Tuberculosis (2007) **87**, 303–311

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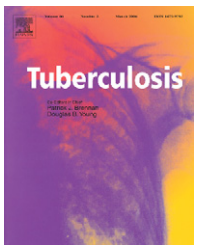

Association of polymorphisms in IL-12/IFN-γ pathway genes with susceptibility to pulmonary tuberculosis in Indonesia

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Received 27 June 2006; received in revised form 5 December 2006; accepted 2 February 2007

KEYWORDS
Susceptibility;
Tuberculosis;
Interleukin-12;
Interleukin-23;
Interferon-gamma;
Polymorphisms

Summary
Upon infection with mycobacteria the IL-12/IFN-γ axis plays an essential role in the activation of cell-mediated immunity required for the elimination of pathogens. Mutations in genes of the IL-12/IFN-γ axis are known to cause extreme susceptibility to infection with environmental mycobacteria, and subtle variations in these genes may influence susceptibility to more virulent mycobacteria. We analyzed the distribution of polymorphisms in four essential genes from the IL-12/IFN-γ axis, *IL12B*, *IL12RB1*, *IFNG* and *IFNGR1*, in 382 pulmonary tuberculosis patients and 437 healthy controls from an endemic region in Jakarta, Indonesia. The *IL12RB1* gene was sequenced in a subset of individuals. Nine known single nucleotide polymorphisms (SNPs) and two new silent variations, 135G>A and 1056C>T, were detected in *IL12RB1*. Six functional SNPs (–2C>T, 467G>A, 641A>G, 1312C>T, 1573G>A, 1781G>A) in *IL12RB1*, an *IL12B* promoter insertion/deletion polymorphism and CA repeats in *IFNG* and *IFNGR1* were analyzed in the cohort. The *IFNGR1* allele CA_{12} ($p = 0.004$) and genotype CA_{12}/CA_{12} ($p = 0.01$; OR 0.5) were associated with protection from pulmonary tuberculosis. Interestingly, *IL12B* promoter heterozygosity

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1472-9792/$ - see front matter
doi:10.1016/j.tube.2007.02.001

was associated with protection from tuberculosis in BCG-vaccinated individuals ($p = 0.03$; OR = 0.6). This new finding supports the role that IL-23—of which *IL12B* encodes a subunit—plays in generation of memory T cells.

Introduction

Tuberculosis (TB) causes nearly 2 million deaths a year, which makes it the second most common cause of death due to an infectious disease (after AIDS).[1] Although one-third of the world population is infected with *Mycobacterium tuberculosis* only 5–10% of infected people develop clinical TB. One of the factors influencing susceptibility to TB is host genetics, with evidence of this first shown in twin studies.[2,3] Many candidate genes have since been investigated for their role in TB, and several have been shown to be associated with TB susceptibility, for instance *HLA*,[4] *VDR*,[5] *NRAMP1*,[6] and *MBL*[7] (reviewed in[8]). These associations are, however, not sufficient to account for the genetic contribution identified in the twin studies.

In the past decade, patients with Mendelian Susceptibility to Mycobacterial Disease (MSMD) were found to have defects in type-1 cytokine pathway leading to severe infections with mainly environmental mycobacteria and salmonellae species. MSMD patients are unable to produce or respond to IFN-γ upon encountering intracellular bacterial pathogens, resulting in the inability to mount an adequate cell-mediated immunity response. These defects resulted from mutations in five genes coding for proteins in the IL-12/IFN-γ axis: *IL12B* (encoding IL-12p40), *IL12RB1* (encoding the β1 chain of the IL-12 receptor), *IFNGR1*, *IFNGR2* (encoding the two chains of the IFN-γ receptor) and *STAT1* (reviewed in[9]). More subtle variations in these genes may account for the variation in susceptibility to more virulent mycobacteria and salmonellae species such as *M. tuberculosis* or *Salmonella typhii*.

Indeed, associations with susceptibility to TB have thus far been detected with four genes from the IL-12/IFN-γ axis: *IL12RB1*, *IL12B*, *IFNG* and *IFNGR1*. One of the two major haplotypes of *IL12RB1*, R214-T365-R378 (RTR) that has been shown to be a lower IL-12 responder,[10,11] was reported to be associated with susceptibility to pulmonary TB in the Japanese population.[10] In Moroccan and Korean patients the major *IL12RB1* haplotypes were not found to be associated with susceptibility to TB.[12,13] In the Moroccan TB patients an association was however found with two polymorphisms in the *IL12RB1* promoter.[12] An insertion/deletion polymorphism in *IL12B* was shown to influence *IL12B* mRNA expression and IL-12p70 production[14,15] and was found to contribute to TB susceptibility in Hong Kong Chinese.[15] A CA repeat in intron 1 of *IFNG* that is linked to IFN-γ production in vitro[16] was also found to be associated with TB in Hong Kong Chinese.[17] In the *IFNGR1* gene a particular allele of the CA repeat in intron 5 has been found to be associated with pulmonary TB in Croatia,[18] although this same polymorphism was not found to be associated with TB in The Gambia.[19]

We investigated whether polymorphisms in the IL-12/IFN-γ axis are associated with susceptibility to pulmonary TB in a case-control study in a highly TB endemic area in Jakarta, Indonesia, a country harboring >10% of all TB cases worldwide. We first identified new variations in *IL12RB1* by sequencing a subset of patients and controls. We analyzed various SNPs in *IL12RB1* as well as the above-described polymorphisms in *IL12B, IFNG*, and *IFNGR1* in order to determine whether any of the alleles or genotypes in these polymorphisms influence susceptibility to pulmonary TB in the Indonesian population.

Materials and methods

Patients and control subjects

In a case-control study design, carried out from June 2001 to December 2004, newly diagnosed pulmonary TB-patients ($n = 382$) aged 15 or older were recruited from an outpatient TB clinic in Central Jakarta, Indonesia. Diagnosis of TB was based on the WHO definition[27] which includes the presence of clinical symptoms, chest X-ray (CXR) examination, and microscopic detection of acid-fast bacilli in Ziehl–Nielsen stained sputum smear or positive culture of *M. tuberculosis*. All patients were tested for HIV serology. Patients with known immunosuppression were excluded (HIV positive, pregnancy or immunosuppressive therapy). HIV infected individuals (<2%) were referred for post-test counseling and appropriate care, according to the national guidelines.

In the same period, community controls ($n = 437$) were recruited, matched by age ($\pm$10%), sex, ethnic background if possible, socio-economic class, and area of residence. Controls were interviewed using the same standard questionnaire and underwent the same physical, blood, and CXR examination as cases. Controls were excluded if they had a history of prior anti-TB therapy, signs and symptoms suggestive of active TB or infiltrates in the CXR. Controls were not tested for HIV.

There were 231 males (60.5%) and 151 females (39.5%) in the patient group with a mean age $\pm$standard deviation (s.d.) of 33.2$\pm$11.9 years and there were 245 males (56.1%) and 192 females (43.9%) in the control group with a mean age $\pm$s.d. of 33.8$\pm$11.5 years. Sex and age were not significantly different between the groups. A BCG scar was found in 39.5% of TB patients and in 48.1% of controls (1% of controls unknown). Self and parental ethnicities were recorded upon recruitment. Of both the patient and control group more than 80% were of Javanese origin, the non-Javanese were born on other Indonesian islands. Written informed consent was obtained from all subjects, and the study was approved by the ethical committees of the Medical Faculty of the University of Indonesia and of the Eijkman Institute.