Journal of Clinical Immunology (2019) 39:287–297
https://doi.org/10.1007/s10875-019-0593-4

ORIGINAL ARTICLE

Molecular, Immunological, and Clinical Features of 16 Iranian Patients with Mendelian Susceptibility to Mycobacterial Disease

Shokouh Azam Sarrafzadeh[1] · Maryam Nourizadeh[1] · Maryam Mahloojirad[1] · Mohammad Reza Fazlollahi[1] · Raheleh Shokouhi Shoormasti[1] · Mohsen Badalzadeh[1,2] · Caroline Deswarte[3,4] · Jean-Laurent Casanova[3,4,5,6,7] · Zahra Pourpak[1] · Jacinta Bustamante[3,4,6,8] · Mostafa Moin[1]

Received: 30 July 2018 / Accepted: 6 January 2019 / Published online: 4 February 2019

Abstract

Purpose Mendelian susceptibility to mycobacterial disease (MSMD) is a rare primary immunodeficiency, triggered by non-tuberculous mycobacteria or Bacillus Calmette-Guérin (BCG) vaccines and characterized by severe diseases. All known genetic etiologies are inborn errors of IFN-γ-mediated immunity. Here, we report the molecular, cellular, and clinical features of patients from 15 Iranian families with disseminated disease without vaccination (2 patients) or following live BCG vaccination (14 patients).

Methods We used whole blood samples from 16 patients and 12 age-matched healthy controls. To measure IL-12 and IFN-γ, samples were activated by BCG plus recombinant human IFN-γ or recombinant human IL-12. Immunological assessments and genetic analysis were also done for the patients.

Results Eight patients affected as a result of parental first-cousin marriages. Seven patients originated from multiplex kindred with positive history of death because of tuberculosis or finding the MSMD-related gene mutations. Two patients died due to mycobacterial disease at the ages of 8 months and 3.7 years. The remaining patients were alive at the last follow-up and were aged between 2 and 13 years. Patients suffered from infections including chronic mucocutaneous candidiasis ($n = 10$), salmonellosis ($n = 2$), and Leishmania (responsible for visceral form) ($n = 2$). Thirteen patients presented with autosomal recessive (AR) IL-12Rβ1 deficiency, meaning their cells produced low levels of IFN-γ. Bi-allelic *IL12RB1* mutations were detected in nine of patients. Three patients with AR IL-12p40 deficiency (bi-allelic *IL12B* mutations) produced low levels of both IL-12 and IFN-γ. Overall, we found five mutations in the *IL12RB1* gene and three mutations in the *IL12B* gene. Except one mutation in exon 5 (c.510C>A) of *IL12B*, all others were previously reported to be loss-of-function mutations.

Conclusions We found low levels of IFN-γ production and failure to respond to IL12 in 13 Iranian MSMD patients. Due to complicated clinical manifestations in affected children, early cellular and molecular diagnostics is crucial in susceptible patients.

Keywords Immunodeficiency · interleukin-12 · interferon-gamma · IL-12Rbeta1

✉ Jacinta Bustamante
jacinta.bustamante@inserm.fr

✉ Mostafa Moin
mmoin@sina.tums.ac.ir; https://www.iaari.tums.ac.ir

[1] Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, 4th Floor, Building no.3, Children's Medical Center, Gharib St, Keshavarz Blvd, 14185-863, Tehran, IR 14194, Iran

[2] Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran

[3] Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Paris, EU, France

[4] Imagine Institute, Paris Descartes University, Paris, EU, France

[5] Howard Hughes Medical Institute, New York, NY, USA

[6] St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller branch, The Rockefeller University, New York, NY, USA

[7] Pediatric Hematology-Immunology Unit, Necker Hospital for Sick Children, AP-HP, Paris, EU, France

[8] Center for the Study of Primary Immunodeficiencies, Necker Hospital for Sick Children, AP-HP, Paris, EU, France

Introduction

Mendelian susceptibility to mycobacterial diseases (MSMD) (OMIM20995) is a rare congenital syndrome. Patients with this condition suffer from infectious disease secondary to *Mycobacterium bovis*–Bacillus Calmette-Guérin (BCG) vaccination [1, 2]. Although it is commonly identified in children, it has been also reported in young adults [3]. The clinical manifestation of these patients is disseminated infections and loco-regional or recurrent infections by intracellular bacteria such as environmental mycobacteria (EM) and *Salmonella*. Patients also suffer from extra pulmonary infection by *M. tuberculosis*. As shown by de Beaucoudrey et al., one-fourth of patients can also develop chronic mucocutaneous candidiasis [4]. Sporadic cases have been reported with rare infections caused by *Listeria*, varicella zoster virus, Epstein-Barr virus (EBV), and herpes virus 8 [5, 6]. To date, 23 genetic etiologies have been found in MSMD patients, 15 of which are autosomal recessive (AR), six autosomal dominant (AD), and two X-linked recessive (XR). These partial or complete defects are located on different genes including *IFNGR1*, *IFNGR2*, *IL12RB1*, *IL12RB2, IL23R, IL12B*, *ISG15*, *STAT1*, *TYK2*, *IRF8*, *CYBB*, *NEMO*, and *SPPL2A* [2, 7, 8]. Complete IL-12Rβ1 deficiency, due to bi-allelic mutations in the *IL12RB1* gene, is the most common type of MSMD; it results in lack of expression or expression of non-functional IL-12Rβ1. Therefore, NK cells and activated T cells are unable to exert an appropriate immune response to intracellular pathogens in these patients [6]. 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 and lead to the secretion of IFN-γ. In turn, ligation of IFN-γ to its receptors on macrophages results to the intracellular killing of the ingested bacteria [9]. The pathogenesis of MSMD is mostly depended on either insufficient production or inadequate response to IFN-γ [9]. The IFN-γ circuit (loop) triggers an immune response to intracellular microbes, and any kind of defects in this loop can lead to complicated infections in these patients [10]. Besides BCG vaccination, these patients are susceptible to infection with other species of environmental mycobacteria [11, 12]. The ability of immune cells to produce IL-12/23 and IFN-γ in an appropriate stimulating setting of whole blood cells is an inexpensive, available, and quick way for early screening of patients who are suspected to suffer from MSMD [12].

In this study, we examined Iranian children with disseminated (BCG-osis) or loco-regional (BCG-itis) infection, following BCG vaccination or a history of previous intracellular infections. Based on the revised pediatric immunodeficiency classification, the phenotypes of BCG-induced disease were classified as BCG-itis with local and/or regional (loco-regional) patterns and BCG-osis with distant or disseminated patterns [13]. To understand the abnormalities in cytokine loop pathways, we measured whole blood IFN-γ/IL-12 cytokine levels in all patients studied here.

Materials and Methods

Patients' Characteristics

Patients who were suspected of MSMD and presented with complicated local or systemic reactions to BCG vaccination including unilateral/bilateral axillary or cervical lymphadenopathy, splenomegaly (identified by abdominal ultrasound evaluation), or a positive family history of BCG-itis/BCG-osis were entered into this study. They were referred to Immunology, Asthma and Allergy Research Institute (IAARI), at Tehran University of Medical Sciences, between 2012 and 2016. A questionnaire asking for demographic characteristics, history of disease onset and manifestations, parental consanguinity, previous admissions, and drug history was completed for each patient. Immunological evaluations for these patients included screening tests to assess humoral immunity (isohemagglutinin, three classes of immunoglobulins, and complements), phagocytic function, and cellular immunity (immunophenotyping and proliferation assay against phytohemagglutinin (PHA), BCG, and *Candida*). The cases with normal immunological function, except BCG-treated peripheral blood mononuclear cells (PBMCs) proliferation (but normal response to PHA) in a lymphocyte transformation test (LTT) setting, were evaluated for IL-12 and IFN-γ secretions. Patients with impaired cytokine release were assumed to have MSMD (16 patients) and cases with other forms of primary immunodeficiencies (PID) were excluded from the research. Finally, a genetic analysis was performed for the MSMD patients to confirm the disease. This study was approved by ethics committee of Tehran University of Medical Sciences (code number: 412/p/171) and informed consent forms were signed by patients or their legal guardians.

Immunological Assessments

Patients were evaluated for immunological elements and responses including the ability to produce immunoglobulins (Igs), oxidation burst activity of neutrophils [nitroblue tetrazolium (NBT) or dihydrorhodamine (DHR) test], complement system compartments, and immunophenotyping of B, T, and NK cells (evaluation of CD3, CD4, CD8, CD19, and CD16-CD56 by flow cytometry). They were also checked for HIV infection (virus copy number with PCR method). HIV negative patients with normal above mentioned immunological assessments were evaluated for polyclonal and specific stimulation of PBMCs using LTT assay. To study proliferation activity of patients' samples compared to the healthy controls, Stimulation index (SI) was determined against PHA (as a polyclonal activator), BCG, and *Candida* (as specific antigens).

PBMCs were isolated using density-gradient centrifugation on Ficoll-Histoprep, from 2 to 3 ml of heparinized blood. Then,

PBMCs were seeded in a 96-well flat bottom (Nunc, Denmark) tissue culture plates at a concentration of 1×10^5 cells/well containing 100 μl of RPMI-1640 (supplemented with 10% FCS) tissue culture medium. Cells were cultured in the presence of either BCG (*M. bovis* BCG, Pasteur® sub-strain) at an MOI of 20 BCG/leukocytes or 10^6 CFU/ml of heat-inactivated (60 °C for 30 min) *Candida albicans* (*C. albicans*) antigens (Strain 1386, DSMZ, Braunschweig, Germany). *C. albicans* were used at an effector/target ratio of 1:1. Positive and negative controls were established using unstimulated cells for negative control and PHA (7 μg/well, Gibco, USA)-stimulated cells as positive control. A healthy control sample was concomitantly tested in the same culture period and stimulation conditions. For every condition, each sample was assayed in triplicates. Then, the plate was incubated at 37 °C, by 5% CO_2 incubator. Cell proliferation was assessed using a colorimetric bromodeoxyuridine (5-bromo-2′-deoxyuridine) [BrdU] proliferation kit according to the manufacturer's instructions (Roche, Germany). In brief, an overnight incubation was done after adding BrdU on day 4. Thereafter, DNA was fixed and HRP-conjugated anti-Brdu antibody was used to detect Brdu incorporation to DNA. Enzyme-substrate interaction was detected as the change of colorless substrate to blue, using 1% sulfuric acid. The absorbance point was read at 450 nm within a maximum period of 30 min after adding stop solution by a micro plate spectrophotometer (Powerwave, Biotek, USA). Final results were calculated as a SI:

SI = OD of stimulated cells by PHA or antigens (mean of triplicates)/OD of unstimulated cells (mean of triplicates)

The cutoff values of LTT setting were determined using statistical analysis of the stimulation indices of 100 healthy BCG-vaccinated children under the age of 13 years (using the remains of their routine checkup examination after obtaining signed informed consent by their guardians). Then, LTT results of patients and age-matched healthy controls were analyzed using the cutoff values, determined as 3.4 (sensitivity = 80%, specificity = 73%), 2.3 (sensitivity and specificity = 99.62%), and 2.8 (sensitivity = 80%, specificity = 73%) for PHA, BCG, and *Candida*-stimulated PBMCs, respectively.

Cytokine Measurement

Since measuring IL-12 and IFN-γ levels are essential for identifying the loop defect, as previously described, we assessed these cytokines in an activated whole blood sample setting. [12]. Three to 5 ml heparinized whole blood samples were drawn from patients and age-matched healthy controls (n = 12; male: 5, female: 7). Then, blood samples were diluted 1:2 in RPMI 1640 supplemented with 100 U/ml penicillin and 100 g/ml streptomycin (Gibco, Thermo Fisher Scientific, USA) at a final volume of 1 ml dispensed into four separate wells of a 24-well plate. Then, cells were incubated 18 h with live BCG at an MOI of 20 BCG/leukocytes plus recombinant human IFN-γ (rhIFN-γ, 5000 IU/ml; Imukin, Boehringer Ingelheim) and 48 h with live BCG plus recombinant human IL-12 (rhIL-12, 20 ng/ml; R&D Systems, USA) for IL-12p70 and IFN-γ assessments, respectively. After finishing the incubation time, using ELISA kits (eBioscience, USA), cell-free supernatants were collected and stored at − 70 °C to measure the cytokines according to the manufacture's instruction. Two wells were also considered for medium alone and live BCG, as controls. The final results were standardized by expressing them as pg/ml/10^6 PBMC. Using blood cell count, the total number of PBMCs was determined on the first day of culture.

Genetic Study

Genomic DNA (gDNA) was obtained from patients, siblings, and parents' whole blood samples when they were available. gDNA (3 μg) of 12 MSMD patients was sheared with a Covaris S2 Ultrasonicator (Covaris). An adaptor-ligated library was prepared with the Paired-End Sample Prep Kit Illumina. Exome sequencing was performed on an Illumina Genome Analyzer to generate 72 base reads. Coding exons of *IL12RB1* or *IL12B* were amplified with specific primers (PCR amplification conditions and primers are available upon request) using Sanger sequencing method. PCR products were analyzed using electrophoresis in 1% agarose gel, sequenced with the Big Dye Terminator cycle sequencing kit (Applied Biosystems) and analyzed on a ABI Prism 3700 (Applied Biosystems).

Statistical Analysis

Data analysis was performed using SPSS version 20 (SPSS Inc. Chicago, IL). Kolmogorov-Smirnov test was applied to examine the normality of quantitative variables. Independent t test was used in order to compare the mean difference between two groups. ROC curve analysis was used to calculate cutoff values. A p value of less than 0.05 was considered as significant. Graphpad prism5 software was used for drawing the graphs.

Results

Clinical and Demographic Findings in 16 Patients with MSMD

In this study, we evaluated 16 patients (11 male and 5 female) from 15 Iranian families (P3 and P4 were siblings). These patients were suspected of having a PID and referred for further immunological evaluations. Demographic features, clinical manifestations, and concomitant infections of the patients are summarized in Table 1. Consanguinity was found in all patients' families. Parents of 8 patients were first-degree relatives, while the parents of the remaining individuals were

second-degree relatives. Family history of PIDs was positive in roughly 81% of the patients (13 out of 16). Patients belonged to five different regions of Iran: northeast ($n = 1$), northwest ($n = 2$), southeast ($n = 4$), southwest ($n = 1$), and central ($n = 8$) regions of the country. The mean age of the patients was equal to 5.1 ± 3.4 years (min = 1, max = 13) when evaluated for PID. Except for two patients with positive family history of MSMD and death of previous siblings (P4 and P6), all the remaining cases had been immunized with BCG vaccine due to lack of early diagnosis. All healthy controls in this study had been vaccinated against BCG without negative effects. Moreover, other infections including non-tuberculosis *Mycobacterium* spp. (P4 and P6), *Salmonella* (P7 and P8), *Leishmania* (responsible for visceral form) (P4 and P15) *Aspergillus* (P1), and *Influenza virus* (P9) were found among the patients. Accordingly, different organs were involved including the gastrointestinal tract (P3, P5, P8, P10, P11, and P13), esophagus (P3, P4, and P16), liver and spleen (all except for P3, P6, and P11), lung (P1, P3, P4, P6, P11, and P15), skin (P4, P7, P9 and P12), bone and joints (P7, P8, P14, and P16), brain (P7, P9, and P16), ear (P6), and nail (P3). Henoch-Schonlein purpura was also detected in P7 and P13 patients. A definitive genetic diagnosis of MSMD could help physicians start the appropriate broad-spectrum antibiotics for managing the infections; especially tuberculosis (isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin) and/or IFN-γ therapy. Accordingly, IFN-γ therapy was done for 7 patients (P3, P4, P5, P7, P8, P9, and P12) with genetically proven IFN-γ deficiency. All patients are now alive except for P10 (death at the age of 3.7 years) and P16 (death at the age of 10 months). P10 died because of disseminated BCG-osis and H1N1 influenza and P16 died with refractory ascites and electrolyte disturbances due to disseminated BCG-osis.

Immunological Findings in MSMD Patients

Laboratory findings of our patients (16 cases) are shown in Table 2. The HIV test results were negative for all patients. Serum levels of immunoglobulins (IgM, IgG, IgA, and IgE), phagocytic activity of neutrophils (normal results for NBT and DHR tests), the percentages, and numbers of B and T cells and CD4/CD8 ratio were normal in all patients.

Regarding the cutoff values of PHA-, BCG-, and *Candida*-stimulated PBMCs (as shown in materials and methods; 3.4, 2.3, and 2.8, respectively), impaired proliferation capacity of T cells was observed in the presence of live BCG, as a regular feature of MSMD. T cell responses were normal as determined using stimulation by PHA, while various responses were obtained among patients for an irrelevant antigen like *Candida*. The SI of PBMC proliferation against *Candida* antigen was below the cutoff values for some patients including P4, P5, P9, P10, P12, P14, and P15. These patients were considered for chronic mucocutaneous candidiasis (CMC)-related gene evaluation. Among 10 patients with clinical symptoms or past history of oral or precutaneous candidiasis, seven had an impaired response to *Candida* (as mentioned above) and three showed normal SI scores (Tables 2 and 3). Due to IFN-γ loop defects in MSMD patients, we evaluated the levels of IL-12p70 and IFN-γ in the cell-free supernatant of whole blood samples stimulated by BCG, with or without IFN-γ and IL-12, respectively (see the Materials and Methods). Cytokine assessment was simultaneously performed in patient samples and the age-matched healthy controls. Patterns of cytokines produced by each patient sample are shown in Table 3.

Only two patients had low levels of IL-12 (P7:1.5 and P15:0.8). This could be due to a genetic defect in *IL12B* gene or *IFNGR* receptor. Unlike IL-12, the majority of patients (13 out of 16) were unable to produce IFN-γ subsequent to IL-12 stimulation, which might be the result of *IL12RB1* gene mutations. LTT and cytokine assay were not done for patient P16 as he died when his parents were referred to IAARI (we had access to the patient's and his parent's DNA samples).

Genetic Analysis in MSMD Patients

Due to inadequate amounts of gDNA in certain cases, we were able to perform whole exome sequencing (WES) for 12 of the patients. Although Sanger sequencing was done for all patients to observe any probable defects in the most involved genes (*IL12RB1* and *IL12B*), no mutation was found in four cases (P1, P2, P6, and P11). As shown in Table 4, the genetic analysis of nine patients (56%) showed a homozygous mutation in *IL12RB1* gene which confirmed the impaired IFN-γ function of immune cells despite triggering by BCG + rhIL-12. We found a homozygous mutation of splice site intron 15 (c.1791+2T>G), in three patients (P3, P4, and P13) with defined mutation in *IL12RB1* (33%).Three patients (P7, P8, and P10) had a missense point mutation in exon 5 (c.517C>T), p.(Arg173Trp).

We evaluated chorionic villus sampling (CVS) for P9 at 10–12 weeks of gestation, using ultrasound-guided CVS in In Vitro Fertilization Research Center (IVFRC), Tehran University of Medical Sciences. Contamination with maternal DNA was excluded from DNA extracted from the fibroblasts obtained during CVS. We found homozygous mutation of the *IL12RB1* gene in the first fetus and the family was referred to Legal Medicine Organization for legal abortion. Fortunately, the second fetus of this family exhibited heterozygous mutation.

Familial segregation was performed for all families in this study. Three of 16 patients (P14, P15, and P16) (19%) showed a homozygous mutation in *IL12B* gene, which resulted in releasing very low amounts of IL-12p40. A small deletion was detected in exon 2 (c.35_44del), p.(Ser12Phe*12) or in exon 5 (c.527_528delCT) p.(Ser176Cysfs*12) of *IL12B* gene in two unrelated patients (P14 and P15), respectively (see

Table 1 Demographic features and clinical manifestations of 16 Iranian MSMD patients

Patients	Sex	Age at diagnosis (years)	Birth city/province	Ethnicities	Family history of PID	Kindred and genetic status	Lymphadenopathy	Consanguinity of parents	Clinical features
P1	F	6	Tehran/Capital city	Fars	Yes/brother and one cousin (died)	Two sisters and one brother (undefined)	Bilateral	Second cousin	BCG-osis, hepatosplenomegaly, aspergillosis, failure to thrive
P2	M	13	Kashan/Isfahan	Fars	No	Two sisters and two brothers (undefined)	Bilateral	Second cousin	BCG-itis, hepatosplenomegaly
P3	M	11	Rafsanjan/Kerman	Fars	Yes/brother	One brother (Homo), parents (Hetero)	Unilateral	Second cousin	Ulcerative colitis, apendicitis, Candidal esophagitis, hematochezia, ulcerative colitis, onychomycosis, pneumonia, inflammatory bowel disease
P4	M	4	Rafsanjan/Kerman	Fars	Yes/brother	One brother (Homo), parents (Hetero)	Bilateral	First cousin	Cutaneous TB, recurrent upper respiratory tract infection, esophageal varices, hepatosplenomegaly, percutaneous candidiasis
P5	F	10	Isfahan/Isfahan	Fars	Yes/cousin (died)	One sister (Hetero), parents (Hetero)	Unilateral	First cousin	BCG-osis, hepatosplenomegaly, diarrhea, inflammatory bowel disease, oral candidiasis
P6	M	4	Zahedan/Sistan and Baluchestan	Baloch	Yes/older sister (died)	Three brothers and two sisters (undefined)	Bilateral	Second cousin	Perforated otitis media, recurrent respiratory infection, pneumonia
P7	M	6	Zanjan/Zanjan	Turk	Yes/cousin (died)	Two sisters (Hetero), parents (Hetero)	Bilateral	First cousin	BCG-osis, hepatosplenomegaly, liver abscess, Henoch-Schonlein purpura, osteomyelitis, seizure, meningitis due to TB
P8	M	4	Shoush/Khuzestan	Arab	No	Two brothers and two sisters (undefined), parents (Hetero)	Bilateral	Second cousin	BCG-osis, pneumonia, salmonellosis, chronic diarrhea, hepatosplenomegaly, failure to thrive, hypo IgG, arthritis, osteomyelitis
P9	M	2	Isfahan/Isfahan	Fars	Yes/two brothers and one cousin (died)	One brother (Homo, died), one brother (Hetero) and cousin (Homo)	Unilateral	Second cousin	BCG-itis, hepatosplenomegaly, sepsis, liver abscess, percutaneous candidiasis, failure to thrive, seizure, meningitis, H1N1 influenza virus infection (cause of death)
P10	M	3	Tehran/Capital city	Fars	No	No brothers and sisters, parents (Hetero)	Unilateral	Second cousin	BCG-osis, hepatosplenomegaly, anemia, diarrhea, malabsorption, HIN1 influenza infection, oral candidiasis
P11	F	5	Mashhad/Razavi Khorasan	Fars	No	One brother (undefined)	Unilateral	First cousin	BCG-itis, chronic diarrhea, malabsorption, oral candidiasis, recurrent upper respiratory tract infection

Table 1 (continued)

Patients	Sex	Age at diagnosis (years)	Birth city/province	Ethnicities	Family history of PID	Kindred and genetic status	Lymphadenopathy	Consanguinity of parents	Clinical features
P12	M	3	Zabul/Sistan and Baluchestan	Baloch	Yes/two sisters, cousin (died)	Two sisters (Hetero)	Bilateral	Second cousin	BCG-osis, hepatosplenomegaly, oral candidiasis, erythroderma, failure to thrive
P13	F	3	Bam/Kerman	Fars	Not available	-undefined	Bilateral	First cousin	BCG-osis, hepatosplenomegaly, vasculitis, oral candidiasis, Henoch-Schönlein purpura, server diarrhea
P14	F	3	Tehran/Capital city	Fars	Yes/cousin (died)	No brothers and sisters, parents (undefined)	Bilateral	First cousin	BCG-itis, osteomyelitis, hepatosplenomegaly, oral candidiasis
P15	M	2	Zahedan/Sistan and Baluchestan	Baloch	Yes/cousin (died)	One brother (Hetero), parents (Hetero), cousin	Unilateral	First cousin	BCG-itis, hepatosplenomegaly, recurrent respiratory infection and pneumonia, oral candidiasis
P16	M	10 M	Tabriz/East Azerbaijan	Turk	Yes/cousin	No brother and sister, parents (Hetero)	Unilateral	First cousin	BCG-itis, failure to thrive, hepatosplenomegaly, septic arthritis, liver abscess, meningitis, sepsis, ascites due to BCG-osis (cause of death)

Homo: homozygous; hetero: heterozygous; PID: primary immunodeficiencies

Table 4 for all mutations). All these mutations have been reported previously.

Patient P16 had a nonsense mutation in exon 5 (c.510C>A), p.(Cys170*) of *IL12B* [1, 2, 14]. This mutation has not been previously reported in the "Human Gene Mutation Database (HGMD®)" and "Leiden Open Variation Database (LOVD)." Therefore, we are reporting a novel mutation in *IL12B* gene (Table 4).

Discussion

According to the Iranian national vaccination program, vaccination with live attenuated BCG sub-strain of *Mycobacterium bovis* (Pasteur1173p2) is required for all newborns except for those who are known to suffer from PIDs or HIV infection [16, 17]. Although BCG vaccination has a vital role in public health, it may endanger the lives of infants with a family history of PID, especially MSMD children who have genetic defects in producing IL-12 and/or IFN-γ, cytokines required to attack mycobacteria by phagocytic cells (monocytes and macrophages) [18, 19]. Although *IL12RB1* gene defect was found in nine MSMD patients (56%), more than 80% of patients were unable to produce IFN-γ (81%). For IL-12 non-responders with no genetic diagnosis, there might be an unknown mutation(s) in related genes that are not routinely checked for these patients. Whole exome sequencing is currently processed for these patients (P1, P2, P6, and P11). Our results showed lower incidence of *IL12B* mutation among our MSMD patients.

These results were in accordance with the report of Jacinta Bustamante *et al.* who showed that by 45% of MSMD patients who have been identified and reported from 16 countries had a gene mutation in one exons of *IL12RB1*, while only 12% of them were categorized in the *IL12B* gene defect group [2, 20, 21]. Also, persistent infectious to *Mycobacterium* together with *Candida* infections caused by IL-12Rβ1 deficiency has been reported in multicenter studies among 30 countries [22, 23]. In our study, concurrent mycobacteria and *Candida* infections were observed in 10 out of 16 MSMD patients.

There are various reports about specific deletion or substitution in the *IL12RB1* gene. As recently reported, some point mutations in intron 15 (c.1791+2T>G) are more common [21, 24]. Apparently, Iranian MSMD patients are mostly presented with *IL12RB1* and *IL12B* gene defects. All mutations have been reported in previously published papers, as loss-of-function (LOF) mutations. *IL12RB1* c.1791+2T>G mutation has been also described in different countries including Sri Lanka, China, Turkey, Saudi Arabia and Pakistan (Asia), Ukraine and Spain (Europe), Mexico (America), and Tunisia (Africa) [6, 9, 24–27, 29, 30]. Considering the distribution in five Asian countries, it might be considered a founder effect mutation. However, a more extensive study is necessary to understand the persistency of this mutation among different

Table 2 Immunological assessments of 16 Iranian MSMD patients

Patients	Age at diagnosis (years)	WBC (NR)	Lymph%* (NR)	CD3 count* (NR)	CD4 count* (NR)	CD8 count* (NR)	CD19 count* (NR)	CD4/CD8 (≥ 1.5)	IgAmg/dL (NR)	IgGmg/dL (NR)	IgM mg/dL (NR)	IgE total IU/ml (NR)	LTT (SI)		
													PHA Cutoffs	BCG	*Candida*
													3.4	2.3	2.8
P1	6	17.8 (5–14.5)	3.5 (1.5–7)	1.96 (1.4–3.7)	1.068 (0.7–2.2)	0.74 (0.49–1.3)	0.96 (0.02–1.4)	1.4	73 (33–202)	1300 (633–1280)	36 (48–207)	76 (1.03–161.3)	3.1	1.5	2.5
P2	13	8.5 (4.5–13.5)	4.5 (1.5–6.5)	1.9 (1–2.20)	2.7 (0.53–1.3)	1.22 (0.33–0.92)	0.75 (0–0.39)	2.2	76 (45–242)	957 (608–1572)	91 (52–242)	52 (2.6–195.2)	2.7	1.7	2.8
P3	11	13.7 (4.5–13.5)	6.1 (1.5–6.5)	3.9 (1.20–2.6)	1.98 (0.65–1.5)	1.52 (0.37–1.1)	0.53 (0–0.74)	1.3	114 (33–202)	1721 (633–1280)	180 (48–207)	360 (98–570.6)	3.9	2.12	ND
P4	4	9.8 (5.5–15.5)	2.6 (2–8)	1.53 (1.4–3. 7)	1.02 (0.7–2.2)	0.6 (0.49–1.3)	0.26 (0.02–1.4)	1.7	11 (25–154)	799 (463–1236)	28 (43–196)	15 (1.07–68.9)	3.9	1.2	1.7
P5	10	12 (4.5–13.5)	5 (1.5–6.5)	3.05 (1.2–2.6)	1.8 (0.65–1.5)	0.9 (0.37–1.1)	1.1 (0–0.74)	2	65	1263 (633–1280)	151 (48–207)	73 (98–570.6)	3.35	0.9	2
P6	4	9.8 (5.5–15.5)	4.7 (2–8)	2.3 (1.4–3.7)	1.41 (0.7–2.2)	0.84 (0.49–1.3)	1.12 (0.02–1.4)	1.7	58 (25–154)	1257 463–1236)	119 (43–196)	219 (1.07–68.9)	3.6	1.4	3.36
P7	6	9.6 (5–14.5)	4.2 (1.5–7)	2.2 (1.4–3.7)	1.19 (0.7–2.2)	0.84 (0.49–1.3)	1.3 (0.02–1.4)	1.4	152 (33–202)	19,300 (633–1280)	26.7 (48–207)	39 (1.03–161.3)	4.5	1.8	2.6
P8	4	9.5 .(5–15.5)	5 (2–8)	2.4 (1.4–3.7)	1.45 (0.7–2.2)	0.9 (0.49–1.3)	1.65 (0.02–1.4)	1.6	66 (25–154)	1584 (633–1280)	259 (43–196)	38 (1.07–68.9)	3.2	1.8	2.8
P9	2	16 (6–17)	9.92 (3–9.5)	6.5 (2.1–6.2)	3.7 (1.3–3.4)	2.2 (0.62–2)	1.57 (0–2.3)	1.7	105 (14–123)	1232 (424–1051)	60 (47–200)	27 (0.31–29.5)	2.8	0.8	2.5
P10	3	8.4 (6–17)	2.68 (3–9.5)	1.5 (1.4–3.7)	1.2 (0.7–2.2)	0.48 (0.49–1.3)	0.56 (0.02–1.4)	2.5	24 (22–159)	1234 (441–1135)	55 (47–200)	6.9 (0.19–16.9)	2.5	1.6	1.2
P11	5	12 (5.5–15.5)	4.8 (1.5–6.8)	3.1 (1.4–3.7)	2.1 (0.7–2.2)	0.86 (0.49–1.3)	1.4 (0.02–1.4)	2.4	89 (25–154)	1566 (463–1236)	90 (43–196)	3.9 (1.07–68.9)	2.7	1.6	3
P12	3	7.2 (6–17)	4.03 (3–9.5)	2.9 (1.4–3.7)	1.46 (0.7–2.2)	0.9 (0.49–1.3)	0.8 (0.02–1.4)	1.6	19 (22–159)	735 (441–1135)	73 (47–200)	0.9 (0.19–16.9)	3	1.9	1.6
P13	3	10 (6–17)	3.7 (3–9.5)	1.86 (1.4–3.7)	1.2 (0.7–2.2)	0.88 (0.49–1.3)	0.51 (0.02–1.4)	1.4	171 (22–159)	2067 (441–1135)	95 (47–200)	118 (0.19–16.9)	3	1.6	3
P14	3	13 (6–17)	7.15 (3–9.5)	3.6 (1.4–3.7)	2.5 (0.7–2.20)	1.5 (0.49–1.3)	1.56 (0.02–1.4)	1.6	19 (22–159)	1100 (441–1135)	73 (47–200)	240 (0.19–16.9)	2.7	1.1	1.8
P15	2	9.6 (6–17)	4.2 (3–9.5)	1.7 (1.4–3.7)	1.3 (0.7–2.2)	0.36 (0.49–1.3)	2.4 (0.02–1.4)	4	58 (14–123)	1057 (424–1051)	119 (47–200)	1.9 (0.31–29.5)	3	1.5	2.1
P16	10 M	12 (6–17.5)	7 (4–10.5)	4.8 (1.9–5.9)	3.2 (1.4–4.3)	1.7 (0.50–1.7)	1 (0.02–2.3)	1.89	19 (16–84)	351 (294–1069)	54 (41–149)	35.1 (0.8–15.2)	–	–	–

NR: normal range, Age-related reference range from The HARRIET LANE HANDBOOK, Jason W. Custer and Rachel E. Rau, Twenty-first Edition

SI, stimulation index

*Absolute counts (number of cells per microliter)

Table 3 IL-12p70 and IFN-γ secretion following stimulation of whole blood samples of MSMD patients in the presence of BCG, BCG plus rhIFN-γ, and BCG plus rhIL-12, respectively

Patients	IL-12p70 concentration after 18 h activation (pg/ml)			IFN-γ concentration after 48 h activation (pg/ml)			LTT (SI)		
	NS	BCG	BCG + IFN-γ	NS	BCG	BCG + IL-12	PHA	BCG	Candida
P1	82	142	1595	97	125	130	3.1	1.5	2.5
P2	55	292	2021	160	135	138	2.7	1.7	2.8
P3	16	237	2960	0	235	112	3.9	2.12	ND
P4	50	96	1186	50	40	68	3.9	1.2	1.7
P5	91	615	4110	54	40	68	3.35	0.9	2
P6	26	168	2600	126	158	93	3.6	1.4	3.36
P7	35	162	2894	16	38	50	4.5	1.8	2.6
P8	12	85	1060	37	47	52	3.2	1.8	2.8
P9	48	150	2015	12	18	22	2.8	0.8	2.5
P10	92	170	1386	13	18	22	2.5	1.6	1.2
P11	35	93	984	16	21	27	2.7	1.6	3
P12	15	51	840	8	17	13	3	1.9	1.6
P13	60	98	1520	32	58	39	3	1.6	3
P14	8	10	15	7	10	73	2.7	1.1	1.8
P15	18	25	23	29	125	820	3	1.5	2.1
P16	Child is not alive								

SI, stimulation index

generations and the common ancestor carries the mutation. In addition to Iran, *IL12RB1* c.517C>T mutation has been reported in Brazil, Venezuela, and Poland where it is not considered a founder effect [].

In this study, we found a novel mutation in the *IL12B* gene (P16). Interestingly, this was the second time we found a novel, un-reported mutation in Iranian MSMD patients. The first one was found in the *IL12RB1* gene and it has been previously published [31].

While the WES helps the geneticists to find the point mutations quickly in the patients, it has some limitations including difficulties in analyzing and interpreting the results and the cost of performing the test. Evidence suggests that one of the main reasons for the prevalence of this *IL12RB1* mutation (as an AR condition) is the high rate of consanguinity in some countries like Iran, Turkey, and Morocco [4]. In the present study, consanguinity was seen in all patients' parents. However, this cannot be the sole explanation, as there are AR forms of IFN-γR1 and IFN-γR2 deficiency, and even the related disorders of STAT1 and IRF8 deficiencies. At any rate, our study indicated that IL-12Rβ1 deficiency is by far the most common form of MSMD in Iran, while IL-12p40 is the second most common etiology, at least when manifested as a BCG disease. It is possible that patients without BCG disease (whether not vaccinated or who did not develop adverse reaction) and with environmental mycobacterial disease have a different genetic/etiological pattern. We confirmed the cytokine release pattern of the patients with their genetic analysis results [2, 23]. Concurrent with our results, previous studies have reported the allelic heterogeneities in *IL12RB1* and *IL12B* genes [3, 32].

When symptoms of infectious diseases manifested following BCG vaccination (11 of 15 cases), we emphasized that their families avoid injecting live vaccines, especially BCG at birth for their future newborns. As shown in the results, most patients suffered from non-tuberculosis infections following BCG vaccination. Although we did not find any alteration in *IFNGR1* or *IFNGR2* gene, it seems that these gene mutations represent a more severe form of disease due to a spectrum of complete to partial deficiency of IFN-γ. This leads to a higher mortality rate compared to other forms of gene defects [33]. A definitive diagnosis of MSMD could help physicians start the either appropriate broad-spectrum antibiotics for managing infections, especially tuberculosis (isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin) and/or IFN-γ therapy for patients with impaired IFN-γ production (as shown in the Results) [6, 34]. Performing the genetic analysis of MSMD-related genes following the cytokine assessment would be a more rapid diagnostic way than WES to determine safety for BCG vaccination.

In conclusion, MSMD is a group of genetic disorder, which can lead to complicated clinical manifestations, even death, in affected patients. Therefore, early prenatal or post-natal diagnosis is crucial in susceptible patients so that the most appropriate treatment protocols could be administered. Since the genome sequencing and genetic analysis of the patients are an expensive and time-consuming process, early screening of patients can be performed using cytokine assessment after excluding other types of PIDs.

Table 4 Genetic analysis of 16 Iranian MSMD patients. Point mutations were found in 9 patients for *IL12RB1* gene and 3 patients for *IL12B* gene

Sample	Gene	Position	Mutation	Result	Status of mutation	References*
Patient 1		No mutation was found in the known MSMD genes				
Patient 2		No mutation was found in the known MSMD genes				
Patient 3	*IL12RB1*	Intron 15	c.1791+2T>G	Splicing site defect	Homozygous	Iran [5, 14, 15], Sri Lanka [15], Spain [5, 15], China [16], Turkey [5], Ukraine [5], Mexico [5, 17], Saudi Arabia [5], Pakistan [18], Tunisia [19]
Father	*IL12RB1*	Intron 15	c.1791+2T>G	Splicing site defect	Heterozygous	
Mother	*IL12RB1*	Intron 15	c.1791+2T>G	Splicing site defect	Heterozygous	
Patient 4	*IL12RB1*	Intron 15	c.1791+2T>G	Splicing site defect	Homozygous	As shown for P3
Father	*IL12RB1*	Intron 15	c.1791+2T>G	Splicing site defect	Heterozygous	
Mother	*IL12RB1*	Intron 15	c.1791+2T>G	Splicing site defect	Heterozygous	
Patient 5	*IL12RB1*	Exon 10	c.1172delC	p.(Pro391Argfs*63)	Homozygous	Iran [20]
Father	*IL12RB1*	Exon 10	c.1172delC	p.(Pro391Argfs*63)	Heterozygous	
Mother	*IL12RB1*	Exon 10	c.1172delC	p.(Pro391Argfs*63)	Heterozygous	
Sister	*IL12RB1*	Exon 10	c.1172delC	p.(Pro391Argfs*63)	Heterozygous	
Patient 6		No mutation was found in the known MSMD genes				
Patient 7	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Homozygous	Iran [21], Brazil [5], Venezuela [5], Poland [5]
Father	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Heterozygous	
Mother	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Heterozygous	
Sister	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Heterozygous	
Sister	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Heterozygous	
Patient 8	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Homozygous	As shown for P7
Father	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Heterozygous	
Mother	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Heterozygous	
Patient 9	*IL12RB1*	Exon 9	c.847C>T	p.(Arg283*)	Homozygous	Faroe Islands [22]
Father	*IL12RB1*	Exon 9	c.847C>T	p.(Arg283*)	Heterozygous	
Mother	*IL12RB1*	Exon 9	c.847C>T	p.(Arg283*)	Heterozygous	
Mother's CVS1	*IL12RB1*	Exon 9	c.847C>T	p.(Arg283*)	Homozygous	
Mother's CVS2	*IL12RB1*	Exon 9	c.847C>T	p.(Arg283*)	Heterozygous	
Patient 10	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Homozygous	As shown for P7
Father	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Heterozygous	
Mother	*IL12RB1*	Exon 5	c.517C>T	p.(Arg173Trp)	Heterozygous	
Patient 11		No mutation was found in the known MSMD genes				
Patient 12	*IL12RB1*	Intron 8	c.783+1G>A	Splicing site defect	Homozygous	Turkey [5, 23–26], Sweden [15], Iran [27]
Father	*IL12RB1*	Intron 8	c.783+1G>A	Splicing site defect	Heterozygous	

Table 4 (continued)

Sample	Gene	Position	Mutation	Result	Status of mutation	References*
Mother	*IL12RB1*	Intron 8	c.783+1G>A	Splicing site defect	Heterozygous	
Sister	*IL12RB1*	Intron 8	c.783+1G>A	Splicing site defect	Heterozygous	
Patient 13	*IL12RB1*	Intron 15	c.1791+2T>G	Splicing site defect	Homozygous	As shown for P3
	Mother and father are not alive					
Patient 14	*L12B*	Exon 2	c.35_44del	p.(Ser12Phe*12)	Homozygous	Iran [28]
Patient 15	*IL12B*	Exon 5	c.527_528delCT	p.(Ser176Cysfs*12)	Homozygous	Iran [28–30]
Father	*IL12B*	Exon 5	c.527_528delCT	p.(Ser176Cysfs*12)	Heterozygous	
Mother	*IL12B*	Exon 5	c.527_528delCT	p.(Ser176Cysfs*12)	Heterozygous	
Patient 16	*IL12B*	Exon 5	c.510C>A	p.(Cys170*)	Homozygous	Novel mutation
Father	*IL12B*	Exon 5	c.510C>A	p.(Cys170*)	Heterozygous	
Mother	*IL12B*	Exon 5	c.510C>A	p.(Cys170*)	Heterozygous	

* References of patients previously reported with the same mutation loss of fonction

Acknowledgments We would like to thank the patients and their families. We also thank Yelena Nemirovskaya, Cecile Patissier, and Céline Desvallées for their assistance. We would like to thank Nastaran Sabetkish and Fatemeh Talebian for English editing of the manuscript. This research was funded in part by a grant from the National Institute of Allergy and Infectious Diseases grant number 5R01AI089970, The Rockefeller University, the St. Giles Foundation, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris Descartes University, Laboratoire d'Excellence Integrative Biology of Emerging Infectious Diseases (ANR-10-LABX-62-IBEID), the French National Research Agency (ANR) under the "Investments for the future" (grant number ANR-10-IAHU-01) and ANR-GENMSMD (ANR-16-CE17-0005-01 for JB). This project has been granted and supported by Immunology, Asthma and Allergy Research Institute under the supervision of Tehran University of Medical Sciences (grant number: 89-33-1/253-1).

Financial Disclosure The authors have no financial relationships relevant to this article to disclose.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Research Involving Human Participants Informed consent for participation in this study was obtained in accordance with local regulations, with approval from ethics committee of Tehran University of Medical Sciences (TUMS). The experiments described here were performed in Iran and France, in accordance with local regulations, and with the approval of the TUMS for Immunology, Asthma and Allergy Research Institute (IAARI), Tehran-Iran; and for Necker Hospital for Sick Children, France.

Informed Consent Written informed consent was obtained from the patients.

References

1. Quispel WT, Stegehuis-Kamp JA, Santos SJ, van Wengen A, Dompeling E, Egeler RM, et al. Intact IFN-gammaR1 expression and function distinguishes Langerhans cell histiocytosis from Mendelian susceptibility to mycobacterial disease. J Clin Immunol. 2014;34(1):84–93. https://doi.org/10.1007/s10875-013-9959-1.
2. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-gamma immunity. Semin Immunol. 2014;26(6):454–70.
3. Mansouri D, Adimi P, Mirsaedi M, Mansouri N, Tabarsi P, Amiri M, et al. Primary immune deficiencies presenting in adults: seven years of experience from Iran. J Clin Immunol. 2005;25(4):385–91.
4. Ouederni M, Sanal O, Ikinciogullari A, Tezcan I, Dogu F, Sologuren I, et al. Clinical features of Candidiasis in patients with inherited interleukin 12 receptor beta1 deficiency. Clin Infect Dis. 2014;58(2):204–13.
5. Aytekin C, Dogu F, Tuygun N, Tanir G, Guloglu D, Boisson-Dupuis S, et al. Bacille Calmette-Guerin lymphadenitis and recurrent oral candidiasis in an infant with a new mutation leading to

interleukin-12 receptor beta-1 deficiency. J Investig Allergol Clin Immunol. 2011;21(5):401–4.
6. de Beaucoudrey L, Samarina A, Bustamante J, Cobat A, Boisson-Dupuis S, Feinberg J, et al. Revisiting human IL-12Rbeta1 deficiency: a survey of 141 patients from 30 countries. Medicine. 2010;89(6):381–402.
7. Kong XF, Martinez-Barricarte R, Kennedy J, Mele F, Lazarov T, Deenick EK, et al. Disruption of an antimycobacterial circuit between dendritic and helper T cells in human SPPL2a deficiency. Nat Immunol. 2018;19(9):973–85.
8. Martinez-Barricarte R, Markle JG, Ma CS, Deenick EK, Ramirez-Alejo N, Mele F, et al. Human IFN-g immunity to mycobacteria is governed by both IL-12 and IL-23. Sci Immunol. 2018; 3(30). https://doi.org/10.1126/sciimmunol.aau6759.
9. Ramirez-Alejo N, Santos-Argumedo L. Innate defects of the IL-12/IFN-gamma axis in susceptibility to infections by mycobacteria and salmonella. J Interf Cytokine Res. 2014;34(5):307–17.
10. Lee WI, Huang JL, Lin TY, Hsueh C, Wong AM, Hsieh MY, et al. Chinese patients with defective IL-12/23-interferon-gamma circuit in Taiwan: partial dominant interferon-gamma receptor 1 mutation presenting as cutaneous granuloma and IL-12 receptor beta1 mutation as pneumatocele. J Clin Immunol. 2009;29(2):2385.
11. Caragol I, Raspall M, Fischi C, Feinberg J, Larrosa MN, Hernandez M, et al. Clinical tuberculosis in 2 of 3 siblings with interleukin-12 receptor beta1 deficiency. Clin Infect Dis. 2003;37(2):302–6.
12. Casanova JL, Abel L. Genetic dissection of immunity to mycobacteria: the human model. Annu Rev Immunol. 2002;20: 581–620. https://doi.org/10.1146/annurev.immunol.20.081501.125851.
13. Hesseling AC, Rabie H, Marais BJ, Manders M, Lips M, Schaaf HS, et al. Bacille Calmette-Guerin vaccine-induced disease in HIV-infected and HIV-uninfected children. Clin Infect Dis. 2006;42(4): 548–58. https://doi.org/10.1086/499953.
14. Carvalho FM, Busser FD, Freitas VL, Furucho CR, Sadahiro A, Kono AS, et al. Polymorphisms on IFNG, IL12B and IL12RB1 genes and paracoccidioidomycosis in the Brazilian population. Infect Genet Evol. 2016; 43:245–51.
15. Prando C, Samarina A, Bustamante J, Boisson-Dupuis S, Cobat A, Picard C, et al. Inherited IL-12p40 deficiency: genetic, immunologic, and clinical features of 49 patients from 30 kindreds. Medicine. 2013;92(2):109–22.
16. Khotaei GT, Sedighipour L, Fattahi F, Pourpak Z. Osteomyelitis as a late complication of Bacille Calmette-Guérin vaccination. J Microbiol Immunol Infect. 2006;39(2):169–72.
17. Yeganeh M, Heidarzade M, Pourpak Z, Parvaneh N, Rezaei N, Gharagozlou M, et al. Severe combined immunodeficiency: a cohort of 40 patients. Pediatr Allergy Immunol. 2008;19(4):303–6.
18. Al-Muhsen S, Casanova JL. The genetic heterogeneity of Mendelian susceptibility to mycobacterial diseases. J Allergy Clin Immunol. 2008;122(6):1043–51.
19. Lee PP. Disseminated Bacillus Calmette-Guerin and susceptibility to mycobacterial infections-implications on Bacillus Calmette-Guerin vaccinations. Ann Acad Med Singap. 2015;44(8):297–301.
20. Fieschi C, Bosticardo M, de Beaucoudrey L, Boisson-Dupuis S, Feinberg J, Santos OF, et al. A novel form of complete IL-12/IL-23 receptor beta1 deficiency with cell surface-expressed nonfunctional receptors. Blood. 2004;104(7):2095–101.
21. Rosain J, Oleaga-Quintas C, Deswarte C, Verdin H, Marot S, Syridou G, et al. A variety of Alu-mediated copy number variations can underlie IL-12Rbeta1 deficiency. J Clin Immunol. 2018.
22. de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M, et al. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. J Exp Med. 2008;205(7):1543–50.
23. van de Vosse E, Haverkamp MH, Ramirez-Alejo N, Martinez-Gallo M, Blancas-Galicia L, Metin A, et al. IL-12Rbeta1 deficiency: mutation update and description of the IL12RB1 variation database. Hum Mutat. 2013;34(10):1329–39.
24. Cleary AM, Tu W, Enright A, Giffon T, Dewaal-Malefyt R, Gutierrez K, et al. Impaired accumulation and function of memory CD4 T cells in human IL-12 receptor beta 1 deficiency. J Immunol (Baltimore, Md : 1950). 2003;170(1):597–603.
25. Fieschi C, Dupuis S, Catherinot E, Feinberg J, Bustamante J, Breiman A, et al. Low penetrance, broad resistance, and favorable outcome of interleukin 12 receptor beta1 deficiency: medical and immunological implications. J Exp Med. 2003;197(4):527–35.
26. Lee PP, Jiang LP, Wang XC, Chan KW, Tu WW, Lau YL. Severe mycobacterial infections in two pairs of Chinese siblings with interleukin-12 receptor beta1 deficiency. Eur J Pediatr. 2008;167(2):231–2.
27. Zahid MF, Ali SA, Jehan F, Billo AG, Casanova JL, Bustamante J, et al. Recurrent salmonellosis in a child with complete IL-12Rbeta1 deficiency. J Immunodefic Disord. 2014;3.
28. Feinberg J, Fieschi C, Doffinger R, Feinberg M, Leclerc T, Boisson-Dupuis S, et al. Bacillus Calmette Guerin triggers the IL-12/IFN-gamma axis by an IRAK-4- and NEMO-dependent, non-cognate interaction between monocytes, NK, and T lymphocytes. Eur J Immunol. 2004;34(11):3276–84.
29. Khamassi I, Ben Ali M, Ben Mustapha I, Barbouche MR, Bejaoui M, Bouyahia O, et al. Salmonella enteriditis inducing cutaneous leucocytoclasic vasculitis: an unusual complication in a patient with an interleukine-12 receptor beta-1 deficiency. La Tunisie medicale. 2015;93(5):328–9.
30. Pedraza-Sanchez S, Herrera-Barrios MT, Aldana-Vergara R, Neumann-Ordonez M, Gonzalez-Hernandez Y, Sada-Diaz E, et al. Bacille Calmette-Guerin infection and disease with fatal outcome associated with a point mutation in the interleukin-12/interleukin-23 receptor beta-1 chain in two Mexican families. Int J Infect Dis. 2010;14(Suppl 3):e256–60.
31. Sarrafzadeh SA, Mahloojirad M, Nourizadeh M, Casanova JL, Pourpak Z, Bustamante J, et al. Mendelian susceptibility to mycobacterial disease due to IL-12Rbeta1 deficiency in three Iranian children. Iran J Public Health. 2016;45(3):370–5.
32. Boisson-Dupuis S, El Baghdadi J, Parvaneh N, Bousfiha A, Bustamante J, Feinberg J, et al. IL-12Rbeta1 deficiency in two of fifty children with severe tuberculosis from Iran, Morocco, and Turkey. PLoS One. 2011;6(4):e18524.
33. Gutierrez MJ, Kalra N, Horwitz A, Nino G. Novel mutation of interferon-gamma receptor 1 gene presenting as early life mycobacterial bronchial disease. J Investig Med High Impact Case Rep. 2016;4(4):2324709616675463.
34. Ying W, Sun J, Liu D, Hui X, Yu Y, Wang J, et al. Clinical characteristics and immunogenetics of BCGosis/BCGitis in Chinese children: a 6 year follow-up study. PLoS One. 2014;9(4):e94485.