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Mendelian susceptibility to mycobacterial disease caused by a novel founder *IL12B* mutation in Saudi Arabia

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Compliance with Ethical Standards

Research involving Human Participants. Informed consent for participation in this study was obtained in accordance with local regulations, with approval from the IRB. The experiments described here were performed in France, in accordance with local regulations, and with the approval of the IRB of Necker Hospital for Sick Children, France.

Informed consent. Written informed consent was obtained from the patients.

Potential conflicts of interest. The authors have no conflict of interest to declare.

Abstract

Purpose—Mendelian susceptibility to mycobacterial disease (MSMD) is a rare primary immunodeficiency predisposing congenitally affected individuals to diseases caused by weakly virulent mycobacteria, such as *Bacillus Calmette-Guérin* (BCG) vaccine strains and environmental mycobacteria (EM). IL-12p40 deficiency is a genetic etiology of MSMD resulting in impaired IL-12- and IL-23-dependent IFN- γ immunity. Most of the reported patients with IL-12p40 deficiency originate from Saudi Arabia (30 of 52) and carry the recurrent *IL12B* mutation c.315insA (27 of 30).

Methods—Whole-exome sequencing was performed on three patients from two unrelated kindreds from Saudi Arabia with disseminated disease caused by a BCG vaccine substrain.

Results—Genetic analysis revealed a homozygous mutation, p.W60X, in exon 3 of the *IL12B* gene, resulting in complete IL12p40 deficiency. This mutation is recurrent due to a new founder effect.

Conclusions—This report provides evidence for a second founder effect for recurrent mutations of *IL12B* in Saudi Arabia.

Keywords

Mendelian susceptibility to mycobacterial disease; *Bacillus Calmette-Guérin* (BCG); disseminated BCG infection, IL-12, interferon

Introduction

Primary immunodeficiencies (PIDs) are inherited disorders with a variable geographic incidence and clinical spectrum, due to variability in both the human genome and the microbial environment across the world (1). The incidence of PIDs is relatively high in Saudi Arabia, due to the high rate of consanguinity in this country (2). Disorders principally affecting innate immunity were found to be less common than those disrupting adaptive immunity in a recent study (1, 3, 4). Recurrent and/or severe infections are the most common clinical feature at PID presentation, and disseminated mycobacterial disease caused by *Mycobacterium bovis*-BCG (BCG-osis) is particularly frequent, in patients with Mendelian susceptibility to mycobacterial disease (MSMD) (3, 5). This PID is characterized by clinical infectious disease due to weakly virulent mycobacteria (BCG and environmental mycobacteria [EM]), *M. tuberculosis*, and non-typhoidal *Salmonella*, in otherwise healthy patients normally resistant to most other infectious agents (5, 6). Allelic heterogeneity for 10 genes (*IFNGR1*, *IFNGR2*, *STAT1*, *IL12RB1*, *IL12B*, *IRF8*, *ISG15*, *TYK2*, *CYBB* and *NEMO*) has been shown to contribute to up to 19 different genetic disorders (5, 7). The products of all the affected genes are involved in interferon (IFN- γ)-mediated immunity, controlling its induction and/or response. The *IL12B* gene encodes IL-12p40, which is common to IL-12 and IL-23. Mutations of *IL12B* have been reported in 52 patients with MSMD from six countries (France, India, Iran, Pakistan, Saudi Arabia and Tunisia) (8–13). Founder effects have been reported in patients of the same ethnic origin. A recurrent mutation, c.526del2, was identified in patients from Iran and shown to have arisen about 600 years ago; a second mutation, g.482+82_856-854del, emerged 700 years ago in India/

Pakistan; a third mutation, c.297del8, arose 1,100 years ago in Tunisia; and a fourth mutation, c.315insA, was identified in Saudi Arabia and found to have arisen about 1,100 years ago. All patients are homozygous for one of these mutations and display a complete deficiency, with no IL-12p40 protein detectable in leukocytes and/or EBV-transformed B cells (EBV-B cells) (8–13). We describe here three new patients from two unrelated kindreds with BCG-osis associated with a previously unknown recurrent mutation in exon 3 of *IL12B* due to another founder effect in Saudi Arabia.

Case report

Patient 1

A 14-month-old boy (Kindred A, II.1) from southern Saudi Arabia was born to a consanguineous couple. The pregnancy and delivery were unremarkable (Figure 1A). He received BCG vaccine at birth. At three months, he presented bilateral cervical and left axillary swellings associated with persistent fever. He was admitted to a general hospital in Riyadh, where analyses of lymph node specimens revealed the presence of acid-fast bacilli (AFB). Treatment with rifampin, isoniazid, ethambutol and streptomycin was initiated, and a mild clinical response to treatment was observed. The family stopped his antibiotic treatment at the age of one year. A few months later, he was readmitted to another hospital, with respiratory distress and generalized lymphadenopathy. He was then transferred to Prince Sultan Military Medical City in Riyadh, Saudi Arabia, for further evaluation and management of BCG-osis. Physical examination revealed a pale, underweight child in respiratory distress, with generalized lymphadenopathy and ascites. A computed tomography (CT) scan of the chest, abdomen and pelvis revealed generalized lymphadenopathy, together with mild bilateral pleural effusion and compression of the smaller airways by the enlarged lymph nodes. Treatment with antituberculous drugs was reinitiated, with a regimen consisting of rifampin, ethambutol, ciprofloxacin, clarithromycin, and amikacin. Analyses of both axillary lymph node biopsy specimens and gastric aspirates revealed the presence of large numbers of AFB. *M. bovis*-BCG strain was obtained in culture. Antituberculous drugs susceptibility testing showed that the patient was infected with a multidrug-resistant strain that was susceptible to ethambutol, streptomycin, amikacin, capreomycin, moxifloxacin, para-aminosalicylic acid and linezolid, but resistant to isoniazid, rifampin, pyrazinamide, and cycloserine. In line with these findings, the treatment was changed to ethambutol, amikacin, capreomycin, moxifloxacin, para-aminosalicylic acid and linezolid. HIV and SCID were excluded (Supplemental table 1). MSMD was suspected and IFN- γ (50 mcg/mm³) was added to the treatment regimen. The patient's clinical condition slowly improved on this treatment and repeated CT scans showed a mild improvement in ascites and a decrease in lymph-node size. After six months of treatment in hospital, the patient was discharged home on four oral antituberculous drugs (ethambutol, moxifloxacin, para-aminosalicylic acid and linezolid) together with IFN- γ subcutaneous injections with regular follow-up visits at an outpatient clinic. This patient is now four years old and his clinical condition has remained stable during outpatient follow-up.

Patient 2

A girl aged four years and seven months, from southern Saudi Arabia, with consanguineous Saudi parents (Kindred B, II.3) received the BCG vaccine at birth. At one month of age, swelling was observed at the BCG vaccine site, followed by left axillary adenomegaly. Lymph-node enlargement gradually progressed to involve the supraclavicular and cervical regions. At the age of four months, the patient underwent an excisional biopsy of the left axillary lymph nodes at the local hospital, revealing granulomatous inflammation and positive staining for AFB. Treatment with rifampin, isoniazid and azithromycin was administered for eight months, resulting in a partial resolution of lymphadenopathies. At the age of 13 months, the patient suffered a recurrence of lymph-node enlargement, involving the supraclavicular, cervical and axillary regions. Treatment was initiated with rifampin, clarithromycin and ciprofloxacin. At 24 months of age, the patient was referred to King Faisal Specialist Hospital, where she underwent extensive investigations, including tests for the diagnosis of PIDs (Supplemental table 1). CT-scans of the chest and abdomen showed multiple enlarged lymph nodes (cervical, axillary on both sides, mediastinum), and small hepatic lesions, with no lung lesions. Resection of the left axillary and right suprasternal lymph nodes revealed chronic and acute inflammation, with AFB. *Mycobacterium bovis* BCG resistant to isoniazid and rifampin grew in culture. Based on the results of susceptibility tests, the treatment was changed to clarithromycin, cycloserine, ethambutol and moxifloxacin. The patient was also found to have extensive oral thrush, which was treated with fluconazole. Her response to antimycobacterial drugs was suboptimal, as, over a period of eight months, a worsening of cervical lymph node enlargement was observed, with draining sinuses. The patient underwent debulking surgery on her enlarged left and right cervical nodes, from which *M. bovis*-BCG resistant to isoniazid and rifampin and susceptible to ethambutol, ciprofloxacin, clarithromycin and PAS was also obtained. IFN- γ (50 mcg/mm³) was added to the treatment regimen, but the response to treatment remained poor. In 2017, the patient was hospitalized due to extensive enlargement and draining of bilateral cervical lymph nodes. Chest X ray showed extensive consolidation of the right upper lobe. Chest CT-scan showed dense consolidation, with cavitation and an air-fluid level in the right upper lobe, with enhanced attenuation of the mediastinum consistent with inflammation. Gastric aspirate tested positive for AFB, and all surgical samples tested positive for *M. bovis*-BCG on culture. *Candida* spp. were detected in mouth samples, and multiplex PCR for *Salmonella* gave negative results with a stool sample. This patient is currently receiving IV amikacin, IV rifampin, IV moxifloxacin, cycloserine, ethambutol, clarithromycin and IFN- γ . Drug levels are monitored. The patient underwent right upper lobectomy, and pathology analyses revealed non-necrotizing granulomatous inflammation with positive staining for AFB. The patient is currently well, but on antibiotics and IFN- γ .

Patient 3

This patient is two and a half years old and is the sister of P2 (Kindred B, II.4). She was vaccinated with BCG at birth. She did well until the age of eight months, when her mother noticed a swelling in the left axillary region, which increased in size, with the overlying skin becoming erythematous. On physical examination, the child appeared well, except for an indurated BCG injection site and an enlarged, erythematous left axillary lymph node. No other lymph nodes were palpable. Functional tests excluded SCID and related PIDs

(Supplemental table 1). The patient underwent surgical resection of the left axillary node. Pathology analyses revealed the presence of a granulomatous inflammation positive for AFB. *M. bovis*-BCG grew in culture and was found to be susceptible to isoniazid, rifampin, ethambutol, ciprofloxacin and clarithromycin. Abdominal ultrasound results were normal. The patient was treated with rifampin, clarithromycin and ethambutol for one year and responded well to treatment. She is currently well and has two other older sisters, aged six and eight years, who have not been tested but are clinically well.

We studied three patients with MSMD, using genetic approach. Whole-exome sequencing (WES) revealed a homozygous mutation in exon 3 of *IL12B* in P1, P2 and P3. A G residue was replaced with an A at nucleotide position 179, resulting in a stop codon in place of a tryptophan codon, leading to premature termination of the protein at position 60 (p.W60X) (Figure 1B). This mutation was confirmed by Sanger sequencing in three patients. The parents and sisters from kindred A and the parents from kindred B were heterozygous for this mutation. No DNA samples were available from the other sisters from kindred B. Interestingly, another three patients from a consanguineous family from Saudi Arabia have been reported to have the same mutation (Figure 1C)(10). We therefore investigated the haplotype of the *IL12B* gene in three patients from three kindreds (Figure 1D). Using the information provided by the use of multilocus markers of two kindreds (P1 and P3), we estimated as long as shared alleles (14). We showed that the W60X mutation was inherited through a common founder effect. The estimated number of generations since the appearance of the common ancestor was 12 (confidence interval: 6–78) corresponding to 300 years, with an estimated generation time of 25 years. We evaluated the IL-12 and IFN- γ responses of peripheral mononuclear cells (PBMCs) in P2. IFN- γ production was similar to that in healthy control cells (data not shown). However, the cells of P2 produced no IL-12p70 in response to BCG plus IFN- γ . These findings are consistent with those observed for other patients with complete IL-12p40 deficiency (10).

DISCUSSION

We describe here three new patients with BCG-osis due to complete IL-12p40 deficiency caused by a novel recurrent homozygous mutation of *IL12B*. This mutation is recurrent due to a second founder effect in Saudi Arabia. The 315_316insA mutation was identified as responsible for a first founder effect in this country (9,10). Despite the increasing number of new patients with IL-12p40 deficiency identified, this PID is still identified essentially in patients of the same ethnic groups as before (from India/Pakistan, Iran, Saudi Arabia, Tunisia)(8–10, 15), although one patient from France has also been reported (12). Clinically, the three patients identified in this study had the same infectious phenotype, characterized by BCG disease, with an onset a few months after BCG vaccination. This vaccine has been routinely administered, at birth, to all babies in Saudi Arabia since 1970. Complications of this vaccination include regional lymph-node enlargement, suppurated lymphadenitis, and disseminated disease in immunocompromised individuals. Disseminated disease is potentially life-threatening and difficult to treat. Based on reports of a low efficacy of the BCG vaccine and adverse effects in patients with PID, several Saudi Arabian studies have recommended postponing vaccination until the child is old enough for immunodeficiencies to be recognized (SCID, CID, or CGD), so as to prevent the devastating complications of

BCG vaccination in patients at high risk (1,2). IL-12p40 deficiency is a rare genetic etiology of MSMD, but this condition is more frequent in Saudi Arabia than previously thought and should be considered in any patient presenting with disseminated BCG infection. BCG vaccination should be re-evaluated and formally contraindicated in newborns with siblings and relatives with a history of MSMD, to prevent life-threatening complications of BCG infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. IL12p40 deficiency

A. Pedigree of two kindreds, showing the index cases, siblings and parents; each generation is designated by a roman numeral (I–II), and an arrow indicates the proband. No gDNA was available for testing for siblings labeled “E?”. **B.** Electropherogram showing the position of the mutation in the *IL12B* gene. **C.** Schematic representation of the coding region of the *IL12B* gene and the mutated alleles (in red). **D.** Shared *IL12B* haplotypes have been reconstructed using SNPs data. SNP rs1019738 corresponds to the last common SNP between P2 and P3 before the mutation. SNP rs7702966 corresponds to the last common SNP between patient reported previously and P2 after the mutation. There are 3413, 3888 and 1847 SNPs in the haplotypes of patient reported previously, P2 and P3 respectively.