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ORIGINAL ARTICLE

# Current Status of the Management of Mendelian Susceptibility to Mycobacterial Disease in Mainland China

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## Abstract

**Purpose** Although many studies have investigated Mendelian susceptibility to mycobacterial disease (MSMD) worldwide, there is no report of the long-term clinical management and prognosis for MSMD in China.

**Methods** This is a cohort study from January 2000 to June 2018. Three hundred and twenty-four patients with bacillus Calmette-Guérin (BCG) infection were diagnosed during this period, and those with MSMD diagnosed by genetic and functional experiments were enrolled in the study. The clinical and genetic characteristics and management of these MSMD patients were summarized.

**Results** Thirty patients diagnosed with MSMD were followed up. The age at the follow-up end point ranged from 5 to 173 months. Among the patients, IL12RB1 mutations were identified in 22, IFNGR1 mutations in 5, STAT1 mutations in 2, and IFNGR2 mutation in 1. The medium age at onset was 3 months. BCG infection involved multiple organs, including regional infection (8/30; 26.7%) or distant or disseminated infection (22/30; 73.3%). Ten percent (30/324) of patients with BCG infection had a confirmed MSMD diagnosis. Protein expression of IL12RB1 or IFNGR1 was decreased in all patients with IL12RB1 or IFNGR1 mutation, respectively, as indicated by flow cytometry. In addition, 77.8% of patients received rhIFN-γ treatment, which can improve the prognosis of patients with IL12RB1 deficiency. Two patients received stem cell transplantation. Twenty-five patients remained alive at the time of publication.

**Conclusion** MSMD is an important cause of BCG infection. Flow cytometric detection of IL12RB1 and IFNGR1 expression is very useful for rapid MSMD diagnosis. rhIFN-γ therapy is effective in patients with MSMD, particularly improving prognosis in those with IL12RB1 deficiency.

**Keywords** *Mycobacterium* infection · immunodeficiency · IFN-gamma · interleukin-12 receptor beta 1 subunit · therapy

## Introduction

Mendelian susceptibility to mycobacterial disease (MSMD) is a rare primary immunodeficiency that confers a selective predisposition toward weakly virulent mycobacteria, such as the bacillus Calmette-Guérin (BCG) vaccine as well as non-tuberculous and environmental mycobacteria (Online Mendelian Inheritance in Man [OMIM] 209950) [1, 2]. The molecular basis of this genetic vulnerability was deciphered by the identification of 13 disease-causing genes, including two X-linked (*CYBB* and *NEMO*) and 13 autosomal (*IFNGR1*, *IFNGR2*, *STAT1* (LOF), *IL12B*, *IL12RB1*, *ISG15*, *IRF8*, *TYK2*, *RORC*, *JAK1* (LOF), *SPPL2a*, *IL-12RB2*, and *IL-23R*) genes [3–10]. Mutations in *JAK1* and *RORC* have been described as responsible for syndromic MSMD [11]. MSMD-causing genes affect IFN-γ immunity, in terms of

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either IL-12/IL-23/ISG15-dependent induction of IFN-γ or IFN-γ cellular responses.

China remains one of 20 countries with a high TB burden recognized by the World Health Organization [12]. Although the prevalence of TB in China fell slightly during the past decade, the country still has the world's second largest population infected with this pathogen [13]. The Chinese Center for Disease Control and Prevention mandates that all infants receive a single dose of the BCG vaccine immediately after birth. For most children, BCG vaccination is harmless; however, infection, even disseminated infection, caused by BCG has been reported in patients with immunodeficiency [11, 14–16]. In contrast to other regions, MSMD remains a great challenge in China due to BCG vaccination [17]. Despite in-depth scientific studies of MSMD, the development of effective approaches for diagnosing and treating MSMD in clinical practice requires further exploration. In addition to antituberculosis drugs, rhIFN-γ therapy is considered to be an effective treatment for improving the prognosis of MSMD [18], yet there are few reports concerning long-term follow-up of the clinical management of MSMD. Here, we summarized the diagnosis, treatment, and prognosis of MSMD patients recruited from a single center in Shanghai from 2000 to 2018.

## Methods

This study was approved by the Ethics Committee of the Children's Hospital of Fudan University. All parents of patients provided written informed consent for enrollment in this study (clinical trial registration No. NCT02590328).

### Patients

Patients with BCG infection who visited the Children's Hospital of Fudan University from January 2000 to June 2018 were enrolled. BCG infection was diagnosed based on clinical course, imaging features, etiology, and pathology. Patients with MSMD among those with BCG infection are reported in this study.

### Diagnostic Criteria for Bacillus Calmette-Guérin Disease

We diagnosed highly probable BCG infections based on a history of BCG vaccination and evidence of dissemination based on pathology/microbiology, including clinical specimens positive for the *Mycobacterium tuberculosis complex* based on culture, acid-fast stain, and histological detection of caseating granuloma. All patients were screened for HIV and excluded "if the test was positive."

### Routine Evaluation of Immunological Function

As previously reported [17], routine immunological function was evaluated, including the following: lymphocyte subsets; immunoglobulins G, A, M, and E; complement factors C3, C4, and CH50; and NADPH oxidase activity in neutrophils (see Supplement 1).

### Functional Evaluation of the IL-12/IFN-γ Pathway

Flow cytometry detection of CD119 and CD212 was performed for all patients with BCG infection (see Supplements 2 and 3 for experimental methods). Production of IFN-γ in whole blood after stimulation with medium alone, LPS, or LPS plus IL-12 respectively was assessed by ELISA in patients with confirmed genetic mutation.

### Gene Sequencing and Analysis

Next-generation sequencing (NGS) of previously identified immunodeficiency genes, including all genes listed in the classification from the International Union of Immunodeficiency Society [16], was performed for all of the patients diagnosed with BCG infection. Reads were mapped to the human reference genome hg19 using Burrows-Wheeler Aligner (BWA). The average coverage sequencing depth for official targets was at least 180× and was higher than 20× for > 99% of the target region. Variants were called in accordance with the gold standard of GATK Best Practices. Deleterious mutations and novel variants detected by NGS were confirmed via Sanger sequencing. PolyPhen-2, sorting intolerant from tolerant (SIFT), and PyMOL (version 1.6.1) were used to predict the pathogenic effects of unreported variants.

### Phosphorylated STAT1 Protein Detection by Western Blotting

Peripheral blood mononuclear cells ($1 \times 10^6$ cells/mL) from patients and a normal control were incubated for 30 min with $10^5$ IU/mL IFN-γ. We used NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, USA) to extract cytoplasmic and nuclear proteins. Equal amounts of cytoplasmic or nuclear extracts were separated on 12% SDS polyacrylamide gels and transferred to PVDF membranes. Phosphorylated STAT1 was detected using STAT1 antibodies (BD Transduction Laboratories, San Jose, USA). Primary antibodies were detected with a horseradish peroxidase-conjugated secondary antibody. Visualization was conducted using an ECL peroxidase substrate.

### Statistical Analysis

Data were analyzed using SPSS 19.0. Categorical variables are displayed as percentiles. Kaplan-Meier survival analysis was applied to compare the effect of IFN-γ treatment. A $P$ value < 0.05 was considered statistically significant.

## Results

### Overview of the Cases

From January 2000 to June 2018, 324 patients with BCG infection were diagnosed at our center. All patients were screened for HIV and excluded if the test was positive. In total, 89 patients were confirmed as having defined primary immunodeficiency disease. Thirty cases (9.25%) were diagnosed with MSMD, 39 (12.0%) with chronic granulomatous disease (CGD), and 20 (6.17%) with other primary immunodeficiency diseases, such as severe combined immunodeficiency (5.5%), hyper-IgM syndrome (0.3%), and hyper-IgE syndrome (0.3%).

Thirty MSMD patients were analyzed in this study. Seventeen (56.7%) were male, and 13 (43.3%) female. The majority of these patients were vaccinated with BCG within 3 days after birth, except for 2 patients whose vaccination was delayed until 42 days after birth due to "wet lung of the newborn" and one patient who was vaccinated at 19 months old because of congenital heart disease. All patients received the Danish strain BCG823. None of these patients had a history of TB contact. Seventy percent (21/30) of the patients exhibited clear evidence of *Mycobacterium bovis* BCG pathogens, based on fine-needle aspirate or swab samples of lymph nodes (17/30), skin pus cultures (10/30), bone marrow cultures (2/30), or pathological indications on bone biopsy (1/30).

### Clinical Characteristics

#### Onset of BCG Infection

The age at onset ranged from 20 days to 19 months, including 73.3% (22/30) of patients with onset at 3 months of age or less and 96.7% (29/30) of patients with onset at 4 months of age or less. Only one patient developed BCGitis at 19 months because the vaccination was delayed until the age of one and a half years to avoid the risk of congenital heart disease. Symptoms at onset included inoculation site infection (26.7%, 8/30) and left axillary lymphadenectasis (73.3%, 22/30) (Table S1).

#### BCG Infection Classification

BCG infection was classified as local, regional, distant, or disseminated according to a previous study [18]. Among the 30 patients, none had local infection, 8 (26.7%) presented regional infection, 3 (10.0%) presented distant infection, and 19 (63.3%) presented disseminated infection.

#### Regional BCG Infection

Among the 30 patients, 8 (26.7%) were diagnosed with regional infection and presented with draining axillary lymph node enlargement. However, 93.3% (28/30) of the patients presented with lymphadenopathy, including distant and disseminated infections (Fig. 1). Lymph node puncture or swab or pus sample collection was performed for 56.7% (17/30) of the patients, and *M. bovis* BCG was isolated from cultures and confirmed by PCR. Lymph node resection was performed in five patients, and incision and drainage were performed in ten. Pathologic findings of biopsies included neutrophil and epithelial cell infiltration, coagulation necrosis, and granuloma formation.

#### Distant BCG Infection

Ten percent (3/30) of patients were diagnosed with distant BCG infection. Two presented with generalized lymphadenopathy, and the other with skin infection. BCG infection was confirmed by right or inguinal lymph node aspiration and skin pus culture.

#### Disseminated BCG Infection

Disseminated BCG infection was diagnosed in 63.3% (19/30) of patients, and 53.3% (16/30) had generalized lymphadenopathy, including cervical lymph node, inguinal lymph node, and right axillary lymph node involvement. Other common infection sites included the lung (36.7%, 11/30), skin and soft tissue (33.3%, 10/30), bone (20.0%, 6/30), peritoneum (13.3%, 4/30), gastrointestinal tract (6.7%, 2/30), central nervous system (6.7%, 2/30), spleen (6.7%, 2/30), and throat (3.3%, 1/30).

#### Other Infectious Diseases

Among the 30 patients in our study, 13.3% (4/30) presented with recurrent thrush caused by *Candida albicans*. Two patients also had *Salmonella enteritidis* infections at 1 month and 3 years old, respectively, with poor response to antibiotic therapy. *Klebsiella pneumoniae* was found in the sputum sample from two patients, and *Escherichia coli* and *Staphylococcus aureus* were cultured from two patients with skin abscesses. Non-tuberculous mycobacteria from either ascites or bone marrow culture were found in two patients.

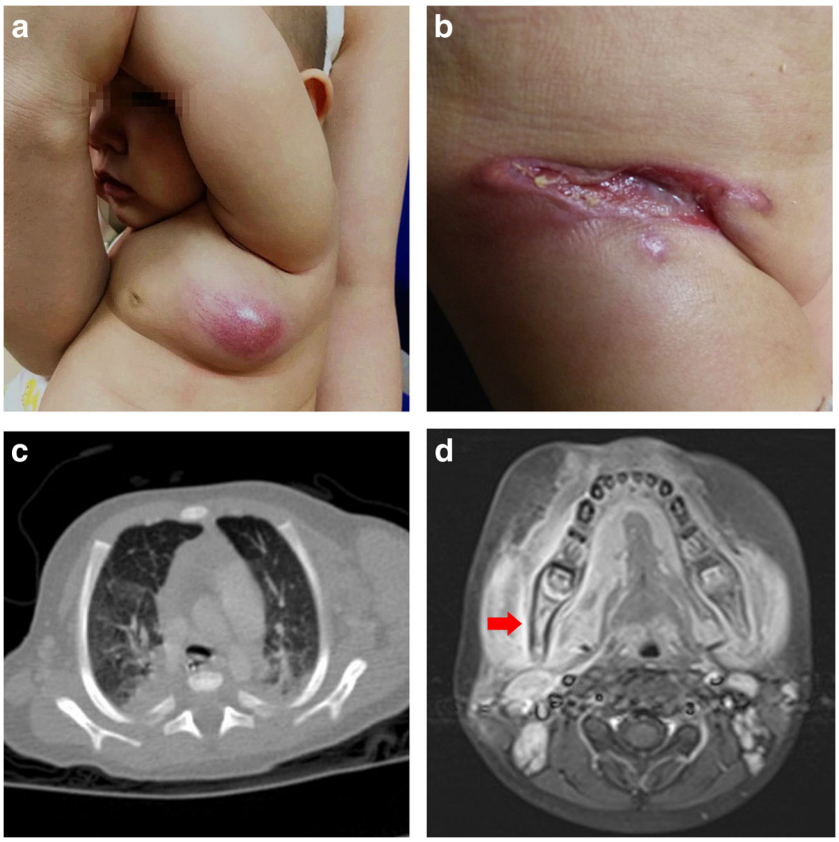


**Fig. 1** Clinical manifestations of MSMD. **a** An enlarged left axillary lymph node with redness and tenderness on the skin surface. **b** Groin abscess. **c** Lung infection on chest CT. **d** Bone destruction of the mandible (arrow)

## Laboratory Examination and Immunologic Characteristics

Among the 30 patients, 60% (18/30) had mild or moderate anemia, and 3.3% (1/30) had thrombocytopenia. Only two patients with cavernous transformation of the portal vein had abnormal liver function. In addition, the purified protein derivative (PPD) test was positive in 33.3% (10/30) of patients. Routine evaluation of immunological function was recorded for 25 patients (Table S2). A decreased CD3+ T cell proportion with a normal absolute count was detected in four patients. Elevated immunoglobulin levels, including IgG, M, A, and E, were observed in approximately half of the patients (43.3%) at the time of diagnosis. NADPH oxidase activity in neutrophils was normal in all patients.

## Gene Sequencing

Among the 30 patients, 22 (73.3%) had IL12RB1 mutations, 5 (16.7%) IFNGR1 mutations, 2 (6.7%) STAT1 mutations, and 1 (3.3%) IFNGR2 mutation (Fig. 2).

Seven patients with IL12RB1 deficiency were homozygous, with the other 15 patients being compound heterozygous. The parents of P16 were consanguineous. Among these patients, 21 mutant alleles were found, and 12 mutations were not reported in the Human Gene Mutation Database (HGMD) [17–25]. These mutations were distributed along the whole gene, including missense (33.3%, 7/21), nonsense (19.0%, 4/21), splicing (19.0%, 4/21), and frameshift (19.0%, 4/21) mutations, as well as large deletions (9.5%, 2/21). Interestingly, 59.1% (13/22) of the IL12RB1 deficiency patients in our cohort carried a mutation at position 211, which occurred at a higher frequency. Expression of IL12RB1 was detected in 20 cases (the other two were not tested) and CD212 was not expressed in any patient with IL12RB1 deficiency (Fig. 4). After 48 h of culture with LPS, IFN-γ levels were low (0.1–21.7 pg/ml) and mildly rescued after the addition of IL-12 (12.3–99.4 pg/ml) (Fig. 3). However, this change was not significant compared to the control, which showed high IFN-γ concentrations after the addition of IL-12 (mean, 921.51 pg/ml).

Five patients were found to have IFNGR1 deficiency, including three with homozygous mutations and two with compound heterozygous mutations. Five mutant alleles are located in the extracellular region, including three missense and two frameshift mutations. G219R and c.114-135del have been reported previously [26, 27]. Disseminated BCG infection developed in 80.0% (4/5) of patients with IFNGR1 deficiency, and expression of IFNGR1 was absent (3/5) or reduced (2/5) in patients with IFNGR1 deficiency (Fig. 4). After 48 h of culture

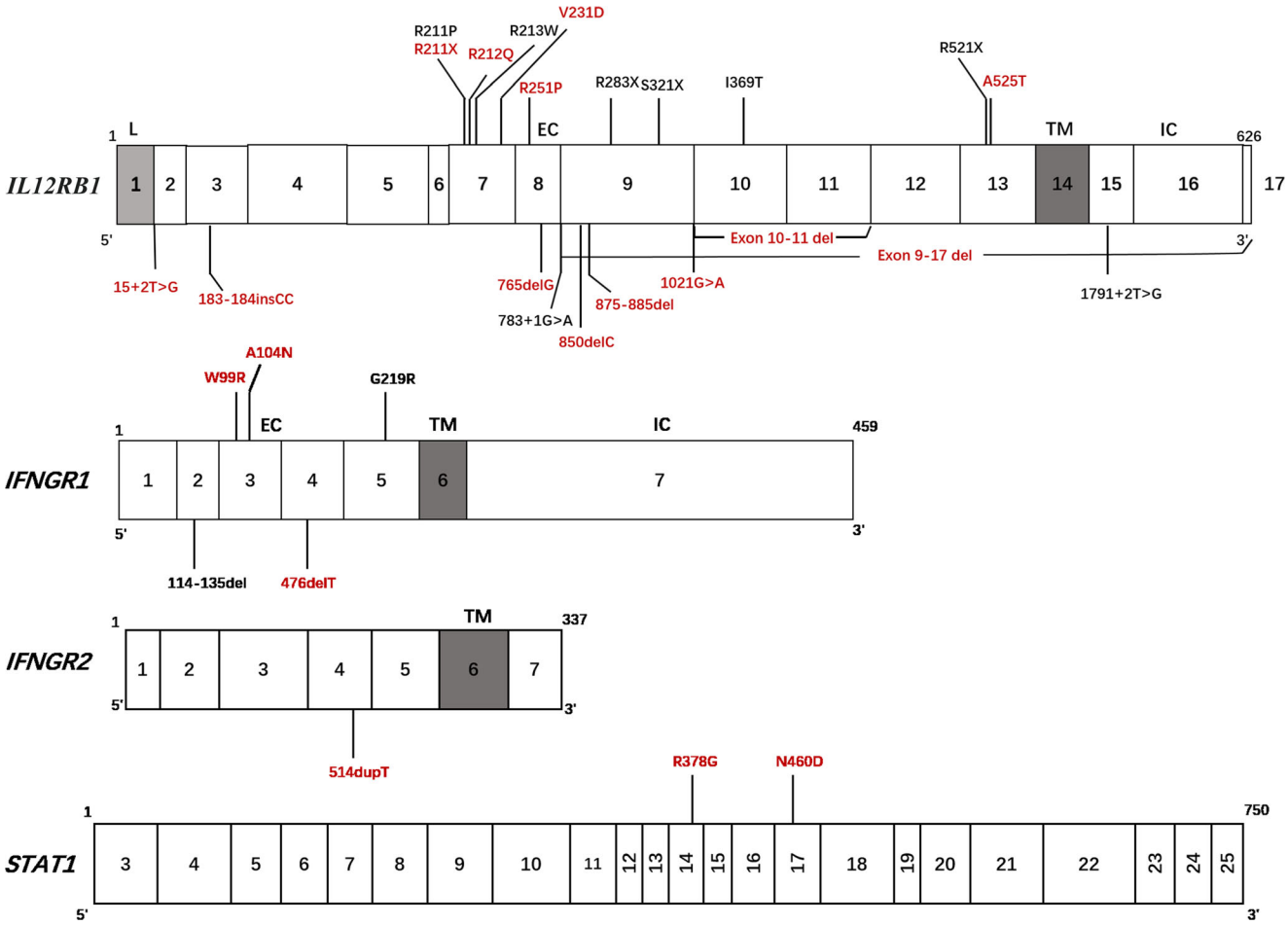


**Fig. 2** Mutated alleles of the IL12RB1 gene. Schematic representation of the coding region of IL-12Rβ1 containing 17 coding exons encoding a 626-amino acid protein with a peptide leader sequence (exon 1, L), an extracellular domain (exons 2–13, EC), a transmembrane domain (exon 14, TM), and an intracellular cytoplasmic domain (exons 15–17, IC). Mutated alleles of the IFNGR1 gene. Schematic representation of the coding region of IFN-γR1 containing 7 coding exons encoding a 459-amino acid protein with an extracellular domain (exons 1–5, EC), a transmembrane domain (exon 6, TM), and an intracellular cytoplasmic domain (exons 7, IC). Mutated alleles of the IFNGR2 genes. Schematic representation of the coding region of IFN-γR2 containing 7 coding exons encoding a 337-amino acid protein with a transmembrane domain (exon 6, TM). Mutated alleles of the STAT1 genes. Schematic representation of the coding region of STAT1 containing 23 coding exons encoding a 750-amino acid protein. Novel mutations are shown in red

with LPS, IFN-γ levels were low (1–4.1 pg/ml) and mildly rescued after the addition of IL-12 (53.4–99.4 pg/ml) (Fig. 3).

In our cohort, one frameshift mutation in IFNGR2 was found. This patient developed a disseminated infection with complete deficiency. Interestingly, low IFN-γ concentrations in the supernatant was observed after stimulation with medium (0.7 pg/ml) and LPS (4.68 pg/ml), with a significant increase after stimulation with LPS plus IL-12 (2261 pg/ml) (Fig. 3).

In addition, two missense mutations in STAT1 were identified. Both of these patients developed disseminated infection involving the vaccination site as well as skin and bone destruction. These two patients displayed recurrent ulceration and granulation hyperplasia at the BCG vaccination site for more than 1 year and still healed slowly after surgical removal of regional granuloma. STAT1 expression was clearly lower in PBMCs from this patient than in PBMCs from a healthy control as shown by Western blotting (Fig. 5). Phosphorylation was reduced after IFN-γ stimulation in the patient. The missense mutations were predicted to be pathogenic base on polymorphism phenotyping version 2 (PolyPhen-2) and SIFT scores. Two STAT1 missense mutations were predicated using the PyMOL software. Wild-type Arg378 is linked to Val457, Thr427, Glu429, and DT-2006 via hydrogen bonding (Fig. S1 B-1). When mutated to glycine, the resulting truncated protein loses its interaction with surrounding amino acids and DNA (Fig. S1 B-2), which is the underlying pathogenicity. Similarly, Asn460 is linked to Ser462, Gln463, DT-1012, and DT-2007 via hydrogen bonding (Fig. S1 C-1), and the protein loses its interaction with the DNA helix with mutation to aspartic acid (Fig. S1 C-2). Thus, we predicted that the amino residues Arg378 and Asn460 may play important roles in maintaining structural stability. Pathogenic variants may result in loss of function, causing disease.

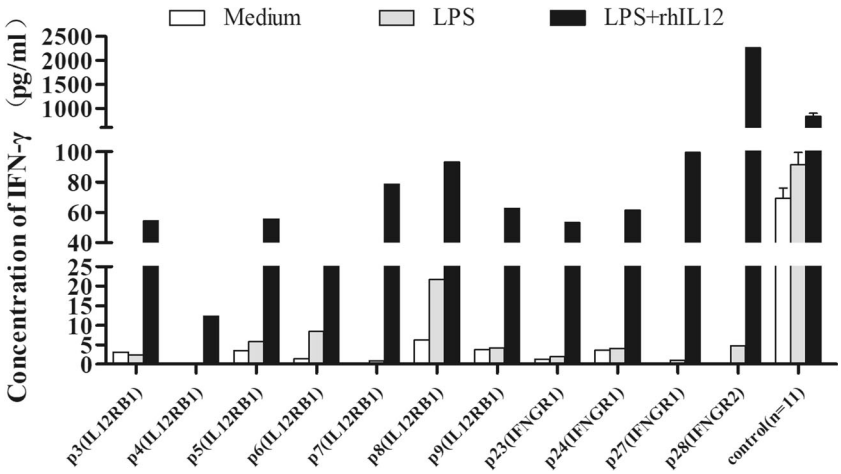


**Fig. 3** Production of IFN-γ was evaluated in 11 patients

## Treatment

All symptomatic patients received antituberculosis medications in the form of double or triple therapies consisting of isoniazide and rifampicin with or without ethambutol or pyrazinamide (until tuberculosis was excluded). Amikacin and linezolid were added when a patient was diagnosed with quadruple therapy (Table 1). The duration of treatment usually lasted for 1.5 years or more. Five of the patients received antituberculosis treatment because of BCGitis early in life

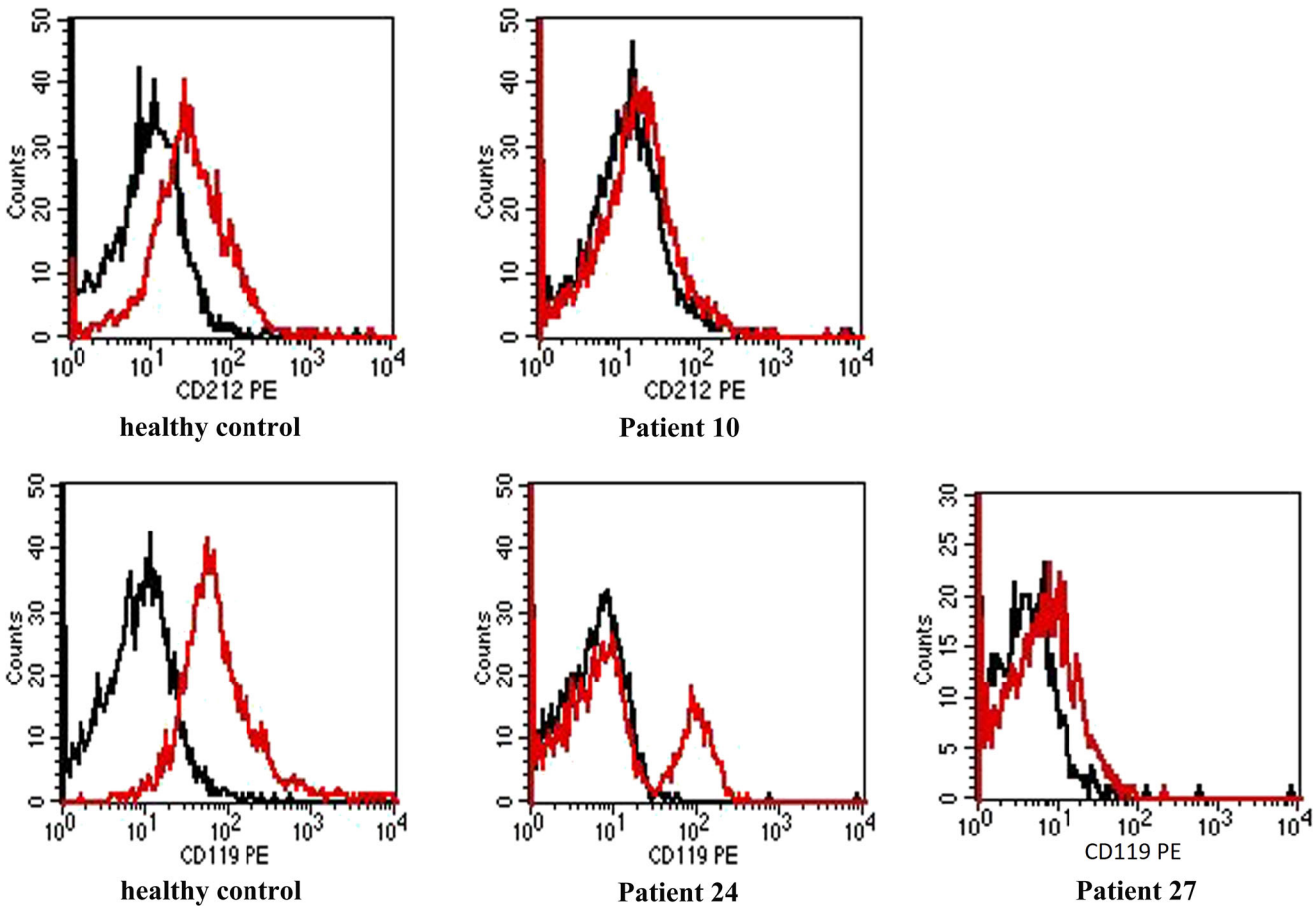


**Fig. 4** The first row shows histograms of IL12Rβ1 expression in a healthy individual (left) and a patient (right). The second row shows expression of IFN-γR1 protein in a healthy individual (left), a patient with decreased expression (middle), and a patient with no expression (right)

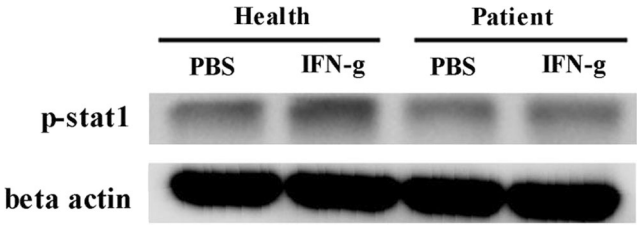


**Fig. 5** Reduced IFN-γ induced phosphorylation of STAT1. Western blot of a total protein extract from PBMCs from the patient and a healthy control probed for phosphorylated STAT1 and beta actin. PBMCs were not stimulated (PBS) or were stimulated with IFN-γ

and the symptoms were relieved within 9 months. However, all of these patients relapsed within 1 year after withdrawal and developed disseminated infection because an underling PID was initially not considered. In addition, 77.8% (21/27) of the patients started rhIFN-γ therapy at 50 μg/m$^2$ subcutaneously once every other day after definitive diagnosis (during infection). When BCG infection was alleviated, rhIFN-γ was administered for prophylaxis (the same dose), and the main adverse reaction was fever. In cases of persistent fever arises, the dose should be reduced appropriately. We did not find other adverse reactions such as abnormal liver function in our patients. In one patient with IL12RB1 deficiency, the medication frequency was gradually extended to every other day after 4 years of INF-γ treatment (parental adjustment) and this patient was followed up for 14 years; the treatment is currently used once every 2 months, and the patient has had no recurrent mycobacterial infections. Two patients with IFNGR1 deficiency underwent hematopoietic stem cell transplantation (HSCT) since 2016.

## Prognosis

Among the 30 patients, 27 were followed up at our hospital until the time of publication or death. The median follow-up

**Table 1** Clinical and genetic features of the patients

| | Gene | Age of onset (months) | Clinical classification | Therapy | | Follow-up time (months) | Outcome |
|---|---|---|---|---|---|---|---|
| | | | | IFN-γ | Antituberculosis | | |
| P1 | *IL12RB1* | 2.5 | Disseminated infection | – | HRZAmK | 12.5 | Died |
| P2 | *IL12RB1* | 2 | Regional infection | + | HR | 171 | AW |
| P3 | *IL12RB1* | 3 | Disseminated infection | – | HRE | 94 | AS |
| P4 | *IL12RB1* | 3 | Regional infection | NA | HRZ | 107 | Lost |
| P5 | *IL12RB1* | 3 | Regional infection | NA | HR | 3 | Lost |
| P6 | *IL12RB1* | 4 | Disseminated infection | + | HRE | 75 | AS |
| P7 | *IL12RB1* | 3 | Distant infection | NA | HR | 90 | Lost |
| P8 | *IL12RB1* | 2.5 | Disseminated infection | + | HRE | 121 | AS |
| P9 | *IL12RB1* | 2 | Disseminated infection | – | HREZ | 24 | Died |
| P10 | *IL12RB1* | 2 | Disseminated infection | + | HREZ | 52 | AS |
| P11 | *IL12RB1* | 4 | Distant infection | + | HRE | 46 | AW |
| P12 | *IL12RB1* | 3 | Regional infection | + | HR | 104 | AW |
| P13 | *IL12RB1* | 2 | Disseminated infection | + | HR | 16 | AS |
| P14 | *IL12RB1* | 3 | Disseminated infection | + | HRE + Lzd | 23 | AS |
| P15 | *IL12RB1* | 3 | Regional infection | + | HREZ | 7 | AS |
| P16 | *IL12RB1* | 4 | Disseminated infection | + | HR | 19 | AS |
| P17 | *IL12RB1* | 3 | Disseminated infection | + | HRE | 38 | died |
| P18 | *IL12RB1* | 4 | Disseminated infection | + | HREZ | 22 | AS |
| P19 | *IL12RB1* | 3 | Regional infection | + | HR | 27 | AW |
| P20 | *IL12RB1* | 2 | Regional infection | + | HRE | 23 | AS |
| P21 | *IL12RB1* | 4 | Disseminated infection | + | HRE + Lzd | 12 | AS |
| P22 | *IL12RB1* | 3 | Distant infection | + | HR | 4 | AS |
| P23 | *IFNGR1* | 2 | Disseminated infection | – | HRE | 89 | HSCT |
| P24 | *IFNGR1* | 3 | Disseminated infection | + | HRE | 81 | AS |
| P25 | *IFNGR1* | 19 | Disseminated infection | + | HRE + Lzd | 58 | HSCT died |
| P26 | *IFNGR1* | 1.5 | Disseminated infection | + | HRE + Lzd | 6.5 | Died |
| P27 | *IFNGR1* | 3 | Regional infection | – | HRZ | 2 | AS |
| P28 | *IFNGR2* | 2.5 | Disseminated infection | + | HRE | 62 | AW |
| P29 | *STAT1* | 2 | Disseminated infection | + | HRE | 19 | AS |
| P30 | *STAT1* | 1 | Disseminated infection | – | HRE | 25 | AS |

*H* isoniazide (15–20 mg/kg/day), *R* rifampicin (20 mg/kg/day), *E* ethambutol (20–25 mg/kg/day), *Z* pyrazinamide (20–25 mg/kg/day), *Amk* amikacin (20 mg/kg/day), *Lzd* linezolid (30 mg/kg/day), *AS* alive symptomatic, *AW* alive well withdrew anti-mycobacterial drugs, *NA* not available, *HSCT* hematopoietic stem cell transplantation

time was 26 months (range, 2 to 171 months) and 18.5% (5/27) of patients died. The median survival time was 26 months (range, 5 to 173 months). Kaplan-Meier survival analysis (Fig. 6) showed that mortality was significantly lower in those with IL12RB1 deficiency who were treated with rhIFN-γ ($P = 0.022$ by the log-rank test) than in those not treated with rhIFN-γ. Among the surviving patients with IL12RB1 deficiency, four were healthy and had stopped using antituberculosis medication. For the remaining 11 patients, the symptoms of *Mycobacterium* infection were controlled after rhIFN-γ treatment. Two patients with IFNGR1 deficiency received HSCT when they were 5 years old. P25 died of a severe infection at 6 months after HSCT; another patient (P23) received HSCT for 5 months and survived well. Umbilical cord blood stem cell transplantation was used in both cases. Among the other deceased patients, three died of severe infections (two with peritoneal infection caused by *M. bovis* and one with severe pneumonia caused by *K. pneumoniae*), and another patient died after treatment was discontinued.

## Discussion

This paper reports the first cohort of patients with MSMD in China. We have previously studied the association of primary immunodeficiency diseases (PIDs) with susceptibility to BCG disease [17]. Among our 324 patients with BCG infection, 1/3 of them had primary immunodeficiency disease. No prominent clinical differences between the genetically determined patients and unidentified patients were found. IFNGR1 deficiency is the first genetic defect identified in patients susceptible to poorly virulent mycobacteria, and the most common genetic etiology of MSMD is IL12RB1 deficiency. In this study, 30 MSMD patients with BCG infection were enrolled over 18 years. Among these patients, 22 had *IL12RB1* defects, accounting for 73.3% of patients. According to Takayuki Hoshina and Lee WI [21, 28], IFNGR1 deficiency may be more common in MSMD among Asian populations, but our results show otherwise.

BCG vaccination is recommended in China because of the high incidence of tuberculosis, and newborns need to be inoculated with the BCG vaccine to prevent tuberculosis meningitis and disseminated tuberculosis. Therefore, BCG infection has become the main clinical characteristic of MSMD in China. We found no significant difference in immunoreaction time at the BCG vaccination site between MSMD patients and normal children. However, due to local recurrent infection, some patients had larger scars. We found that the onset of BCG infection occurred within 4 months after vaccination, earlier than previously reported (before 3 years of age) [29]. This difference was likely caused by early encounters with the BCG vaccine in China.

The lymph nodes, lungs, skin, bones, and peritoneum were the most common sites of infection in our study. Different genotypes exhibited different degrees of infection. Among the patients with IL12RB1 deficiency, 6/22 had infection involving only the lymph nodes, whereas 4/5 patients with IFNGR1 deficiency and 2/2 patients with STAT1 deficiency exhibited disseminated infection. Recently, it was found that IFN-γ plays a role in inhibiting osteoclastogenesis in human cells and that this inhibition is reduced in osteoclasts from patients with IFNGR1 or STAT1 deficiency [30]. This finding may explain the bone destruction that developed in patients with these two types of genetic defects.

In this study, salmonellosis and candidiasis were found in patients with IL12RB1 deficiency, but the incidences were significantly lower than those of 43% and 25%, respectively, reported in the literature [3, 31]. It has also been reported that IL12RB1-deficient patients are prone to clinical disease caused by mycobacteria, *Salmonella*, and other intracellular pathogens, likely because of impaired interleukin 12-dependent interferon-γ production, and mucocutaneous candidiasis may be due to impaired interleukin 23-dependent interleukin 17 immunity [31].

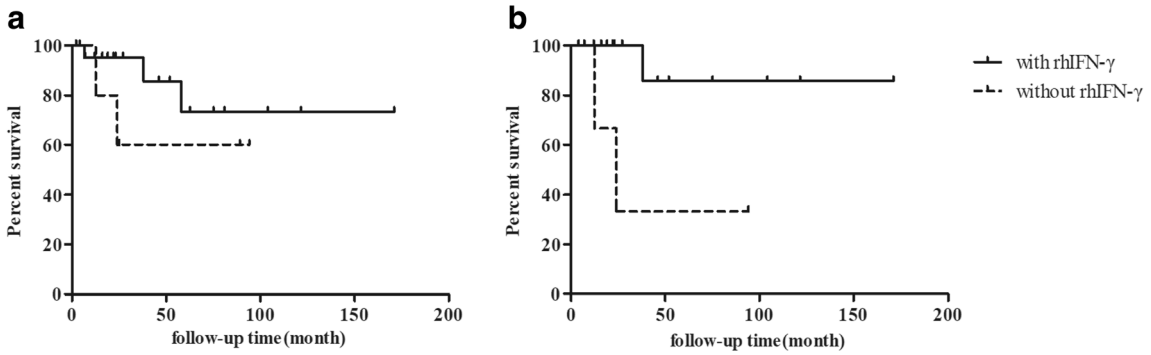


**Fig. 6** **a** Kaplan-Meier estimate of survival for patients with MSMD treated with (solid line) or without (dashed line) IFN-γ (log-rank $\chi^2$ 0.998; $P = 0.31$). **b** Kaplan-Meier estimate of survival for patients with IL12RB1 treated with (solid line) or without (dashed line) IFN-γ (log-rank $\chi^2$ 5.247; $P = 0.022$)

In previous studies, cavernous transformation of the portal vein and congenital hearing impairment were not found in patients with IL12RB1 deficiency. Because intestinal and peritoneal infections had occurred in these two patients, the portal vein abnormalities and abnormal liver function observed may have been secondary to infection.

Multiple new pathogenic variants were detected in four genes, including 12 mutations in *IL12RB1*, 3 in *IFNGR1*, 1 in *IFNGR2*, and 2 in *STAT1*. Flow cytometry is increasingly being used for clinical diagnosis, and in recent years, the application of flow cytometry to determine protein expression has been used to verify the pathogenicity of mutant genes [24, 32]. IL12RB1 protein expression was absent in all patients with deficiency in this gene, and the IFNGR1 protein was either absent or reduced in IFNGR1-deficient patients. Flow cytometric detection of IL12RB1 and IFNGR1 protein expression and genetic consistency results suggest that this method can be used as a means of rapid clinical diagnosis for MSMD. Furthermore, in vitro stimulation tests indicated that the cells from patients with such genetic deficiency have an impaired but not absent response to IL-12, resulting in the production of IFN-γ. This may suggest that our patients were partial deficient. A novel c.514 duplication that results in frameshift mutation was detected in patients with IFNGR2 deficiency.

Pyrazinamide is ineffective for BCG infection due to natural resistance. Regardless, some patients in our cohort received pyrazinamide treatment in local hospitals because of lack of knowledge of this resistance pattern. In our study, treatment with antimycobacterial drugs alone was not effective and the prognosis was poor in those admitted during the late stage of BCG infection and in those who did not receive rhIFN-γ treatment. P1 died of disseminated BCG and NTM infection, this patient was diagnosed in 2004 when awareness of this disease was poor. After treatment with rhIFN-γ, most of these infections are controllable, and quality of life improves. Nonetheless, the dose and frequency of rhIFN-γ have not yet been well defined. For P2, who had IL12RB1 deficiency, the medication frequency was gradually extended to once per month, with no recurrent mycobacterial infections. This may indicate that the dose and frequency of IFN-γ treatment should be further explored.

In contrast to the patients with IL12RB1 deficiency, the clinical phenotype of the patients with IFNGR1 deficiency was characterized by early-onset, disseminated, and life-threatening infections with mycobacteria, and these patients account for 29% of patients with MSMD [33]. Two genetic forms of AR IFNGR1 deficiency have been described, with or without surface expression of the receptor (complete type: IFNGR1 is not expressed, and no IFN-γ is produced; partial type: IFNGR1 is expressed, with weak IFN-γ production) [34]. Due to the cellular phenotype of AR, complete IFNGR1 deficiency is characterized by a lack of response to IFN-γ, and IFN-γ treatment cannot alleviate symptoms in these patients. Thus, the only cure is stem cell transplantation. However, among 11 affected children who underwent stem cell transplantation, five died within 1 year after transplant (four cases of rejection, one case of severe infection). Conversely, transplantation was successful in five cases, and the patients were followed up for 1~6 years without apparent infection [32, 34, 35]. These five patients had an early age of onset, disseminated infection, decreased IFN-γ production, and reduced or no expression of the IFNGR1 protein. According to Esteve-Solé et al. [36], more experiments such as assessments of cytokine production and STAT1 phosphorylation in response to IFN-γ should be performed to distinguish partial or complete deficiency. These analyses were not performed and this is a limitation of our study. Two of our patients received HSCT after failed treatment with IFN-γ. P25 died from rejection and severe infection after transplant, whereas P23 remained alive at 5 months. Overall, mycobacterial infection is controllable, and the curative effect requires further observation. P26 died because of admission at a later stage of BCG infection. Another variation, p.G219A, was present in a homozygous state in two non-consanguineous patients who received antituberculosis therapy and exogenous IFN-γ supplementation for 3.5 years and 2 months, respectively. Surprisingly, one patient experienced control of disseminated infection after exogenous IFN-γ therapy, and protein expression was reduced rather than absent, another patient with the same mutation had regional BCG infection and no protein expression. This discrepancy may have occurred because the follow-up time was very short; hence, the patient's progression and prognosis need further observation.

## Conclusions

In summary, MSMD is an important cause of BCG infection in the Chinese population, and IL12RB1 deficiency was found to be common. Flow cytometric detection of IL12RB1 and IFNGR1 can be used as a means of rapid diagnosis. rhIFN-γ therapy is effective and improves prognosis, particularly in patients with IL12RB1 deficiency.

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**Authors' Contributions** Dr. Ying participated in study design, collected data, performed data analyses, drafted the manuscript, and incorporated revisions, and takes responsibility for the manuscript as a whole.

Prof Wang and Prof Sun conceptualized and designed the study, supervised data collection, and reviewed and revised the manuscript.

Dr. Liu and Dr. Dong support technical and material experiment and performed the statistical analysis.

Dr. Wang, Dr. Hou, Dr. Yao, Dr. Hui, Dr. Zhou, and Dr. Sun collected data and reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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## Compliance with Ethical Standards

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

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