

Inherited IL-12p40 Deficiency: Genetic, Immunologic, and Clinical Features of 49 Patients From 30 Kindreds

Prando et al

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SUPPLEMENTAL PATIENTS AND METHODS

This Supplemental Digital Content contains the description of Subjects and Kindreds referred to in the full version of this article, which appears in the March 2013 issue of *Medicine*.

Subjects and Kindreds

Kindred 1

The parents are consanguineous, originating from Pakistan and currently living in the United Kingdom. The proband (01.II.1) suffered from bacille Calmette-Gu[acute]erin (BCG) and *Salmonella enteritidis* infections at the ages of 3 months and 3.5 years, respectively. IL-12p40 deficiency¹ has been diagnosed, and the patient is now aged 22 years and doing well. The current treatment includes ciprofloxacin and exogenous interferon (IFN)- γ .

Kindred 2

The parents are consanguineous, originating from the Gujarat region of India. Their first daughter (02.II.1) was BCG vaccinated at birth and died of disseminated BCG infection at age 5 years.⁴ A lymph node biopsy showed tuberculoid granulomas with multiple visible acid-fast bacilli (AFB). Her brother (02.II.2), who was also vaccinated with BCG, suffered no adverse effects and is now a healthy 22-year-old. The youngest sister (02.II.3) did not receive BCG vaccination, and at about 2-4 years of age suffered 4 episodes of disseminated *S. enteritidis* infection. She received antibiotic treatment with clinical improvement. The patient was lost to follow-up, and we do not know her current clinical status.

Kindred 3

The parents are consanguineous, originating from and living in Saudi Arabia.⁴ Their first, second, third, fifth, seventh, and eighth children received BCG vaccines and are healthy. Sibs 11-15 did not receive BCG vaccination and are healthy. Their fourth child (03.II.4) had axillary adenitis on the side of BCG infection, was treated with isoniazid and rifampin and fully recovered with no further infections. The sixth child (03.II.6) was vaccinated with BCG shortly after birth and developed disseminated BCG infection and responded well to antimycobacterial drugs. Lymph node biopsy showed mature granuloma formation and multiple AFB. She presented with *Nocardia asteroides* pleurisy at age 8 years, which responded well to antibiotics. Homozygous IL12B mutation was diagnosed. The patient is now aged 20 years and is well. The ninth child was BCG vaccinated at birth and died of disseminated BCG infection at the age of 2 years. No genetic diagnosis was performed. The proband (03.II.10) was vaccinated with live BCG at birth and developed disseminated BCG infection 6 months later. A lymph node biopsy showed no mature granuloma formation and multiple AFB. Upon antimycobacterial therapy a full recovery was achieved, and she is now a healthy 13-year-old.

Kindred 4

The parents are consanguineous, originating from and living in the Kingdom of Saudi Arabia.⁴ The proband, similar to the second child (04.II.1 and 04.II.2, respectively), was vaccinated with BCG at birth and presented with axillary BCG adenitis at the age of 1 year. Both responded well to the antibiotics, recovered fully, and are now aged 21 and 20 years, respectively. The third sib was healthy. The fourth sib (04.II.4) was vaccinated with live BCG at birth and presented with axillary BCG adenitis 12 months later. She was successfully treated with antibiotics and by surgical excision. At the age of 15 months, she presented with *Salmonella* type C gastroenteritis. At age 2.6 years she developed occipital lymphadenitis due to *Mycobacterium tuberculosis*. She was successfully treated with antibiotics but had a relapse at the age of 4 years. She is now aged 16 years and is well. The 4 younger sibs are healthy and are currently aged 15, 14, 11, and 9 years, respectively.

Kindred 5

The parents are consanguineous, originating from and living in the Kingdom of Saudi Arabia.⁴ The proband (05.II.2) was vaccinated with live BCG at birth and developed disseminated BCG infection 8 months later. After surgical excision, she was treated with antibiotics and exogenous IFN- γ , but did not respond to treatment and died of disseminated infection at the age of 2 years. The histology of a biopsy from diseased lymph nodes showed tuberculoid lymphadenitis with poorly formed granuloma and numerous visible AFB. The proband's older brother (05.II.1) was vaccinated with live BCG, with no adverse effect. The 2 younger sibs did not receive BCG vaccines and are healthy.

Kindred 6

The parents are consanguineous, originating from and living in the Kingdom of Saudi Arabia.⁴ The first 2 sibs were BCG vaccinated with no adverse effects. The third child (06.II.3) was vaccinated with BCG at birth and presented with disseminated BCG infection 3 months later. She also suffered from *Salmonella* adenitis. She was treated with antibiotics for a total of 18 months. She remained well, with no treatment, for 8 years. At age 11 years, she developed meningoencephalitis, which was not bacteriologically confirmed and proved fatal. The fourth child (06.II.4) was vaccinated with BCG at birth and at 7 months of age suffered from disseminated BCG infection, which responded to the antibiotics. She has subsequently suffered from recurrent oral candidiasis. The proband, the fifth child (06.II.5), was not vaccinated with BCG at birth. She developed disseminated *M. chelonae* infection at age 3 years, did not respond to treatment, and died of disseminated infection at age 5 years. She also presented with *Salmonella* group B adenitis at the age of 3 years. The other 3 children were not vaccinated and are currently healthy.

Kindred 7

The parents are not consanguineous, originating from and living in Tunisia. The first child in the family was BCG vaccinated and experienced no adverse effects.² The next children in the family were dizygotic twins. One dizygotic twin (07.II.02) was BCG vaccinated at birth and developed axillary lymphadenitis at 3 months. The antituberculosis treatment led to clinical improvement, but after cessation of treatment, the patient suffered from relapse. The antituberculosis treatment

was re-initiated; however, due to hepatotoxicity, it had to be interrupted. The patient subsequently developed disseminated infection with AFB. Quadruple antituberculosis treatment was administered for 2 months followed by dual therapy for 1 year. The patient developed hepatic toxicity in response to this treatment and accordingly the drugs were replaced by clofazimine. When the patient was aged 3 years, portal-vein cavernous angioma was diagnosed. Later the patient suffered from multiple fistulous adenopathies, hepatosplenomegaly, abdominal masses, and scrotal swelling. Peritoneal biopsy showed moderately well-circumscribed and differentiated tuberculoid granuloma with epithelioid and multinucleated giant cells without central caseous necrosis. Microscopy identified intra- and extracellular AFB; however, the cultures were negative. Histology of liver biopsy also revealed epithelioid granuloma with rare giant cells and no necrosis. The patient received quadruple-drug antituberculosis therapy and clofazimine, which resulted in clinical stabilization for a period of 3 years. However, the patient later succumbed to fulminant varicella zoster infection. The patient's dizygotic twin brother was BCG vaccinated and suffered no adverse effects. The fourth child in the family was BCG vaccinated at birth. At 3 months she suffered from axillary adenopathy with fistulization. Antituberculosis therapy has been administered, and the patient has improved. She also had varicella infection and fully recovered. She is now 12 years old.

Kindred 8

The parents are consanguineous, originating from and living in Tunisia. The first 2 children were BCG vaccinated with no adverse effects.² The proband 08.II.3 received the BCG vaccine at birth and developed bilateral fistulizing axillary adenitis and hepatosplenomegaly at 3 months. *M. bovis* BCG strain was cultured bacteriologically. The patient's clinical status improved upon antituberculosis treatment. However, she later developed ascitis, portal-vein cavernous angioma, and portal hypertension. Furthermore, she developed tuberculosis of the left humerus with subperiosteal abscess and abscess of iliac fossa. AFB were identified in the pus. Despite the antituberculosis treatment and exogenous IFN- γ , the patient died at the age of 2 years.

Kindred 9

The parents are consanguineous, originating from and living in Iran. The proband was BCG vaccinated at the age of 7 years.³ Four months later he developed disseminated BCG infection with bacteriologic confirmation. Histology of axillary and cervical lymph nodes, performed at the ages of 7 and 18 years, showed widespread macrophage and polymorphonuclear infiltration without granuloma formation, suggestive of necrotizing lymphadenitis. Microscopy for AFB was negative. He was treated with antituberculosis therapy but had multiple relapses. The patient was hospitalized due to *S. enteritidis* infection, was treated, and then presented with disseminated salmonellosis relapse 1 year later. Despite the treatment with exogenous IFN- γ , the patient succumbed to the infection. Two other sibs died at earlier ages due to acute gastroenteritis and acute abdomen. All other sibs are healthy.

Kindred 10

The parents are consanguineous, originating from and living in the Kingdom of Saudi Arabia. The proband (10.II.3) was vaccinated with BCG at birth and presented with localized BCG infection 6 months later. She was treated with antibiotics and had a relapse of BCG infection at

12 months, which was treated successfully. Her brother (10.II.4) was BCG vaccinated at birth, and 6 months later developed localized BCG infection. Treatment with antibiotics resulted in full recovery. The proband's sister (10.II.5) carries the same mutation as the proband. She did not receive BCG vaccination and remains healthy. The 2 other sibs, 10.II.6 and 10.II.7, are wild-type and heterozygous, respectively, and are healthy at ages 12 and 3 years.

Kindred 11

The parents are consanguineous, originating from and living in the Kingdom of Saudi Arabia. The proband (11.II.5) was vaccinated with BCG at birth and 3 months later presented with disseminated BCG infection. He was treated with antibiotics and succumbed to the infection at 16 months. The proband, as well as the younger 3 sibs, suffered from mental retardation. Three of 4 sibs are deceased, for reasons which are not known, and no further information is available for the oldest child in the family.

Kindred 12

The parents originate from and live in the Kingdom of Saudi Arabia. The consanguinity is unknown. The proband (12.II.1) was BCG vaccinated at birth, developed disseminated BCG infection at 5 months, received antituberculosis treatment and fully recovered. At the age of 2 years, the patient developed disseminated salmonellosis with *Salmonella* group D, was treated but suffered multiple relapses. At the age of 4 years the patient developed *M. chelonae* lymphadenitis and fully recovered after treatment.

Kindred 13

The parents are consanguineous, originating from Tunisia and living in France. The proband (13.II.5) was BCG vaccinated at 10 months, developed localized BCG infection, was treated, and fully recovered. At the age of 4 years and 5 months he developed salmonellosis, which relapsed after 6 months of treatment. At the last follow-up, 3 years ago, the patient was 7 years old and had had no infection, despite the fact that he had not received any prophylactic drugs. During family segregation analysis we identified that the older sib in the family, 13.II.1, carries the same homozygous mutation in IL12B gene; however, he has never had any clinical symptoms. The BCG vaccination status of this sib is unknown. The parents and all other sibs were heterozygous for the mutation.

Kindred 14

The parents are consanguineous, of Indian origin but living in Malaysia. The proband (14.II.6) was BCG vaccinated at birth and suffered from disseminated BCG infection at the age of 3 months. He received 18 months of antituberculosis treatment, which led to clinical remission. However, at the age of 2.5 years he presented with a relapse of the BCG infection. He received antituberculosis treatment and had no clinical follow-up after the family moved back to India. At the age of 4.5 years, the patient returned to Malaysia and was admitted to the hospital with symptoms of disseminated mycobacteria infection affecting his mediastinal lymph nodes, chest wall, spine, and lungs. Bacteriologic confirmation of BCG infection was obtained. Despite extensive antituberculosis, broad-spectrum antibiotic and exogenous IFN- γ treatment, the patient

eventually succumbed to the progressive respiratory disease, spinal cord compression, and paraplegia. Three older sibs are healthy.

Kindred 15

The parents are consanguineous, originating from and living in Iran. The proband (15.II.1) was BCG vaccinated at birth and 3 months later developed disseminated BCG infection. He was treated with antituberculosis therapy and exogenous IFN- γ ; however, he suffered multiple relapses and severe side effects of treatment, in particular malabsorption and severe ascitis. The patient remains on antituberculosis treatment.

Kindred 16

The parents are consanguineous originating from and living in the Kingdom of Saudi Arabia. The proband (16.II.2) was BCG vaccinated at birth and developed disseminated BCG infection at the age of 1 year. She underwent surgical debridement of the infected areas, and antituberculosis and exogenous IFN- γ treatments were administered. Despite these therapeutic measures, the disease progressed. At the age of 3-4 years, the patient suffered from disseminated salmonellosis. Furthermore, at the age of 9 years she was diagnosed with *Escherichia coli* urinary tract infection and systemic candidiasis. The patient is still on antituberculosis and antibiotic treatment and suffers from severe side effects. The proband's younger sister (16.II.5) was BCG vaccinated at birth, and developed disseminated BCG infection during the first year of her life. This patient received both antituberculosis and surgical treatment and suffers from severe side effects. Significantly, both the proband and her younger sib received recombinant IFN- γ treatment without notable improvement. The oldest sib in the family (16.II.1) had regional lymphadenitis after BCG vaccination, received 6 months of antituberculosis treatment, and has been healthy ever since. Genetic investigation of this sib was not possible due to a lack of biologic material. However, we do know that he remains healthy. The parents and 4 remaining sibs are heterozygous.

Kindred 17

The parents are consanguineous, originating from and living in the Kingdom of Saudi Arabia. The proband (17.II.2) was BCG vaccinated at birth and developed disseminated BCG infection at 9 months. Antituberculosis treatment was administered and resulted in observed clinical improvement. The proband also suffered from portal hypertension after portal vein thrombosis.

Kindred 18

The parents are consanguineous, originating from and living in Iran. The proband (18.II.1) received BCG vaccination at the age of 3 years. When the patient was aged 5 years, disseminated tuberculosis was diagnosed, with bacteriologic confirmation. *Candida albicans* infection was diagnosed and was treated with both antituberculosis and antifungal medications. Despite the treatment, mycobacterial infection persisted and spread further into the bone marrow. Histologic examination of a bone marrow biopsy showed noncaseating granulomas.

Kindred 19

The parents are not consanguineous, originating from and living in Tunisia. The proband (19.II.1) was BCG vaccinated at birth and developed disseminated BCG infection at 3 months of age. He received antituberculosis treatment which resulted in clinical remission. The younger sib, 19.II.4, has the same homozygous mutation as the proband, but was not BCG vaccinated and remains healthy.

Kindred 20

The parents are consanguineous, originating from Pakistan and currently living in the United Kingdom. The proband (20.II.3) was BCG vaccinated and first developed localized BCG infection, which, despite antituberculosis treatment, developed into disseminated infection, which was bacteriologically confirmed. A second lymph node biopsy was cultured and *M. tuberculosis* growth was detected. Antituberculosis and exogenous IFN- γ treatment was administered and led to clinical improvement. However, 3 months later biliary cirrhosis was diagnosed, which, based on histologic examination, was due to neither drug hepatotoxicity nor to mycobacterial assault of the hepatic tissue. Due to advanced cirrhosis the patient underwent orthotopic liver transplantation. During the posttransplantation period the patient did not suffer from any bacterial infections related to Mendelian susceptibility to mycobacterial disease (MSMD), but has suffered from ongoing Epstein-Barr virus and varicella infections as a consequence of the solid-organ transplantation. The younger sib, 20.II.4, has the same homozygous mutation as the proband, but was not BCG vaccinated and remains healthy.⁵

Kindred 21

The parents are consanguineous, originating from and living in the Kingdom of Saudi Arabia. The proband (21.II.2) was BCG vaccinated at birth and presented with localized BCG infection at the age of 6 months. Antituberculosis treatment was administered followed by observed clinical improvement. The older sib in the family was BCG vaccinated and remains healthy.

Kindred 22

The patient is a boy who was born in 2004 in Iran. His parents are nonconsanguineous. The patient, 23.II.1, received BCG at birth and presented with disseminated BCG disease soon after. The diagnosis of IL-12p40-deficiency was established 5 years later; in the meantime, the patient presented with 2 relapses of BCG disease before the diagnosis was established. For treatment of BCG disease he received antibiotics, and no recombinant human IFN- γ was administered during infections. At the last update in November 2010, the patient was alive and had not presented with any other infections.

Kindred 23

The parents are consanguineous, originating from and living in the Kingdom of Saudi Arabia. The proband (22.II.1) was BCG vaccinated at birth and developed localized BCG infection at age 12 months. Antituberculosis treatment was administered followed by observed clinical improvement. At the age of 4 years, the patient developed persistent lymphadenitis. *Nocardia brasiliensis* was cultured from the lymph node biopsy. The 8 younger sibs in the family are healthy.

Kindred 24

A Persian female patient (25.II.1) was born in 2008 in Iran. Her parents are first-degree consanguineous. BCG vaccine was administered at birth. Within 4 months the patient developed disseminated BCG. The disease was controlled but not cleared, and relapse of the infection occurred multiple times. Antibiotic and recombinant human IFN- γ were administered for treatment and continually used for prophylaxis. The patient was last seen by the physicians in Iran at the age of 2 years. She was free of infections and had not presented any other MSMD-related infection.

Kindred 25

A female patient (26.II.1) was born in Iran in 2002. The parents are first-degree consanguineous. She received BCG at birth and developed a localized BCG reaction 6 months later. Peripheral lymph nodes corresponded to the site of infection, which was treated with antibiotics and IFN- γ . IL-12p40 deficiency was diagnosed 6 months after the onset of infection. In July 2007, the patient presented with pyoderma gangrenosum, which was treated with corticosteroids and broad-spectrum antibiotics. At the time of the last update the patient was aged 8 years and had not had other MSMD-related infections.

Kindred 26

The parents are first-degree consanguineous originating from and living in Saudi Arabia. The patient 27.II.1 is a boy born in 2005. He received BCG at birth and had an immediate normal reaction to the vaccine. At 6 months the patient developed disseminated BCG infection. BCG was isolated from multiple sites, including the peripheral, mediastinal, and abdominal lymph nodes; lung; liver; and spleen. IL-12p40-deficiency was diagnosed 6 months after the onset of infection. Antibiotics and recombinant human IFN- γ were used for treatment and were continued as prophylaxis. Surgery was performed for the resection of peripheral and abdominal lymph nodes, pneumonectomy, and chest wall abscess drainage. Multiple episodes of relapse occurred, and the patient died at age 5 years. The patient has 2 healthy sisters who were not BCG vaccinated.

Kindred 27

The patient is a Tunisian boy who was born in 2007 to nonconsanguineous parents. He received BCG at birth and developed disseminated BCG disease 3 months later. The infection was treated with specific antibiotics and recombinant human IFN- γ . Despite treatment, the infection was not cleared, and the patient had relapses at ages 14 months, 24 months, and 29 months. The last follow-up occurred 7 months after the last BCG disease relapse, and the patient was free of infection. As for his family history, we noted that his mother had localized BCG disease after vaccine. The older brother and 1 maternal aunt were treated for tuberculosis, and both fully recovered from the disease.

Kindred 28

The patient (28.II.7) is a Saudi Arabian boy aged 2 years, born to consanguineous parents. The patient received BCG vaccine at birth. Clinical symptoms started when the child was 3 months old as left axillary lymphadenitis spontaneous rupture within 3 months of disease. Despite antituberculosis treatment there was no improvement of symptoms. When the patient was 10 months old he presented with bacteremia. Two months later he was hospitalized due to

gastroenteritis. Culture of tissue extracted from a biopsy of the left axillary lymph node identified *Salmonella* group D. At age 18 months the patient presented with disseminated disease with generalized lymphadenopathy with central necrosis in chest and abdomen as well as multiple splenic abscesses. The patient is alive and currently receiving antibiotics. One older brother (28.II.6) presented with adverse reaction to BCG vaccine as left axillary lymphadenitis at age 3 months. He was successfully treated with antituberculosis therapy.

Kindred 29

A female patient (29.II.1) was born in Saudi Arabia in 2010. Her parents were consanguineous. She received BCG vaccine at birth and developed disseminated BCG disease 2 months after the vaccination. Antituberculosis treatment was initiated with no improvement of symptoms. The patient died 1 year later. A younger brother (29.II.2) was born in 2011. He has the same homozygous mutation as the proband, but was not BCG vaccinated and remains asymptomatic.

Kindred 30

The patient (30.II.5) is a Saudi Arabian boy born to consanguineous parents in 2010. He received BCG at birth. At age 4 months the patient developed disseminated BCG disease with involvement of mediastinal lymph nodes, spleen, liver, and bones. Antibiotics and IFN- γ were administered and the patient recovered with no further infections. All 4 older sibs were BCG vaccinated with no adverse reaction.

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