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No Evidence for Association Between the Functional rs1862513 Polymorphism in the Promoter Region of the Resistin Gene (RETN) and Pulmonary Tuberculosis in Northern Spain

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Abstract

Background: Elevated levels of circulating Resistin, a peptide hormone produced by adipocytes in rodents but also by monocytes and macrophages in humans, has been reported in patients with tuberculosis infection. To date, only a few studies mainly focused in conditions such as diabetes, obesity, and cardio-metabolic risk have treated to analyse the role of single nucleotide polymorphisms in the Resistin Gene (RETN). However, polymorphisms in this gene have not been previously tested in pulmonary tuberculosis susceptibility.

Methods: The rs1862513 (-420C>G), a functional polymorphism in the RETN gene, which has been found to increase circulating resistin levels, was analysed in 192 patients with pulmonary tuberculosis, all of them HIV negative, and in 245 healthy individuals from a well genetically conserved Spanish population.

Results: Significant differences in genotype and allele frequencies of the rs1862513 functional polymorphism were not observed between patients and normal controls.

Conclusion: Our results would indicate that RETN rs1862513 would not play a major role in the susceptibility to pulmonary tuberculosis at least in our population in a well-powered study.

Keywords: Resistin; RETN; Single nucleotide polymorphism; Pulmonary tuberculosis; Susceptibility

Abbreviations

TB: Tuberculosis, Mtb: *Mycobacterium tuberculosis*, PTB: Pulmonary Tuberculosis, SNP: Single Nucleotide Polymorphism

Introduction

Tuberculosis (TB) is still a leading cause of death in humans and therefore remains a global health problem. *Mycobacterium tuberculosis* (MTB) infects approximately one-third of the human population. In 2013, there were an estimated 9.0 million cases and 1.5 million deaths [1]. Among people with pulmonary TB (PTB), those from South-East Asia, Western Pacific and African regions account for three quarters. Spain is one of the European countries with the highest rates of incidence and prevalence of TB. Approximately, 10% of individuals infected with MTB develop active pulmonary disease, suggesting that there exist differences in susceptibility or resistance to progression.

Susceptibility to TB seems to be multifactorial, resulting of complex interactions between host, pathogen, environmental and genetic factors. To date, numerous host genes have been reported to be involved in this process [2-4].

Resistin, acronym derived from resistance to insulin, was initially found to be expressed in adipocytes in mice, and described as an adipokine known as adipose tissue-specific secretory factor, involved in lipid and glucose metabolism [5-7]. Further studies, revealed that in humans, resistin was predominantly expressed and secreted by monocytes and macrophages acting as a pro-inflammatory cytokine [8]. Moreover, plasma concentrations of resistin and resistin-like molecules have been found significantly increased in different types of infections and it has been proposed as a biomarker of sepsis severity [9-13].

Regarding TB, a few studies have been conducted focused on correlating circulating resistin levels with severity, or as a surrogate biomarker for monitoring TB disease [14-16]. Several studies have investigated the different SNPs of the RETN gene identifying the rs1862513 SNP (-420 C/G) in the promoter region of RETN as the main determinant of circulating resistin concentration in humans [17,18].

Due to the lack of previous studies regarding the role of this SNP in PTB, the aim of this work was to elucidate if this SNP could be

associated with susceptibility to PTB in a well genetically conserved population (Cantabria, Northern Spain).

Materials and Methods

Study population

Through a case-control study, patients with PTB (n=192) and age and sex matched healthy controls (n=245) entered this research. Both controls and patients were of Caucasian background, all of them from Cantabria region (Northern Spain). Diagnosis of PTB was clinically confirmed by X-rays, and bacteriologically assessed using both sputum smear examination and culture. The presence of active or latent infection in the control group was ruled out by performing an interferon-gamma release assay (QuantiFERON-TB Gold In-Tube). All subjects were HIV-negative and they did not presented any autoimmune disease.

Procedures used in the study conformed to the principles outlined in the Declaration of Helsinki. All samples were collected with the written consent of the participants. The study protocol was accepted and approved by the Research Ethics Board of the Hospital.

Genotyping

DNA samples were prepared from whole blood by using the Maxwell 16 Blood DNA Purification Kit (Promega Biotech Ibérica, S.L., Spain) following the manufacturer's instructions.

Genotyping of the RETN rs1862513 (-420 C/G), was performed by a TaqMan SNP Genotyping Assays (Life Technologies S.A., Madrid, Spain)) in an ABI PRISM 7900 HT sequence detection systems (Life Technologies).

Satistical analysis

For the statistical analysis, the results were processed with the SPSS computer package (version 12). Allele and genotype frequencies were estimated by direct counting, and the χ^2 test was used to identify significant departure from the Hardy-Weinberg equilibrium. Comparison of frequencies between PTB patients and healthy controls was carried out using the χ^2 test with Yates correction. The multiple inheritance models (dominant, recessive or log-additive models) were analysed by using the web tool SNPStats [19].

Statistical power of the present study sample size was calculated by using the PS Power and Sample Size Calculations software version 3.0.

Clinical profile	PTB patients (n=192)	Controls (n=245)
Age (y), mean (range)	42.7 (19-64)	38.8 (18-65)
Sex (male/female)	120/72	152/93
Origin	Cantabria (Northern Spain)	Cantabria (Northern Spain)

Table 1: Clinical and demographic features of PTB patients and healthy controls included in the study.

Results

Clinical and demographic characteristics of both PTB patients and healthy controls are shown in Table 1. Allele and genotype frequencies were all in Hardy–Weinberg equilibrium (controls χ^2=2.21, p=0.14, patients χ^2=0.11, p=0.73). The statistical power for detecting an odds ratio (OR) ≥ 2. Allele frequencies are given in Table 2 and Table 3.

Allele	PTB patients (n=192)	Controls (n=245)	p	OR	95% CI
-420 C	273 (71.09)[a, b]	330 (67.35)	0.26[c]	1.19	0.89-1.59
-420 G	111 (28.91)	160 (32.65)			

Table 2: Allelic frequencies of RETN rs1862513 SNP in PTB patients and healthy controls. [a]The data are expressed as absolute numbers and percentages in parenthesis. [b]The sum of the absolute numbers of the two alleles of each SNP is twice the number of patients and controls. [c]The study has 99% power for detecting an odds ratio (OR) ≥ 2.

RTN Genotype	PTB patients (n=192) n%	Controls (n=245) n%	p	OR	95% CI
CC	98 (51.04)	106 (43.27)	0.13	1.37	0.94-2.00
CG	77 (40.10)	118 (48.16)	0.11[a]	0.72	0.49-1.06
GG	17 (8.86)	21 (8.57)	0.95	1.04	0.53-2.02

Table 3: Comparison of genotype frequencies of RETN rs1862513 SNP in patients with PTB and in healthy controls. [a]The study had >90% power for detecting an odds ratio (OR) ≥ 2 for the CC and CG genotypes, and 60% for the GG genotype.

We did not find a significant difference in the distribution of the two alleles between PTB patients and controls (Table 1). In the same way, no significant association was found when we compared genotypes (Table 2) neither when we analysed the dominant, recessive nor log-additive models (data not shown).

Discussion

In this study, we have investigated for the first time the role of the functional SNP, rs1862513 (-420 C/G) in the promoter region of RETN in conferring susceptibility or resistance to PTB. Although we did not find any association, our results showed to be statistically well-powered.

To date, resistin levels or the role of this SNP have been studied and associated with different conditions, such as cardiovascular diseases, insulin resistance, type 2 diabetes, periodontal inflammation and serious infections among others [5,7,9-13,18,20-22]. In these studies, the G allele and/or the GG genotype were associated with higher circulating resistin levels.

Regarding type-2 diabetes and obesity, two recent meta-analysis have demonstrated a lack of correlation of this SNP with this disease [23,24]. The authors conclude that this lack of association might be due to a small pooled sample size.

Another explanation of this lack of association could be due to the fact that circulating resistin levels would be influenced by other genetic variants in different genes [25].

Regarding PTB a few studies have focused on the relationship between resistin and the disease. One of them studied the utility of measuring circulating resistin levels as a sensitive biomarker to determine TB treatment end points concluding that this molecule would be a surrogate marker for TB treatment. Furthermore, resistin could be useful as an early prognostic biomarker for monitoring TB disease onset [16]. Another study suggested that increased resistin levels in patients with diabetes mellitus and severe TB might act directly in macrophages suppressing the Mtb-induced inflammasome activation through inhibiting reactive oxygen species production by leukocytes [14]. This could explain an increased susceptibility to pulmonary tuberculosis.

Interestingly, an increased expression of RETN in lung tissue has been observed in Glässer's disease in pigs (a respiratory disease produced by *Haemophilus parasuis* infection which can affect other organs) [26]. The results of these studies would support the role of resistin in conferring susceptibility or resistance against pulmonary infection, probably mediated through an inhibition of macrophage function.

To our knowledge, this is the first report on investigating the relationship between a functional SNP in the RETN gene and susceptibility to PTB. We could conclude that this SNP would not influence susceptibility or resistance to PTB, at least in our population. However, it should be consider the influence of ethnic differences in genetic polymorphisms which could determine their functions in the different populations. Thus, the relationship between this SNP and PTB would need further studies to elucidate if it would play a role in the pathogenesis of PTB.

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