

Eight years of follow-up experience in children with mendelian susceptibility to mycobacterial disease and review of the literature

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Abstract

Background: Mendelian susceptibility to mycobacterial disease (MSMD) is a rare primary immunodeficiency, caused by non-tuberculous mycobacteria or Bacillus Calmette-Guerin (BCG) vaccine and characterized by severe diseases in childhood.

Objective: In this study, we examined eight years followed-up 12 Turkish children with genetically proven MSMD and we tried to evaluate the survival rate with successful disease management, rate of consanguinity, molecular, cellular and clinical features of patients. In addition, we wanted to emphasize the importance of early diagnosis before administration of BCG vaccine in countries where this vaccine is routinely used.

Methods: Twelve patients diagnosed with molecular studies [IFN γ R1 complete (n = 1), IFN γ R2 partial (n = 3), IL-12R β 1 (n = 6), NEMO (n = 1), STAT1 mutation (n = 1)] were included.

Results: Ten patients (83%) were born from consanguineous parents and frequency of family history for the primary immunodeficiency was 58% (n = 7). All the cases had been immunized with BCG vaccine (*Mycobacterium bovis*) due to lack of early diagnosis. Two patients had BCG-itis and four patients had "BCG-osis". Survival rate was 75% after successful disease management with antibiotics, anti-tuberculous agents and recombinant IFN- γ .

Conclusion: It was concluded that MSMD must be differentiated from different forms of primary immunodeficiencies, so clinicians should be aware of MSMD especially in patients with BCG vaccine complications and non-tuberculous mycobacterial infection.

Key words: mendelian susceptibility to mycobacterial disease, primary immunodeficiency, consanguinity, BCG vaccine, IL12R β 1 deficiency

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Introduction

Mendelian Susceptibility to Mycobacterial Disease (MSMD) is a rare inherited condition classified on the IUIS (International Union of Immunology Societies) Primary Immunodeficiency Disease (PID) classification as VIth group (Intrinsic and Innate Immunity) and characterized by selective susceptibility to weakly virulent mycobacteria which includes Bacillus Calmette-Guerin (BCG) vaccines, non-tuberculous environmental mycobacteria (EM), and salmonella.^{1,2}

The disease mostly begins in childhood and has various clinical manifestations, ranging from regional to disseminated infections with one or more mycobacterial species, mostly with environmental mycobacteria and infant BCG vaccination complications. Immunosuppressed patients are vulnerable to adverse complications of the BCG vaccine, a condition ranging from regional disease (BCG-itis) to disseminated disease (BCG-osis).³ As some PIDs are remained undiagnosed until

the appearance of the presumed complications, clinicians should be alert for MSMD warning signs after the most common PIDs predisposing to mycobacterial diseases (severe combined immunodeficiency, combined immunodeficiency, and chronic granulomatous disease) were ruled out.⁴ MSMD patients are also vulnerable to extrapulmonary infections by *Mycobacterium tuberculosis* and mild forms of chronic mucocutaneous candidiasis.^{5,6} Infections caused by various bacteria, fungi, parasites, and viruses have also been reported.¹

The pathogenesis mostly depends on gene mutations lead to either insufficient production or inadequate response to IFN- γ which is mandatory for an efficient immune response to Mycobacteria species.^{7,8} An intact IL12/23-IFN γ circuit phosphorylates macrophage intracytoplasmic signal transducers and activators of transcription-1 (STAT1) to upregulate specific genes to kill mycobacteria. Infected macrophages of healthy individuals produce IL-12 and IL-23 which bind to their receptors on the surfaces of T and NK cells with high affinity, leading ultimately to IFN- γ secretion by these cells. IFN- γ then binds its receptor on the macrophage and activates genes including IL-12 and the respiratory burst NADPH oxidase system which will allow the macrophage to kill the ingested bacteria.^{9,10} The mutations which lead to a defect in IFN- γ or receptors or signal transduction pathways on IL12/23 axis will lead to an incomplete response to mycobacterial infections.^{11,12}

Since the discovery of its first genetic etiology in 1996, 23 genetic etiologies have been found in MSMD patients (15 autosomal recessive- AR, 6 autosomal dominant- AD, and 2 X-linked recessive- XR) which are partial or complete defects located on different genes (IFN γ R1, IFN γ R2, IL-2R β 1, IL12R β 2, IL23R, IL-12 β , ISG15, STAT1, TYK2, IRF8, CYBB, NEMO, SPPL2A).^{1,13,14,15} Human mutations in IFN γ R1, IFN γ R2, and STAT1 impair the cellular response to IFN- γ , whereas mutations in IL12 β and IL12R β 1 impair the IL-12/23-dependent production of IFN- γ .²

In general, the spectrum of the diseases may range from milder defects as IL12R β 1 deficiencies in which patients can be treated with “exogenous human recombinant IFN- γ ” in addition to antibiotics, to complete IFN γ R deficiencies that can result in death if hematopoietic stem cell transplantation (HSCT) is not performed. So, herein, we aimed to present clinical, laboratory and molecular characteristics of twelve Turkish MSMD patients, to draw attention to the importance of early diagnosis to prevent the BCG vaccine complications and we tried to evaluate the survival rate with successful disease management in long term follow-up.

Patients and Methods

Twelve patients (8 boys and 4 girls) that had been followed-up for various PID clinical phenotypes and diagnosed as MSMD in Department of Pediatric Immunology, Ege University, Turkey were evaluated. All recorded data such as demographic characteristics (name, sex, date of birth, age at onset of symptoms, age at admission, age at diagnosis), history of PID disease symptoms, family history, consanguinity, molecular examinations, treatments, and prognosis were collected. Patients with atypical mycobacterial infections,

mycobacterial disease resistant to therapy, microbiological evidence of Salmonella, and BCG vaccination complication history had been included in this study group. Patients with lymph node involvement alone without systemic involvement were considered to have BCG-itis and those with both lymph node involvement and systemic involvement were considered to have BCG-osis.

Laboratory investigations at admission including whole blood count (Cell-Dyn Ruby hemocounter; Abbott Diagnostics, USA), immunoglobulin levels (IgG, IgM, IgA by BNII nephelometry, Siemens, Germany), immunophenotyping of T, B, and Natural Killer cells (CD3%, CD4%, CD8%, CD19%, and CD16-CD56% by FACS Calibur flow cytometry, Becton Dickinson, USA) were performed in Immunology Laboratory and bacteriological/ serological analyses were performed in Laboratories of Microbiology Department, Ege University, Turkey. Genetic analyses (sequence analysis n = 6, whole exome sequencing n = 4, targeted next generation sequencing n = 2) were performed to confirm the disease in different centers. The study was approved by the ethics committee and no financial support was received. The written informed consent was obtained from parents.

Statistical analysis

Statistical analyses were performed by using SPSS Windows Version 16.0 (SPSS Inc., USA). Descriptive and frequency analyses were used, the results were expressed as mean \pm standard deviation and percentage.

Results

In this study, twelve MSMD patients (8 boys and 4 girls) with a mean age of 12.0 \pm 7.36 years (1.5-26) were enrolled and have been followed-up for various PID phenotypes. The mean follow-up time of the patients was about 92.2 \pm 62.6 (3-218) months. **Table 1** summarizes all demographic features, clinical and laboratory manifestations of patients. The mean ages at initiation of symptoms, at admission and at diagnosis were 16.2 \pm 26.6 (1-96), 42.5 \pm 38.6 (4-120) and 47.3 \pm 39.6 (12-120) months, consecutively.

Ten patients (83%) were born from consanguineous parents and the frequency of family history for the primary immunodeficiency was 58% (n = 7). All patients (100%) had a recurrent respiratory infection since early infancy and 33% of patients (n = 4) had chronic diarrhea. 3 of the patients (25%) had failure to thrive. On physical examination, the most frequent pathological findings were lymphadenitis (75%), hepatosplenomegaly (66%), generalized lymphoproliferation (41%), eczema (8%) and edema (8%). All the cases had been immunized with the BCG vaccine (*Mycobacterium bovis*) due to a lack of early diagnosis. Two patients had BCG-itis and four patients had “BCG-osis” due to disseminated infection (**Table 1**, case 1, 2, 8 and 12). Laboratory findings at the admission of all patients were summarized in **Table 2** and 11 patients had shown hypergammaglobulinemia. Molecular diagnostic distributions were also as follows; IFN γ R1 complete defect (n = 1), IFN γ R2 partial defect (n = 3), IL12R β 1 defect (n = 6), NEMO defect (n = 1) and STAT1 defect (n = 1) (**Table 1**), respectively.

Table 1. Demographic features, clinical and laboratory manifestations of patients

No	Sex	Age (months)		Family history of PID	Consanguinity	Gene defect	Genetic mutation	Genetic analysis	Pathogen microorganisms	Clinical complications	Final state	Follow-up (months)
		Beginning of symptoms	Diagnosis									
1	M	6	10	no	yes	IFN- γ R1	106insT homozygous	sequence analysis of IFN- γ R1 gene	<i>M. bovis</i> (LN, BM) <i>M. avium intracellulare</i> (LN) <i>M. fortuitum</i> (LN)	granulomatous dermatitis hypalbuminemia BCGosis	died at the age 6 (pseudomonas sepsis 9 months after HSCIT)	67
2	F	7	12	yes	yes	IFN- γ R2 partial	G141R homozygous	whole exome sequencing	<i>M. bovis</i> (LN)	pleural effusion BCGosis	follow up with prophylactic anti-tuberculous treatment	62
3	M	2	90	yes	yes	IFN- γ R2 partial	G141R homozygous	whole exome sequencing	<i>M. elephantis</i> (LN)	(-)	follow up with prophylactic anti-tuberculous treatment	125
4	M	2	18	yes	yes	IFN- γ R2 partial	G141R homozygous	sequence analysis of IFN- γ R2 gene	<i>M. bovis</i> (LN)	BCGitis	follow up with prophylactic anti-tuberculous treatment	168
5	M	30	84	no	no	IL12R β 1	C.557_563delins8 homozygous	sequence analysis of IL12RB1 gene	<i>Salmonella group B</i> (blood, feces)	cutaneous vasculitis	follow up with prophylactic anti-tuberculous and rIFN γ treatment	96
6	M	18	24	yes	yes	IL12R β 1	C198R homozygous	sequence analysis of IL12RB1 gene	<i>Salmonella enteritidis</i> (blood) <i>M. tuberculosis complex</i> (LN)	(-)	follow up with prophylactic anti-tuberculous and rIFN γ treatment	149
7	M	96	120	yes	yes	IL12R β 1	R173P homozygous	sequence analysis of IL12RB1 gene	<i>M. gardanea</i> (fasting gastric fluid) <i>M. avium intracellulare</i> (LN, liver) <i>S. aureus</i> (LN) <i>S. chelonae</i> (LN)	granulomatous hepatitis hypersplenism splenectomy	followed up with prophylactic anti-tuberculous and rIFN γ treatment died at the age 17 (sepsis)	84
8	F	1	102	no	yes	IL12R β 1	R173P homozygous	sequence analysis of IL12RB1 gene	<i>M. chelonae</i> (LN) <i>M. bovis</i> (abscess swap) <i>Salmonella enteritidis</i> (LN)	leucocytoclastic vasculitis hypersplenism (splenectomy) BCGosis	follow up with prophylactic anti-tuberculous and rIFN γ treatment	218
9	M	1	36	no	no	IL12R β 1	C684-685insT homozygous	Whole-exome sequencing	<i>Mollicoccus contagiosum</i> (skin)	dermatitis	anti-fungal, antibiotic IVIG replacement therapy	52
10	F	8	12	no	yes	IL12R β 1	C261 homozygous	Targeted next generation sequencing	<i>M. bovis</i> (LN)	BCGitis	follow up with prophylactic anti-tuberculous and rIFN γ treatment	6
11	F	6	30	yes	yes	STAT1	C.1154-p-Thr385Met de novo	Targeted next generation sequencing	<i>M. tuberculosis</i>	chronic lung disease	Anti-fungal, antibiotic IVIG replacement therapy	64
12	M	5	24	no	no	NEMO	c.74_77delACGT hemizygous	Whole-exome sequencing	<i>M. bovis</i> (LN, liver, skin)	brain abscess BCGosis	follow up with prophylactic anti-tuberculous and rIFN γ treatment died at the age of 2 (sepsis)	19

LN: Lymph node, BM: Bone marrow F: Female, M: Male

Table 2. Laboratory assessments of patients at diagnosis (with normal reference ranges)

No	Age at diagnosis (months)	WBC (K/ μ L)	Absolute lymphocyte (K/ μ L)	IgG (mg/dL)	IgM (mg/dL)	IgA (mg/dL)	IgE (kU/L)	CD3 (%)	CD19 (%)	CD4 (%)	CD8 (%)	NK (%)
1	10	15.9 (6-17)	4.50 (0.6-3.4)	2200 (512-874)	474 (45.8-126.4)	292 (16.2-89.6)	2816 (0-100)	78 (51-79)	11 (14-44)	41 (26-55)	33 (10-31)	8 (5-23)
2	12	27.7 (6-17)	5.42 (0.6-3.4)	1610 (512-874)	231 (45.8-126.4)	95 (16.2-89.6)	-	57 (51-79)	28 (14-44)	32 (26-55)	21 (10-31)	14 (5-23)
3	90	4.72 (5-13)	2.04 (0.6-3.4)	1670 (838-1244)	45 (54.7-140.5)	14 (66.1-150.7)	-	67 (57-81)	14 (10-27)	35 (24-47)	27 (17-37)	8 (8-28)
4	18	14.7 (6-17)	8.20 (0.6-3.4)	1240 (575-974)	240 (58.0-138.6)	109 (25.8-62.4)	65 (0-100)	59 (51-77)	29 (17-41)	30 (29-55)	30 (15-33)	10 (4-15)
5	84	16.2 (5-13)	2.53 (0.6-3.4)	2570 (838-1244)	187 (54.7-140.5)	225 (66.1-150.7)	-	59 (57-81)	14 (10-27)	25 (24-47)	33 (17-37)	24 (8-28)
6	24	13.1 (6-17)	3.50 (0.6-3.4)	2680 (575-974)	128 (58.0-138.6)	174 (25.8-62.4)	390 (0-100)	54 (51-77)	67 (17-41)	37 (29-55)	18 (15-33)	10 (4-15)
7	120	7.20 (5-13)	2.89 (0.6-3.4)	1628 (824-1302)	68 (63.6-141.2)	98 (72.8-158.8)	-	37 (57-81)	9 (10-27)	9 (24-47)	26 (17-37)	4 (8-28)
8	102	3.13 (5-13)	1.60 (0.6-3.4)	3520 (824-1302)	456 (44.6-143.2)	143 (70.8-162.6)	-	64 (57-81)	16 (10-27)	16 (24-47)	37 (17-37)	12 (8-28)
9	36	8.51 (5-13)	2.12 (0.6-3.4)	1620 (614-1031)	35 (58.6-126.4)	160 (26.7-80.3)	4561 (0-100)	69 (55-79)	23 (11-31)	34 (26-49)	30 (9-35)	5 (5-28)
10	12	13.3 (6-17)	9.65 (0.6-3.4)	867 (512-874)	32 (45.8-126.4)	95 (16.2-89.6)	-	73 (51-79)	18 (14-44)	47 (26-55)	21 (10-31)	7 (5-23)
11	30	11.4 (5-13)	3.69 (0.6-3.4)	1320 (614-1031)	149 (58.6-126.4)	98 (26.7-80.3)	<1 (0-100)	71 (55-79)	23 (11-31)	40 (26-49)	28 (9-35)	5 (5-28)
12	24	14.0 (6-17)	4.2 (0.6-3.4)	2640 (575-974)	145 (58.0-138.6)	20 (25.8-62.4)	2.8 (0-100)	74 (51-77)	21 (17-41)	18 (29-55)	53 (15-33)	1.6 (4-15)

Microorganisms that were isolated from lymph nodes, bone marrow, blood, and other tissues during follow-up were summarized in **Table 1**. *Mycobacterium bovis* was isolated from three patients with IFN- γ defect (cases 1, 2, 4), two patients with IL12R β 1 (cases 8, 10) and NEMO defect (case 12) Salmonella infections were detected mainly in IL12R β 1 defected patients (cases 5, 6, 8). Other viral and parasitic infections were also detected in some of the cases. *Varicella-Zoster virus* (case 1), *Cytomegalovirus* (case 2) and *Giardia* (case 7) infections were also seen. Leukocytoclastic vasculitis was observed in two cases (IL12R β 1 defect; cases 5, 8) and granulomatous dermatitis was found in one patient (IFN γ R1 defect; case 1). Splenectomy has been performed in two patients because of hypersplenism (cases 7, 8). One IL12R β 1 defected male patient had common neck lesions and molluscum contagiosum was isolated from his skin culture (case 9).

Patients were followed-up with prophylactic anti-tuberculous treatment and five IL12R β 1 and one NEMO defected patients underwent regular recombinant IFN- γ treatment. One patient who had symptoms of Hyper IgE syndrome on admission found to have IL12R β 1 defect (case 9) and benefited from regular IVIG therapy. One female STAT1 defected patient (case 11) who was included in the HSCT scanning program also had regular IVIG replacement. Three out of twelve patients died; one case with IFN γ R1 complete defect (case 1) deceased after hematopoietic stem cell transplantation at the age 6, one IL12R β 1 defected patient (case 7) died after sepsis at the age of 17 years and one NEMO defected patient (case 12) deceased after disseminated BCGosis and sepsis at the age of 2 years.

Discussion

The communication between innate and adaptive immunity, mediated by IFN- γ and IL-12, plays a very important role in the control of infections by mycobacteria and other intracellular bacteria. MSMD, or now known as ‘inborn defects of the IL-12/IFN- γ axis’ have warning signs ranging from regional to disseminated infections with one or more mycobacterial species.³ National vaccination programs that have a vital role in public health may endanger the lives of PID infants especially in countries with high rate consanguineous marriages as in our country. BCG is a live attenuated bacterial vaccine that protects children from miliary tuberculosis and tuberculosis meningitis. BCG inoculation is necessary for countries with a high incidence of tuberculosis but has high mortality in patients with PID. So, awareness is particularly important in countries with high BCG coverage at birth and a high prevalence of consanguinity.^{3,4,16}

In this study, we summarized our twelve patients that have been followed-up for PID for many years (mean 92.2 \pm 62.6 months) and genetically diagnosed as MSMD disease. Ten of them were born from consanguineous parents and all of the cases had been immunized with the BCG vaccine due to lack of early diagnosis. Early-onset of the symptoms attracted attention [16.2 \pm 26.6 (1-96) months], but the delay time for diagnosis was about 33.3 \pm 33.7 (5-101) months. As a result, patients had been exposed to many infections during this period. All patients had recurrent respiratory infections

since early infancy and 33% of patients had chronic diarrhea. Two patients had BCG-itis and four patients had ‘‘BCG-osis’’ history and clinical findings following vaccination.

In our patient group, the most frequent molecular defect was IL12R β 1 defect (n = 6) (**Table 1**) similar to literature, but IFN γ R2 partial defect (n = 3) was more seen than IFN γ R1 complete defect (n = 1) unlike current literature.^{1,17}

AR complete IL12R β 1 deficiency is the most common type of MSMD and our six patients had IL12R β 1 defect.^{1,17} Tan et al. had previously reported eighteen IL12R β 1 defected children from Turkey in 2016.¹⁸ This defect results in a lack of expression or non-functional IL12R β 1 and therefore, NK cells and activated T cells are unable to exert an appropriate immune response to intracellular pathogens in these patients.⁹ Literature suggests the presence of IL-12R β 1 deficiency should be determined in children with mycobacterial infections at least in countries with a high prevalence of parental consanguinity.¹⁹ On the other hand, as IL-12R β 1 deficiency was also the first PID to be associated with classic pediatric tuberculosis in children with normal resistance to BCG and EM, therefore, should probably be suspected in any patient with an unusual infection with intracellular pathogens even in the absence of parental consanguinity.^{9,20} In a Chinese cohort, 73% of the MSMD patients with BCG infection had IL-12R β 1 defect and only one patient was born from consanguineous parents.²¹ *M. tuberculosis complex*, *M. gardeniae*, *M. avium intracellulare*, *M. chelonae*, *M. bovis* were isolated from our patients (**Table 1**). IL12R β 1 defected patients commonly suffer from salmonella and, to a lesser extent, candida infections, but not usually present with viral infections.^{1,9,22} In this study group, non-typhoidal salmonella infection was observed mainly in three of our patients (cases 5, 6, 8) and giardia infection was detected in one child (case 7).

The clinical phenotype of the patients with AR complete IFN γ R1 deficiency is characterized by early-onset, disseminated, life-threatening infections with BCG and/or EM (including species such as *M. chelonae*, *M. fortuitum*, etc.) and infections typically begin before three years of age.²² Our patient (case 1) had symptoms at sixth months. *M. bovis*, *M. avium intracellulare* and *M. fortuitum* were isolated in lymph node preparations (**Table 1**). This group of patients are more prone to viral diseases and our case had *Varicella-Zoster virus* infection.¹ The overall prognosis is poor and HSCT is the only known curative treatment.²² Our male case had granulomatous dermatitis during follow-up and deceased after HSCT complicated by a sepsis attack at the age of six years.

On the other hand, AR partial IFN γ R1 deficiency patients express the receptor on their cell surface but display an impaired response to high concentrations of IFN- γ with less severe clinical phenotype than that of the completed form and treatment with antibiotics and IFN- γ for several years is necessary to control the infections.^{1,23} The clinical features of the AD partial IFN γ R1 deficiency are less severe than completed form also and in the 72% of the patients, the infections affect the bone and some patients even develop osteomyelitis with no other organ involvement.²² In completed IFN γ R2 deficiencies, the response to IFN- γ is abolished and the clinical presentation resembles that of completed IFN γ R1 deficiency,

manifests in early childhood, with poorly defined and multi-bacillary granulomas and HSCT is the only curative treatment for these patients.²²

In partial IFN γ R2 deficiencies, patients have weak IFN γ R2 expression on the cell surface and mycobacterial infections are caused mostly by BCG, *M. abscessus*, *M. bovis*, *M. elephantis*, *M. fortuitum*, and *M. simiae*.²² In our patients, *M. bovis* (cases 2, 3, 4) and *M. elephantis* (case 3) were isolated from lymph nodes (Table 1). Case 2 had also Cytomegalovirus infection during follow-up. Antibiotics are an effective treatment for infection in these patients with or without recombinant IFN- γ . Our patients were treated with antibiotics and anti-tuberculous treatment.

During clinical follow-up, various complications were also observed. One patient (case 7) had granulomatous hepatitis. Leukocytoclastic vasculitis was observed in two cases (IL-12R β 1 defect; cases 5, 8) and splenectomy was performed in two patients because of hypersplenism (cases 7, 8). One IL-12R β 1 defected male patient who had symptoms of Hyper IgE syndrome on admission had common neck lesions (case 9). *Molluscum contagiosum* virus was isolated from the skin culture and this patient benefited from regular IVIG therapy. The other five IL12R β 1 defected patients followed-up with prophylactic anti-tuberculous and regular recombinant IFN- γ treatment and one of them (case 7) deceased after sepsis attack at the age of 17 years.

AD STAT1 deficiency was first described in 2001 in two kindreds with MSMD and STAT1 gain of activity was first described in 2011, in patients with chronic mucocutaneous candidiasis.¹ The defect of the cellular IFN- γ response is partial, accounting for the relatively good prognosis of infections and the disease outcome is good, as no death related to MSMD has been reported in patients with AD STAT1 mutations.¹ Our case with STAT1 GOF mutation (case 11) had presented with a combined immunodeficiency phenotype at admission with a predisposition to various infections with bacteria, viruses, and fungi.²⁴ As IFN- γ and IFN- α induced STAT1 phosphorylation is enhanced in GOF STAT1 mutations, impaired response to IFN restimulation may be the cause of increased susceptibility to infections.²⁴ Positive mycobacterial evidence could not be confirmed during follow-up and molecular genetic analyses finally revealed the Th17-IL17 pathway defect. Sometimes mycobacterium isolation may be impossible in patients with immune deficiency; so, in this case, clinical, laboratory, and X-ray findings which show regression with anti-tuberculous treatment may help the diagnosis of mycobacterial disease.²⁵ Our female case is included in unrelated donor searching for HSCT because of recurrent infections.

XR NEMO deficiencies have been reported to have relatively mild disease and better prognosis.^{26,27} But our male case (case 12) showed initial symptoms in the fifth month, and during follow-up, he had brain abscess and deceased after a sepsis attack at the age of 2 years.

The mean follow-up time for these patients was approximately eight years and 75% survived after successful disease management with antibiotics, anti-tuberculous agents and recombinant IFN- γ . In conclusion, MSMD may be masked with different forms of PIDs, so clinicians should be aware of MSMD especially in patients with BCG vaccine complications

and non-tuberculous mycobacterial infection. The detection of the genetic defect is necessary to offer the best treatment options and genetic counseling, and therefore to decrease mortality. An increasingly-used approach for the evaluation of MSMD is to start with next-generation sequencing either with a gene panel or with whole exome/genome sequencing and then to perform functional tests to confirm the observed mutations. Early HSCT should be performed in IFN γ R completed defects before the development of complications and the patients with other MSMD must be followed-up and treated regularly to obtain a high survival rate.

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