

## Clinical and genetic features of IL12R $\beta$ 1 deficiency: Single center experience of 18 patients

Çağman Tan<sup>1</sup>, Deniz Çağdaş-Ayvaz<sup>1</sup>, Ayşe Metin<sup>2</sup>, Özlem Keskin<sup>3</sup>, İlhan Tezcan<sup>1</sup>, Özden Sanal<sup>1</sup>

<sup>1</sup>Division of Immunology, Department of Pediatrics, Hacettepe University Faculty of Medicine, <sup>2</sup>Dışkapı Children's Hospital, Ankara, and <sup>3</sup>Division of Immunology and Allergy, Department of Pediatrics, Gaziantep University Faculty of Medicine, Gaziantep Turkey. E-mail: cagmantan@gmail.com

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**SUMMARY:** Tan Ç, Çağdaş-Ayvaz D, Metin A, Keskin Ö, Tezcan İ, Sanal Ö. Clinical and genetic features of IL12R $\beta$ 1 deficiency: Single center experience of 18 patients. Turk J Pediatr 2016; 58: 356-361.

Mendelian susceptibility to mycobacterial disease (MSMD) is characterized by infections with weakly virulent mycobacteria (BCG and environmental mycobacteria), *M. tuberculosis*, Salmonella, candida and some other intracellular microorganisms. Nine different genetic defects have been defined to cause MSMD and IL-12R $\beta$ 1 deficiency is the most common form. We present here the clinical and genetic features of 18 patients with IL12R $\beta$ 1 deficiency diagnosed by surface expression of IL-12R $\beta$ 1 and Sanger's sequencing. Seventeen patients showed classical presentation (infections with BCG, salmonella and candida) while one patient experienced recurrent leishmaniasis. In all patients the percentage of activated lymphocytes with surface expression of IL12R $\beta$ 1 was <1% indicating that it is an effective method for the screening of these patients. Three recurrent mutations were responsible for 85% of our families. Prognosis was good in patients, in whom specific antimicrobial therapy was given before dissemination occurs, as well as prophylactic antimicrobial treatment when needed and IFN- $\gamma$  therapy for severe infectious episodes.

**Key words:** IL-12R $\beta$ 1, MSMD, BCG, IL-12R $\beta$ 1 deficiency.

Mendelian susceptibility to mycobacterial disease (MSMD), which is one of the disorders of Innate Immunity, is a rare disorder in which the patients are susceptible to severe diseases with weakly virulent mycobacteria, such as *Mycobacterium bovis* in Bacille Calmette-Guerin (BCG) and environmental mycobacteria (EM), also known as atypical or nontuberculous mycobacteria in otherwise healthy individuals<sup>1,2</sup>. These patients are also susceptible to Salmonella, Candida and *M. tuberculosis* species<sup>2,3</sup>. Nine disease-causing genes have been described, including three genes controlling the response to IFN- $\gamma$  (*IFNGR1*, *IFNGR2*, *STAT1*), four involved in IFN- $\gamma$  production (*IL12B*, *IL12RB1*, *NEMO*, *ISG15*), one involved in the IFN- $\gamma$  dependent induction of IL-12 (*IRF8*) and another gene controlling the macrophage respiratory burst, which can be triggered by IFN- $\gamma$  (*CYBB*)<sup>2,4-8</sup>. The considerable allelic heterogeneity at some

of these loci has resulted in the definition of up to 18 different inborn errors of immunity<sup>2</sup>. However, the genetic etiology of about half the cases of MSMD has not been identified yet<sup>2</sup>.

IL-12R $\beta$ 1 deficiency is the most common form of MSMD<sup>2</sup>. IL-12R $\beta$ 1 is a common receptor chain of the IL-12 and the IL-23 receptors and deficiency of IL-12R $\beta$ 1 causes a profound defect in both IL-12 and IL-23 signaling<sup>2,7,8,9</sup>. IL-12 is an important cytokine for the development of IFN- $\gamma$  producing T cells and also induces IFN- $\gamma$  production by Natural killer (NK) and Natural Killer T (NKT) cells<sup>9</sup>. The lack of IL-12 stimuli impairs the production of IFN- $\gamma$ <sup>2</sup>. In IL-12R $\beta$ 1 deficient patients the development of IL-17 producing T cells (Th17 cells) was also found to be impaired<sup>10</sup>. Although the exact involvement of IL-23 and Th17 cells is not clear, IL-23 and IL-17 are clearly implicated in various autoimmune and inflammatory disorders and are important for adaptive host defense against

Table 1. Clinical and Genetic Features of the Patients

NO	Age (years)	Sex	BCG Vaccinated	Local BCG Inf.	Candidiasis	Salmonella Infection	Other Clinical characteristics	Mutation	Age of onset (months)	Initial Complaints	Consanguinity	Outcome	Ref.
1	16	M	-	-	-	+	Recurrent Leishmaniasis	R175W	18	Salmonella infection	+	alive	[16]
2	23	M	+	+	-	-	Recurrent systemic BCG Inf.	c.592T>C	2	Local draining BCG adenitis	+	alive	[17]
3-1	Died at age 4	M	+	+	+	-	Disseminated BCG Inf.	c.783+1G>A	4	Draining BCG adenitis	+	deceased	[17]
3-2	19	F	-	-	+	+	BCG infection? Leucocytoclastic vasculitis	c.783+1G>A	9	Multiple granulomatous lymphadenitis	+	alive	[17]
4-1	Died at age 7	F	+	+	+	-	Disseminated BCG Inf.	c.783+1G>A	4	Draining BCG adenitis	+	deceased	[17]
4-2	Died at age 3	M	+	+	+	-	Disseminated BCG Inf.	c.783+1G>A	4	Draining BCG adenitis	+	deceased	[17]
5-1	Died at age 4	F	+	+	+	-	Disseminated BCG Inf.	NA	18	Draining BCG adenitis	+	deceased	[18]
5-2	19	M	-	-	-	+	Leucocytoclastic vasculitis	c.783+1G>A	6 years	Acinetobacter adenitis, Salmonella infection	+	alive	[3]
6	12	M	+	+	-	-	Systemic BCG Inf.	R175W	11	Draining BCG adenitis	+	alive	
7	14	F	+	No BCG scar PPD was +	-	+	Recurrent Salmonella inf.	R175W	30	Salmonella infection	+	alive	[19]
8	12	M	+	+	-	-	E. histolytica Multiple draining BCG adenitis	R175W	30	Local draining BCG adenitis	+	alive	
9	12	M	+	+	-	-	Multiple draining BCG adenitis	c.64+2T>G	8	Local draining BCG adenitis	+	alive	
10	12	M	+	+	-	-	Multiple draining BCG adenitis	c.64+2T>G	9	Local draining BCG adenitis	+	unknown	
11	9	M	+	+	-	-	Multiple draining BCG adenitis	c.64+2T>G	8	Local draining BCG adenitis	+	alive	
12	9	M	+	+	-	-	Bacterial pneumonia Multiple draining BCG adenitis	c.64+2T>G	8	Local draining BCG adenitis	+	alive	
13	5	F	+	+	-	-	Bacterial pneumonia Multiple draining BCG adenitis	R175W	8	Local draining BCG adenitis	+	alive	
14	25	M	+	- after 1st vaccination + after 2nd vaccination at age 7	-	+	Recurrent Salmonella inf.	R173P	5 years	Salmonella infection	+	alive	[3]
15	6	F	+	+	-	-	Recurrent multiple Salmonella adenitis	c.783+1G>A	8	Cervical adenitis	+	alive	

extracellular bacteria and *Candida albicans*<sup>11,12</sup>. In this study, we present the clinical features and genetic characteristics of 18 patients with IL12Rβ1 deficiency.

### Material and Methods

The patients included in the study were diagnosed at and followed up at Hacettepe İhsan Doğramacı Children's Hospital Pediatric Immunology Division between 1998-2013. Eighteen patients from 14 families (11 boys, 7 girls) who have symptoms suggestive for MSMD (BCG infection and recurrent salmonellosis) or history of sibling death due to BCG infection were evaluated. The expression of the IL-12Rβ1 was detected on the lymphocyte surface by flow cytometry after the lymphocytes were stimulated with PHA in vitro. Mutation analyses were done by Sanger sequencing and all coding exonic fragments and the exon-intron boundaries of the IL12RB1 gene was amplified and sequenced for detection of mutations. All flow cytometric analysis was done in the Pediatric immunology laboratory of Hacettepe Children's Hospital as well as Sanger sequencing in families 6, 8-13 and 15. Mutation analysis in the rest of the families were done in different centers and published previously.

### Results

All index cases were presented clinically either with BCG or salmonella infections. The first clinical infection occurred at ages 2 months

to 6 years. All 14 patients except one who were BCG vaccinated developed BCG infection (Table I).

BCG infection (draining lymphadenitis developed after BCG vaccine or systemic or disseminated BCG infection) was present in 14, isolated salmonellosis in 3 patients, a combination of BCG and salmonella infections in 2, Salmonella and recurrent Leishmania infections in 1 patient.

Three patients presented with salmonella infection, another two patients had concomitant salmonella infection. One patient who was not BCG vaccinated presented with Henoch-Schonlein purpura (HSP) and dramatic and abrupt improvement was observed with antibiotics against salmonella, another patient developed leukocytoclastic vasculitis during follow up. Candidiasis was the accompanying feature in five patients. One patient experienced recurrent leishmaniasis along with salmonellosis. The follow up period was between 3 to 21 years (8 families reported earlier, Table I). Patients were given prolonged specific antimicrobial agents, prophylaxis when needed and IFN-γ particularly during severe infectious episodes. Four patients died of BCG infections who had advanced or disseminated mycobacterial infection when first admitted. The percentage of lymphocytes with surface IL12Rβ1 expression was found to be less than %1 in all patients (Fig 1).

Fourteen families had 5 different homozygous mutations, the most common mutation was

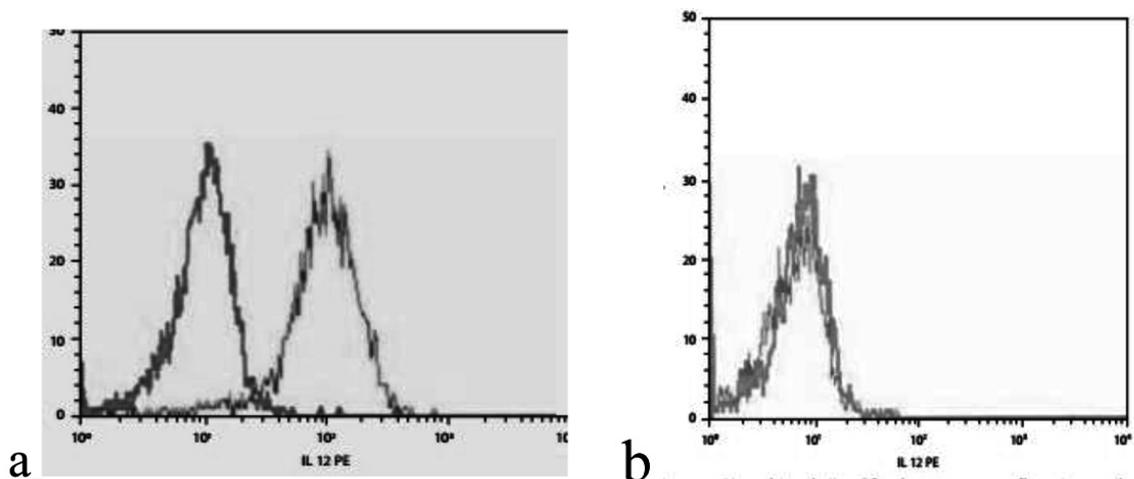


Fig.1. The histograms of IL-12Rβ1 expression a) in a healthy individual b) in a patient

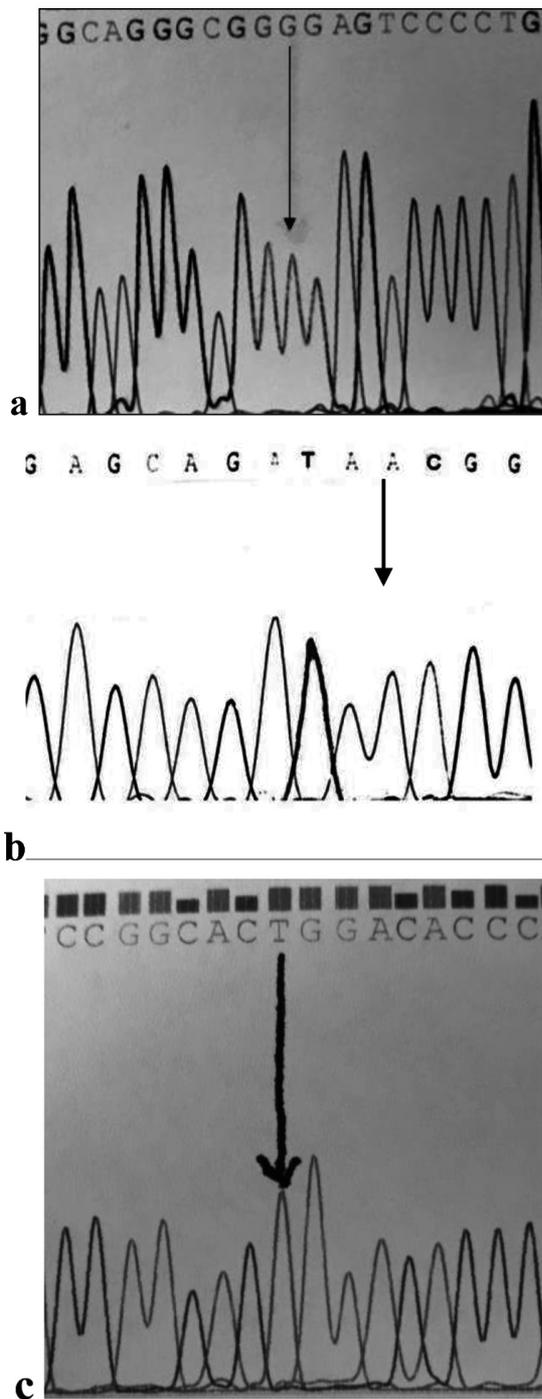


Fig.2. The most frequent mutations identified in our patients

a)Homozygous c. 64+2T>G b) Homozygous c.783+1G>A c)Homozygous R175W

c.523C>T (p.R175W) (in 6 families) followed by 783+1G>A (R173P) (in 4 families) and 64+2T>G (in 2 families). One family each had c.592T>C- and c.518G>C mutations (Fig. 2), (Table I).

### Discussion

Mendelian susceptibility to mycobacterial diseases (MSMD), which is one of the disorders of Innate Immunity, is a rare immunodeficiency syndrome characterized by a narrow vulnerability to poorly virulent mycobacteria, such as BCG and EM<sup>1,2</sup>. These patients are also susceptible to salmonella, candida and *M. tuberculosis* species<sup>2,13,14</sup>. Some other intracellular microorganisms have been reported in a small number of patients<sup>13</sup>. Thus the infectious phenotype extends beyond susceptibility to mycobacterial diseases.

IFN- $\gamma$ R1 deficiency is the first genetic defect identified in these patients while the most common genetic etiology of MSMD is IL-12R $\beta$ 1 deficiency<sup>2,4,13</sup>.

This study consists of 18 patients with IL12R $\beta$ 1 deficiency. Fourteen patients out of 15 who were BCG vaccinated presented with BCG infection. The most common and earliest infection occurred was BCG infection probably because of the early encounter with these microorganisms<sup>13</sup>. Three patients presented with salmonella infections, another two were an associated infection. Five patients had associated candidiasis and one patient experienced recurrent leishmaniasis. None of our patients developed infection with *M. tuberculosis*.

One patient first presented with leukocytoclastic vasculitis and responded dramatically to antibiotic treatment against salmonella, another case of leukocytoclastic vasculitis was observed during follow up period. Vasculitic skin involvement has been reported previously in patients with MSMD<sup>13</sup>. Three patients with IL-12/IL-23 deficiency out of 141 patients (2.1%) developed vasculitic skin lesions and was considered to be caused by antigenic overload or a defect in immune complex clearance<sup>13</sup>.

Candidiasis was observed as an associated feature in 5 (28%) patients. In the literature, mucocutaneous candidiasis has been reported in 32 of 132 (25%) symptomatic IL-12R $\beta$ 1

deficient patients<sup>13</sup>. Although oropharyngeal candidiasis is the most common form, esophageal, vulvovaginal and invasive candidiasis have also been documented<sup>14</sup>. First candida infection episode may appear even before BCG infection<sup>14</sup>. Impaired interleukin 23-dependent interleukin 17 immunity along with IFN- $\gamma$  seems to be responsible from predisposition to candida infection<sup>10,14</sup>.

Infections with some other intracellular pathogens (nocardia, klebsiella, histoplasma, paracoccidiomycose, coccidioides, toxoplasma, listeria) have been described mostly in single cases<sup>13</sup>. One of our patients experienced recurrent leishmaniasis<sup>16</sup>. He responded well to lyposomal amphotericin B treatment, however since he showed recurrences after the cessation of therapy he was given prophylactic Amp B on which he did well for the last 9 years.

The patients were followed-up up to 21 years. The prognosis was poor only in patients admitted at later stages of BCG infection. Four of our patients who were admitted with and died of disseminated BCG infection were diagnosed during the 1990s when the awareness of the disease was poor.

Three mutations were recurrent and covered 12 out of 15 families (85%). In 27 Turkish families reported in Beaucoudrey's<sup>13</sup> study, these 3 mutations covered 48% of the Turkish families.

The results of our patients showed that the analysis of the surface expression of IL-12R $\beta$ 1 on activated lymphocytes in patients with features suggestive for this defect (e.g. patients with BCG infection, recurrent salmonella infections) and in family members is an effective method for screening of this entity. However, to prevent missing novel mutations which are associated with intact IL-12R $\beta$ 1 expression, it may be advisable to measure IFN- $\gamma$  production by activated T cells<sup>7</sup>. Other genetic etiologies for MSMD should also be searched. Among our patients IL-12R $\beta$ 1 is the most common etiology of MSMD, up until now only two IFN- $\gamma$ R1 and one Interferon Stimulating Gene 15 (ISG15) deficiency were identified in our center<sup>15</sup>. Infectious phenotype of these genetic disorders continues to expand and it may be advisable to investigate patients with recurrent

leishmaniasis and severe infections due to other infectious agents, although rarely described in these patients, for IL-12R $\beta$ 1 deficiency.

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