Case Report: Interferon-Gamma Receptor-1 Gene Promoter Polymorphisms and Susceptibility to Leprosy in Children of a Single Family

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Abstract. The autosomal recessive disorder, because of a single mutation in interferon- γ receptor-1 (IFNGR1) at position –56, was found to be associated with susceptibility to leprosy in children of the same family. The existence of such heterozygous carriers might explain the crucial role of IFNGR1 in the host defense against intracellular pathogens such as *Mycobacterium leprae*. The single nucleotide polymorphisms (SNPs) in major candidate genes, i.e., natural resistance-associated macrophage protein 1 (NRAMP1), vitamin D receptor (VDR), tumor necrosis factor- α (TNF- α), interleukin-10 (IL-10), interleukin-12-receptor 1 (IL-12R1), were not found to be associated with this disease.

INTRODUCTION

Leprosy is an infectious disease caused by the *Mycobacterium leprae*.¹ Clinically, leprosy is manifested as a disease spectrum with two polar forms, lepromatous-LL and tuberculoid-TT.² The spectrum is further divided into infectious multibacillary, paucibacillary, and transitional borderline forms.² The precise mechanism of transmission of *Mycobacterium leprae* is unknown. Recent molecular studies have begun to explain the importance of immune response to *M. leprae* at two fundamental levels. In the first level the overall susceptibility and resistance to this organism have been described, and at the second level a range of cellular immunity and delayed hypersensitivity have been shown among individuals who are infected.^{3,4} Generally, they revealed a predominance of interleukin-2 (IL-2), tumor necrosis factor- α (TNF- α), interferon-gamma (IFN- γ) transcripts in tuberculoid lesions, and IL-4, IL-5, and IL-10 in lepromatous ones.^{2,3} Alternatively, in cytokine genes several single nucleotide polymorphisms (SNPs) have been confirmed to play a critical role in triggering host-pathogen interactions.⁵ In this study, the influence of major candidate genes, i.e., SNPs in the natural resistance-associated macrophage protein 1 (NRAMP1) gene, vitamin D receptor (VDR), TNF- α , IL-10, interleukin-12-receptor 1 (IL-12R1), and IFNGR1 were investigated within infected children of a single family.

In 2010, an Iranian boy, 12 years of age, was admitted to a Department of Pediatric in National Research Institute of TB and Lung Diseases, Tehran, Iran. He presented with massive lesions with localized drainage on the left and right foot and complaining about edema (Figure 1). The problem started 8 years ago with the appearance of a small blister on his toes. A presumptive diagnosis of fungal infection was made at local consultation that did not improve despite topical and systemic antifungal therapy. On admission to our hospital, nerve trunks of the upper and lower limbs were normal. The routine laboratory tests showed, erythrocyte sedimentation rate (ERS) of 110 mm/h with 9.5g/dL of hemoglobin. Antinuclear antibodies (ANAs) and rheumatoid factor (RF) were nega-

tive. Radiological investigation was normal (thorax and skeleton). Histopathological examination of skin biopsy stated the chronic lymphohistiocytic process associated with vasculitis. The result of smear examination for acid-fast bacillus (AFB) was reported to be scanty positive. Bacterial DNA was prepared by a microbead method and 7 μ L of the supernatant was used for polymerase chain reaction (PCR) using primers Tb11 (5'-ACCAACGAT GGTGTCCAT) and Tb12 (5'-CTTGTCGAACCGC-ATACCCT).⁶ The PCR amplified

FIGURE 1. Infection with *Mycobacterium leprae*.

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a 439-bp fragment of *hsp65*. Digestion of PCR product upon restriction enzymes (*Bst*II and *Hae*III) produced fragments of 315, 135 bp and 265,130 bp, respectively. Therefore, the PCR-restriction fragment length polymorphism (PCR-RFLP) using *hsp65* is simple, fast, and useful for the positive identification of *M. leprae*, which still remains the only non-cultivable *Mycobacterium*.⁶

The treatment was initiated according to World Health Organization (WHO) recommendations, with 100 mg/d of dapsone, 50 mg/d of clofazimine, and rifampicin 600 mg/month.⁷ After only 1 month of treatment the skin lesions became smaller and flatter.⁷ Further classical investigation showed two more cases of leprosy in family members (his maternal uncle and his sister). They were registered in different hospitals. They had already developed deformities in their feet and hands. In one of them, the foot was spontaneously and in another one was surgically amputated. Genetic investigation was performed on all three cases and other family members that were in close contact, but did not develop leprosy (mother, father, brothers, sisters, uncle, nephew, and nieces). The SNPs at INT4, D543, 3'UTR of NRAMP1 gene, SNPs in restriction sites of *Bsm*I and *Fok*I of VDR gene, and SNPs of TNF- α at -238, -308, -244, -857, -863 positions, SNPs of IL-10 at position -1082, -819, -592, SNPs of IL-12 RB1 at +705, +1158, +1196, +1637, +1664, and IFNGR1 at position

-179 and -56 were investigated using PCR-RFLP.⁸ The frequency of the genotypes were estimated by direct gene counting and the data analyzed using Pearson's χ^2 test. The results were compared with previously published data⁸. Allele frequencies of NRAMP1 gene polymorphisms for INT4, D543, and 3'UTR were the same in the patient and his close family members with or without leprosy (G/G; G/G; TGTG+/+). Similarly, no difference was observed between allele frequencies of VDR for *Fok*I and *Bsm*I gene polymorphisms. The observed allele frequencies of IL-10 (-1082 G to A; -819 C to T; -592 C to A), IL-12 (-705A to G; -1158 T to C; -1196 C to T; -1637 G to A; -1665 C to T), and TNF- α (-238 G to A; -308 G to A) were also not statistically significantly (Table 1). Although, all three cases that developed leprosy had an SNP at IFNGR1 at -56 positions. This type of mutation was absent in other family members and in healthy control population of Iran (Table 1).

The IFN- γ induces cellular activation by binding to a receptor complex consisting of at least two subunits: the IFN- γ binding subunit (IFNGR1) and a chromosome 21-encoded transmembrane accessory factor (IFNR2).^{9,10} Recently, Newport and others,¹¹ showed mutations in the gene for IFN- γ are able to limit the growth of mycobacteria *in vitro*, and there has been a synergistic effect of IFN- γ and TNF- α . It was interesting to note that the investigated patients in this study had no other infections

TABLE 1
Gene polymorphisms in leprosy patients and their close family members. The results were compared with healthy populations

SNPs	Genotype	Patients (N = 3)	Close family member (N = 12)	Healthy populations* (N = 60)	P value	χ^2
TNF ⁻²³⁸	G/G	3 G/G (100%)	8 G/G (66%)	57 G/G (95%)	0.007	$\chi^2 = 9.8$
	G/A or A/A	0	4 G/A (33%)	3 G/A (5%)		
TNF ⁻³⁰⁸	G/G	2 G/G (66%)	1 G/G (8.3%)	56 G/G (93%)	0.001	$\chi^2 = 43.32$
TNF ⁻²⁴⁴	G/A or A/A	1 G/A (33%)	11 G/A (91%)	4 G/A (6%)	—	—
	G/G	3 G/G (100%)	12 G/G (100%)	60 G/G (100%)		
TNF ⁻⁸⁵⁷	C/C	3 C/C (100%)	10 C/C (83%)	42 C/C (70%)	0.1	$\chi^2 = 4.57$
TNF ⁻⁸⁶³	C/T or T/T	0	2 C/T (16.6%)	18 C/T (30%)	0.5	$\chi^2 = 1.36$
	C/C	3 C/C (100%)	5 C/C (41%)	39 C/C (65%)		
	C/A or A/A	1 G/A (33%)	7 C/A (58%)	21 C/A (35%)		
IFN ⁻¹⁷⁹	G/G	3 G/G (100%)	12 G/G (100%)	60 G/G (100%)	—	—
	G/T or T/T	0	0	0		
IFN ^{-56†}	T/T	0	12 T/T (100%)	60 T/T (100%)	—	—
	T/C or C/C	3 C/C (100%)	0	0		
IL-10 ⁻¹⁰⁸²	G/G	0	0	8 G/G (13.3%)	0.32	$\chi^2 = 2.24$
	G/A or A/A	2 G/A (66%), 1 A/A (33%)	11 G/A (91%), 1 A/A (8.3%)	45 G/A (75%), 7 A/A (11%)		
IL-10 ⁻⁸¹⁹	C/C	1 C/C (33%)	1 C/C (8%)	5 C/C (8%)	0.34	$\chi^2 = 2.14$
	C/T or T/T	2 C/T (66%)	7 C/T (58%), 4 T/T (33%)	43 C/T (71%), 12/T (20%)	0.34	$\chi^2 = 2.14$
IL-10 ⁻⁵⁹²	C/C	1 C/C (33%)	1 C/C (8.3%)	5 C/C (8%)		
	C/A or A/A	2 C/A (66%)	7 C/A (58%), 4 A/A (33%)	40 C/A (66%), 20 A/A (33%)		
IL-12 ⁻⁷⁰⁵	A/A	3 A/A (100%)	12 A/A (100%)	60 A/A 100%	0.34	$\chi^2 = 2.14$
	A/G or G/G	0	0	0	0.34	$\chi^2 = 2.14$
IL-12 ⁻¹¹⁵⁸	T/T	1 T/T (33%)	1 T/T (8.3%)	5 T/T (8%)		
	T/C or C/C	2 C/A (66%)	7 C/A (58%) 4 A/A (33%)	45 C/A (75%), 10 A/A (16%)		
IL-12 ⁻¹¹⁹⁶	C/C	1 C/C (33%)	1 C/C (8%)	5 C/C (8%)	0.34	$\chi^2 = 2.14$
	C/T or T/T	2 C/A (66%)	7 C/A (58%) 4 A/A (33%)	45 C/A (75%), 10 A/A (16%)		
IL-12 ⁻¹⁶³⁷	G/G	3 G/G (100%)	12 G/G (100%)	60 G/G (100%)	—	—
	G/A or A/A	0	0	0		
IL-12 ⁻¹⁶⁶⁵	C/C	3 C/C (100%)	12 C/C (100%)	60 C/C (100%)	—	—
	C/T or T/T	0	0	0		
NRAMP INT4	G/G	3 G/G (100%)	12 G/G (100%)	60 G/G (100%)	—	—
	G/C or C/C	0	0	0		
NRAMP D543	G/G	3 G/G (100%)	12 G/G (100%)	44 G/G (73%)	0.08	$\chi^2 = 5.0$
	G/A or A/A	0	0	16 G/A (27%)		
NRAMP 3'UTR	TGTG+/+	3 +/+ (100%)	12 +/+ (100%)	57 +/+ (95%)	0.68	$\chi^2 = 0.78$
	+del or del/del	0	0	3 +del (5%)		

SNPs = single nucleotide polymorphisms.

* Healthy population: here referred to those cases that had no mycobacterial-infection, using purified protein derivative (tuberculin) (PPD), smear, and x-ray chest examination.

† These heterozygous carriers (T to C at IFN⁻⁵⁶ position) might explain differences in susceptibility to *M. leprae*.

with pathogenic bacteria or fungi and have coped with viral infections normally. The specificity of the defect in these children suggests that there must be a redundancy of immunologic mechanisms against most of these pathogens but not against *M. leprae*. Therefore, present cases not only provide important information on the role of the IFNGR1 Pathway in human immunity, but also identify a gene with a potentially crucial role in determining susceptibility to *M. leprae*. Few investigators showed an association between mutation of IFNGR1 and lepromatous leprosy patient, but others did not support the idea.^{10,12} Generally, the diverse genetic results of the disease acquisition might be explained by distinct genetic susceptibility to the disease among different populations.² Clearly, the complete absence of IFNGR1 on cell surface induces so profound an immune defect that such mutations are unlikely to explain *M. leprae* susceptibility in the general population. However, the existence of heterozygous carriers of the defect or more subtle variations in this gene might explain differences in *M. leprae* susceptibility within the population.

In our study IFNGR1 at position -56 T/C polymorphism was positively associated with an increased susceptibility to leprosy. Although polymorphisms of NRAMP, VDR, and TNF- α have been associated with increased susceptibility to leprosy in some studies, we did not find a mutation in these genes.^{2,13,14} Additionally, we could not find a link between IL10 and IL12R1 and the disease in our study. Overall, there are many literatures about clinical manifestations and patient's immune response of adult leprosy. However, the molecular basis of the genetic vulnerability of leprosy in children is still limited. The association of IFNGR1 polymorphism with susceptibility to leprosy should be studied with a larger sample size to elucidate the exact role of IFNGR1 polymorphisms in this disease.

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