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Genetic susceptibility to different clinical forms of tuberculosis in the Peruvian population

C.A. Taype [a,*], S. Shamsuzzaman [a], R.A. Accinelli [b], J.R. Espinoza [c], M.-A. Shaw [a]

[a] Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, University of Leeds, UK
[b] Laboratory of Respiration, Research Institute of Altitude, Faculty of Medicine, Universidad Peruana Cayetano Heredia, Peru
[c] Molecular Biotechnology Unit, Laboratories for Research and Development (LID), Faculty of Science and Philosophy, Universidad Peruana Cayetano Heredia, Peru

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ABSTRACT

Racial variation, twin studies, segregation analyses, linkage and association studies all suggest that genetic factors play an important role in predisposition to tuberculosis. Many previous studies have been performed with pulmonary TB patients, as the most prevalent form of clinical TB (nearly 95%), and very few of them have considered extrapulmonary TB. The present study evaluates the effects of variation in eight candidate genes (*LTA*, *TNF*, *IL1B*, *IL1RN*, *IL10*, *TGFB1*, *TIRAP* and *P2X7*) with pulmonary, pleural, miliary and other extrapulmonary forms of TB in a Peruvian population from the North of Lima. 626 TB cases and 513 healthy controls were enrolled in this study. LTA_{+368} and $IL10_{-592}$ were associated with different clinical forms of TB ($P < 0.05$). LTA_{+368} genotype A/A was protective for pleural TB, LTA_{+368} G/A was correlated with susceptibility to miliary TB. Genotypes A/A and G/A were associated with protection and susceptibility respectively when considering all extrapulmonary TB forms *versus* either healthy controls or pulmonary TB patients. Carriers of $IL10_{-592}$*C were under-represented among those with pulmonary TB and all TB forms ($P < 0.001$). $IL10_{-1082}$–$IL10_{-592}$ haplotypes showed different distributions among patients with pulmonary TB and all TB forms ($P < 0.01$) when compared to healthy controls. In addition, $IL10_{-1082}$–$IL10_{-592}$ haplotypes showed differences between pleural, miliary and all forms of extrapulmonary TB when compared with pulmonary TB ($P < 0.05$). All findings are consistent with an under-representation of the $IL10_{-1082}$*A–$IL10_{-592}$*A haplotype in pulmonary TB patients. These results suggest that the polymorphisms LTA_{+368} and $IL10_{-592}$, or variants in strong linkage disequilibrium, variably affect susceptibility to the differing clinical forms of TB in Peruvians.

1. Introduction

TB (tuberculosis) is a global public health problem and is the infectious disease with the highest mortality in the world. One-third of the earth's population is infected with TB and is at risk of progression to active disease. In 2006, Peru reported an incidence rate of 110 per 100,000 inhabitants, while the incidence of smear-positive pulmonary TB was 67 per 100,000 inhabitants. The mortality rate was 3.5 per 100,000 inhabitants (MINSA, 2006).

TB is a disease with a spectrum of clinical forms ranging across pulmonary, extrapulmonary and disseminated cases. Once the primary infection has occurred, more than 90% of individuals will be asymptomatic and can only be found by measuring the tuberculin skin test conversion from negative to positive. Patients infected with *Mycobacterium tuberculosis* (MTB) can develop the disease at any time through reactivation of a previously acquired, but latent infection, or through exogenous reinfection (Dale, 2003).

Since less than 10% of people infected with MTB will have clinical disease, and only a small fraction of them will have an obvious identifiable risk factor for developing TB, a role for host factors regulating disease expression is likely (Israel et al., 1941). Racial variation, twin studies, segregation analyses, linkage and association studies all suggest that genetic factors play an important role in predisposition to tuberculosis (Stead et al., 1990; Shaw et al., 1997; Hill, 2006; Li et al., 2006; van der Eijk et al., 2007).

Many genes implicated in the control of susceptibility to infectious diseases are located in the major histocompatibility complex. Patients carrying particular HLA (human leukocyte antigen) class II alleles show differential immune responses to tubercle bacilli and thus are variably prone to developing the disease. Different case–control studies for HLA genes and pulmonary TB have been performed in a number of populations. *HLA-DRB1*1501* and *-DQB1*0601* alleles were associated with TB in

* Corresponding author at: St. Barnabas Hospital, Albert Einstein College of Medicine, 4422 Third Avenue, Mills Building, Bronx, NY 10457, United States. Tel.: +1 718 960 9000.
E-mail address: carmentaype@yahoo.com (C.A. Taype).

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India (Ravikumar et al., 1999), while in a Vietnamese population, the associated allele was *HLA-DQB1*0503* (Goldfeld et al., 1998). *HLA-DQB1*05* and *-DQB1*02* have been found to be over-represented and under-represented, respectively, in pulmonary TB in Poland (Dubaniewicz et al., 2003). In Iran, *HLA-DRB1*07* and *-DQA1*0101* were associated with susceptibility to TB, while *HLA-DQA1*0301* and *-DQA1*0501* were associated with protection (Amirzargar et al., 2004). In a Mexican study *HLA-DQA1*0101*, *-DQB1*0501* were implicated (Teran-Escandon et al., 1999), and in Korea *HLA-DRB1*0803* and *-DQB1*0601* were associated with disease progression (Kim et al., 2005). Such highly variable observations might indicate a number of contributory effects from the MHC region.

TNF-α (tumor necrosis factor) is important in the host-mediated damage seen in extensive pulmonary TB (Bekker et al., 2000). *TNF* and *LTA* loci are in the MHC class III region. The TNF_{-308} polymorphism has been associated with extensive pulmonary TB in Russia (Bikmaeva et al., 2002). However, a preliminary case control study in Peru failed to find any association between TNF_{-308} and TB, although TNF-α production was increased in pulmonary TB patients (Castro et al., 2003). An Indian study evaluating *LTA* polymorphisms in TB patients failed to find any association, although *HLA-A1*, *-B17*, *-B21* and *-DR7* in combination with TNF_{-308}*A and LTA_{+368}*A were associated with protection against pulmonary TB and with susceptibility to bacteriological relapse (Selvaraj et al., 2001).

Even though *MHC* genes have a clear role in the development of TB, they are not sufficient to account for disease susceptibility. Non-*HLA* genes including *SLC11A1* (solute carrier family 11 member A1), *INFG* (interferon-γ), *SP110* (nuclear body protein sp110), *VDR* (vitamin D receptor), *CR1* (complement receptor 1), *IL10* (IL-10), *IL12RB1* (IL-12 receptor β1 subunit), *INFGR1* (interferon-γ receptor 1) and others have been examined in a variety of studies (Hill, 2006; Taype et al., 2006).

In the Gambia, individuals heterozygous for $IL1RN_{VNTR}$*2 (the IL-1 receptor antagonist gene) were protected against pulmonary TB, while $IL1B_{-511}$ alleles were not associated (Bellamy et al., 1998). Another study in the Gambia found that heterozygous and $IL1B_{-511}$*C allele carriers show protection against pulmonary TB (Awomoyi et al., 2005). Homozygous individuals for $IL1B_{-511}$*C failed to show an increase in IL-1β production after stimulation, suggesting a key role for this cytokine in TB (Awomoyi et al., 2005). Conversely, $IL1RN_{VNTR}$*2 has been connected with susceptibility to pulmonary TB in Russians (Freidin et al., 2006).

IL-10 is a down-regulatory cytokine involved in suppressing cell-mediated immunity (Moore et al., 2001). Similarly, TGF-β1 (transforming growth factor beta 1) plays important role in the modulation of cellular growth and differentiation, immunoregulation and extracellular matrix formation (Wenner and Yan, 2003). Both are major immunomodulators in TB (Toossi and Ellner, 1998). An increase in the active TGF-β1 form has been observed in both macrophages and lung washings from patients with pulmonary TB (Toossi et al., 1995; Bonecini-Almeida et al., 2004). Although no association between *IL10* polymorphisms and TB was found in Gambians (Bellamy et al., 1998), heterozygous $IL10_{-1082}$ and $IL10_{-1082}$*G allele carriers were associated with susceptibility to pulmonary TB in Cambodia and Turkey (Delgado et al., 2002; Ates et al., 2007). $IL10_{-592}$*A was associated with protection against pulmonary TB in Korea (Shin et al., 2005), and $IL10_{-1082}$*A has been correlated with susceptibility to pleural diseases in Colombia (Henao et al., 2006). Strong linkage disequilibrium (LD) is seen for three *IL10* polymorphisms, $IL10_{-1082}$, $IL10_{-819}$ and $IL10_{-592}$ (Moreno et al., 2007). Association studies between $TGFB1_{+869}$ and pulmonary TB have failed to find any significant relationship in China, Hong Kong, Colombia and Turkey (Niimi et al., 2002; Henao et al., 2006; Oral et al., 2006; Mak et al., 2007). However, in Iran there was a significant negative association between $TGFB1_{+869}$*T and pulmonary TB (Amirzargar et al., 2006).

TIRAP encodes the Toll-interleukin 1 receptor domain-containing adaptor protein, also known as Mal or MyD88 adapter-like protein. TIRAP is a membrane protein, located on the cytoplasmic side, implicated in TLR2 (Toll-like receptor) and TLR4 pathways (Khor et al., 2007). In a Vietnamese population, both heterozygosity and homozygosity for $TIRAP_{C558T}$*T were associated with meningeal TB, but not pulmonary TB, suggesting a role for this polymorphism in the phenotypic expression of TB (Hawn et al., 2006). Recently, $TIRAP_{C558T}$*T has been associated with protection against pulmonary TB and systemic lupus erythematosus in Colombia (Castiblanco et al., 2008).

The P2X7 (purinergic receptor P2X, ligand-gated ion channel, 7) is an ATP-gated cation channel that is highly expressed in macrophages (Rassendren et al., 1997) that are infected with *Mycobacteria*, resulting in the induction of apoptosis in the pathogen (Ferrari et al., 1999). $P2X7_{E496A}$*C has been associated with extrapulmonary diseases but not pulmonary TB in Southeast Asia (Fernando et al., 2007). However, the same allele has been associated with pulmonary TB in a Mexican Mestizo population (Nino-Moreno et al., 2007). Further work is required to understand these different associations for candidate loci, considering both genetic heterogeneity and linkage disequilibrium patterns in the studied populations.

Many of the previous studies have been performed with pulmonary TB patients, as the most prevalent form of clinical TB (nearly 95%) and very few of them have evaluated extrapulmonary TB. The present study will evaluate the relationship of LTA_{+368}, TNF_{-308}, $IL1B_{-511}$, $IL1RN_{VNTR}$, $IL10_{-1082}$, $IL10_{-592}$, $TGFB1_{+869}$, $TIRAP_{S180L}$ and $P2X7_{E496A}$ polymorphisms between pulmonary, pleural, miliary and other extrapulmonary forms of TB and healthy controls in a Peruvian population of the North of Lima.

2. Materials and methods

2.1. Patients and controls

Patient and control samples were collected between 1999 and 2002 and previously described (Taype et al., 2006). The TB samples were collected from the North of Lima city. By 2005, this area had 6250 new cases of TB and a morbidity rate of 217.69 per 100,000 inhabitants, an incidence rate of 185.24 per 100,000 inhabitants and a mortality rate of 8.71 per 100,000 inhabitants (MINSA, 2006). People from Lima are mainly a mix of European (mostly Spanish) and Amerindian and called Mestizos. Cases and controls were chosen sequentially and were unrelated. Controls were from the same geographical area as the cases. The Mestizo character of a representative Peruvian subpopulation has been studied in the past by confirming the presence of typical Caucasian and Amerindian HLA haplotypes (de Pablo et al., 2000).

This study included cases of TB in adult people (≥15 years old) who were diagnosed with pulmonary, pleural, miliary or other extrapulmonary forms of TB. A total of 626 patients were included and the mean ± S.D. age was 29.01 ± 11.42 years and 611 were male (97.6%). The patient collection area consisted of the Pulmonary Division of the Hospital Nacional Cayetano Heredia and its surrounding medical centres. Enrolled patients were recently diagnosed with TB or relapse/reinfection cases but all were infected with drug-sensitive strains of MTB. In the present set of samples, 75 of 504 (14.9%) pulmonary TB cases were relapse/reinfection (73 patients had 1 previous episode while 2 patients had two previous episodes of TB). In the case of pleural, miliary and extrapulmonary TB, 2 of 78, 2 of 35 and 1 of 9 had one previous episode of TB respectively. Considering all 626 TB cases, 80 of them (12.8%) had a