Interleukin-17F single-nucleotide polymorphism (7488T>C) and its association with susceptibility to leprosy

V. S. Chaitanya*, R. S. Jadhav†, M. Lavania†, M. Singh§, V. Valluri‖ & U. Sengupta**

Summary

The objective of this study was to investigate the association, if any, between the interleukin-17F (7488T>C) (rs763780) polymorphism and susceptibility to leprosy and to elucidate the relationship between IL-17F genotypes and clinical profile of the disease. DNA was extracted from the peripheral venous blood of leprosy cases (n = 140), which were classified as per WHO classification into paucibacillary (PB) (n = 53) and multibacillary (MB) (n = 87) categories and healthy controls (n = 84) without any signs and symptoms of leprosy. The IL-17F (7488 T/C) polymorphism was genotyped using amplification refractory mutation system – polymerase chain reaction (Allele-specific amplification). In both PB and MB categories of leprosy cases, the homozygous TT genotype frequency was significantly higher than that of the healthy controls (78.70% vs. 29.76%, P < 0.05). The heterozygous TC genotype was higher in the controls than in the leprosy cases (57.14% vs. 17.68%, P < 0.05). TT genotype was more associated with the type 1 reactional states and tuberculoid/borderline tuberculoid groups in leprosy than the TC genotype. This study reveals that the IL-17F (7488T>C) single-nucleotide polymorphism is associated with susceptibility to leprosy and polymorphism confers decrease in risk of contracting leprosy in the north Indian cohort.

Introduction

Leprosy is a chronic infectious disease mainly affecting the skin and the peripheral nervous system. It is caused by infection with Mycobacterium leprae (M.leprae), and the disease continues to be a significant public health problem in the developing countries. Multidrug therapy (MDT) can cure the infection, but leprosy reactions of type 1 and type 2 may occur and neuropathy may develop, which will lead to disability and deformity. It is important that the manifestations of the condition are recognized as early as possible so that early nerve damage can be identified and treated rapidly (Walker & Lockwood, 2006). Identification of a genetic marker, which determines susceptibility to leprosy, may aid in developing diagnostic tools for early detection of leprosy. Invasion of M.leprae elicits a cascade of immunological responses essential for the control of infection, which include generation of antigen-specific T-cell responses and activation of infected macrophages, involving either up-regulation or down-regulation of pro-/anti-inflammatory cytokines (Garcia et al., 2001). Single-nucleotide polymorphisms (SNPs) in the coding regions of these cytokine coding genes may have an impact on the expressed proteins leading to either alterations in the amount of cytokine produced or change in the functional role (Turner et al., 1997). Polymorphic variants of these cytokine coding genes may therefore be used as prognostic genetic markers to determine the susceptibility to this disease (Moraes et al., 2004).

Only a subset of the individuals who are exposed to M.leprae develop the clinical disease and also present a broad clinical and immunological spectrum. The outcome of the infection is in part influenced by the host genes that regulate the host-specific immune responses and control the initial infection. So far around 12 genes were implicated in leprosy susceptibility, and a genomewide association study has led to the identification of at least one positional candidate, the human leucocyte antigen (HLA class 1), coding gene in the major histocompatibility complex (MHC) site. Evidence that host genetic factors contribute to susceptibility to leprosy comes from epidemiological data, segregation and twin studies. Thus far, all the...
genes suggested to have a role in the susceptibility to leprosy either act to directly modulate development of the adaptive response (HLA, major histocompatibility complex class I, polypeptide-related sequence A (MICA), transporter 2, ATP-binding cassette, subfamily B (TAP2), cytotoxic T lymphocyte-associated antigen (CTLA4), vitamin D receptor (VDR)) or may bridge the innate and adaptive responses (solute carrier family 1 member 1 (SLC11A1), toll-like receptor 2 (TLR2), heat shock protein 70 (HSP70), tumour necrosis factor alpha (TNFα), macrophage mannose receptor-1 (MRC1)). Although the genomewide linkage studies reach statistical significance, it seemed possible that infectious diseases like that of leprosy are too polygenic to have enough power to detect linkage using genomewide approach (Fitness et al., 2002). IL-17F is one of the novel candidates that is to be explored in this context to identify its relevance as a genetic marker of susceptibility to leprosy.

Interleukin-17F is an important member of a group of cytokines called the interleukin-17 family. It acts as a potent mediator in the delayed-type hypersensitivity reactions to recruit monocytes and neutrophils to the site of inflammation by increasing chemokine production in various tissues, which is similar in function to interferon-gamma (Kawaguchi et al., 2007). It is produced by the T helper cells and is induced by IL-23, which results in destructive tissue damage in delayed-type hypersensitivity reactions (Iwakura & Ishigame, 2006). Interleukin-17 as a family functions as a proinflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen’s cellular matrix (Bettelli et al., 2008). It is an active recruiter of neutrophils to inflammatory sites, and IFN-gamma regulates the induction of Th17 cells. This may explain the damaging inflammatory response seen during mycobacterial infection of IFN-gamma-deficient mice, indicating that IFN-gamma and IL-17 may counter regulate with each other during chronic mycobacterial infections (Cruz et al., 2006).

Owing to the capabilities of IL-17F to potentially induce chemokine expression and recruit cells to parenchymal tissues and the involvement of Th1 and Th17 responses that cross-regulate during mycobacterial infections (Khader & Cooper, 2008), we have chosen to study the coding region sequence variant single-nucleotide polymorphism of the IL-17F (7488 T/C, rs763780) gene in the context of leprosy to identify its association, if any, with susceptibility to leprosy. This coding SNP has been proved to have a strong functional role by antagonizing the normal function of wild-type IL-17F, thereby conferring protection in the asthma model (Kawaguchi et al., 2006).

The objective of this study was to perform a case-control association analysis to identify the possible association between IL-17F single-nucleotide polymorphism (7488 T/C, rs763780) and its role in conferring susceptibility to leprosy. We determined the percentage of each polymorphic variant (genotype) within the study groups and identified the differences in their occurrence within cases and controls. Further, we associated the genotypes within the leprosy group with various clinical and immunological manifestations of the disease so as to delineate the possible functional effects of this polymorphism within the disease model.

**Methods**

**Patients and controls**

The study population consisted of active untreated leprosy cases (n = 140) attending the Out Patient Department of The Leprosy Mission Community Hospital - Shahdara, New Delhi and healthy controls from the same ethnic background (n = 84) without any signs and symptoms of leprosy. The leprosy cases were classified based on the number of skin lesions present into Paucibacillary (PB) (n = 53) and Multibacillary (MB) (n = 87) (Pardillo et al., 2007). Under this method of classification, a case with ≤5 lesions is considered as PB and a case with > 5 lesions is considered as MB. The demographic and clinical data of all the subjects were assessed based on the complaints presented and the clinical investigations. All the subjects enrolled in the study belong to New Delhi (National Capital Region) and surrounding areas in Uttar Pradesh (North India), indicating no significant variation in the geographical locations. All the study subjects share the same ethnicity, and there are no significant variations in the age and gender distribution within the cases and controls (Table 1). Cases were enrolled immediately on diagnosis and before MDT treatment and were followed up during the course of treatment to monitor the development of reactions. Cases in Type 1 reaction indicate leprosy cases with increased cell-mediated immune response against *M. leprae* which is often:

**Table 1. Basic characteristics of the study population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Paucibacillary leprosy</th>
<th>Multibacillary leprosy</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>53</td>
<td>87</td>
<td>84</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>69</td>
<td>65</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>34.1 ± 14.3</td>
<td>29.9 ± 11.8</td>
<td>33.4 ± 12.6</td>
</tr>
<tr>
<td>Range</td>
<td>7–79</td>
<td>9–66</td>
<td>7–76</td>
</tr>
<tr>
<td>Cases in type 1 reaction</td>
<td>44</td>
<td>46</td>
<td>–</td>
</tr>
<tr>
<td>RJ classification*</td>
<td>50 (84.3)</td>
<td>38 (43.7)</td>
<td>–</td>
</tr>
<tr>
<td>TT/BT (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RJ classification*</td>
<td>3 (5.7)</td>
<td>49 (56.3)</td>
<td>–</td>
</tr>
<tr>
<td>BL/LL (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*There were no BB cases in leprosy study group, and hence, the BB group was not included under RJ classification.
manifested by localized tissue damage, acute inflammation and neuritis resulting in permanent nerve damage (Manandhar et al., 2002). All the cases in type 1 reaction \((n = 90)\) were clinically determined by experienced clinicians at the base hospital. As a part of the clinical diagnosis, histopathological analysis was performed and Ridley–Jopling classification (RJ classification) was determined by the clinicians. The leprosy cases were classified based on RJ classification into tuberculoid/borderline tuberculoid \((TT/BT)\) \((n = 88)\) and borderline lepromatous/lepromatous \((BL/LL)\) \((n = 52)\) groups (Ridley & Jopling, 1966). This is a histopathological classification that determines the immunological state of the patient. The spectrum of clinical manifestations of leprosy includes 5 groups from tuberculoid pole to the lepromatous pole namely tuberculoid \((TT)\), borderline tuberculoid \((BT)\), borderline borderline \((BB)\), borderline lepromatous \((BL)\) and lepromatous \((LL)\). The tuberculoid pole is characterized by the increased cell-mediated immunity \((CMI)\) and low bacillary load, which gradients towards the lepromatous pole which is characterized by decreased CMI and increased bacillary load and with comparatively greater humoral immunity (Ridley, 1974). Two millilitre of peripheral venous blood was collected after informed consent for participation was signed by all the study subjects. The study protocol was approved by the ethics committee of the Leprosy Mission Trust India.

Genotyping of polymorphism

Genomic DNA was isolated from 2 mL of peripheral venous blood using DNeasy Blood and Tissue Kit from Qiagen (Hilden, Germany) (Cat No:69581), and IL-17F gene polymorphism \((7488T>C)\) was genotyped using amplification refractory mutation system – polymerase chain reaction (Germer et al., 2000). ARMS-PCR amplified the 2 alleles in 2 different PCRs (Hizawa et al., 2004). The region flanking the mutation is amplified by a common \((outer)\) primer, producing a non-allele-specific common amplicon. Two allele-specific \((inner)\) primers are designed in opposite orientation and, in combination with the common primer, can simultaneously amplify both the wild-type and the mutant amplicons. Primers for human growth hormone gene \(\text{Forward primer:} 5’\text{GCCCTTCCAACCATT C CCTTA 3’ and reverse primer:} 5’\text{TCACGGATTTCTG T TGTGTTTC 3’}\) are used to amplify a sequence of 426 bp, which is used as internal control so as to assess the specificity of PCR amplifications (Bittar et al., 2006).

The primers were designed to specifically amplify either the \(+7488\ T\) or the \(+7488\ C\) alleles in two separate polymerase chain reactions. The forward primer for T-specific allele is \(5’\text{GGATATGCACCTCTTAC TGCACCT} 3’\) and for C allele is \(5’\text{GGATATGC ACCTCTTACGTACCT} 3’\) \((\text{Nucleotides corresponding to SNP position are underlined})\). The common reverse primer is \(5’\text{CACCAAGGCTGCTTGT} 3’\).

IL-17F \((7488T>C)\) SNP and leprosy

Further analysis of the distribution of genotypes within the study groups revealed that the homozygous TT genotype was significantly higher in PB group than the control group \((79.2\% \text{ vs.} 29.8\%, \text{ }P < 0.05)\) and the heterozygous genotype TT is significantly lower in the PB group when compared with the control group.
(16.9% vs. 57.1%, \( P<0.05 \)). In the MB group, again the homozygous genotype TT was significantly higher than the control group (78.2% vs. 29.8%, \( P<0.05 \)) and the heterozygous genotype TC is significantly lower in MB group when compared with the control group (18.4% vs. 57.1%, \( P<0.05 \)). The allelic frequency distribution was significantly different between the leprosy groups (PB and MB) and the control group. The type 1 reaction includes cases with skin reactions. The type 1 reaction includes cases with skin reactions.

**Figure 1.** In the ARMS-PCR model, allele-specific primers were used which amplify a sequence of 106 bp; however, the C-specific primer will amplify only if ‘C’ is present at 7488 location in IL-17F gene, and T-specific forward primer will amplify only if ‘T’ is present at the above-mentioned position. As both these allele-specific forward primers have a common reverse primer, the size of the amplicon is same. Two PCRs were set for each sample where one contains T allele-specific forward primer and common reverse primer, and the other reaction tube contains C allele-specific forward primer and a common reverse primer. Both the amplicons were electrophoresed on a 2% agarose gel in adjacent lanes and amplification in only C-specific PCR, and no amplification in T-specific PCR indicates a CC homozygous mutant genotype, which is shown in lanes 1 and 2. The amplification in T-specific PCR and no amplification in C-specific PCR indicate a TT homozygous wild genotype, which is shown in lanes 3 and 4. The amplification in both the allele-specific PCR indicates a TC heterozygous mutant genotype as shown in lanes 5 and 6. Lane 8 has a 2000-bp ladder, and lane 10 is a negative control lane.

**Figure 2.** Distribution of IL-17F 7488T>C polymorphic variants (genotypes) and allele frequency distribution across the study groups.

**Association between the IL-17F genotypes and clinical phenotypes of leprosy**

We have also associated the IL-17F polymorphic variants (genotypes) with the clinical manifestations of the disease such as type 1 reversal reactions and with the two groups of histopathological classification of leprosy (RJ Classification), the tuberculoid/borderline tuberculoid (TT/BT) group and borderline lepromatous/lepromatous (BL/LL) groups. We have identified that the homozygous TT genotype is significantly higher in the tuberculosis/borderline tuberculoid (TT/BT) group than the heterozygous TC genotype (70.0% vs. 36.0%, \( P<0.05 \)). However, homozygous TT genotype distribution was significantly lower in borderline lepromatous/lepromatous (BL/LL) group when compared with the heterozygous TC genotype (30.0% vs. 64.0%, \( P<0.05 \)) (Table 3). Of the 110 cases with TT genotype, 82 (74.5%) cases developed type 1 reaction, whereas of 25 cases with TC genotypes, 8 (32.0%) cases developed type 1 reaction (74.5% vs. 32.0%, \( P<0.05 \)). This indicated that the subjects with TT genotype are more likely to develop type 1 reversal reactions. The type 1 reaction includes cases with skin reaction and/or nerve reaction.

**Discussion**

Our current findings demonstrate that TT genotype of IL-17F 7488 T/C polymorphism is associated with susceptibility to leprosy on contrary to TC genotype, which confers a decrease in risk of contracting leprosy.

**Table 2.** The genotypes and allele frequency distribution of IL-17F (7488) T>C in Leprosy patients and controls. (CI = Confidence Interval, OR =Odds Ratio

<table>
<thead>
<tr>
<th>Group</th>
<th>T/T genotype (%)</th>
<th>T/C genotype (%)</th>
<th>C/C genotype (%)</th>
<th>T/C allele (%)</th>
<th>C allele (%)</th>
<th>P-value (1 + 2 vs. 3)</th>
<th>OR (95%CI) (1 + 2 vs. 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Leprosy PB (n = 53)</td>
<td>42 (79.2)</td>
<td>9 (16.9)</td>
<td>2 (3.8)</td>
<td>88</td>
<td>12</td>
<td>(T/T) &lt;0.0001</td>
<td>(T-allele) 5.31 (CI:2.6–10.9)</td>
</tr>
<tr>
<td>(2) Leprosy MB (n = 87)</td>
<td>68 (78.2)</td>
<td>16 (18.4)</td>
<td>3 (3.4)</td>
<td>87</td>
<td>13</td>
<td>(T/C) &lt;0.0001</td>
<td>(C-allele) 0.20 (CI:0.1–0.4)</td>
</tr>
<tr>
<td>(3) Controls (n = 84)</td>
<td>29 (29.8)</td>
<td>48 (57.1)</td>
<td>11 (13.1)</td>
<td>58</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Single-nucleotide polymorphisms (SNPs) within IL-17F gene were studied in various models (Paradowska-Go
grycka et al., 2010; Guo et al., 2013; Lv et al., 2013) to evaluate their use as predictive and prognostic
genetic markers of disease susceptibility. We performed a case–control association analysis initially between the leprosy and control groups and later between various clinical manifestations within the leprosy group to evaluate the use of this SNP as the genetic marker of susceptibility to leprosy as such and also to identify its association with various clinical phenotypes. A genetic marker conferring susceptibility to leprosy is required to detect and treat early cases before the manifestation of symptoms and avoid nerve damage and subsequent deformities. In this context, various studies on cytokine gene polymorphisms and their association with susceptibility to leprosy were carried out, and they revealed varying odds of developing the disease. The IL-10 haplotype −3575T/−2849G/−2763C/−1082A/−819C/−592C was associated with resistance to leprosy with an odds ratio of 0.5 (Malhotra et al., 2005). The interferon-gamma receptor-1 gene promoter polymorphism at position −56 T/C was positively associated with an increased susceptibility to leprosy (Velayati et al., 2011). The TNF α-308 G>A polymorphism revealed a protective effect of the -308A allele with an odds ratio of 0.7 (Cardoso et al., 2010). The interferon-gamma gene polymorphism at the loci +874 T>A is shown to have a protective effect for +874T carriers (OR (adjusted) = 0.7; P = 0.005) (Cardoso et al., 2010).

We identified a statistically significant low association of TC genotype with leprosy and significantly high association with controls conferring a protective effect of C allele with an odds ratio of 0.2 (P < 0.001). Later, the genotypes were associated with clinical conditions in leprosy as this polymorphism is a coding region sequence variant SNP causing a His to Arg substitution at the amino acid 161 (H161R) within the peptide sequence leading to alterations in the functions of IL-17F. Functional effects of this amino acid substitution have been studied with recombinant IL-17F proteins produced from genes with TT and TC genotypes in asthma model and results suggested that the IL-17F protein encoded by gene with TC genotype has less ability to induce allergic inflammation (Kawaguchi et al., 2006).

Initially, we associated the genotypes with PB and MB classification of the disease, which is the indirect measure of disease severity (based on number of lesions) to explore the possibility of association of the specific genotypes with disease severity. It was observed that 79.2% of the TT genotypes are associated with PB leprosy in comparison with 78.2% in MB leprosy. There is no statistically significant difference between the two groups. However, significant difference was observed in the association of genotypes between the tuberculoid and lepromatous groups (RJ Classification) where TT genotypes is significantly associated with tuberculoid form when compared with lepromatous form. It has been proposed that tuberculo
doid leprosy involves activation of proinflammatory cytokines (Modlin, 1994), and it has also been demonstrated that the IL-17F encoded by gene with TC genotype lacks the ability to activate the mitogen-activated protein kinase kinase cascade (MEK) leading to low levels of inflammatory response. Studies revealed that inhibition of MEK pathway in the in vivo conditions resulted in the anti-inflammatory responses (Jaffee et al., 2000). Based on these studies, our observations suggest that cases with TT genotype are significantly associated with tuberculoid forms of the disease where there is an increased cell-mediated immune response.

We identified a significantly low association of heterozygous genotype TC with the type 1 reactions in leprosy cases (74.54% vs. 32.00% P < 0.05, TT vs. TC) where there is an increased Th1 response leading to inflammation (Dockrell et al., 1996; Little et al., 2001). However, the TT genotype is highly associated with type 1 reactions indicating that the encoded IL-17F protein may play a role in the regulation of MEK pathway leading to inflammatory reactions in leprosy (Verhagen et al., 2000). Further assessment needs to be carried out in the context of type 1 reactions to understand the actual role of MEK pathway and its regulation of inflammatory responses. This may help in deciphering the role of IL-17F proteins coded by the (7488T>C) polymorphic variants in the regulation of inflammatory responses associated with type 1 reactions in leprosy.

A large multicenteric cohort needs to be studied to confirm the validity of the results. Despite the limitations of sample numbers, this study has adequate relevance to test the hypothesis that IL-17F gene polymorphism is a genetic factor associated with leprosy.

In conclusion, the TT genotype of IL-17F (7488T>C) polymorphism is associated with susceptibility to leprosy, and TC genotype confers a decrease in risk of contracting leprosy in the north Indian Cohort. This polymorphism also influences the clinical phenotypes in leprosy.

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Declaration of interest

The authors declare that they have no conflict of interest. Authors alone are responsible for the content and writing of the manuscript.

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