Approximately two billion individuals globally are infected with Mycobacterium tuberculosis, yet only 10% develop clinical tuberculosis (TB). Nonetheless, TB, especially in its pulmonary form, remains an important cause of morbidity and mortality worldwide. M. tuberculosis is an intracellular parasite and cell-mediated immunity is crucial for containment of infection.

Activation of infected macrophages by interferon-gamma (IFN-γ) derived from T cells and natural killer cells is the principal antimycobacterial effector mechanism. The importance of IFN-γ in human mycobacterial immunity was established by the identification of mutations in the gene encoding the IFN-γ receptor ligand binding chain (IFNGR1, MIM #107470) as a cause of Mendelian susceptibility to mycobacterial infection (MSMI).

Mutations in the genes encoding the IFN-γ receptor-1 gene (IFNGR1) result in increased susceptibility to mycobacterial infection, including TB, in affected families. The role of genetic variation in IFNGR1 in susceptibility to common mycobacterial diseases such as pulmonary TB in outbred populations has not previously been investigated.

**Methods:** The association between IFNGR1 and susceptibility to pulmonary TB was investigated in a Gambian adult population sample using a case-control study design. The coding and promoter regions of IFNGR1 were sequenced in 32 patients with pulmonary TB, and the frequencies of six common IFNGR1 polymorphisms were determined using PCR based methods in 320 smear positive TB cases and 320 matched controls. Haplotypes were estimated from the genotype data using the expectation-maximisation algorithm.

**Results:** There was no association between the IFNGR1 variants studied and TB in this Gambian population sample. Three common haplotypes were identified within the study population, none of which was associated with TB.

**Conclusions:** These data represent an important negative finding and suggest that, while IFNGR1 is implicated in rare Mendelian susceptibility to mycobacterial disease, the common variants studied here do not have a major influence on susceptibility to pulmonary TB in The Gambian population.

**Background:** Tuberculosis (TB) is a major global cause of mortality and morbidity, and host genetic factors influence disease susceptibility. Interferon-γ mediates immunity to mycobacteria and rare mutations in the interferon-γ receptor-1 gene (IFNGR1) result in increased susceptibility to mycobacterial infection, including TB, in affected families. The role of genetic variation in IFNGR1 in susceptibility to common mycobacterial diseases such as pulmonary TB in outbred populations has not previously been investigated.

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10 ml of venous blood was collected into 3.8% sodium citrate and DNA extracted using a standard phenol/chloroform protocol. Sequencing of the IFNGR1 promoter region between −1400 and +100 relative to the translation start site has been reported elsewhere. Further sequencing between −768 relative to the translation start site and the first exon/intron boundary, the coding regions, and splice sites for exons 2–7 of IFNGR1 was conducted in 32 patients. These regions were amplified by polymerase chain reaction (PCR) and products sequenced using the ABI Big Dye Terminator v2 kit and an ABI 3700 capillary sequencer (ABI; Foster City, CA, USA). PCR and sequencing primers designed from NCBI sequence data are shown in Table 1.

The polymorphisms at positions −470, −270, and +95 were genotyped using ARMS PCR as described elsewhere. The −611 and −470 promoter polymorphisms and the E7+189 SNP were genotyped using Invader (Third Wave Technologies; Madison, WI, USA) as described elsewhere. Attempts to genotype the −56T>C SNP using ARMS PCR, RFLP and Invader failed, possibly because it lies within a GC rich region. This SNP was successfully genotyped using the multiplex SNAPSHOT kit (Applied Biosystems; Foster City, CA, USA). The primers 5′-CGGTCAGGAAGTGCCTGA and 5′GGATCTCTCCCTCCCTCTC were used to PCR amplify a 238 base pair region of the IFNGR1 gene which includes the −56 and +95 SNPs. The primers 5′-AGGCCGGGGCAGGAG-GGCGG and 5′-CGGCGGAGCCCGCCAAGCGGGA were used as internal SNAPSHOT primers to genotype the −56 and +95 SNPs respectively, following the SNAPSHOT protocol supplied by the manufacturers. SNAPSHOT products were analysed using the ABI 3100 DNA sequencer (Applied Biosystems). Typing the −470 and the +95 polymorphisms by two different methods allowed us to validate the genotyping performed for this study.

Fisher’s exact test was used to compare allele frequencies between cases and controls. The software UNPHASED (http://www.hgmp.mrc.ac.uk/~fudbrid/software/unphased/) was used to construct haplotypes from the genotype data and to compare haplotype frequencies between the case and control groups. This program calculates association statistics for multilocus haplotypes in case-control data using the expectation-maximisation algorithm to estimate haplotype odds ratios across multiple categories, giving a likelihood ratio test of homogeneity.

## RESULTS

The presence of the −470, −270, −56, and +95 polymorphisms identified previously was confirmed. Two additional SNPs were identified in this Gambian sample at positions −611 A>G relative to the transcriptional start codon and at E7+189 G>C (rs1327474 and rs11914, respectively, http://www.ncbi.nlm.nih.gov/SNP/). The exon 1 V14M polymorphism previously reported to be associated with systemic lupus erythematosus in a Japanese population17 and the H318P and L450P polymorphisms associated with anti- Helicobacter pylori IgG concentrations in a Northern Senegalese population14 were not present in this Gambian sample. Allele and genotype frequencies for the −470 and +95 SNPs obtained by two different methods were concordant (correlation 0.99 and 0.98, respectively).

Genotype and allele frequencies at six IFNGR1 loci are shown in table 2. There was no significant difference in allele or genotype frequencies between cases and controls for any of the polymorphisms. Genotype distribution of all polymorphisms did not deviate significantly from Hardy-Weinberg equilibrium. A total of 11 haplotypes were estimated to be present in this population, of which three major haplotypes with frequency >4% account for 90% of all chromosomes (table 3). There was no significant difference in haplotype frequencies between the two groups, indicating that there was no association between variation in IFNGR1 and pulmonary TB in this study.

## DISCUSSION

IFN-γ is required for host defence against a broad range of pathogens and is especially critical for mycobacterial immunity. Children lacking either chain of the IFN-γ receptor are highly susceptible to mycobacterial disease and fail to upregulate in vitro monocyte function in response to IFN-γ. Heterozygous carriers have an intermediate phenotype, suggesting that more subtle variation in the IFN-γ response pathway may underlie susceptibility to TB in outbred human populations. Since the IFNGR1 mutations responsible for MSMI are rare and often lethal, it is unlikely they play a role in pulmonary TB. Nonetheless, it is possible that more common genetic variants such as promoter region polymorphisms that influence gene expression are associated with disease. However, we have failed to show an association between six IFNGR1 variants and pulmonary TB in a Gambian population sample.

### Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>Primer name</th>
<th>Primer sequence (5' to 3')</th>
<th>PCR product (bp)</th>
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<tbody>
<tr>
<td>Promoter</td>
<td>IFNGR1-PPr</td>
<td>CAAGGTCAGGAAAGTGCCATGA</td>
<td>588</td>
</tr>
<tr>
<td>Promoter</td>
<td>IFNGR1-PPr2</td>
<td>CACCCCATCTGCTGGAATCTC</td>
<td></td>
</tr>
<tr>
<td>Exon 1</td>
<td>IFNGR1-E1g</td>
<td>GTGACGACTATGCTCCCTC</td>
<td>107</td>
</tr>
<tr>
<td>Exon 1</td>
<td>IFNGR1-E1h</td>
<td>GCCAAGCAGCCTGACCTGA</td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>IFNGR1-E2u</td>
<td>TATCGGCCCATGTGGCATC</td>
<td>301</td>
</tr>
<tr>
<td>Exon 2</td>
<td>IFNGR1-E2d</td>
<td>ACGTGGAGAAGCGGTAGAAA</td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>IFNGR1-E3u</td>
<td>TTCTACCCGCTTGTGCTG</td>
<td>498</td>
</tr>
<tr>
<td>Exon 3</td>
<td>IFNGR1-E3d</td>
<td>ATTCCTGCTAGTGGGATC</td>
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</tr>
<tr>
<td>Exon 4</td>
<td>IFNGR1-E4u</td>
<td>GTTATTAAAGCAGCCCGAGA</td>
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</tr>
<tr>
<td>Exon 4</td>
<td>IFNGR1-E4d</td>
<td>TGATCTGGTGATCTGCTGGA</td>
<td></td>
</tr>
<tr>
<td>Exon 5</td>
<td>IFNGR1-E5u</td>
<td>TGCATTAGTCTGCTGCTTA</td>
<td>502</td>
</tr>
<tr>
<td>Exon 5</td>
<td>IFNGR1-E5d</td>
<td>GGAAATGGCAATGCAATCAATG</td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>IFNGR1-E6u</td>
<td>GCTGGAAGAGAACCATATTC</td>
<td>503</td>
</tr>
<tr>
<td>Exon 6</td>
<td>IFNGR1-E6d</td>
<td>TGGTCCAAGCCAAGAACAGCA</td>
<td></td>
</tr>
<tr>
<td>Exon 7</td>
<td>IFNGR1-E7u</td>
<td>GCCATGCTTGCTGCTTTG</td>
<td>1461</td>
</tr>
<tr>
<td>Exon 7</td>
<td>IFNGR1-E7d</td>
<td>TGTCGTGACGAGCTGACTAC</td>
<td></td>
</tr>
<tr>
<td>Exon 7</td>
<td>IFNGR1-E7l</td>
<td>CAATAATAAAGGTTGAAATATAAA</td>
<td>Internal</td>
</tr>
<tr>
<td>Exon 7</td>
<td>IFNGR1-E72</td>
<td>CAGTAGAAGAAGACAGTATCC</td>
<td>Internal</td>
</tr>
</tbody>
</table>
Our negative findings have a number of possible interpretations. Firstly, variation in IFNGR1 may contribute to the susceptibility to TB, but the effect is small and a much larger sample size is required to detect it. Furthermore, we only sequenced gene regions that are most likely to harbour functionally relevant variants—that is, the promoter region, exons, and intron/exon boundaries. We therefore cannot exclude a causal variant elsewhere in the gene. A weak association between the intron 6 IFNGR1 A/T SNP in the first intron of the IFN-γ receptor-1 (IFNGR1) gene polymorphisms and tuberculosis 293 has been reported in a Croatian population, 20 suggesting that the TT genotype which is associated with lower IFN-γ production confers susceptibility to pulmonary TB. Secondly, we have investigated pulmonary TB which is caused by reactivation of latent infection. Mycobacterial infections in MSMI are generally disseminated, which suggests that IFNGR1 may influence the course of primary infection in childhood or the development of disseminated TB (for example, osteomyelitis and meningitis). However, IFN-γ is still required for control of pulmonary disease and pulmonary TB occurs in IFNGR1 deficient individuals. Alternatively, sequence variation in other genes in this pathway may affect susceptibility to pulmonary TB. Two recent studies have reported an association between the +874 A/T SNP in the first intron of the IFN-γ gene and pulmonary TB, 22 23 suggesting that the TT genotype which is associated with lower IFN-γ production confers susceptibility to pulmonary TB.

Table 3 Estimated haplotype probabilities for six polymorphisms in the interferon-γ receptor-1 (IFNGR1) gene

<table>
<thead>
<tr>
<th>Haplotype number</th>
<th>Haplotype</th>
<th>Estimated haplotype frequencies*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls, n (%)</td>
<td>Cases, n (%)</td>
</tr>
<tr>
<td>1</td>
<td>A, insTT, C, T, T, T</td>
<td>189 (47.6)</td>
</tr>
<tr>
<td></td>
<td>A, insTT, C, C, C, T</td>
<td>124 (31.1)</td>
</tr>
<tr>
<td>2</td>
<td>A, delTT, C, C, C, T</td>
<td>50 (12.6)</td>
</tr>
<tr>
<td>Others</td>
<td>35 (8.8)</td>
<td>41 (10.0)</td>
</tr>
</tbody>
</table>

Markers are shown in chromosomal order (i.e. IFNGR1-611, IFNGR1-470, IFNGR1-270, IFNGR1-56, IFNGR1-95, and IFNGR1 E7-189).

*Only haplotypes with an estimated probability of >4% are shown.

p value = 0.13 (NS)
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