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Association between *SLC11A1* polymorphisms and susceptibility to different clinical forms of tuberculosis in the Peruvian population

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Abstract

Polymorphism in *SLC11A1* has been implicated in host susceptibility to tuberculosis. We have studied associations between INT4, D543N, and 3′UTR polymorphisms of *SLC11A1* and different clinical forms of TB. Analysis used 507 patients with pulmonary TB, 123 with extra pulmonary TB and 513 controls. INT4 and D543N showed allelic association with pulmonary TB ($P = 0.02$ and 0.03 respectively). INT4–D543N–3′UTR haplotypes showed an association with pulmonary TB ($P = 0.03$). No association of *SLC11A1* with miliary TB was observed, and a possible association of D543N to the pleural form ($P = 0.08$) was suggested. These results support association between *SLC11A1* and TB, particularly to the common pulmonary form.

Keywords: Tuberculosis; *SLC11A1*; Allelic association; Pulmonary; Pleural; Miliary

1. Introduction

Tuberculosis (TB), caused by the human pathogen *Mycobacterium tuberculosis*, is the leading cause of morbidity and mortality caused by infectious agents worldwide (Dolin et al., 1994). One-third of the world population is infected with *M. tuberculosis*, and is at risk of developing the disease during their lifetime (Dye et al., 1999). The latest global data suggests that 8.3 million new TB cases were detected in 2000. Nine percent of all new TB cases in adults (aged 15–49 years) were attributable to HIV infection. There were an estimated 1.8 million deaths from TB, of which 12% were attributable to HIV. Tuberculosis was the cause of 11% of all adult AIDS deaths (Corbett et al., 2003).

In Peru, the National TB Control Program reported in 2003 a morbidity rate of 119.9 per 100,000. In the same period 18,504 patients with smear positive pulmonary TB were diagnosed, giving an incidence rate of 68.8 per 100,000 (Ministerio de Salud, Peru, 2004). There were reported HIV prevalences from 1% to 1.5% in TB patients during 1996–1999 (Ministerio de Salud, Peru, 2000).

TB is an infectious disease with a spectrum of clinical forms ranging across pulmonary, extrapulmonary and disseminated cases. The distribution of different clinical forms in 4483 patients that attended the Program of Control of Tuberculosis of Hospital Nacional Cayetano Heredia, Lima, Peru and some of its surrounding centers, between 1980 and 1990, was: 90% pulmonary TB, 7.9% non-pulmonary TB, and 2.1% patients presenting with both pulmonary and non-pulmonary TB (Vigil, 1991).

Studies on animal models, and a variety of twin, linkage and association studies in human populations support the role of host genetics in susceptibility to TB. However, most of these studies are related to pulmonary forms of the disease and no

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exhaustive study regarding extrapulmonary disease and genetic background of patients has been reported. A number of candidate genes such as *SLC11A1*, HLA and cytokines genes are currently implicated in susceptibility to TB.

Many of the genes that control the susceptibility and resistance to infectious diseases are located in the major histocompatibility complex. Association studies between HLA and pulmonary TB have been carried out in different populations. *HLA-DRB1*1501* and *DQB1*0502* have been reported to be more common in pulmonary TB patients than in healthy controls in Asian Indians (Meyer et al., 1998; Ravikumar et al., 1999). The allele *HLA-DQB1*0503* has been associated with tuberculosis in Vietnam (Goldfeld et al., 1998). A preliminary report found association between alleles *HLA-DQB1*05* and *DQB1*02* and pulmonary tuberculosis in Poland (Dubaniewicz et al., 2003).

Selvaraj et al. (2001) studied polymorphisms *TNF* and *LTA* and susceptibility to tuberculosis. *TNF* and *LTA* were not associated with tuberculosis, but *TNF–LTA* combined haplotypes were associated with protection against pulmonary tuberculosis and bacteriological relapse. Delgado et al. (2002) studied four polymorphisms located in the promoter region of *TNF* in a Cambodian population, but there was no significant association between these markers and pulmonary TB, and recently, Fitness et al. (2004) failed to find any significant association between *TNF* polymorphisms and TB in Northern Malawi.

MHC genes are not sufficient in conferring susceptibility or resistance to disease. Non-HLA genes have also been inplicated in TB susceptibility. Polymorphisms in the Vitamin D receptor gene (*VDR*) have been associated with TB in The Gambia (Bellamy et al., 1999). Recently, in three West Africa countries (The Gambia, Guinea, and Guinea-Bissau), Bornman et al. (2004) conducted a family-based study and found significant association of TB with *VDR* haplotypes. *IFNG* has been associated with TB in Sicily and Spain (Lio et al., 2002; Lopez-Maderuelo et al., 2003). Variants in the *IL1* gene cluster have been associated with susceptibility to TB in The Gambia and Hindu residents of London, identified as being of Gujarati origin (Bellamy et al., 1998a; Wilkinson et al., 1999). *IL12B* polymorphisms have been associated with TB in China (Tso et al., 2004), although Puzyrev et al. (2002) failed to find any association in Slavic population of Siberia.

Solute carrier family 11 member a1 (Slc11A1) is a proton/divalent cation antiporter that is more familiar by its former designation Nramp1/NRAMP1 (natural resistance associated macrophage protein 1). It was originally described for its roles in regulating resistance and susceptibility to *Salmonella typhimurium*, *Leishmania donovani*, and *Mycobacterium bovis BCG* in mice. A number of recent studies now provide evidence that polymorphism in *SLC11A1* is involved in determining autoimmune and infectious disease susceptibility, and innate and adaptive immune responses to mycobacterial products (Blackwell et al., 2003).

Several studies on *SLC11A1* support its role in susceptibility to TB in different human populations. In a case-control study in the Gambia, Bellamy et al. (1998b) showed association between $5'(CA)_n$, INT4, D543N and 3′UTR polymorphisms of *SLC11A1* and pulmonary TB. Greenwood et al. (2000) found significant linkage between *SLC11A1* and TB in a large Aboriginal Canadian family. Cervino et al. (2000) studied 160 individuals from 44 families in Guinea-Conakry. Only a single base change in intron 4 showed an association with TB, whereas $5'(CA)_n$ and 3′UTR polymorphisms did not, and haplotype analysis did not reach statistical significance. Delgado et al. (2002) also evaluated polymorphisms in *SLC11A1* and showed that D543N and 3′UTR were associated with susceptibility to pulmonary TB in Cambodians. Associations between *SLC11A1* and pulmonary TB have also been reported in people from Japan and Korea (Gao et al., 2000; Ryu et al., 2000), and in the Chinese Han population. INT4, D543N and 3′UTR were associated with susceptibility to pulmonary TB (Liu et al., 2004). In South Africa $5'(GT)_n$ was associated with protection against TB (Hoal et al., 2004). Recently, in Karonga district, northern Malawi, *SLC11A1* polymorphisms were evaluated in patients with tuberculosis. $5'(CA)_n$ and 3′UTR were not associated with tuberculosis, however, a new CAAA insertion/deletion polymorphism in the 3′-untranslated region was associated with protection against tuberculosis (Fitness et al., 2004). However other studies have failed to find association (Abe et al., 2003; El Baghdadi et al., 2003; Liaw et al., 2002).

All these studies were made in pulmonary TB patients, the most prevalent form of TB. Kim et al. (2003) evaluated *SLC11A1* in pleural patients, finding significant association with INT4 and 3′UTR polymorphisms. This paper presents a study of the association of *SLC11A1* polymorphisms with pulmonary, pleural, miliary and other extrapulmonary forms of TB in the Peruvian population.

2. Materials and methods

2.1. Patients and controls

The cases included in this study were adult patients ($\geq$15 years old) with diagnosis of pulmonary, pleural, military or other extrapulmonary forms of TB from the North Region of Lima (Pulmonary Service of Hospital Nacional Cayetano Heredia and its surrounding centres). In the sampling area of Lima and to the north the population is mainly Mestizo. Patients were enrolled in the study after giving informed consent. The study was approved by the joint ethics committee of Universidad Peruana Cayetano Heredia. DNA samples were collected from 630 patients. Their mean $\pm$ S.D. age was 29.01 $\pm$ 11.42 years. Pulmonary TB was diagnosed from detection of *M. tuberculosis* in sputum by smear and culture, and pleural TB by pleural biopsy. All the pleural biopsies showed granulomatous chronic inflammation with giant cells. The diagnosis of miliary TB was made clinically. Other extrapulmonary forms of TB were diagnosed by direct visualization of *M. tuberculosis* in biopsies from the affected tissue. A total of 507 patients had a diagnosis of acid-fast bacilli positive pulmonary TB, 78 pleural TB as diagnosed by biopsy, 36 miliary TB and 9