Cryptococcal osteomyelitis in a child with a novel compound mutation of the *IL12RB1* gene

Orathai Jirapongsananuruk,[1] Voravich Luangwedchakarn,[2] Julie E. Niemela,[3] Punchama Pacharn,[1] Nualanong Visitsunthorn,[1] Charin Thepthai,[2] Pakit Vichyanond,[1] Surapon Piboonpocanun[4] and Thomas A. Fleisher[3]

Summary

The IL-12p40/IL-12Rβ1 and IFN-γR1/IFN-γR2/STAT1 signaling pathways are important for clearing intracellular bacteria. Genetic defects within these pathways are associated with increased susceptibility to intracellular pathogens. Among these, IL-12Rβ1 deficiency is the most common defect and leads to infections with *Salmonella* and *Mycobacterium* spp.

We report a child who presented with *Cryptococcal* osteomyelitis and history of disseminated *Mycobacterial* infection and recurrent *Salmonella* septicemia. Flow cytometry showed defective expression of IL-12Rβ1. Mutation analysis revealed a novel compound heterozygous mutation of *IL12RB1*, c.625C>T, p.Q209X was found in exon 7 on the paternal allele and c.710delC, p.P237HfsX5 was found in exon 8 on the maternal allele. As these mutations each result in a stop codon before the last spliceable exon, the transcripts likely underwent nonsense mediated decay, leading to a lack of IL12Rβ1 expression on the cell surface and eradicating signaling via the IL12 signaling pathway. *(Asian Pac J Allergy Immunol 2012;30:79-82)*

Key words:* *Cryptococcal* *osteomyelitis, IL-12Rβ1 deficiency, Mycobacterial infection, recurrent* *Salmonella* *infection

From the 1. Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
2. Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
3. Department of Laboratory Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, MD, USA
4. Institute of Molecular Biology and Genetics, Mahidol University, Nakorn Pathom,Thailand
Corresponding author: Orathai Jirapongsananuruk
E-mail: siojr@mahidol.ac.th
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Introduction

Interleukin (IL)-12 is a major cytokine for initiating a response to intracellular organisms.[1] IL-12 consists of 2 subunits, p40 and p35 chains, and is produced by antigen presenting cell upon contact with bacteria or bacterial products.[1] IL-12 binds to its receptor on activated T cells and NK cells inducing the production of IFN-γ, the major cytokine for Th1 mediated immunity.[1] The IFN-γ receptor consists of IFN-γR1 and IFN-γR2 that are constitutively expressed on monocytes and macrophages. Activation of IFN-γR induces the phosphorylation and homo-dimerization of the signal transducer and activator of transcription 1 (STAT1). The IL-12 receptor consists of IL12Rβ1 and IL12Rβ2 chains[1,2] and T cells that fail to express IL-12Rβ1 do not produce IFN-γ upon exposure to IL-12.[1,2]

Both the IL-12p40/IL-12Rβ1 and IFN-γR1/IFN-γR2/STAT1 systems are important for clearing intracellular bacteria.[1] Mutations of *IL12P40*, *IL12RB1*, *IFNGR1*, *IFNGR2* and *STAT1* have all been reported in patients susceptible to *Mycobacterium* spp.[2] Among these defects, *IL12RB1* deficiency is the most common genetic etiology observed.[2,3] The clinical phenotype of *IL12RB1* deficient patients includes infections with *Mycobacterium* spp. and *Salmonella* spp.[1,2,4]

We report a child who presented with *Cryptococcal* osteomyelitis as well as a history of disseminated *Mycobacterial* infection and *Salmonella* septicemia. Investigation revealed a lack of IL12Rβ1 on the cell surface and mutation analysis confirmed novel mutations affecting *IL12RB1*.

Case Report

A 3 year-old boy was referred to Siriraj Hospital, Mahidol University for suspected primary immunodeficiency. He had a history of swelling and tenderness of the right ankle for 6 months and had been diagnosed as having chronic osteomyelitis of

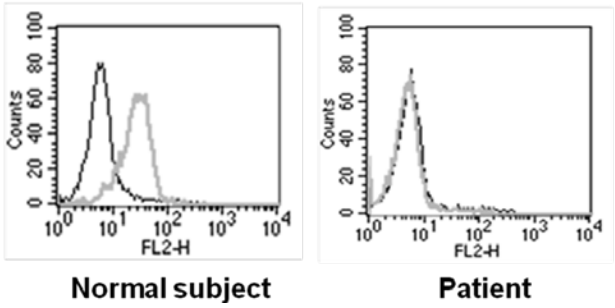

Figure 1. Detection of IL-12Rβ1 expression by flow cytometry on PHA-stimulated PBMCs of the patient and a normal subject. The black line represents isotype control and the grey line represents IL-12Rβ1 staining. The stimulated PBMCs of the normal subject showed a clear signal for IL-12Rβ1 expression while the patients' activated PBMCs demonstrate absence of IL-12Rβ1 surface expression.

the right calcaneous with septic arthritis of the subtalar joint. The cultures from open drainage at the site of infection obtained by curettage and draining pus from the right calcaneous revealed *Cryptococcus neoformans.* Amphotericin B was given for 9 weeks and he was then switched to oral fluconazole 100 mg daily. He had previously been diagnosed with disseminated *Mycobacterial* infection involving the lungs and right axillary lymph nodes when he was 9 months of age. The chest X-ray at that time revealed alveolar infiltrates of both lungs. The PPD skin test showed induration of 18 mm but the sputum was negative for AFB. A lymph node biopsy showed inflammation with granulomas and numerous acid fast bacilli. He was successfully treated with anti-tuberculous drugs for 6 months. He also had a history of recurrent *Salmonella* group D septicemia 3 times between 18 and 36 months of age. The patient is the only child in the family and there was no history of consanguinity. The parents have not had frequent infections or any known risk of HIV infection and the family history did not reveal anything suggestive of primary immunodeficiency.

Physical examination revealed an afebrile Thai boy with the weight and height in the 10th percentile. Multiple cervical and inguinal lymph nodes were palpable at 1.5 cm. in diameter without tenderness but there was no hepatosplenomegaly. The right ankle was not swollen and demonstrated a healed surgical scar.

The complete blood count showed a hemoglobin of 8.3 g/dL, hematocrit of 27%, white blood cell count of 25,300/mm^3 with 76% neutrophils, 13% lymphocytes, 10% monocytes and 1% basophils, and a platelet count of 324,000/mm^3. The erythrocyte sedimentation rate was 76 mm/hr and the anti-HIV antibody test was non-reactive. The immunoglobulin levels and CD3, CD4, CD8, CD19 and NK cell counts were normal as well as the dihydrorhodamine assay. The cell surface expression of IFN-γR1 and IL-12Rγ1 were studied by flow cytometry. The IFN-γR1 expression of the patient's monocytes was normal. However, the IL-12Rγ1 expression of PHA-stimulated mononuclear cells (activated T cells) was absent as shown in Figure 1 and a preliminary diagnosis of IL-12Rγ1 deficiency was made. Mutation analysis confirmed this with the finding of compound heterozygous mutations of *IL12RB1* consisting of c.625C>T, p.Q209X (exon 7) and c.710delC, p.P237HfsX5 (exon 8). Mutation analysis of *IL12RB1* in the mother and father revealed the c.710delC, p.P237HfsX5 (exon 8) mutation and the c.625C>T, p.Q209X (exon 7) mutations, respectively (Figure 2)

The *Cryptococcal* osteomyelitis was treated with fluconazole for 2 years. After that, he was switched to itraconazole for fungal prophylaxis and also maintained on cotrimoxazole for *Salmonella* prophylaxis and azithromycin for *Mycobacterium* prophylaxis. He has been well without serious infection for the past 3 years up to the time of this report.

Discussion

In a patient with unusual, severe infections caused by poorly pathogenic *Mycobacterium* and *Salmonella* spp., a defect in the IFN-γ and IL-12 pathways should be considered.[5] IL-12Rβ1 deficient and IL-12p40-deficient patients have a history of infection with *Salmonella* spp. (about 50%) more frequently when compared to IFN-γR1/IFN-γR2/STAT1-deficient patients. The latter are consistently infected with *Mycobacterium* spp. but only infrequently infected with *Salmonella* spp.[1-3] Other infectious organisms which have been occasionally reported in IL-12Rβ1 deficiency include *Klebsiella* spp., *Citrobacter freundii*, *Paracoccidioides brasiliensis*, *Toxoplasma gondii*, *Histoplasma* spp., *Leishmania* spp., and *Nocardia* spp..[4,6]

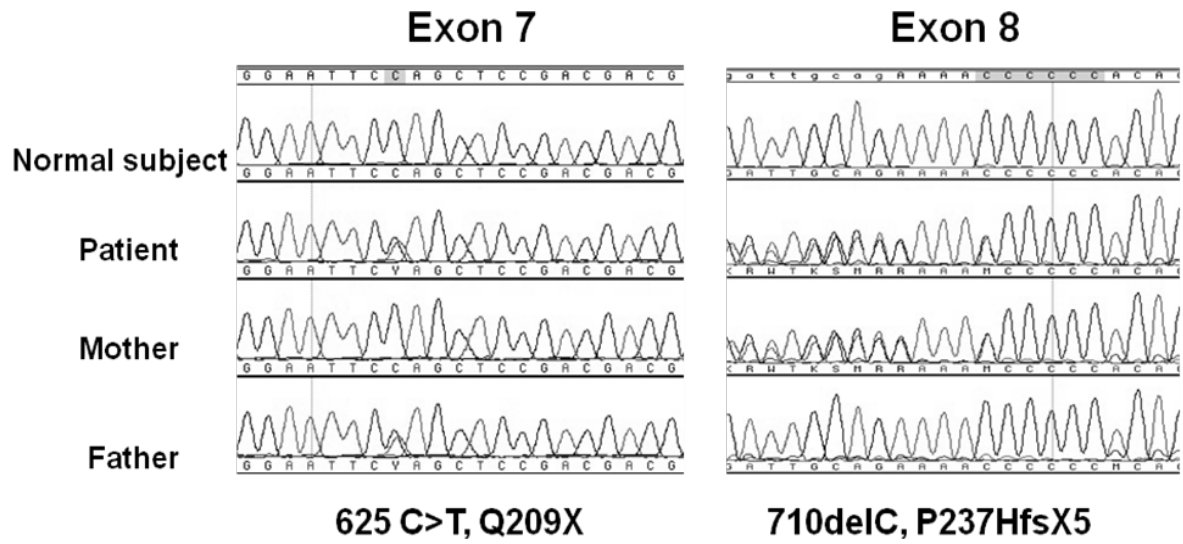

Figure 2. Mutation analysis of *IL12RB1* exon 7 and 8 of a normal subject, the patient and his mother and father. Where the A of the ATG start codon is numbered +1, the sequence of *IL12RB1* exon 7 of the patient and his father reveals a substitution of C with T at cDNA position 625, which results in an immediate stop codon at amino acid Q209. In exon 8 (reverse sequencing data shown), deletion of C at cDNA position 710 in the patient and his mother results in a frameshift at amino acid P237 and a stop codon at position 5 in the shifted reading frame.

Cryptococcal osteomyelitis is an unusual presentation for IL-12Rβ deficiency. *Cryptococcal neoformans* is a yeast found in soil contaminated with bird droppings.[7] The central nervous system and the lung are most frequently infected.[8] There are infrequent reports of *Cryptococcal* arthritis, cellulitis, hepatitis, prostatitis, vaginitis, infection of the urinary tract and the intestine.[7] *Cryptococcal* osteomyelitis is a rare disease occurring in 5-10% of disseminated *Cryptococcal* infection.[7-9] Infection with *C. neoformans* is observed most frequently in immunocompromised patients with phagocyte or severe T cell immunodeficiency.[10] The most common among these are patients infected with human immunodeficiency virus.[7] In primary immunodeficiency, *Cryptococcus* has been reported as a cause of infection in X-linked hyper-IgM syndrome, hyper-IgE syndrome, idiopathic CD4 lymphopenia and X-linked agammaglobulinaemia.[7,10-14] Disseminated *Cryptococcal* infection was first reported in a child with IL-12Rβ1 deficiency in Iran[15] and to our knowledge the case reported here is the second case of IL-12Rβ1 deficiency associated with *Cryptococcal* infection.

In this patient, IL-12Rβ1 deficiency was confirmed by mutation analysis of *IL12RB1*. The *IL12RB1* gene includes 17 exons, encoding a gp130-like protein, formed by an extracellular domain (with cytokine binding region), a transmembrane domain and an intracellular domain[3,5] Various types of *IL12RB1* mutations have been reported, including nonsense, missense, splice mutations, microdeletions, microinsertions, microduplications, and large deletions.[2,3] All of these mutations are recessive and result in loss of receptor function.[1,3] In this patient, the mutation in exon 7, c.625C>T affecting the paternal allele resulted in a stop codon at amino acid Q209 (Figure 2). The mutation in exon 8, c.710delC affecting the maternal allele resulted in a frameshift at P237 ending with a stop codon at position 5 in the shifted reading (Figure 2). Both mutations resulted in stop codons before the last spliceable exon, thus the transcripts likely underwent nonsense mediated decay resulting in a lack of IL12Rβ1 on the cell surface.[5,16] To our knowledge, these mutations have not been previously reported.[4,5,17] As is expected in this autosomal recessive disorder, the parents who have only one defective allele, are asymptomatic.

In conclusion, we report a patient who presented with *Cryptococcal* osteomyelitis and history of disseminated *Mycobacterial* infection and recurrent *Salmonella* septicemia. Flow cytometry showed defective expression of IL-12Rβ1. Mutation analysis revealed novel mutations of *IL12RB1* in exon 7 and 8 resulting in a lack of IL12Rβ1 expression on the cell surface with subsequent defective IL12

signaling and increased susceptibility to *Mycobacterial* and *Salmonella* infection as well as *Cryptococcal* infection.

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