

Variable presentation of primary immune deficiency: Two cases with CD3 gamma deficiency presenting with only autoimmunity

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Abstract

Background: CD3 chain expression defects including CD3 gamma, epsilon, delta, and zeta chain subunits, are autosomal recessive inherited severe combined immunodeficiencies (SCID). The phenotype is usually T-B+NK+ SCID with lymphopenia where the clinical findings may be mild (CD3 γ) or severe (CD3 δ , ϵ , ζ) owing to the underlying molecular defect. There is limited information about the disease in literature.

Methods: Here, we present two siblings from non-consanguineous family with autoimmunity including Evans syndrome, autoimmune hepatitis, nephrotic syndrome, and Hashimoto's thyroiditis and with no previous history of infections. To define the molecular basis of the disease, we performed linkage analysis around the CD3 receptor cluster and found consistent linkage to this region.

Results: The patient one displayed low TCR $\alpha\beta$ expression, low IgG, low IgA, low IgM, low CD3, low CD4, low CD8. The patient two also displayed low TCR $\alpha\beta$ expression and low anti-HBs titer. We went onto identify a homozygous splicing mutation (IVS2-1G>C) in the two affected individuals in the CD3 γ gene.

Discussion: To date, only four cases have been reported with CD3 γ deficiency. Occasionally, the patients present with only autoimmunity including autoimmune hemolytic anemia, vitiligo, Hashimoto's thyroiditis, and autoimmune enteropathy. However, Evans syndrome, autoimmune hepatitis, and nephrotic syndrome have not been reported in previous cases. We believe that our cases will contribute to the literature.

Primary immune deficiency diseases (PID) comprise a genetically heterogeneous group of disorders that affect distinct components of the innate and adaptive immune system, such as neutrophils, macrophages, dendritic cells, complement proteins, NK cells, as well as T and B lymphocytes (1). Genetic disorders that affect T-cell development may display a range of phenotypes from severe combined immunodeficiency (SCID), through partial immunodeficiency with dysregulation, to predominant autoimmunity (1, 2). CD3 chain deficiencies, a heterogeneous group of autosomal recessive immunodeficiencies, are responsible for only a small proportion of SCID. The phenotype is usually T-B+NK+ with lymphopenia where the clinical findings may be mild (CD3 γ) or severe (CD3 δ , ϵ , ζ) owing to the underlying molecular defect (3).

Patients with CD3 γ deficiency usually present with recurrent infections. However, autoimmunity may be only manifestation of the disease without severe infection history. Only four cases have been reported to date (4–8). First described cases with CD3 γ deficiency were two affected brothers with a different clinical course. They had recurrent respiratory infections and autoimmune diseases in different severity (5). The third case with of Turkish origin who presented with a milder phenotype characterized by upper respiratory tract infections and asthma (7). Turul et al. (4) described a fourth patient who had severe gastrointestinal involvement manifested with intractable diarrhea and perianal–rectal fistulas in 2008. All four presented with infection and autoimmunity, and only one was diagnosed with autoimmunity because of a sibling history. Characteristics of CD3 γ deficient patients are summarized in Table 1.

Methods

Here, we present two siblings with autoimmunity and no previous history of infection. The histories, clinical and laboratory findings of the patients are summarized below. Complete blood count, serum immunoglobulin levels, and CD25 expression in lymphocytes stimulated by phytohemagglutinin were evaluated. Isohemagglutinin titer, peripheral blood lymphocyte subset analysis, and direct Coombs test were performed. To define the molecular basis of the disease, we performed linkage analysis around the CD3 receptor cluster by genotyping microsatellite markers (D11S908 and D11S1998) on chromosome 11q23 in all family members. Following establishment of compatible linkage, mutation screening of the 38 coding exons and exon–intron boundaries of CD3 δ , CD3 γ , and CD3 ϵ were PCR-amplified with specific intronic primers in genomic DNA of affected patients. Sequencing was performed using standard methods on an ABI 3730 automated sequencer.

Patients and Results

A 1-year-old girl from non-consanguineous parents presented with epistaxis and thrombocytopenia. Her physical examination was normal except hepatosplenomegaly. Complete blood count revealed leukopenia, lymphopenia, anemia, and thrombocytopenia (Table 2). Peripheral blood smear showed normoblastemia, spherocytic erythrocytes, anisocytosis, and thrombocytopenia. Direct Coombs test was positive (3+), and reticulocyte count was increased (10%) consistent with AIHA. Bone marrow examination revealed that normoblastic erythroid hyperplasia, thrombocytopenia with increased megakaryocytic cells, without malign infiltration. Serum levels of total bilirubin (8.5 mg/dl), direct bilirubin (5.2 mg/dl), aspartate aminotransferase (223 U/l), and alanine transferase (235 U/l) were elevated. Viral markers were negative. Autoantibodies including anti-nuclear antibody, anti-double-stranded DNA, anti-smooth muscle antibody, anti-liver–kidney mitochondrial antibody, and anti-neutrophil cytoplasmic antibody were negative. Serum levels of complements 3 and 4, vitamin B12, folic, and albumin were in normal range. CD3-positive and double-negative portion of T cells were normal (3%). *In vitro* lymphocyte apoptosis test was normal. Mutational analysis for autoimmune lymphoproliferative syndrome was not available. Abdominal ultrasonography showed hepatosplenomegaly. Liver biopsy was consistent with autoimmune hepatitis. Nephrotic proteinuria (80 mg/m²/hour) and pretibial edema (+++) were detected. Kidney biopsy was consistent with minimal change nephrotic syndrome. Steroid therapy was effective for treating autoimmune hepatitis and nephrotic syndrome. The patient was relatively well for following period of 5 yr. She was admitted to hospital because of epistaxis at 11 yr of age. Her laboratory findings were consistent with Evans syndrome.

Immunologic evaluation, which was carried out due to lymphopenia, revealed low levels of IgA, IgG level, low specific antibody response, and low TCR $\alpha\beta$ expression (Table 2) (Fig. 1). When lymphocytes of those patients were stimulated by phytohemagglutinin, there was no increase in CD25

expression showing insufficient activation of T cell. Laboratory findings of the patient suggested hemolytic anemia and T-cell deficiency. We performed linkage analysis around the CD3 receptor cluster and found consistent linkage to this region. We went onto identify a homozygous splicing mutation (IVS2-1G>C) in the two affected individuals in the CD3 γ gene (Fig. 2). There was no obvious gene defect in TCR alpha subunit constant gene (TRAC).

To control Evans syndrome, steroid therapy was given at a dose of 2 mg/kg/day. Cyclosporine A therapy was added to steroid therapy because of persistent thrombocytopenia and anemia. The patient experienced two pneumonia episodes when she was receiving high-dose corticosteroid therapy to control AIHA at follow-up. Intravenous immunoglobulin replacement therapy was started at dose of 0.4 gr/kg/every 3 wk to control infections.

During hospitalization, we noticed that the patient's 20-year-old sister had growth retardation with no history of infection and chronic disease. She was identified as having AIHA and Hashimoto's thyroiditis while she was evaluating growth retardation. Her immunologic evaluation is shown in Table 2. Mutation analysis confirmed the same mutation as her sister. The patients' non-consanguineous parents were all healthy but heterozygous for the same mutation (Fig. 2).

Discussion

CD3 γ deficiency is a very rare autosomal recessive immunodeficiency caused by mutations in the gene coding for T-cell surface glycoprotein CD3 γ (gamma) chain precursors. There is limited information in the literature about the disease, because only four patients have been described to date. Clinical features of the reported patients are generally mild and consistent with recurrent infections presenting early in life (3). Autoimmunity may be the first and only manifestation of the disease. All previously reported patients with CD3 γ deficiency had experienced various autoimmune diseases including AIHA vitiligo, Hashimoto's thyroiditis, and autoimmune enteropathy. However, Evans syndrome, autoimmune hepatitis, and nephrotic syndrome have not been reported in previous cases. Our index case was admitted to our hospital due to autoimmunity including Evans syndrome. Autoimmune hepatitis and nephrotic syndrome were transient manifestations, but Evans syndrome requiring hospitalization was persistent in our patient. During the hospitalization, her older sister was diagnosed as CD3 γ deficiency due to AIHA and growth retardation. She was also diagnosed as Hashimoto's thyroiditis while she was evaluating for growth retardation. The underlying mechanism for these organ-specific autoimmune diseases is still unclear. Genetically mediated canceling of the immunologic tolerance toward the self-antigens resulting in a T-cell-driven autoimmune response may be a reason for autoimmunity in those patients.

Immunologic findings may be variable in CD3 γ -deficient patients. B cells are variably affected. The most common laboratory finding is a TCR/CD3 expression defect on CD4 and CD8-positive lymphocytes. They all show variable degrees of lymphopenia, normal or decreased levels of serum immunoglobulins, specific antibody response to polysaccha-

Table 1 Characteristics of CD3- γ -deficient patients

Authors	Publication year	Number of cases	Parent consanguinity	Age of diagnosis	Clinical features	Immunologic features	Genetic analysis
Arnaiz-Villena et al.	1992	2		1 yr (male)	Recurrent pulmonary infections, intractable diarrhea, failure to thrive, autoimmune enteropathy, severe AIHA, fatal outcome in infancy	Normal lymphocyte count, selective IgG2 deficiency, serum auto antibodies against mitochondria, smooth muscle and intestinal epithelial cells	Homozygous splicing mutation (IVS2-1G>C) in the in the CD3 γ gene
Van Tol M et al.	1997	1	Yes	15 months (male) 4 yr (male)	Mild respiratory distress, vitiligo, autoimmune thyroiditis. Mild clinical course, infrequent episodes of gram-positive and gram-negative bacterial infections (easily controlled), asthma, diarrhea, vitiligo, atopic eczema, viral meningitis,	Low IgG2 levels, abnormal T-cell phenotype and function, antithyroid antibodies ND	ND
Ozgur TT et al.	2008	1	No	10 months (male)	mild dilated cardiomyopathy Intractable diarrhea, failure to thrive, recurrent pneumonias, recurrent oral moniliasis, chronic pruritus, skin desquamation, inflammatory bowel disease.	CD3 lymphopenia, low TCR alpha/beta expression	Homozygous A to T transversion at nucleotide 205 in exon 3 of CD3 γ gene
Tokgoz H et al.	2012	2	No	12 yr (female)	Evans syndrome, autoimmune hepatitis, nephrotic syndrome	Lymphopenia, leukopenia, anemia, low IgA, low IgG, low IgM levels, low anti-HBs, low CD3, low CD4, low CD8, low TCR alpha/beta expression	Homozygous splicing mutation (IVS2-1G>C) in the in the CD3 γ gene
				20 yr (female)	Hashimoto's thyroiditis, autoimmune hemolytic anemia	Low anti-HBs, low TCR alpha/beta expression	

ND: not define.

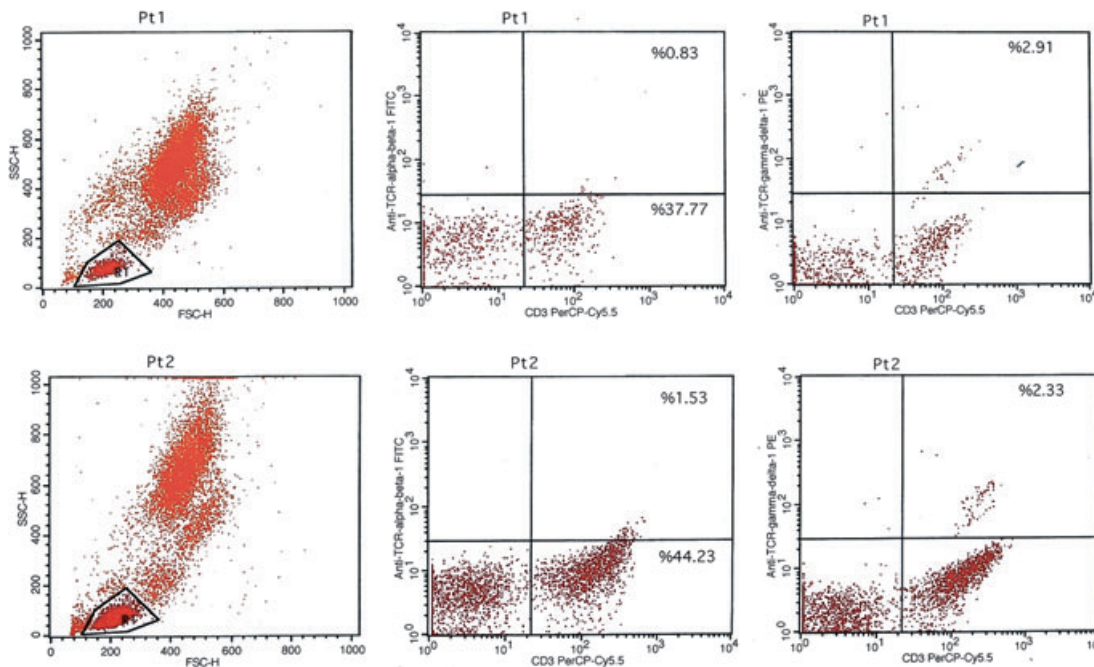


Figure 1 Expression of surface $\alpha\beta$ and $\gamma\delta$ TCR complex on peripheral T cells in the patients.

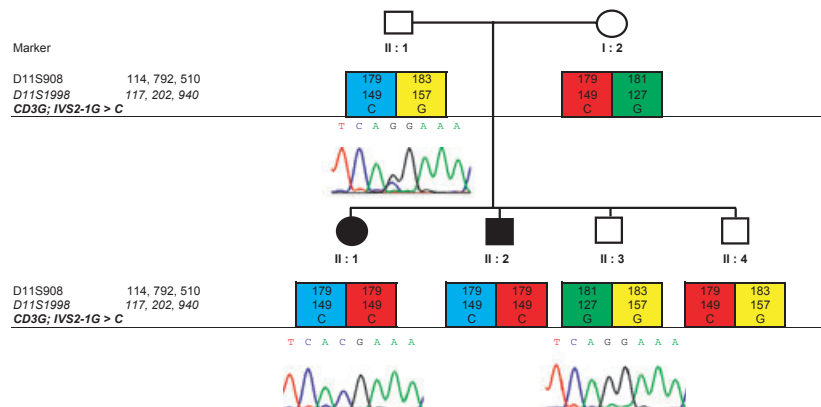


Figure 2 Pedigree of family with a CD3-gamma chain deficiency. Linkage analysis in family members using microsatellite markers spanning the CD3G gene locus and sequencing electropherograms showing a parental carrier, affected individual homozygous mutant, and unaffected non-carrier for the IVS2-1G>C splice-site mutation. The solid symbols represent the affected individuals.

rides and proteins. Isohemagglutinin titer may be normal or low. We did not do any immunologic evaluation in our index case when she was admitted for AIHA at first year of age because she did not have any recurrent infections and additional hematologic abnormality. Her immunologic analysis at 11 yr of age revealed mild lymphopenia, hypogammaglobulinemia, and low levels of TCR/CD3 $\alpha\beta$ expression.

CD3 γ is essential for development of both the pre-TCR complex and mature TCR $\alpha\beta$ -CD3 complex due to its association with the TCR β chain. Developmental step that is

under the control of the pre-TCR complex cannot occur without the CD3 γ chain. Maturation of thymocyte precursors from the CD4-CD8- double-negative (DN) to the CD4+ CD8+ double-positive (DP) stage of development requires productive rearrangement of the gene segments encoding the TCR β chain. Transition from the CD44- CD25+ to the CD44- CD25- DN stage is severely impaired in CD3 γ -/- mice, with only very few cells progressing to the DP and single-positive (SP) stage. Small numbers of SP T cells reach the peripheral lymphoid organs in CD3 γ -/- mice, but

Table 2 Immunologic findings of the patients

Count Blood Cells	Patient 1 (11 yr)	Normal range	Patient 2 (20 yr)	Normal range
WBC (mm ³)	3710 (low)	4–10	7120	4–10
ANS (mm ³)	2020	1.1–4.1	3720	1.1–4.1
ALS (mm ³)	970 (low)	1.5–5.7	2900	1.5–5.7
Hb (g/dl)	7.5 (low)	12.1–17.2	9.1 (low)	12.1–17.2
Hct%	22.3 (low)	36.1–50.3	32.1 (low)	36.1–50.3
PLT (mm ³)	20000 (low)	150000–40000	288000	150000–40000
Immunoglobulins and specific antibody response				
IgA (mg/dl)	6.6 (low)	96–465	165	139–378
IgM (mg/dl)	50.4 (low)	83–282	219	88–322
IgG (mg/dl)	491 (low)	907–1958	1660	913–1884
Anti-HBs (mIU/ml)	Negative	>10	1.24 (low)	>10
Isohemagglutinin titer (Blood group)	Negative (AB Rh+)	Negative	1/16 (B Rh+)	>1/10
Lymphocyte subset analysis				
Absolute lymphocyte numbers (mm ³)	Patient 1 (11 yr)	Normal range	Patient 2 (20 yr)	Normal range
CD3%	970 (low)	1.5–5.7	2900	1.5–5.7
CD3+4+%	40.32 (low)	58–82	42.07 (low)	58–82
CD3+8+%	22.52 (low)	26–48	30.34	26–48
CD3+8+%	12.30 (low)	16–32	7.00 (low)	16–32
CD19%	14.92	10–30	28.49	10–30
CD16–56%	39.05	8–30	16.91	8–30
TCR α - β %	0.83 (low)		1.53 (low)	
TCR γ - δ %	2.91		2.33	
CD45RA%	55.70	66–95	0.82 (low)	66–95
CD45RO	37.55	14–44	42.75	14–44

these mature T cells, like immature thymocytes, express approximately 10-fold lower levels of CD3 ϵ and TCR $\alpha\beta$ or TCR $\gamma\delta$ compared with normal T cells (9). Consistent with these results, TCR expression is dramatically reduced in a CD3 γ -negative variant of the human T-cell line Jurkat (10). CD3 γ thus clearly affects the surface expression levels of both the signaling modules and the TCR-module of the TCR–CD3 complex and might already contribute to T-cell development in that way. Thus, it is not surprising results to find TCR $\alpha\beta$ expression as low in patients with CD3 γ deficiency.

Those findings were consistent with CD3 γ deficiency. To define mutation leading the disease, we performed linkage analysis around the CD3 receptor cluster and found clear linkage to this region. We went onto identify a homozygous splicing mutation (IVS2-1G>C) in the two affected individuals in the CD3 γ gene. This mutation has already been reported in a heterozygous state by Arnaiz-Villena et al. (5).

Treatment of CD3 γ deficiency is symptomatic in milder cases such as regular infusions of intravenous immunoglobulin (IVIG), appropriate antimicrobial treatment of infections, and prophylaxis for infections. Hematopoietic stem cell transplantation (HSCT) may be considered in severe cases (4), which may contribute the recovery of autoimmune manifestations.

Our index case had no infection in her past history. She suffered two pneumonia episode requiring hospitalization under steroid and Cyclosporine A therapy. She was started IVIG and trimethoprim–sulfamethoxazole prophylaxis to controlling infections. Under the prophylaxis, she did not experience any episodes of severe infection. Her elder sister did not given any prophylaxis due to no history of severe infection.

CD3 γ deficiency is very rare disease presenting with variable clinic and immunologic features. Autoimmunity may be only manifestation in these patients. Patients with autoimmunity should evaluate for CD3 γ deficiency before start steroid therapy especially if they have recurrent infection or family history of autoimmunity. Determination of TCR/CD3 expression defect on CD4- and CD8-positive lymphocytes will be useful to define the disease. Understanding the cause of autoimmunity may provide a useful model for the management of CD3 γ -deficient individuals.

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References

1. Al-Herz W, Bousfiha A, Casanova JL, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol* 2011; **2**: 54.
2. Liston A, Enders A, Siggs OM. Unravelling the association of partial T-cell immunodeficiency and immune dysregulation. *Nat Rev Immunol* 2008; **8**: 545–58.
3. Regueiro JR, Espanol T. CD3 and CD8 deficiencies. In: Ochs HD, Smith CIE, Puck JM (Eds). *Primary Immunodeficiency*

- Diseases: A Molecular and Genetic Approach*, second edition. New York, Oxford: Oxford University Press; 2007, 216–26.
4. Ozgur TT, Asal GT, Cetinkaya D, et al. Hematopoietic stem cell transplantation in a CD3 gamma-deficient infant with inflammatory bowel disease. *Pediatr Transplant* 2008; **12** : 910–3.
 5. Arnaiz-Villena A, Timon M, Corell A, Perez-Aciego P, Martin-Villa JM, Regueiro JR. Brief report: primary immunodeficiency caused by mutations in the gene encoding the CD3-gamma subunit of the T-lymphocyte receptor. *N Engl J Med* 1992; **327** : 529–33.
 6. Allende LM, Garcia-Perez MA, Moreno A, Ruiz-Contreras J, Arnaiz-Villena A. Fourteen years' follow-up of an autoimmune patient lacking the CD3 gamma subunit of the T-lymphocyte receptor. *Blood* 2000; **96** : 4007–8.
 7. Van Tol MJD, Sanal O, Langlois Van Den Bergh R, et al. CD3 g chain deficiency leads to a cellular immunodeficiency with mild clinical presentation. *Immunologist* 1997; **1** (Suppl): 41–3.
 8. Regueiro JR, Arnaiz-Villena A, Ortiz de Landazuri M, et al. Familial defect of CD3 (T3) expression by T cells associated with rare gut epithelial cell autoantibodies. *Lancet* 1986; **1** : 1274–5.
 9. Haks MC, Krimpenfort P, Borst J, Kruisbeek AM. The CD3gamma chain is essential for development of both the TCR alpha beta and TCR gamma delta lineages. *EMBO J* 1998; **7**: 1871–82.
 10. Geisler C. Failure to synthesize the CD3-gamma chain. Consequences for T cell antigen receptor assembly, processing, and expression. *J Immunol* 1992; **8**: 2437–45.