, otitis media, and sinusitis.

We also evaluated the findings of chest imaging and bronchiectasis, as an indicator of the inflammatory process, with no or detectable findings. We also observed increased skewness in the patients with pneumonia and bronchiectasis. Other infections were also associated with specific clones. Similarly, Wong et al demonstrated a decrease in repertoire diversity and requirement of more antibiotic courses per year in CVID patients with moderate-to-severe bronchiectasis[12]. T-cell repertoire diversity assessment might predict infection risk better than numerical T-cell count. These parameters could be useful in predicting clinical prognosis if their relevance is demonstrated in larger studies.

We also found evidence of clonal TCR-vβ restriction in patients with lymphoproliferation. Autoimmunity was also associated with specific TCR-vβ clones. However, the accuracy of these correlations is limited by patient heterogeneity. In addition, pediatric patients have not yet been able to develop autoimmune disease. There is limited evidence in the literature of an association between these clinical entities and the TCR repertoire. In CVID patients, TCR repertoire limitation is not related to the presence or absence of autoimmunity[43,47,48]. However, there are several hypotheses that autoreactive TCRs and BCRs cause autoimmunity due to impaired selection in patients with hypomorphic mutations[49]. Furthermore, the appearance of autoantibodies often precedes the appearance of clinical findings of autoimmune disease, and the same process has been hypothesized for TCRs[50]. Therefore, clonotypic blood analysis may warn of the development of autoimmunity in the future, which is a very valuable tool for people with antibody deficiency[41].

In summary, we observed skewed clonal proliferation of most TCR-vβ clones in DNA repair defects, especially AT. In addition, our study demonstrated that some TCR-vβ clones might be predictive of some clinical entities. In a previous study, Fang et al[7] evaluated this skewness as an immunological "signature". However, a match of the specific vβ families in the skewed clones was not found in the studies. In addition, it should be noted that the oligoclonality in repertoire diversity in our study was due to a case-control study with limited sample size. A case-control study may show different patterns with respect to some repertoire parameters in an independent large population. Also, the CD4 and CD8 T cell repertoire was not evaluated in this study.

Future studies should describe normal repertoire diversity in age-matched healthy populations. In addition, patients with DNA repair defects should be evaluated by examining complete T-cell epitopes. However, this identification still seems distant and difficult. Measuring the TCR repertoire is an important step for this identification. To further investigate the impact of the diversity of the TCR repertoire on the clinical phenotype, future studies should focus on the analysis of naïve and memory T cells, the detection of the source of oligoclonality, and the relationship between clonality and clinical entities.

Declarations

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CONFLICT OF INTEREST

The authors declare no relevant financial or non-financial interests to disclose.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

AUTHORSHIP CONTRIBUTIONS

All authors contributed to the study conception and design. Betul Gemici Karaaslan, Isilay Turan, Zeynep Hizli Demirkale, Zeynep Meric, Sezin Aydemir, Esra Yucel, Ayca Kiykim, Haluk Cokugras, Cigdem Aydogmus followed patients, collected samples and provided clinical data. Material preparation, and data analysis were performed by Betul Gemici Karaaslan, Zeynep Hizli Demirkale, Isilay Turan, Nihan Burtecene, Birol Topcu and Ayca Kiykim. The first draft of the manuscript was written by Zuleyha Taskin, Ozgur Can Kilinc and Betul Gemici Karaaslan all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Istanbul University-Cerrahpasa, Faculty of Medicine (Date: 08.07.2022/ No: 431711).

CONSENT TO PARTICIPATE

Informed consent was obtained from the parents of all individual participants included in the study.

CONSENT FOR PUBLICATION

Not applicable.

References

1. Rojas LAmaya-UribeM, Azizi G, Anaya J-M, Eric M, Gershwin. Primary immunodeficiency and autoimmunity: A comprehensive review. J Autoimmun. 2019;99:52–72.
2. Fischer A. Human primary immunodeficiency diseases: a perspective. Nat Immunol. 2004;5:23–30.
3. IJspeert H, et al. Strategies for B-cell receptor repertoire analysis in primary immunodeficiencies: from severe combined immuno- e2. deficiency to common variable immunodeficiency. Front Immu- nol. 2015;6:157.
4. Minervina A, Pogorelyy M, Mamedov I. T-cell receptor and B-cell receptor repertoire profiling in adaptive immunity. Transpl Int. 2019;32:1111–23.
5. Marrack P, Kappler J. The T cell receptor. Sci 1987 Nov 20;238(4830):1073–9.
6. Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, Teyton L, Wilson IA. An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex. Science. 1996 Oct 11;274(5285):209 – 19.
7. Fang M, Su Z, Abolhassani H, Zhang W, Jiang C, Cheng B, Luo L, Wu J, Wang S, Lin L, Wang X, Wang L, Aghamohammadi A, Li T, Zhang X, Hammarström L, Liu X. T Cell Repertoire Abnormality in Immunodeficiency Patients with DNA Repair and Methylation Defects. J Clin Immunol. 2022 Feb;42(2):375–93.
8. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem. 2010;79:181–211.
9. Bakr A, et al. Involvement of ATM in homologous recombination after end resection and RAD51 nucleofilament formation. Nucleic Acids Res. 2015;43(6):3154–66.
10. Lee YN et al. Characterization of T and B cell repertoire diversity in patients with RAG deficiency. Sci Immunol. 2016;1(6).
11. Wu J, et al. T-cell receptor diversity is selectively skewed in T-cell populations of patients with Wiskott-Aldrich syndrome. J Allergy Clin Immunol. 2015;135(1):209–16.
12. Wong GK, et al. Accelerated loss of TCR repertoire diversity in common variable immunodeficiency. J Immunol. 2016;197(5):1642–9.
13. Moeini Shad T, Yazdani R, Amirifar P, Delavari S, Heidarzadeh Arani M, Mahdaviani SA, Sadeghi-Shabestari M, Aghamohammadi A, Rezaei N, Abolhassani H. Atypical Ataxia Presentation in Variant Ataxia Telangiectasia: Iranian Case-Series and Review of the Literature. Front Immunol. 2022 Jan;14:12:779502.

14. Qin B, Yu J, Nowsheen S, Zhao F, Wang L, Lou Z. STK38 promotes ATM activation by acting as a reader of histone H4 ufmylation. Sci Adv. 2020 Jun 3;6(23):eaax8214.
15. Shrivastav M, Miller CA, De Haro LP, Durant ST, Chen BP, Chen DJ, Nickoloff JA. DNA-PKcs and ATM co-regulate DNA double-strand break repair. DNA Repair (Amst). 2009 Aug 6;8(8):920-9.
16. Chen L, Morio T, Minegishi Y, Nakada S, Nagasawa M, Komatsu K, Chessa L, Villa A, Lecis D, Delia D, Mizutani S. Ataxia-telangiectasia-mutated dependent phosphorylation of Artemis in response to DNA damage. Cancer Sci. 2005 Feb;96(2):134–41.
17. Amirifar P, Ranjouri MR, Yazdani R, Abolhassani H, Aghamohammadi A. Ataxia-telangiectasia: A review of clinical features and molecular pathology. Pediatr Allergy Immunol. 2019 May;30(3):277–88.
18. Rawat A, Tyagi R, Chaudhary H, Pandiarajan V, Jindal AK, Suri D, Gupta A, Sharma M, Arora K, Bal A, Madaan P, Saini L, Sahu JK, Ogura Y, Kato T, Imai K, Nonoyama S, Singh S. Unusual clinical manifestations and predominant stopgain ATM gene variants in a single centre cohort of ataxia telangiectasia from North India. Sci Rep. 2022 Mar 8;12(1):4036.
19. Zielen S, Duecker RP, Woelke S, et al. Simple Measurement of IgA Predicts Immunity and Mortality in Ataxia-Telangiectasia. J Clin Immunol. 2021;41:1878–92.
20. Crawford TO, Skolasky RL, Fernandez R, Rosquist KJ, Lederman HM. Survival probability in ataxia telangiectasia. Arch Dis Child. 2006 Jul;91(7):610–1.
21. Giovannetti A, Mazzetta F, Caprini E, Aiuti A, Marziali M, Pierdominici M, Cossarizza A, Chessa L, Scala E, Quinti I, Russo G, Fiorilli M. Skewed T-cell receptor repertoire, decreased thymic output, and predominance of terminally differentiated T cells in ataxia telangiectasia. Blood. 2002 Dec 1;100(12):4082-9.
22. van Dongen JJ, Langerak AW, Bruggemann M, Evans PA, Hummel M, Lavender FL, Delabesse E, Davi F, Schuuring E, Garcia-Sanz R, van Krieken JH, Droese J, Gonzalez D, Bastard C, White HE, Spaargaren M, Gonzalez M, Parreira A, Smith JL, Morgan GJ, Kneba M, Macintyre EA. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98- 3936, Leukemia 17 (12) (2003) 2257e2317.
23. Langerak AW, Groenen PJ, Bruggemann M, Beldjord K, Bellan C, Bonello L, Boone E, Carter GI, Catherwood M, Davi F, Delfau-Larue MH, Diss T, Evans PA, Gameiro P, Garcia Sanz R, Gonzalez D, Grand D, Hakansson A, Hummel M, Liu H, Lombardia L, Macintyre EA, Milner BJ, Schuuring S, Spaargaren M, Hodges E. J.J. van Dongen, Euro- Clonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferation, Leukemia 26 (10) (2012)
24. Alamyar E, Duroux P, Lefranc MP, Giudicelli V. IMGT((R)) tools for the nucleotide analysis of immunoglobulin (IG) and T cell receptor (TR) V-(D)-J repertoires, polymorphisms, and IG mutations: IMGT/V-QUEST and IMGT/ HighV-QUEST for NGS, Methods Mol. Biol. 2012;882:569e604.
25. Mamedov IZ, Britanova OV, Zvyagin IV, Turchaninova MA, Bolotin DA, Putintseva EV, Lebedev YB, Chudakov DM. Preparing unbiased T-cell receptor and antibody cDNA libraries for the deep next generation sequencing profiling. Front Immunol. 2013;4:456.
26. Salameire D, Le Bris Y, Fabre B, Fauconnier J, Solly F, Pernollet M, Bonnefoix T, Leroux D, Plumas J, Jacob MC. Efficient characterization of the TCR repertoire in lymph nodes by flow cytometry. Cytom A. 2009;75(9):743–51.
27. Ozturk E, Catak MC, Kiykim A, Baser D, Bilgic Eltan S, Yalcin K, Kasap N, Nain E, Bulutoglu A, Akgun G, Can Y, Sefer AP, Babayeva R, Caki-Kilic S, Tezcan Karasu G, Yesilipek A, Ozen A, Karakoc-Aydiner E, Baris S. Clinical and Laboratory Factors Affecting the Prognosis of Severe Combined Immunodeficiency. J Clin Immunol. 2022 Jul;42(5):1036–50. Epub 2022 Apr 22. Erratum in: J Clin Immunol.
28. Ulgen E. Kiykim A, Saygili S, Burtecene Nihan, Cokugras H, Caliskan S. Impaired T cell rerceptor repertoire in children with chronic kidney disease. Pediatr Nephrol (2022). 37;2803–2969.
29. Seidel MG, et al. The European Society for Immunodeficiencies (ESID) Registry working definitions for the clinical diagnosis of inborn errors of immunity. J Allergy Clin Immunol Pract. 2019;7(6):1763–70.
30. Kiykim A, Ogulur I, Dursun E, Charbonnier LM, Nain E, Cekic S, et al. Abatacept as a long-term targeted therapy for LRBA deficiency. J Allergy Clin Immunol Pract. 2019;7(8):2790–800. e15.
31. Ogulur I, Kiykim A, Baser D, Karakoc-Aydiner E, Ozen A, Baris S. Lymphocyte subset abnormalities in pediatric-onset common variable immunodeficiency. Int Arch Allergy Immunol. 2020;181(3):228–37.
32. Yu X, Almeida JR, Darko S, van der Burg M, DeRavin SS, Malech H, Gennery A, Chinn I, Markert ML, Douek DC, Milner JD. Human syndromes of immunodeficiency and dysregulation are characterized by distinct defects in T-cell receptor repertoire development. J Allergy Clin Immunol. 2014 Apr;133(4):1109–15.
33. Driessen GJ, et al. Antibody deficiency in patients with ataxia telangiectasia is caused by disturbed B- and T-cell homeostasis and reduced immune repertoire diversity. J Allergy Clin Immunol. 2013;131(5):1367–75e9.
34. Nikolich-Zugich J. The twilight of immunity: emerging concepts in aging of the immune system. Nat Immunol. 2018;19(1):10–9.
35. Gennery AR, Slatter MA, Grandin L, Taupin P, Cant AJ, Veys P, Amrolia PJ, Gaspar HB, Davies EG, Friedrich W, Hoenig M, Notarangelo LD, Mazzolari E, Porta F, Bredius RG, Lankester AC, Wulffraat NM, Seger R, Gungor T, Fasth A, Sedlacek P, Neven B, Blanche S, Fischer A, Cavazzana- M, Calvo P, Landais B Inborn Errors Working Party of the European Group for, Marrow T. and I. European Society for, Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? J. Allergy Clin. Immunol. 126 (3) (2010) 602e610 e1-11.
36. Yu X, Almeida JR, Darko S, van der Burg M, DeRavin SS, Malech H, Gennery A, Chinn I, Markert ML, Douek DC, Milner JD. Human syndromes of immunodeficiency and dysregulation are characterized by distinct defects in T-cell receptor repertoire development. J Allergy Clin Immunol. 2014;133(4):1109e1115.

37. [15] D, Zennaro E, Scala D, Pomponi E, Caprini D, Arcelli E, Gambineri G, Russo A, Mari. Proteomics plus genomics approaches in primary immunodeficiency: the case of immune dysregulation, polyendocrinopathy, enteropathy, X- linked (IPEX) syndrome, Clin. Exp Immunol. 2012;167(1):120e128.
38. [16] AE, O'Connell S, Volpi K, Dobbs C, Fiorini E, Tsitsikov H, de Boer IB, Barlan JM, Despotovic FJ, Espinosa-Rosales IC, Hanson MG, Kanariou R, Martinez-Beckerat A, Mayorga-Sirera C, Mejia-Carvajal N, Radwan AR, Weiss SY, Pai YN, Lee LD, Notarangelo. Next generation sequencing reveals skewing of the T and B cell receptor repertoires in patients with wiskott-Aldrich syndrome. Front Immunol. 2014;5:340.
39. Staines Boone AT, Chinn IK, Alaez-Versón C, Yamazaki-Nakashimada MA, Carrillo-Sánchez K, García-Cruz MLH, Poli MC, González Serrano ME, Medina Torres EA, Muzquiz Zermeño D, Forbes LR, Espinosa-Rosales FJ, Espinosa-Padilla SE, Orange JS. Lugo Reyes SO. Failing to Make Ends Meet: The Broad Clinical Spectrum of DNA Ligase IV Deficiency. Case Series and Review of the Literature. Front Pediatr. 2019 Jan 21;6:426.
40. Cunniff C, Bassetti JA, Ellis NA. Bloom's Syndrome: Clinical Spectrum, Molecular Pathogenesis, and Cancer Predisposition. Mol Syndromol. 2017 Jan;8(1):4–23.
41. Wong GK, et al. Immune dysregulation in immunodeficiency disorders: The role of T-cell receptor sequencing. J Autoimmun. 2017. http://dx.doi.org/10.1016/j.jaut.2017.04.002.
42. Hathcock KS, et al. ATM influences the efficiency of TCRbeta rearrangement, subsequent TCRbeta-dependent T cell develop- ment, and generation of the pre-selection TCRbeta CDR3 repertoire. PLoS ONE. 2013;8(4):e62188.
43. Ramesh M, Hamm D, Simchoni N, Cunningham-Rundles C. Clonal and constricted T cell repertoire in Common Variable Immune Deficiency. Clin Immunol. 2017 May;178:1–9.
44. Hingorani R, Choi IH, Akolkar P, et al. Clonal pre-dominance of T cell receptors within the CD8 CD45RO subset in normal human subjects. J Immunol. 1993;151:5762–9.
45. Marashi SM, et al. Inflammation in common variable immunodeficiency is associated with a distinct CD8(+) response to cytomegalovirus. J Allergy Clin Immunol. 2011;127(6):1385–93. [PubMed: 21536322].
46. Lev A, Simon AJ, Broides A, Levi J, Garty BZ, Rosenthal E, Amariglio N, Rechavi G, Somech R. Thymic function in MHC class II-deficient patients. J Allergy Clin Immunol. 2013 Mar;131(3):831–9. 10.1016/j.jaci.2012.10.040.
47. Jones J, et al. Human autoimmunity after lymphocyte depletion is caused by homeostatic T-cell proliferation. Proc Natl Acad Sci U S A. 2013;110(50):20200–5. [PubMed: 24282306].
48. 30, Qi Q et al. Mechanisms shaping the naive T cell repertoire in the elderly — thymic involution or peripheral homeostatic3 proliferation? Exp Gerontol. 2014.
49. Grimbacher B, Warnatz K, Yong PF, Korganow AS, Peter HH. The cross- roads of autoimmunity and immunodeficiency: lessons from polygenic traits and monogenic defects, J Allergy Clin Immunol 137 (1) (2016) 3e17 quiz 18.
50. Attaf M, Huseby E, Sewell AK. Alphabeta T cell receptors as predictors of health and disease. Cell Mol Immunol. 2015;12(4):391e399.

Figures

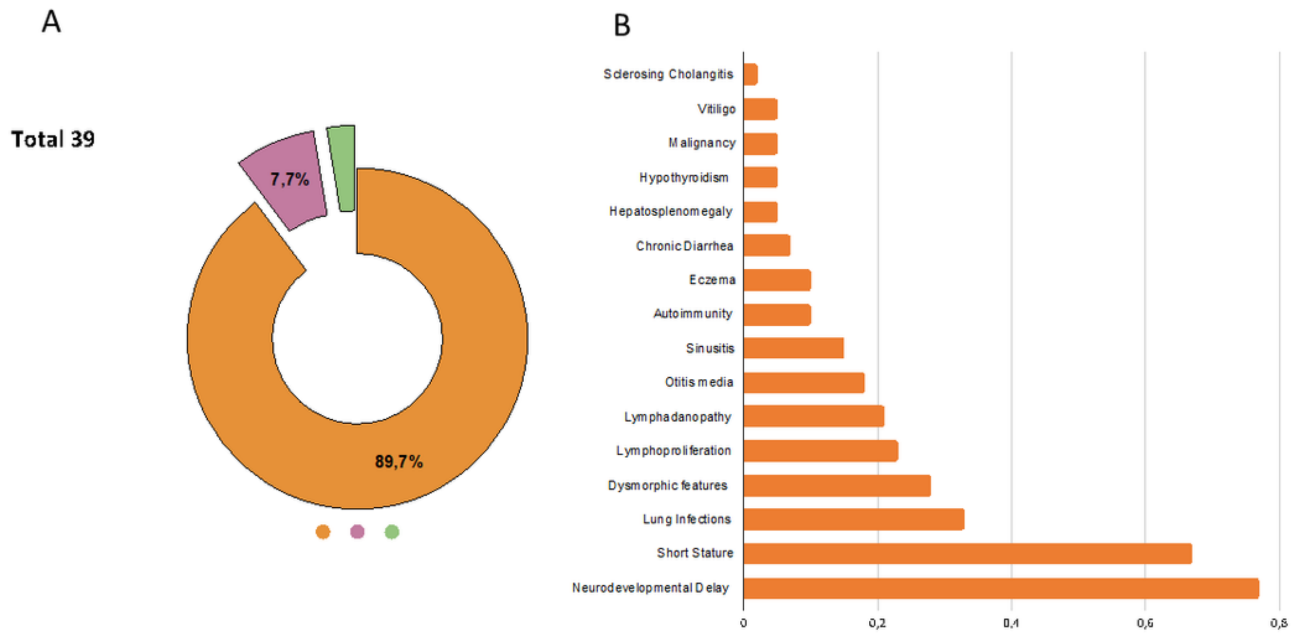

Fig1

Figure 1

The Genetic Diagnosis and Clinical Findings of the Patients

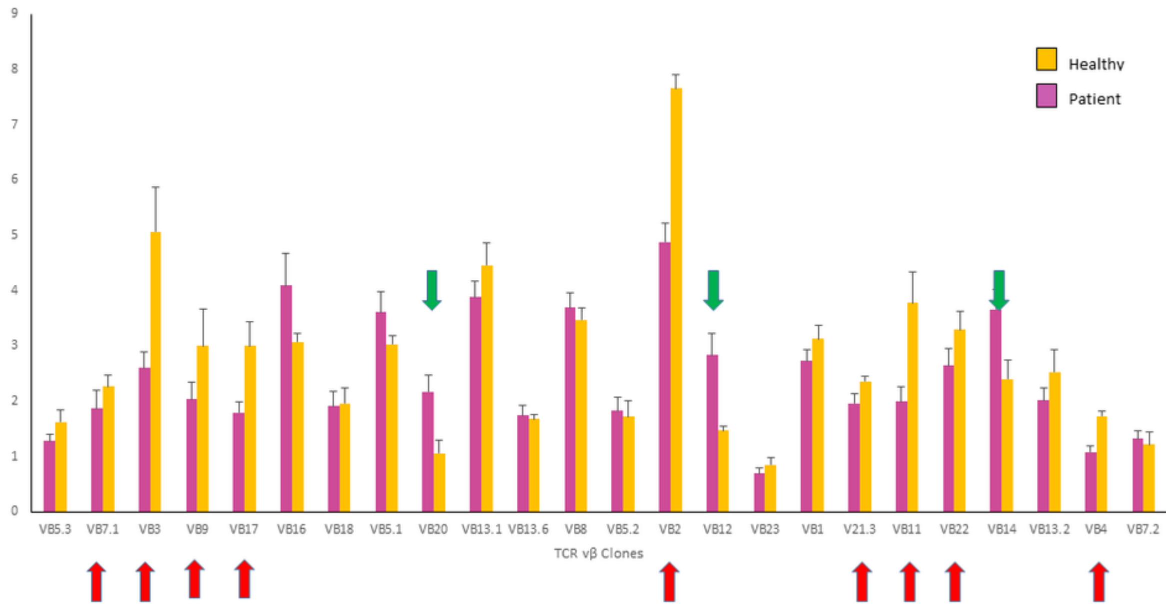

Fig2

Figure 2

TCR-Vβ Repertoire Comparing Between Healthy Controls and Patient Group. The red arrow shows statistically significantly diminished clones, and the green arrow indicates statistically significantly expanded ones.

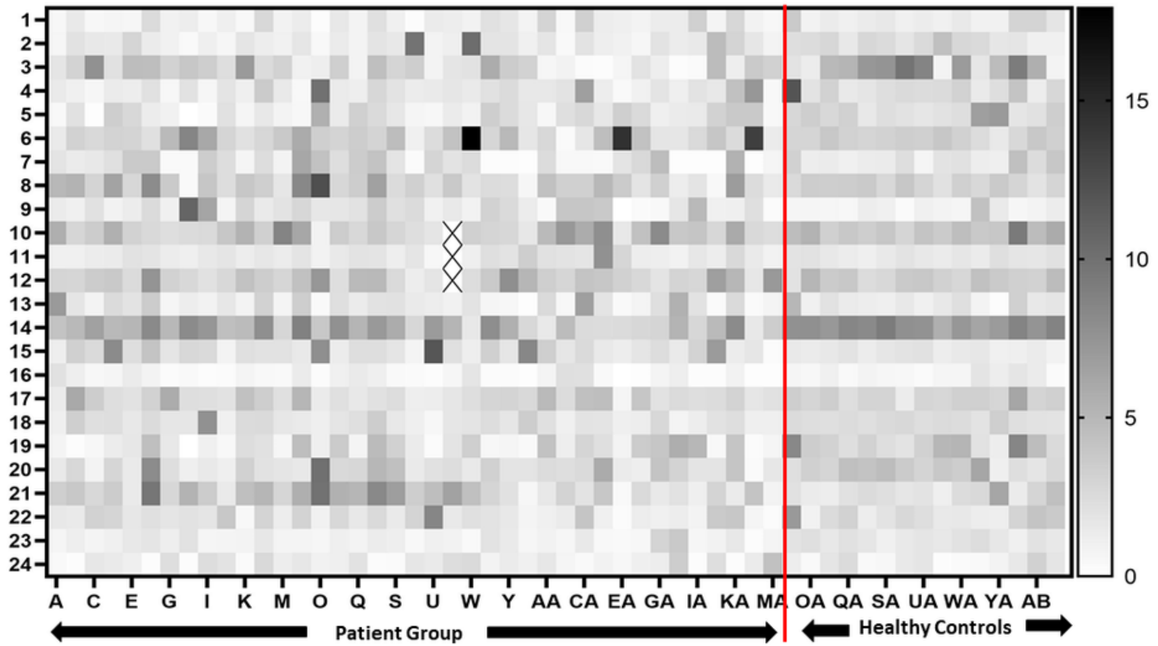

Fig3

Figure 3

The heat map (grayscale) graphic shows the diversity of TCRvβ repertoires from patient groups and healthy controls. Each rectangle indicates a specific TCRvβ clone, and the color of the rectangle corresponds to its frequency. Patient group (n=39), Healthy control (n=15).

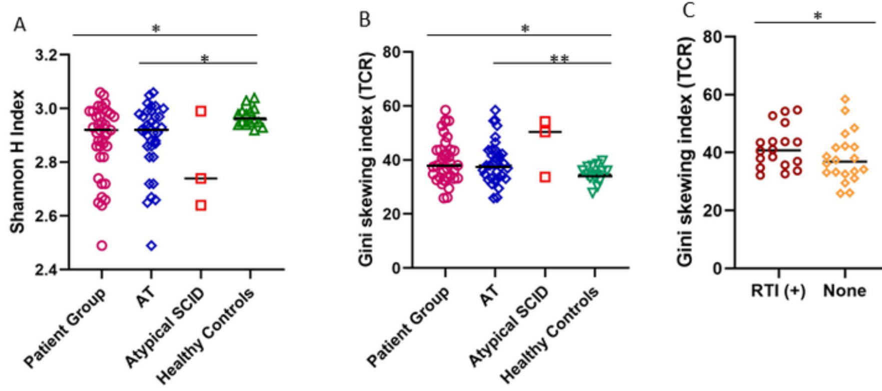

Fig4

Figure 4

TCR-vβ repertoire diversity correlates with the clinical phenotype. Scatter plot of Shannon's H diversity index (A), Gini skewing index of unevenness (B), Gini skewing index of unevenness in the patients with lower respiratory tract infections (RTI)(C), The asterisk indicates the significance tests between each group and healthy controls, and the asterisk above the line are p-values between the two groups in the ends of the line ($p \leq 0.05$ *, $p \leq 0.01$**)

Fig5

Figure 5

Oligoclonality of TCR repertoire correlates with some clinical phenotype. A Proportion of clones associated with different clinical phenotypes, including LRTI **(A1),** LRTI inside AT patients**,** the patients that consolidation, atelectasis, bronchiectasis, and nodules, were detected in thorax imaging (TFI+) **(B1),** AT patients with TFI or without TFI**(B2)** , bronchiectasis **(C)**, sinusitis and otitis media**(D1)**, otitis media inside AT **(D2),**autoimmunity **(E1),** AT patients with autoimmunity or without autoimmunity**(E2)** were visualized. LRTI: Lower respiratory tract infections, OM: otitis media, TFI: thorax findings at imaging. The asterisk above each group indicates the significance tests between each group is phenotype (+) or (-) patients ($p \leq 0.05$ *, $p \leq 0.01$**).

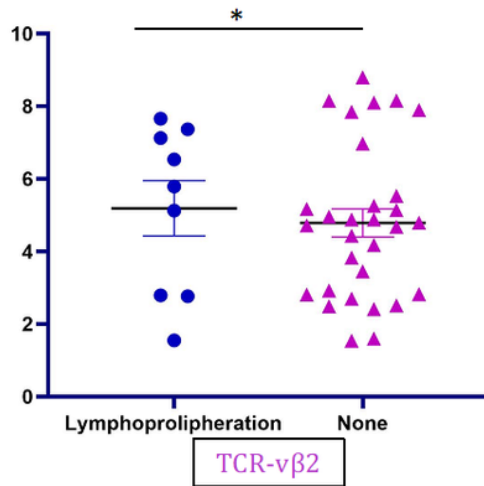

Fig6

Figure 6

Oligoclonality of TCR repertoire correlates with lymphoproliferation. The asterisk above each group indicates the significance tests between each group is phenotype (+) or (-) patients ($p \leq 0.05$ *)

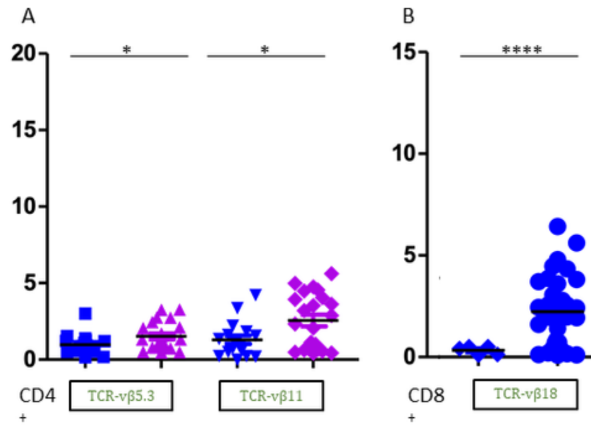

Fig7

Figure 7

TCR-vβ repertoire diversity correlates with the immunological phenotype. The scatter plot shows the abnormal distribution of specific TCR- vβ clones of the patients group with CD4+ (A) and CD8+ (B) lymphopenia compared to the normal lymphocyte group. Asterisk above each group indicate the significance of tests between the two groups ($p \leq 0.05$ *, $p \leq 0.0001$ ****).

Supplementary Files

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- SUPPLEMENTARYMATERIALTCRVB.docx