Running title: T- cell receptor defects

Advances in the genetics of TCR inborn errors of immunity

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Abstract

Inborn errors of immunity affecting the T-cell receptor (TCRIEI) comprise a rare and heterogeneous group of genetic disorders caused by germline mutations in genes encoding components of the TCR/CD3/CD247 complex isoforms. This complex is essential for proper T-cell development in the thymus as well as for antigen recognition, and intracellular signaling of mature T cells. TCR defects thus impair thymic selection and peripheral T-cell function, resulting in clinical phenotypes ranging from severe combined immunodeficiency (SCID) to milder forms with immune dysregulation or autoimmunity. Pathogenic variants have been identified in the *CD3E, CD3D, CD3G, CD247, TRAC* and *PTCRA* involving nonsense, frameshift, splice-site mutations and large deletions in more than 80 patients. These alterations cause complete or partial blocks in αβ and/or γδ T-cell development and are associated with marked clinical variability caused mostly by αβ T-cell numbers. Deficiencies in CD3ε, CD3δ and (less so) CD247 typically present early in life with profound T-cell lymphopenia and SCID, the latter with *CD247* somatic mutations. In contrast, TCRα, CD3γ and particularly preTα defects show later onsets and a broader clinical spectrum, including "leaky" immunodeficiency and autoimmunity. Diagnosis of TCRIEI relies on an integrated approach combining neonatal screening by T-cell receptor excision circles, immunophenotyping by flow cytometry, and genetic testing using next-generation, whole-exome, or whole-genome sequencing. Hematopoietic stem cell transplantation remains the main curative treatment for severe forms, with outcomes influenced by the affected gene, clinical severity, and age at diagnosis. Improved understanding of TCRIEI enhances early diagnosis, prognostic stratification, and personalized clinical management, and uncovers unexpected aspects of T-cell immunology.

1. TCR structure and function

T-cell receptor (TCR) is a crucial molecular complex found on the surface of T lymphocytes. Its primary functions are antigen recognition and signal transduction into the cell. TCR is composed of a single variable heterodimer formed by either the TCRα and TCRβ (TCRαβ) or the TCRγ and TCRδ chains (TCRγδ) together with two invariant

heterodimers constituted by CD3ε, CD3δ, CD3γ proteins (CD3εδ and CD3εγ) and a single invariant homodimer of CD247 (also known as ζ) proteins (Morath & Schamel, 2020). All these proteins are required for the correct assembly and exportation of the TCR on the cell surface. The TCRα, TCRβ, TCRγ, and TCRδ proteins present two main regions: a variable region, which contains the complementarity-determining regions (CDRs), and a constant region. However, these chains lack the necessary domains for signal transduction. This function is carried out by Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) located in the cytoplasmic tails of the CD3 and CD247 chains (Fig. 1) (Shah et al., 2021).

Most human mature T cells (95%) express TCRαβ. These cells play a fundamental role in adaptive immunity. Their functions include responding to inflammatory signals, detecting and eliminating pathogens via their Human Leukocyte Antigen (HLA)-restricted TCR, and signaling for assistance from other cells, such as recruiting non-resident leukocytes to fight infection (Marin et al., 2025). Before expression of the TCRαβ, developing thymocytes express a pre-TCR. This precursor complex consists of a TCRβ chain paired with an invariant preTα chain, associated with the CD3 molecules (Yang & Mariuzza, 2015). During a process known as β-selection, the pre-TCR promotes a burst of proliferation and differentiation into $CD4^{+}CD8^{+}$double positive (DP) thymocytes (Materna et al., 2024).

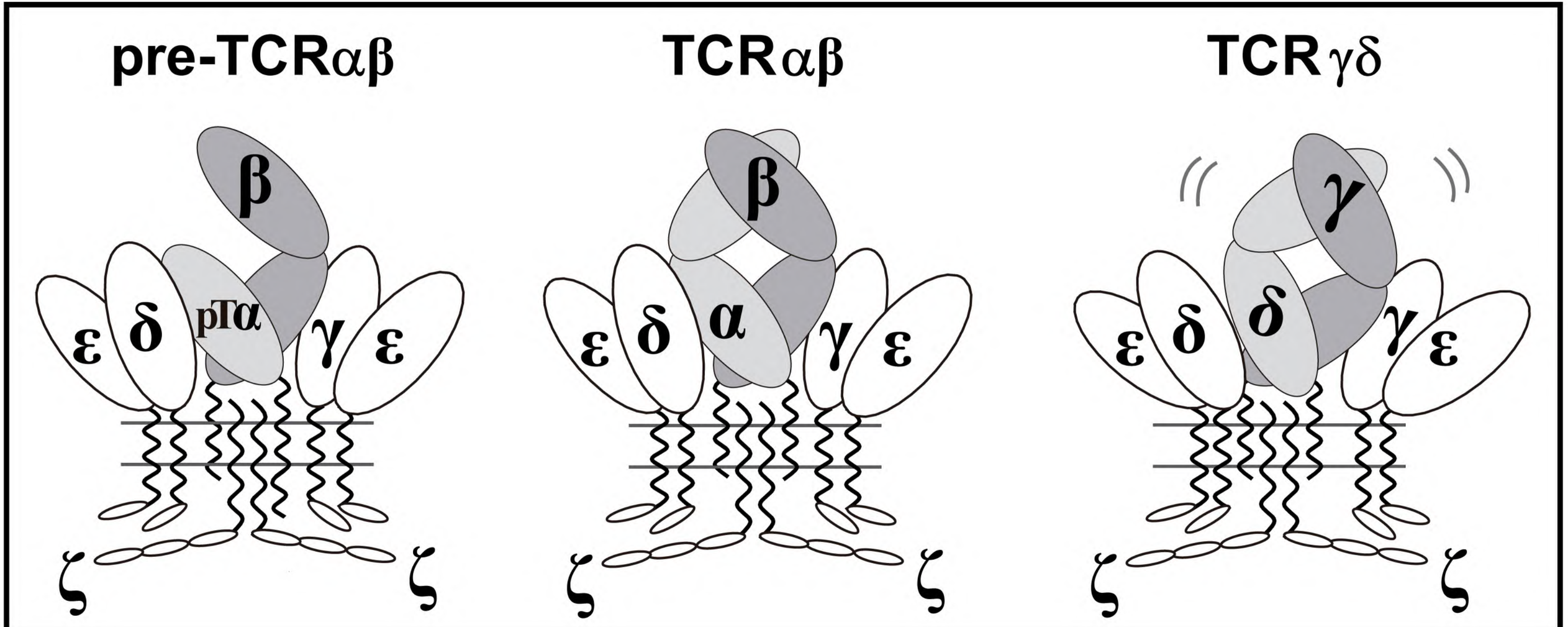
While most blood T cells express TCRαβ, a small percentage (5%) expresses the TCRγδ isotype. γδ T cells are also involved in defense against pathogens and provide a first line of defense in a tissue-specific fashion by innate and adaptive (TCR) surface receptors, although their real-life biological role is unclear to date (Marin et al., 2025). γδ T cells directly seed peripheral tissues and respond to diverse microbial stimuli independently of HLA-peptide recognition (Gray et al., 2024). Since the TCRαβ plays a fundamental role in adaptive immunity, any defect in its components can lead to TCR inborn errors of immunity (TCRIEI).

[Figure 1]

Figure 1. Human T-cell receptor (TCR) isotypes. Note: Used with permission from (Marin et al., 2025).

2. TCR genes and mutations: TCRIEI

TCRIEI are rare genetic disorders caused by defects in the expression of the TCR/CD3 complex, which impair thymocyte selection in the thymus and disrupt effector and T-cell function in the periphery (Rowe et al., 2018). These conditions manifest with peripheral T-cell lymphocytopenia, leading to increased susceptibility to infections and frequently progressing to severe combined immunodeficiency (SCID) and/or autoimmune manifestations (Garcillán et al., 2015). TCR diversity, generated through gene rearrangement and somatic recombination, is fundamental for adaptive immunity. Mutations in *CD3E, CD3D, CD247, TRAC*, *CD3G* and *PTCRA* can critically impair the assembly of the TCR and pre-TCR complexes. Therefore, comprehensive genetic and


pre-TCRαβ
TCRαβ
TCRγδ
β
pTα
ε
δ
γ
ε
ζ
ζ
β
α
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γ
ε
ζ
ζ
γ
δ
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δ
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ζ

functional evaluation of TCRIEI is essential for accurate diagnosis, prognosis, and targeted treatment.

[Figure 2]

TCRIEI shows clinical features at onset that vary depending on the affected subunit. Homozygous mutations in *CD3E* and *CD3D* block T-cell development at the ETP (double-negative to double-positive) thymocyte transition (Fig.2), leading to profound T-cell depletion, near-complete absence of $CD3^+$ lymphocytes and life-threatening susceptibility to infections early in life (Fischer et al., 2005).

CD3ε is crucial for correct T-cell development as it is part of the two invariant heterodimers that form the TCR complex (Fig. 1). The first CD3ε deficiency was identified in 1993 by Soudais et. due to homozygous c.IVS7+2T>C mutation in *CD3E* (Fig. 3) characterized by the absence of peripheral T cells despite normal B and NK cell counts. The affected two-year-old patient presented with recurrent severe viral respiratory infections, markedly reduced expression of the TCR on circulating lymphocytes and impaired T-cell functional responses.

[Figure 3]

Figure 3. Graphical representation of germline mutations causing TCRIEI and their predicted protein. LP, leader peptide; EC extracellular; TM, transmembrane; IC, intracellular; ITAM, Immunoreceptor Tyrosine-based Activation Motif. *: Mutations present only in another isoform: CD247 isoform 1. See Table 1 for clinical details.

[Table 1]

Table 1. Germline mutations of *CD3E, CD3D, CD247, TRAC, CD3G* and *PTCRA*.

In 2004 a study characterizing a complete CD3ε deficiency among consanguineous European families revealed that homozygous deletions (c.128_129del) induced an early frameshift and premature stop codon, resulting in severe SCID with blockade of peripheral T-cell development (de Saint Basile et al., 2004). Moreover, in 2017 next generation sequencing (NGS) was used to detect a novel homozygous frameshift deletion (c.173delT) in two affected siblings with CD3ε deficiency (Firtina et al., 2017). In 2020 a patient with a homozygous nonsense mutation (c.176G>A) in *CD3E* that caused a stop codon in the beginning of the extracellular domain was described. This patient had a complete lack of CD3+ T cells, suggesting that the mutation causes a complete absence of the CD3ε chain (Erman et al., 2020).

CD3δ deficiency is a rare autosomal recessive form of SCID characterized by profound T-cell lymphopenia (T−B+NK+) resulting from homozygous or compound heterozygous mutations in *CD3D*. In 2004, two *CD3D* mutations (c.279C>A, c.202C>T) were reported, both introducing a premature stop codon in the extracellular domain and causing early protein truncation, which similarly disrupted both αβ and γδ T-cell development while sparing B and NK cells (de Saint Basile et al., 2004). In 2011, two unrelated cases with a leaky splicing mutation in *CD3D* (c.IVS2+5G→A splice-site mutation) were reported. These patients were characterized by Tαβ-$T\gamma\delta^+B^+NK^+$ SCID phenotype, low CD3 expression, impaired proliferative responses to T-cell mitogens, severe lymph-node T-cell depletion indicating selective αβ T-cell arrest while preserving γδ T cells. Additional splice-site mutations as c.274+5G>A have been

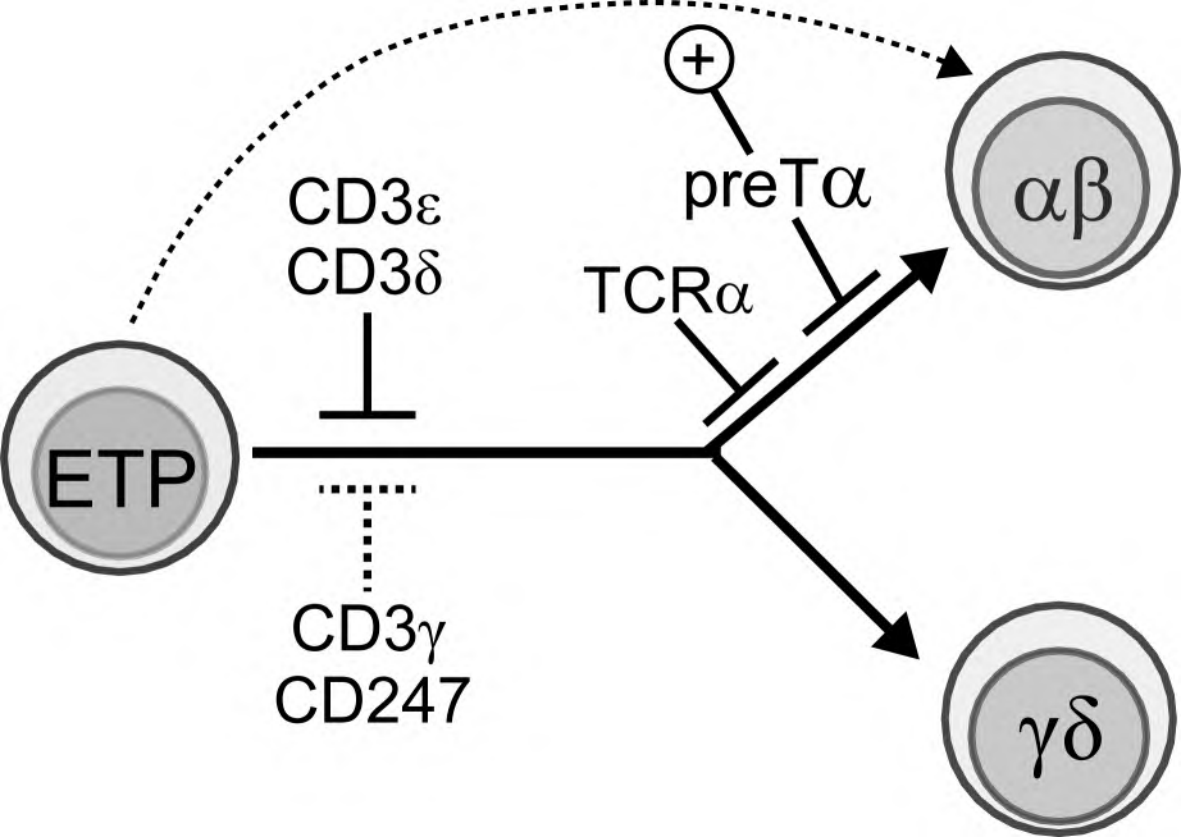

+
preTα
CD3ε
CD3δ
TCRα
αβ
ETP
CD3γ
CD247
γδ

FIGURE LEGENDS

Figure 1. Human T-cell receptor (TCR) isotypes. Note: Used with permission from (Marin et al., 2025).

Figure 2. TCRIEI causes complete (solid T`s) or partial (dashed T) impairments of human αβ and/or γδ T-cell development. Isolated greek letters stands for CD3, CD247(ζ) or TCRα TCRIEI. + indicates non-canonical biased differentiation (Materna et al., 2024). ETP: Early Thymic Progenitor. Adapted from (Marin et al., 2025).

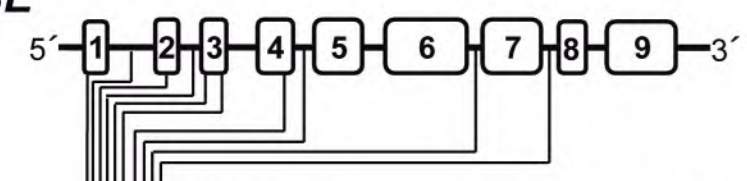
Figure 3. Graphical representation of germline mutations causing TCRIEI and their predicted protein. LP, leader peptide; EC extracellular; TM, transmembrane; IC, intracellular; ITAM, Immunoreceptor Tyrosine-based Activation Motif. See Table 1 for clinical details.

Figure 4. Graphical Representation of *CD247* somatic mutations. LP, leader peptide; EC extracellular; TM, transmembrane; IC, intracellular; ITAM, Immunoreceptor Tyrosine-based Activation Motif.

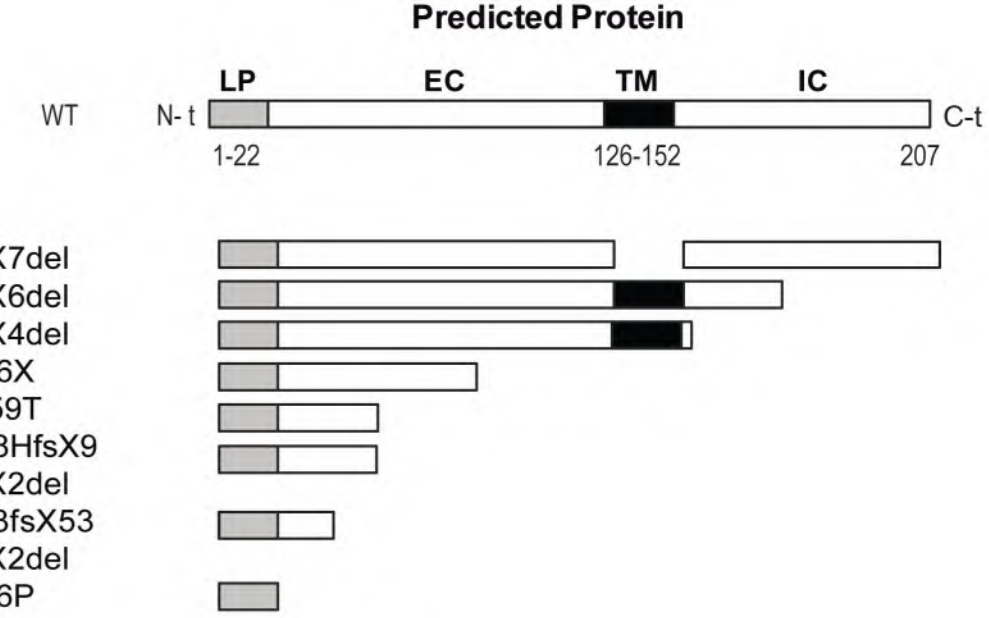
Figure 5. Prenatal (left) or postnatal (right) diagnosis strategy for TCRIEI. TRECS, T-cell receptor excision circles; WB, western blot; FC, flow cytometry.

Figure 6. Age at diagnosis (years) for reported TCRIEI patients, ordered by clinical severity (Table 3). Red dots denote patients who have undergone hematopoietic stem cell transplantation (HSCT) and empty dots indicate exitus. One-way Anova Kruskal-Wallis test was performed (**** = $p < 0.0001$). Exitus and HSCT numbers and % are indicated below each gene.

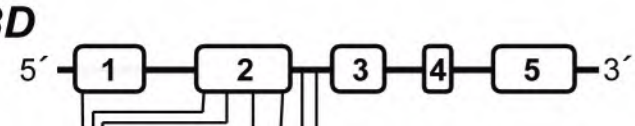
CD3E



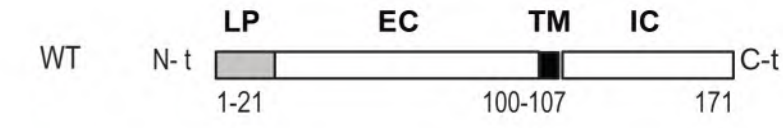
c.IVS7+2T>C	Exon 7 skipping	p. EX7del
c.567+42_567+73del	Exon 6 skipping	p. EX6del
c.352+1G>A	Exon 4 skipping	p. EX4del
c.288T>A	Frameshift	p.Y96X
c.176G>A	Stop codon	p.W59T
c.173delT	Frameshift	p.L58HfsX9
c.IVS2+1G>C (c.128+1G>C)	Exon 2 skipping	p. EX2del
c.128_129del	Frameshift	p.T43fsX53
c.104-1G>A	Exon 2 skipping	p. EX2del
c.46T>C	LP mutation	p.S16P



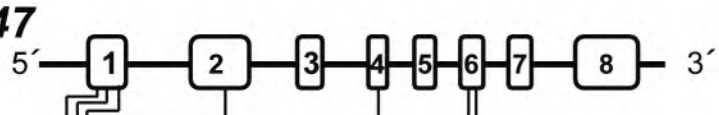
CD3D



c.IVS2-2A>G	Exon 3 skipping	p.EX3del
c.IVS2+5G>A (leaky)	Exon 2 skipping	p.EX2del
c.279C>A	Stop codon	p.C93X
c.247G>T	Stop codon	p.G83T
c.202C>T	Stop codon	p.R68X
c.158C>A	Stop codon	p.C53X
c.56-1G>T	Frameshift	p.S19fsX

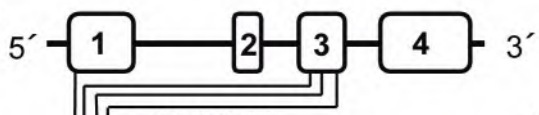


CD247

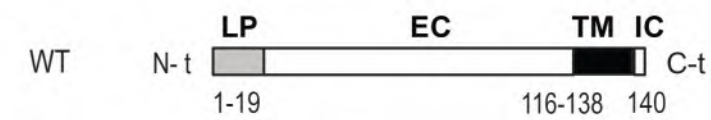


c.456C>A	Stop codon	p.Y152X
c.411insC	Frameshift	p.D138fsX272
c.301C>T	Stop codon	p.Q101X*
c.208C>T	Stop codon	p.Q70X
c.62C>T	LP mutation	p.A21V
c.43_44delCA	Frameshift	p.Q15Vfs
c.2T>C	Loss of start codon	p.M1T

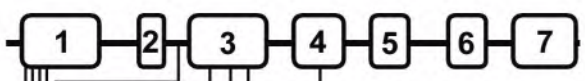
TRAC



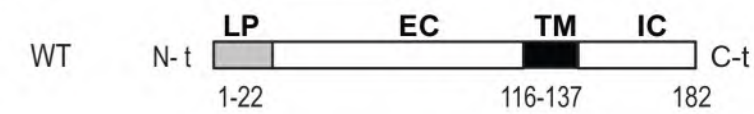
c.361C>T	Stop codon	p.R121X
c.347C>G	Frameshift	p.S116X
c.*1G>A	Exon 3 skipping	p.T107LfsX56
c.192_205del	Frameshift	p.W65X



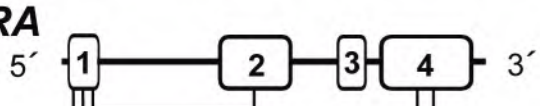
CD3G



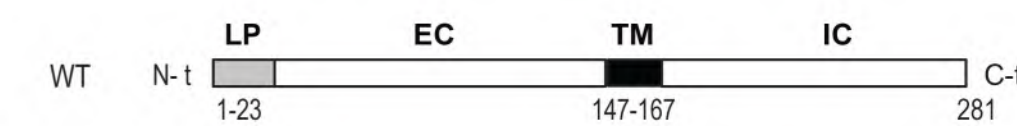
c.390_391delTGinsCT	Indel in-frame	p.V131L
c.213delA	Frameshift	p.R71EfsX42
c.205A>T	Stop codon	p.K69T
c.242A>T	Missense	p.D81V
IVS2-1G>C; c.1A>G	New splice site	p.N28V;H29X
c.80_96del17	Frameshift	p.N28VfsX20
c.80-1G>C /IVS2-1G>C	Exon 1 skipping	p.EX1del
c.18delC	Frameshift	p.L7fsX44
c.1A>G	Loss of start codon	p.M1V



PTCRA



c.446G>A	Stop codon	p.W149X
c.446G>A;c.429delA	Framehift	p.P144RfsX24
c.296_299del	Frameshift	p.L99HfsX68
c.58+5G>A	New splice site	p.G20R
c.58G>C; Δex1-3	Exon 1-3 skipping	Δex1-3
c.42T>A	Stop codon	p.C14X



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730 TABLES

731 Table 1

LOCUS	Sequence variation	Clinical Significance (Clinvar)	nº of cases	Age range at testing (months or years)	Onset (age at years old)	HSCT	Exitus	Reference
CD3E	c.IVS7+2T>C	No data	1		5	0/1	0/1	(Soudais et al., 1993)
	c.567+42_567+73del	No data	1	5m	<1	1/1	1/1	(Sonmez et al., 2025)
	c.352+1G>A	No data	1	7m				(Vignesh et al., 2020)
	c.288T>A	Pathogenic	1	8m				
	c.176G>A	Pathogenic	1	4m	<1	1/1	0/1	(Erman et al., 2020)
	c.173delT	Pathogenic	7	0-1y	<1	1/7	1/7	(Sonmez et al., 2025) (Platt et al., 2021);(Firtina et al., 2017)
	c.IVS2+1G>C	No data	1	2m	<1	1/1		(Fuehrer et al., 2014)
	c.128_129del	Pathogenic	2	1m	<1	1/2	2/2	(de Saint Basile et al., 2004)
	c.104-1G>A	No data	1	0m				(Platt et al., 2021)
	c.46T>C	No data	1	3m	<1		1/1	(Setia et al., 2021)
CD3D	c. IVS2-2A>G	No data	3	0-6m	<1	2/3	1/3	(Vignesh et al., 2020) (Takada et al., 2005)
	c.IVS2+5G>A (leaky)	No data	2	6-13m	<1-1	2/2	1/2	(Gil et al., 2011) (Garcillán et al., 2014)
	c.279C>A	Pathogenic	2	0-5m	<1	1/2	2/2	(de Saint Basile et al., 2004)
	c.247G>T	No data	1	0m				(Platt et al., 2021)
	c.202C>T	Pathogenic	6	0-1y	<0-1	2/6	5/6	(Alsalamah et al., 2015) (de Saint Basile et al., 2004) (Dadi et al., 2003)
	c.158C>A	No data	1	5m				(Vignesh et al., 2020)
	c.56-1G>T	No data	1	1y	1	1/1	0/1	(Sonmez et al.,

								2025)
CD247	c.456C>A	No data	1	8 y	3			(Briones et al., 2024)
	c. 411insC	No data	1	10 m	<1	1/1	0/1	(Roberts et al., 2006)
	c.301C>T	No data	2	1 m -50 y	<1			(Briones et al., 2024)
	c.208C>T	No data	1	10 m	<1	1/1	0/1	(Rieux-Laucat et al., 2006)
	c.62C>T	No data	1	2y	2		1/1	(Setia et al., 2021)
	c.43_44delCA	No data	1	22 m	<1	0/1	0/1	(Kaiser et al., 2021)
	c.2T>C	Pathogenic	1	11 m	<1	1/1	1/1	(Marin et al., 2017)
TRAC	c.361C>T	No data	1	<1m			1/1	(Materna et al., 2025)
	c.347C>G	No data	1	1m	<1			(Garkaby et al., 2022)
	c.*1G>A	No data	5	3m-9 y	≤ 1	2/5	3/5	(Rawat et al., 2021) (Morgan et al., 2011)
	c.192_205del	No data	1	<1m		1/1	0/1	(Materna et al., 2025)
CD3G	c.390_391delTGinsCT	No data	1	1y	1	1/1		(Sonmez et al., 2025)
	c.213delA	Pathogenic	2	9-15y	4-15			(Lin et al., 2024) (Lee et al., 2019)
	c.205A>T	Pathogenic	3	1-4y	<1-1	0/3	1/3	(Sonmez et al., 2025) (Recio et al., 2007) (Sanal et al., 1996)
	c.242A>T	No data	1	4 y	<1			(Gokturk et al., 2014)
	c.IVS2-1G>C; c.1A>G	Pathogenic	2	3-24y	<1-1	0/2	1/2	(Arnaiz-Villena et al., 1992) (Recio et al., 2007)
	c.80_96del17	No data	1		<1		1/1	(Arnaiz-Villena et al., 1992)
	c.80-1G>C	Pathogenic	4	6-23y	1-10			(Rowe et al., 2018)
	c.IVS2-1G>C	Pathogenic	7	1-24y	<1-20	1/7	0/7	(Gokturk et al., 2014) (Tokgoz et al., 2013)
	c.18delC	No data	1	3y				(Platt et al., 2021)

	c.1A>G	Pathogenic	1	1y	<1	1/1	1/1	(Delmonte et al., 2021)
PTCRA	c.446G>A	Pathogenic	1	31y	<20			(Materna et al., 2024)
	c.446G>A; c.429delA	No data	2	65-66y	25-65			
	c.296_299del	Pathogenic	3	8-19y	8-15		1/3	
	c.58+5G>A	No data	1	2y	<1			
	c.58G>C; Δex1-3	No data	2	4-7y	<1			
	c.42T>A	No data	1	2y	<1			

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733 Table 2

LOCUS	**Sequence variation**	**Clinical Significance**	**nº of cases**	**Age range at testing (months)**	**Reference**
***CD247* (Somatic)**	c.209A>T	Pathogenic	1	10	Rieux-Laucat et al., 2006
	c.208_209inv	Pathogenic	1	10	
	c.208_210delinsTAT	Pathogenic	1	10	
	c.2T>C>T	No data	1	11	Marín et al., 2017
	c.-8A>T	No data	1	11	

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735 Table 3

Affected gene	**Phenotype range**	**Age of onset range**	**Clinical features**
CD3E		Neonatal	Recurrent severe infections (respiratory, otitis media, rhinosinusitis, candidiasis), enteropathy, failure to thrive, abnormal thymic development, and autoimmunity.
CD3D		Neonatal	Recurrent severe infections (respiratory, otitis media, rhinosinusitis, candidiasis), enteropathy, failure to thrive, abnormal thymic development, and autoimmunity.
CD247	CID to SCID	Neonatal / childhood	Recurrent severe infections (respiratory, otitis media, rhinosinusitis, candidiasis), eosinophilia, and eczema.
TRAC	Mild ID to SCID	Neonatal / adolescenc	Recurrent infections (respiratory, otitis media, rhinosinusitis, candidiasis),

documented in other populations. Patients typically present life with severe opportunistic infections and failure to thrive within the first 6 months of. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the definitive curative approach for these deficiencies (Gil et al., 2011).

CD247 is essential for TCR assembly and signaling though its three ITAM domains (Dexiu et al., 2022). To date, eight patients have been diagnosed with SCID caused by homozygous null mutations in *CD247*. Germline mutations identified englobed nonsense (c.301C>T, c.456C>A, c.208C>T, c.62C>T) and frameshifts (c.411insC, c.43_44delCA) that truncate essential ITAM domains, as well as start codon mutations (c.2T>C) that prevent translation, being c.301C>T the most documented variant, found both in homozygosis (causing SCID) and heterozygosis (with dominant-negative effects and reduced surface CD3 expression) (Fig. 4). As an example, a patient with homozygous *CD247* Q70X mutation showed profound T-cell dysfunction, although some T cells acquired somatic mutations allowing partial TCR expression (Rieux-Laucat et al., 2006). Even heterozygous mutations show dominant-negative effects, leading to a reduction in surface CD3 expression (Briones et al., 2024).

[Figure 4]

Figure 4. Graphical Representation of *CD247* somatic mutations. LP, leader peptide; EC extracellular; TM, transmembrane; IC, intracellular; ITAM, Immunoreceptor Tyrosine-based Activation Motif.

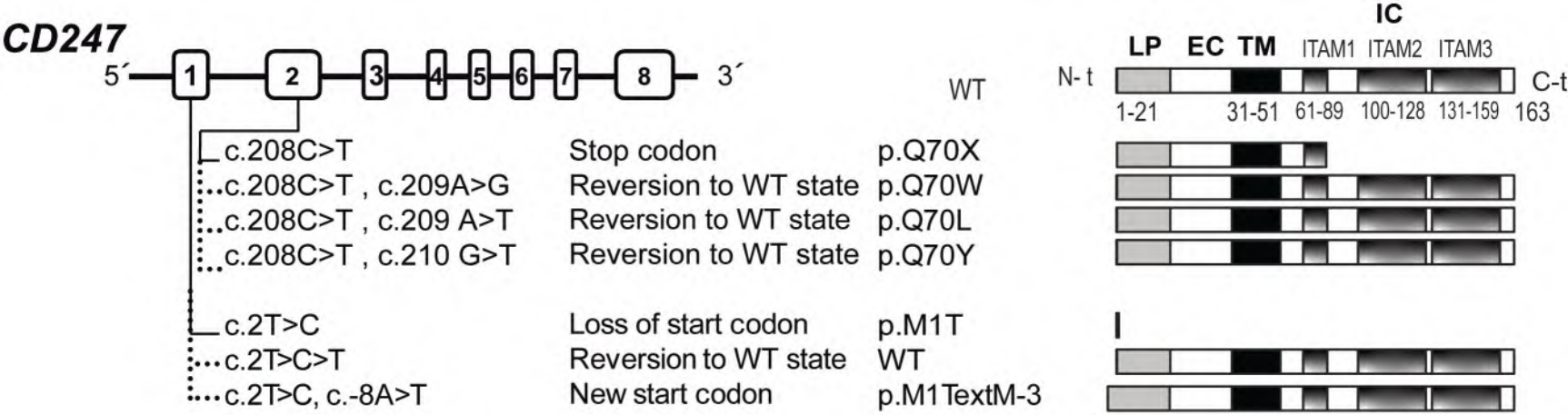
Concurrently, multiple compensatory somatic mutations (c.209A>T, c.208_209inv, c.208_210delinsTAT, c.2T>C>T, c.-8A>T) were identified that restore the reading frame or function as stop codon suppressors, enabling partial or complete TCR expression in the mutated T cells' progeny. These somatic reversions demonstrate remarkable but clinically limited cellular plasticity and ultimately require HSCT for definitive immune reconstitution (Table 2).

[Table 2]

Table 2. Somatic mutations of the *CD247*.

According to the NCBI Reference Sequence database, the canonical form of *CD247* encodes an isoform composed of 163 amino acids (NP_000725.1). All mutations described in this study were located within this canonical isoform, except for the c.301C>T (p.Q101X) variant that occurs in an alternative *CD247* transcript differing in its coding length (164 amino acids) and thereby affecting a distinct isoform of the CD247 chain. Notably, this alternative isoform diverges from the canonical sequence at position 101, where an additional glutamine (Q) residue is inserted.

TCRα deficiency represents a rare autosomal recessive IEI caused by mutations in *TRAC*, leading to absent TCRαβ expression and combined immunodeficiency that debuts in infancy or childhood with recurrent viral and bacterial infections, immune dysregulation, hepatosplenomegaly, lymphadenopathy and autoimmune features (Morgan et al., 2011; Garkaby et al., 2022). In 2011, the first cases of TCRα chain deficiency were reported. These patients involved two consanguineous families with a


CD247
5´
1
2
3
4
5
6
7
8
3´
WT
c.208C>T
Stop codon
p.Q70X
c.208C>T , c.209A>G
Reversion to WT state
p.Q70W
c.208C>T , c.209 A>T
Reversion to WT state
p.Q70L
c.208C>T , c.210 G>T
Reversion to WT state
p.Q70Y
c.2T>C
Loss of start codon
p.M1T
c.2T>C>T
Reversion to WT state
WT
c.2T>C, c.-8A>T
New start codon
p.M1TextM-3
IC
LP
EC
TM
ITAM1
ITAM2
ITAM3
N-t
C-t
1-21
31-51
61-89
100-128
131-159
163

LOCUS	Sequence variation	Clinical Significance	nº of cases	Age range at testing (months)	Reference
CD247 **(Somatic)**	c.209A>T	Pathogenic	1	10	Rieux-Laucat et al., 2006
	c.208_209inv	Pathogenic	1	10	
	c.208_210delinsTAT	Pathogenic	1	10	
	c.2T>C>T	No data	1	11	Marín et al., 2017
	c.-8A>T	No data	1	11	

homozygous c.*1G>A mutation in exon 3, causing impaired mRNA splicing and loss of the transmembrane and cytoplasmic domains of the TCRα chain. This mutation prevents surface expression of the TCRαβ complex, resulting in the absence of TCRαβ$^+$ T cells, increased susceptibility to infection and autoimmunity (Morgan et al., 2011). The most severe form was a SCID patient with profound T cells lymphopenia and absent TCRαβ$^+$ T cells due to a nonsense homozygous c.347C>G mutation in exon 3 of *TRAC*, which introduces a premature stop codon and abolishes functional TCRα chain expression, impairing TCRαβ expression and T-cell development (T$^-$B$^+$NK) (Garkaby et al., 2022). Three siblings from a non-consanguineous family with a previously reported *TRAC* variant who developed combined immunodeficiency, where the youngest developed non-Hodgkin lymphoma in infancy, were described (Rawat et al., 2021). To date, most patients identified with TCRα chain deficiency carry the same c.*1G>A mutation, but recently two patients were described with a SCID-like clinical phenotype, showing normal NK and B lymphocytes with a predominance of γδ T cells and a complete absence of TCRαβ$^+$ T cells, due to their mutations. One patient carried the c.192_205del mutation and the other the c.361C>T mutation, both introduce a premature stop codon and result in a complete loss of TCRα chain protein expression (Materna et al., 2025).

In contrast, *CD3G* mutations cause milder phenotypes with partial T-cell immunodeficiency primarily characterized by autoimmunity rather than severe immunodeficiency. Patients with CD3γ defects exhibit reduced regulatory T-cell diversity, increased clonality, and diminished suppressive function which increases self-reactivity and ultimately leads to a milder, autoimmune phenotype (Rowe et al., 2018). To date, ten patients have been reported with *CD3G* mutations leading to immunodeficiency. Five patients from two families were reported with homozygous *CD3G* mutations (c.80-1G>C), presenting with T-B+NK+ immunodeficiency and prominent autoimmune features including thyroiditis, hemolytic anemia, and hepatitis. Notably, heterozygous carriers also frequently developed autoimmunity (67%), suggesting *CD3G* as a candidate gene for autoimmune disorders (Gokturk et al., 2014).

The pre-TCR, formed by TCRβ and preTα chains associated with CD3 and CD247 dimers (Fig. 1), is essential for rescuing thymocytes from programmed cell death and enabling their maturation into αβ T cells (von Boehmer & Fehling, 1997). *PTCRA* mutations lead to a rare immunodeficiency characterized by disrupted pre-TCR signaling during early αβ T-cell development. Biallelic loss-of-function variants (including c.296_299del mutation) result in markedly reduced naive αβ T-cell populations from birth, with variable preservation of memory αβ T cells and preserved or increased γδ T-cell counts. Consequently, most patients remain clinically healthy into adulthood, despite variable susceptibility to infection (Materna et al., 2024). In contrast, hypomorphic variants, which are more prevalent in certain populations, are predominantly associated with autoimmune manifestations rather than increased infection susceptibility. Unexpectedly, patients with pre-Tα deficiency show normal to low levels of functional αβ T cells, probably due to increased non-canonical differentiation from ETP. A cohort of ten patients carrying rare biallelic loss-of-

function *PTCRA* variants was analyzed (Materna et al., 2024). Despite having small thymuses and low circulating naive αβ T-cell counts, these patients exhibited normal αβ memory T-cell numbers confirming that pre-Tα is not strictly essential for αβ T-cell development in humans. The authors also identified two common homozygous hypomorphic *PTCRA* variants (the same common partial mutation in both alleles, less severe, more frequent, and associated with mild or late autoimmunity): p.Asp51Ala (found in ~1/4000 individuals from the Middle East and South Asia) and p.Tyr76Cys (found in ~1/73,000 individuals from Africa). Homozygous *PTCRA* variants leading to partial pre-Tα deficiency showed high circulating naive γδ T-cell counts and higher risk of autoimmunity (Materna et al., 2024).

3. TCRIEI diagnosis

The diagnosis of TCRIEI patients will depend on the local diagnosis services available at each hospital, as summarized in Fig. 5. Guthrie or "Heel Prick" test is routinely performed on neonatal blood samples (typically at 48–72 hours of age) to screen for a variety of known genetic disorders. This often includes quantification of T-cell excision cycles (TRECS), which may appear reduced due to impaired T-cell production. Subsequently, clinical exome sequencing can provide insights into potentially pathogenic variants in genes involved in the immune system. Homozygous variants in genes encoding for TCR components should be further evaluated if in silico mutation prediction suggests pathogenicity. Alternatively, some infants who did not undergo Guthrie test may present immunodeficiency symptoms. In such cases, clinical exome sequencing is recommended if feasible. If the symptoms and clinical signs are compatible with TCRIEI, TCR analysis by flow cytometry and/or western blot can help to identify the molecular defect. As noted, mutations in TCR components can also be found in adults presenting autoimmunity or immunodeficiency, for which clinical exome sequencing or targeted sequencing of *TRAC* and *PTCRA* can support a definitive diagnosis.

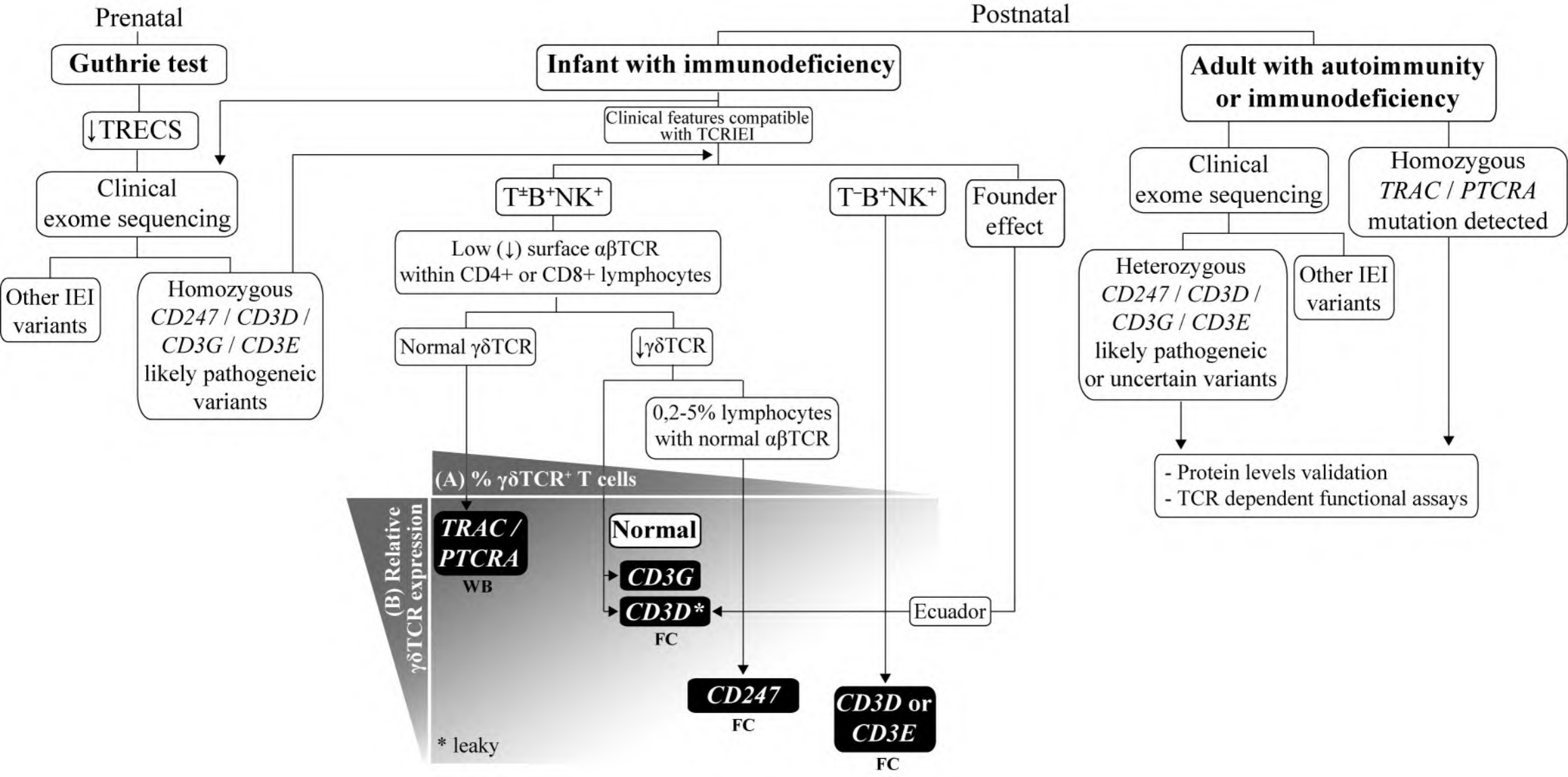
[Figure 5]

Figure 5. Prenatal (left) or postnatal (right) diagnosis strategy for TCRIEI. TRECS, T-cell receptor excision circles; WB, western blot; FC, flow cytometry.

3.1 Clinical management

The Expert Committee of the *International Union of Immunological Societies* (IUIS), in its classification of IEI, includes each of the described disorders affecting the genes involved on the TCR complex (*CD3D, CD3E, CD247, CD3G, TRAC,* and *PTCRA*) and categorizes most of them as combined (impairing both cellular and humoral) immunodeficiencies. The sole exceptions are loss-of-function (LOF) and hypomorphic variants in *PTCRA*, which have been sorted among combined immunodeficiencies with syndromic features as published in the latest of the IUIS classification (Poli et al., 2025), and *CD3G* variants, which are categorized as combined immunodeficiencies less profound than classic SCID forms.

Since the first report of CD3 defect as a cause of immunodeficiency (Regueiro et al., 1986), more than 150 pathogenic variants have been described in these genes. These variants consist of single nucleotide variants (SNVs; missense, nonsense, and splice site mutations), copy number variants (CNVs; deletions and duplications), and large-scale rearrangements (translocations and insertions), all of them having an impact on gene function (Marin et al., 2015). Defects in T-cell development usually result in SCID phenotype. However, pathogenic variants of the encoding gene for the TCRα constant subunit (*TRAC*) give rise to a wide clinical spectrum, ranging from SCID to milder immunodeficiencies associated to immune dysregulation and/or hematological malignancies or autoimmune manifestations (e.g., hemolytic anemia, eczema,


Prenatal
Postnatal
Guthrie test
↓TRECS
Clinical exome sequencing
Other IEI variants
Homozygous CD247 / CD3D / CD3G / CD3E likely pathogeneic variants
Infant with immunodeficiency
Clinical features compatible with TCRIEI
T±B+NK+
Low (↓) surface αβTCR within CD4+ or CD8+ lymphocytes
Normal γδTCR
↓γδTCR
0,2-5% lymphocytes with normal αβTCR
T−B+NK+
Founder effect
Ecuador
Adult with autoimmunity or immunodeficiency
Clinical exome sequencing
Homozygous TRAC / PTCRA mutation detected
Heterozygous CD247 / CD3D / CD3G / CD3E likely pathogeneic or uncertain variants
Other IEI variants
- Protein levels validation
- TCR dependent functional assays
(A) % γδTCR+ T cells
(B) Relative γδTCR expression
TRAC / PTCRA
WB
Normal
CD3G
CD3D*
FC
CD247
FC
CD3D or CD3E
FC
* leaky

hypereosinophilia, vitiligo, alopecia areata), reflecting defective positive and negative selection (Morgan et al., 2011; Rawat et al., 2021). Patients with hypomorphic variants may exhibit longer-term survival than featured in standard SCID cases due to the expansion of $CD3^{lo}TCR\alpha\beta^{lo}$ lymphocyte subset and an increased proportion of γδ T cells (Materna et al., 2025).

Lately, homozygous loss-of-function variants of pre-TCRα gene (*PTCRA*) have been shown to disrupt thymic differentiation, leading to low circulating naïve TCRαβ T cells during childhood or adolescence, mild immunodeficiency, autoimmunity, and a higher rate of lymphoproliferative disorders. Susceptibility to infectious diseases often improves in adulthood, being simultaneous with the accumulation of TCRαβ memory T cells with skewed TCRαβ repertoires. This finding suggests a non-canonical thymic differentiation pathway that partially corrects this defect (Materna et al., 2024).

Patients with deficiencies in the CD3ε, CD3δ, and CD247 subunits typically show lower circulating T-cell counts and early-onset combined or severe combined immunodeficiency, defined by recurrent respiratory infections, otitis, enteropathy, failure to thrive, abnormal thymic development, and autoimmunity (Dadi et al., 2003; de Saint Basile et al., 2004; Soudais et al., 1993; Tokgoz et al., 2013). As shown in TCRα defects, eosinophilia and eczema have also been reported in CD247 deficiency (Rieux-Laucat et al., 2006).

Genetic disturbances in CD3γ exhibit a highly variable clinical picture, ranging from lethal SCID, associating enteropathy and autoimmune anemia, to asymptomatic individuals with normal lymphocyte counts but diminished TCR expression (Arnaiz-Villena et al., 1992; Recio et al., 2007). The relatively mild course observed in some patients may be partially explained by the formation of TCR complexes with atypical stoichiometry with the CD3δ subunit partly compensating for the absence of CD3γ (Zapata et al., 2004). A summary of the clinical features shown by all of these disorders in TCR genes is included in Table 3.

[Table 3]

Table 3. Summary of clinical spectrum and age of onset of the different IEI related to genes of the TCR complex, ordered from greatest to milder severity.

The prognosis of survival for those patients who develop a SCID phenotype is usually poor. Although stem cell gene edition therapy is becoming an increasingly important alternative for some SCID causing IEI, there are no data in TCRIEI. Thus HSCT is the main option for these patients. The age at diagnosis, HSCT and exitus of TCRIEI patients are shown in Fig. 6. There are no specific recommendations for HSCT in TCRIEI, mainly due to its low incidence. Despite this, the Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation and the European Society for Immune Deficiencies (EBMT/ESID) published a common HSCT guideline for IEI that provides some recommendations for *CD3D*, *CD3E* and *CD247* (Lankester et al., 2021).

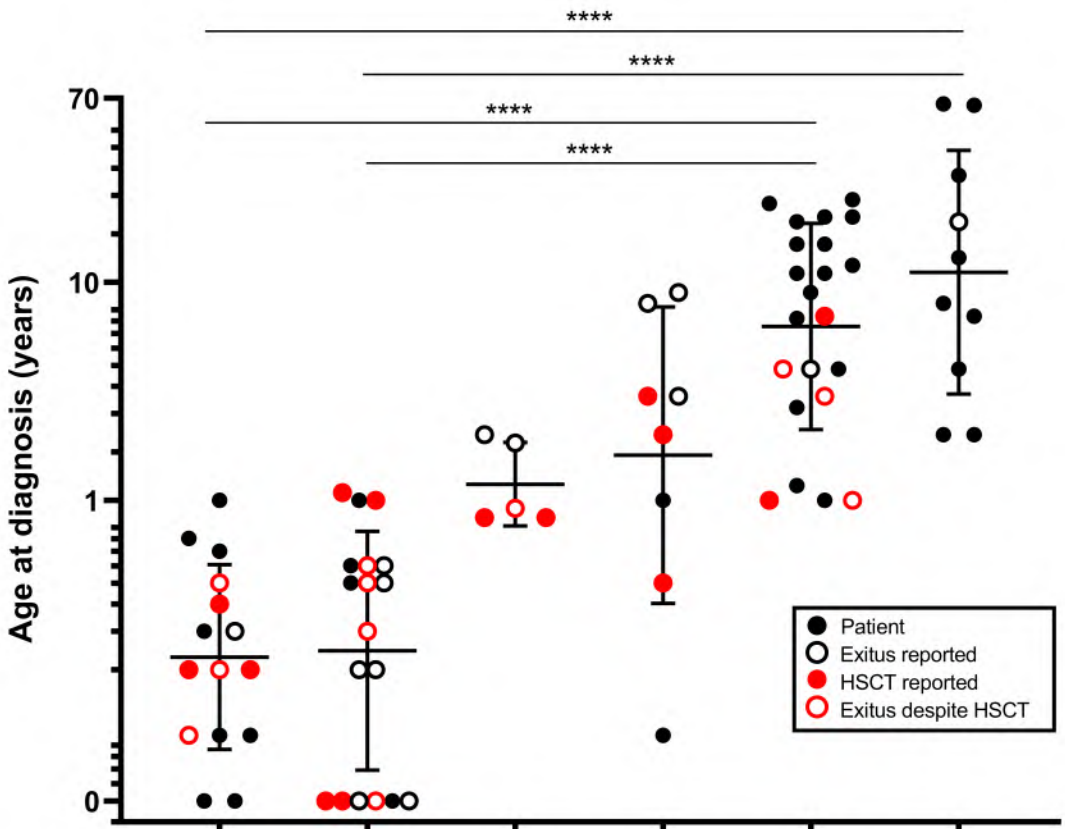
HLA identical siblings are the first donor choice in most cases, but the improvement in HLA typing and HSCT complications have brought new donor options with non-related and mismatched donors (Shah et al., 2018). HSCT for SCID can be performed without any conditioning regimen, but it facilitates donor engraftment and allows a better T and B reconstitution (Haddad & Hoenig, 2019). For matched unrelated donors, mismatched unrelated donors or mismatched family donors, EBMT/ESID suggests Busulfan i.v. (AUC = 60–70 mg*h/L) and Fludarabine (160–180 mg/m^2) or Treosulfan (30–42 g/m^2) and Fludarabine (150–160 mg/m^2). Same regimens are recommended for matched sibling donors and matched related donors, but no conditioning is also considered an option (Lankester et al., 2021).

The outcome of HSCT is directly related to infections as active infection at conditioning has been associated with poor survival (Haddad & Hoenig, 2019), autoimmunity, specific organ damage and age at transplant. Establishing recommendations for HSCT in adolescent or adult patients or in cases with CID phenotype can be challenging. The latency in the diagnosis and the evolution of the IEI can lead to comorbidities such as severe autoimmune conditions or organ damage. Considering the post-HSCT survival data in TCRIEI such as CD3γ deficiency, these situations could explain how, in theoretically less severe conditions, the success after HSCT might nonetheless be more limited. Thus, it is essential to achieve the earliest diagnosis, closely to monitor complications, and always individualize the indication and timing for transplantation (Greco et al., 2025).

[Figure 6]

Figure 6. Age at diagnosis (years) for reported TCRIEI patients, ordered by clinical severity. Black dots denote individual patients; Red dots denote patients who have undergone hematopoietic stem cell transplantation (HSCT) and empty dots indicate exitus. One-way Anova Kruskal-Wallis test was performed (**** = $p < 0.0001$). Exitus and HSCT numbers and % are indicated below each gene.

Affected gene	Phenotype range	Age of onset range	Clinical features
CD3E	SCID	Neonatal	Recurrent severe infections (respiratory, otitis media, rhinosinusitis, candidiasis), enteropathy, failure to thrive, abnormal thymic development, and autoimmunity.
CD3D	SCID	Neonatal	Recurrent severe infections (respiratory, otitis media, rhinosinusitis, candidiasis), enteropathy, failure to thrive, abnormal thymic development, and autoimmunity.
CD247	CID to SCID	Neonatal / childhood	Recurrent severe infections (respiratory, otitis media, rhinosinusitis, candidiasis), eosinophilia, and eczema.
TRAC	Mild ID to SCID	Neonatal / adolescence	Recurrent infections (respiratory, otitis media, rhinosinusitis, candidiasis), enteropathy, eosinophilia, eczema.
CD3G	Asymptomatic to CID	Infancy / adulthood	Immune deficiency and autoimmunity of variable severity (Evans syndrome, autoimmune hepatitis, thyroiditis).
PTCRA	Asymptomatic to CID	Childhood / adulthood	Recurrent mild infections, higher risk of lymphoproliferation, and/or autoimmunity, hypoplastic/absent thymus.



	CD3E	CD3D	CD247	TRAC	CD3G	PTCRA
Exitus	4/15; 27%	10/18; 55%	3/5; 60%	3/8; 38%	4/22; 18%	1/10; 10%
HSCT	6/15; 40%	8/18; 44%	3/5; 60%	3/8; 38%	5/22; 23%	0/10; 0%
Exitus in HSCT	3/6; 50%	4/8; 50%	1/3; 34%	0/3; 0%	3/5; 60%	-

3.2 Genomic diagnosis

Genomic testing is pivotal for IEI diagnosis (Bousfiha et al., 2022). Up to now, 555 IEI due to variants in over 500 genes have been identified (Poli et al., 2025), a number that continues to grow thanks to advances in sequencing technologies and variant interpretation (Notarangelo et al., 2020; Seth et al., 2021). This reason, along with the fact that the clinical phenotype of patients with the same and different defects may be both overlapping and variable, even more when it involves mutations with few cases reported, is why, in the context of a suspected IEI, experts' committees recommend genetic diagnostic using techniques that cover a larger proportion of the genome (Bousfiha et al., 2025).

In clinical practice, mutations in TCR complex genes (including *CD3D, CD3E, CD3G, CD247, and TRAC*) form an established subset of IEI and are routinely incorporated into targeted NGS gene panels used worldwide for diagnostic testing (Bousfiha et al., 2022; Tangye et al., 2022; Bousfiha et al., 2025). As stated above, the inclusion of *PTCRA gene* in the 2024 updated classification of genes causing IEI provided by IUIS (Poli et al., 2025), has led to its incorporation into NGS genetic panels. Gene panels are the cheapest and first-line NGS approach, allow high sequencing depth for targeted genes, although its yield can show a broad range due to low coverage of certain coding regions, and presence of pseudogenes, copy number variation (CNV) or DNA regions with nucleotide repeats (Yska et al., 2019; Meyts et al., 2016).

In silico panels, which restrict reviewed variants to well-established genes, may miss rare pathogenic changes in less conventional loci (Bousfiha et al., 2022). Variant annotation continues to improve as new disease genes and genotype–phenotype correlations emerge (Demirdag et al., 2021). One important approach to raise the diagnostic yield is iterative reanalysis (periodic re-examination of existing genetic data, integrating updated gene lists, new variant knowledge, and superior bioinformatic tools). This strategy has been shown to substantially increase the number of genetic diagnoses in other Mendelian diseases, and similar benefits are predicted for inborn errors of immunity (Wenger et al., 2017; Boycott et al., 2019).

Whole exome sequencing (WES) has become a cornerstone for genetic testing in IEI, enabling rapid screening of hundreds of disease-associated genes in parallel (Arts et al., 2019). While the diagnostic yield for WES averages around 30%, it can range more broadly depending on the clinical cohort, composition, underlying phenotype, and the genes included in silico diagnostic panels (Yska et al., 2019; Vorsteveld et al., 2021). Whole genome sequencing (WGS) offers benefits over WES as a complementary strategy when standard panels yield negative or ambiguous results, especially in atypical cases (Vorsteveld et al., 2021; Boycott et al., 2019). WGS provides information about structural rearranges, introns, regulatory domains, and intergenic regions not reachable by WES, while it ensures a more uniform coverage. On the other hand, it increases the cost, risk of incidental findings and data analysis complexity (Meyts et al., 2016).Molecular diagnosis historically involved analyzing T-cell RNA to detect mutations in CD3 and related genes, along with cDNA amplification and sequencing to

identify splicing defects, point mutations, or deletions. Such mutations exhibit a spectrum of functional impact, from complete TCR absence to "leaky" partial deficiencies, occasionally showing secondary mutations that partially restore expression in T-cell precursors (Marin et al., 2015). V(D)J gene segments (*TRAV, TRBV, TRDV, TRGV*)—responsible for TCR diversity—are not currently recognized as monogenic causes of TCRIEI and thus are generally excluded from diagnostic panels. Instead, analysis of these segments is mainly reserved for TCR repertoire profiling and clonality assessments in research and oncology (Hodges et al., 2003).

3.3 Proteomics

Thanks to advances in protein prediction algorithms, most TCR genetic defects are not routinely validated at the protein level, particularly when the specific mutation has already been described in previous patients or is predicted to be pathogenic and occurs in homozygosity. Nevertheless, it is highly recommended to include proteomics as part of the assessment to better understand patient disease. TCR proteins can be assessed using extracellular or intracellular flow cytometry and/or western blot, as recommended in the literature and summarized in Table 4.

[Table 4]

Table 4. Summary of techniques to assess TCR components at the protein level. FC: flow cytometry; SSC: side scatter; FSC: forward scatter; MFI: mean fluorescence intensity; SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis.

4. Conclusions

TCR complex genes mutations may cause TCRIEI and must be prioritized in molecular diagnoses, including IEI and standard NGS panels and closely followed in genotype-phenotype studies. The variable region segments remain the domain of research due to the lack of evidence supporting their involvement in IEI. These practices, integrating neonatal screening and flow cytometry validation, ensure efficient, sensitive, and clinically relevant genetic testing for TCRIEI patients, facilitating prompt diagnosis, management, and genetic counseling (Bousfiha et al., 2022; Hodges et al., 2003).

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References

		e	enteropathy, eosinophilia, eczema.
CD3G	Asymptomatic to CID	Infancy / adulthood	Immune deficiency and autoimmunity of variable severity (Evans syndrome, autoimmune hepatitis, thyroiditis).
PTCRA	Asymptomatic to CID	Childhood / adulthood	Recurrent mild infections, higher risk of lymphoproliferation, and/or autoimmunity, hypoplastic/absent thymus.

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737 Table 4

Objective	Target	Detailed epitope	Antibody clones	Technique	Strategy	References
To identify generic TCR level defects within T cells	CD3	CD3εδ/εγ	UCHT-1, OKT-3	Extracellular FC	Evaluate CD3 MFI within SSC vs FSC lymphocytes in CD4+ or CD8+ gated cells.	(Marin et al., 2017)
To identify specific TCR component defects		CD3γ	EPR4517	Intracellular (and extracellular suggested) FC	Evaluate CD3 chains or CD247 MFI within SSC vs FSC lymphocytes. Extracellular CD4 and CD8 staining are recommended to ensure T-cell evaluation.	
		CD3δ	EP4426			
		CD3ε	EPR5361(2)			
	CD247	CD247	6B10.2			
	TCRα	TCRα	H-1 (sc-515719)	SDS-PAGE followed by immunoblot	Resolve whole protein lysate by 15% SDS-PAGE and develop with indicated antibody in PVDF membrane.	(Chen et al., 2022)
	preTα	preTα	K5G3	Extracellular FC	Evaluate preTα MFI within SSC vs FSC thymocytes in CD4+, CD8+ or double positive gated cells.	(Ramiro et al., 2001)
	TCRβ	TCRβ	H-197 (sc-9101)	SDS-PAGE followed by immunoblot	Resolve whole protein lysate by 15% SDS-PAGE and develop with indicated antibody in PVDF membrane.	(Zhong et al., 2004)

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