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Association of the Presence of the *IS6110* Gene and the Polymorphisms of the Receptor of the Bactericide $P2X_7$ (A1513C and -762 C/T) in Mexican Patients with Takayasu's Arteritis and Tuberculosis. Is the Vasculitis A Manifestation of Extrapulmonary Tuberculosis?

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Abstract

Background and objective: Takayasu's arteritis is a disease that has been associated with tuberculosis, based upon the presence of Langerhans giant cells and granulomas. ATP treatment of mycobacteria infected macrophages induces apoptosis mediated via the $P2X_7$ pathway. Here we evaluated if the polymorphisms of the $P2X_7$ receptors are associated to the presence of tuberculosis in Mexican patients with Takayasu´s arteritis.

Methods: 63 aortic samples, 33 from Takayasu´s and 30 from tuberculosis subjects were studied. Histological analysis was made and the sequence of the gene *IS6110* which identifies the presence of *Mycobacterium tuberculosis* was amplified. Polymorphisms of the A1513C and -762 C/T receptors for the *$P2X_7$* gene were analyzed.

Results: The analysis showed a high percentage of extra pulmonary lesions (36.3%) in the Takayasu´s group. No significant differences between the studied genotypes were found in any of the models analyzed or in any of the two $P2X_7$ polymorphisms analyzed. Moreover, an increase in the 1513C allele in presence of *IS6110 gene* in the Takayasu´s group was observed when comparing with the group of tuberculosis.

Conclusion: We found a high percentage of extra pulmonary tuberculosis in subjects with Takayasu´s arteritis; this increase was associated with a high frequency in the allele 1513C in the presence of *IS6110 gene*. Thus, these alleles may confer a significant susceptibility at the aortic level in the Mexican population having these diseases.

Keywords: $P2X_7$ receptor polymorphism; Takayasu´s arteritis; Tuberculosis; *IS6110 gene*; *Mycobacterium tuberculosis*

Introduction

Takayasu´s Arteritis (TA) is an inflammatory and stenotic disease that affects medium and large-sized arteries and is characterized by a strong predilection for the aortic arch and is therefore often referred to as the "aortic arch syndrome". This disease promotes the inflammation and damage of vessel walls, as well as the development of non-specific clinical manifestations, such as fever, night sweats, malaise, weight loss, arthralgia, myalgia, and mild anemia [1-3]. As the inflammatory process progresses, stenosis develops and the characteristic features of the disease become apparent, influenced by the development of collateral circulation. The etiology of TA is unknown, but an association with tuberculosis has been reported [4]. The association between TA and tuberculosis (TB) was described 50 years ago, based upon the presence of Langerhans giant cells and granulomas similar to those found in tuberculosis lesions [5]. Hyper reactivity to tuberculin application has been documented [6,7]. Although information remains controversial, there are reports showing the presence of *Mycobacterium tuberculosis* in histological preparations of arterial lesions [8,9]. *Mycobacterium tuberculosis* is an airborne pathogen and the hallmark of the infection is the presence of granulomas within the lungs, starting with a transient influx of neutrophils to the site of the infection which is followed by activated macrophages and lymphocytes. However, the migration of the pathogen through the blood stream has been demonstrated and that richly oxygenated tissues, such as the aorta may become an alternative host organ for *Mycobacterium* accommodation [10].

Tuberculosis is primarily caused by *Mycobacterium tuberculosis*, and the DNA from this agent can be present in tissues for long periods during the latent infection. Furthermore, it has been reported that analysis of the inserts of the sequence of *IS6110* can be used for identifying the presence of various species of mycobacteria such as *M. bovis and M. tuberculosis*.

In a previous study, we demonstrated that patients with Takayasu's arteritis and tuberculosis lesions in the aortic tissues had the *IS6110* gene, demonstrating that in both diseases the mycobacterium was present [11].

Some reports have mentioned that extracellular ATP treatment of mycobacteria infected macrophages induces apoptosis and death mediated via the $P2X_7$ pathway. ATP exerts its bactericidal activity through activation of the $P2X_7$ purinergic receptor [12]. The $P2X_7$ receptor is a ligand-gated cation channel highly expressed by human

and murine macrophages [12]. Activation of $P2X_7$ by adenosine triphosphate (ATP) promotes the opening of a cation-selective channel, allowing the influx of Ca^{2+} and Na^+ and the efflux of K^+. These events have downstream effects such as caspase activation, resulting in apoptosis and the activation of phospholipase D (PLD), and promoting the phagosome/lysosome fusion that finally leads to mycobacterium lysis [13,14].

Genetic factors play an important role in the functional phenotype of the $P2X_7$ receptor, and the $P2X_7$ receptor genes are highly polymorphic showing several single nucleotide polymorphisms (SNPs) that lead to the loss of the receptor's function [15]. The most common of these SNPs is the A1513C polymorphism, where the substitution of Glu-496 by an Ala residue in the intracellular C-terminal tail is produced. This leads to a near-complete loss of $P2X_7$ receptor function and, as a result, the lack of ATP-induced mycobacterial lysis [16]. This replacement results in the expression of a non-functional $P2X_7$ receptor in macrophages from subjects homozygous for the 1513 C allele, while heterozygous individuals at this locus have impaired $P2X_7$ receptor function [17]. In addition, a -762T → C SNP in a promoter region of $P2X_7$ has been described as a protective change in a Gambian population with tuberculosis [18]. However, the functional role of this SNP is not yet determined and there are controversial results depending on ethnic populations.

Due to discrepancies in the relationship existing between the two diseases, a way to define and better understand the disease is through genetic studies. Therefore, here we studied the association of $P2X_7$ receptor polymorphisms (A1513C and -762 C/T) and the presence of *IS6110* gene as a proof of the presence tuberculosis in Mexican patients with Takayasu's arteritis.

Methods

A total of 63 aortic samples embedded in paraffin were chosen from 57,560 autopsy files that were reviewed in the pathology department of the National Institute of Cardiology ¨Ignacio Chávez¨. Thirty-three samples from patients with Takayasu's arteritis, diagnosis were assessed when patients met four or more criteria from the American College of Rheumatology. Cases were excluded if: a) patients did not fill the clinical diagnostic criteria, or b) if it was not possible to obtain the right aortic tissue or c) if DNA could not be suitably extracted or d) if expedients were incomplete. The second group consisted of thirty samples from patients with tuberculosis (TB), diagnosed by positive cultures of secretions or tissue samples and confirmed by bacilloscopy. Tuberculosis was also confirmed by culture at the time of the autopsy.

DNA extraction

DNA extraction from the paraffin embedded tissues was performed using a commercial kit (Illustra Nucleon Genomic, GE Healthcare). Nucleon TM resin contained within the Nucleon genomic extraction kit was used to extract DNA from difficult samples. This system is designed to give high yields of pure DNA from paraffin sections. The procedures have been optimized to allow maximum recovery of high molecular weight DNA. This system removes proteins effectively without the use of phenol.

Bacteria genome

To confirm the presence of *Mycobacterium tuberculosis* in the sample tissues and to be able to relate it to previous infection with the disease, the sequence of the gene *IS6110* (123 bp) was amplified as a genetic marker for *M. tuberculosis*. The primers used in the amplification were: *IS6110* Forward (5'-CCTGCG AGC GTA GGC, GTC GG-3') and IS6110 Reverse (5´-CTC GTC CAG CGC CGC TTC GG-3') accord to Negi SS [19]. The positive control for the analysis was a sample of *M. tuberculosis* obtained from cell lines extracted from strains of *M. tuberculosis HRv37*, provided by the Department of Cellular Biology of the National Institute of Cardiology (Figure 1).

$P2X_7$ polymorphisms

An allele-specific PCR assay was used to detect the C/T polymorphisms at position -762 according to Mokrousov I [20]. Two outer primers were used to flank the entire $P2X_7$OF (5´-GAAACAGGGCCCTGGGTCCTC-3´) and $P2X_7$OR (5´-TGGTGGGGGTGGAGGGGC-3´) region and amplified a 373 bp fragment in all strains. Two inner primers were also used, $P2X_7$IF (5´-GGTGTCCCTCACTGAATAGGTCAAT-3´) and $P2X_7$IR (5´-GGCAGTCCAACAAAGTTAGGTTTG-3´). To detect the -762C allele, a 235 bp fragment was amplified using the outer forward ($P2X_7$OF) and inner reverse ($P2X_7$IR) primers. For -762T allele, a 186 bp was amplified using the inner forward ($P2X_7$IF) and the outer reverse ($P2X_7$OR) primers. The amplified PCR fragments were subjected to electrophoresis in acrylamide gels and stained with nitrate silver. The A1513C SNP (rs: 3751143) was genotyped using 5' exonuclease TaqMan assay on a 7900HT Fast real-time PCR system, following the manufacturer's instructions (Applied Biosystems, Foster City, USA). Each SNP (allele and genotype) was manually and automatically defined with the allelic discrimination software (7300 System SDS Software® by Applied Biosystems).

Histological analysis

Tissues were stained with hematoxylin-eosin, Schiff's periodic acid, and Masson stain, and Auramine Rhodamina. The tissues were assessed by a certified pathologist, who verified the presence of fibrosis and inflammatory infiltrates (Figure 1).

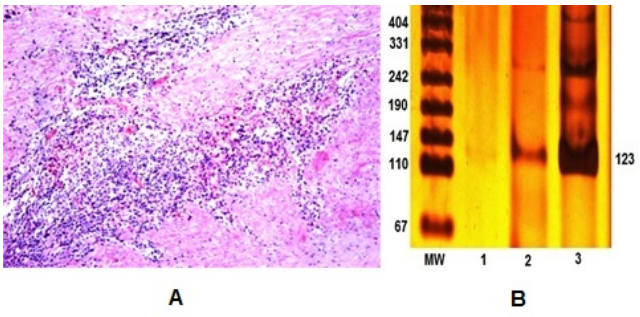

Figure 1: A. Tissue stained with hematoxylin-eosin, showing the presence of fibrosis and inflammatory infiltrates. B. Acrylamide gel stained with silver nitrate for the detection of *Mycobacterium tuberculosis* through *IS6110*Molecular weight marker; Lane 1: negative control; Lane 2: Bacillus of *M. tuberculosis* in aortic tissue; Lane 3: positive control of *M. tuberculosis* from cell culture (HRv37).

Calculation of the sample size

Considering that the Takayasu´s arteritis is an uncommon disease, the estimation of the size sample required in our hypothesis was of 31, the statistical power was more than 90%, P >0.05. However, in the studies where the size sample is unknown, the number used in epidemiology that has shown to have a normal distribution is of at least 30. The formula used for estimation of sample size to calculate proportions in independent samples (cases and controls) was:

$$\frac{poqo\left[Z \propto + Z\beta\sqrt{\frac{piqi}{poqo}}\right]2}{(pi-po)^2}$$

It was calculated from the approximate tuberculosis incidence, from extra-pulmonary tuberculosis (0.25) and tuberculosis (.05) Δ = (0.20)

$$\frac{(.25)(.75)\left[1.96+1.28\sqrt{\frac{p(.05)(.95)}{(.25)(.75)}}\right]2}{((.25)-(.05))2}$$

Sample power:

$$\left[\sqrt{{poqo}/{p1q1(Z\alpha/2)}}+\frac{||po-p1\sqrt{n}}{\sqrt{poqo}}\right]\alpha=0.05po=0.25p1=0.05n=33=0.964$$

Statistical analysis

Allele and genotype frequencies of the studied polymorphisms in patients and controls were obtained by direct counting. The significance of the differences between groups was determined using Mantel-Haenzel chi-squared analysis, which was combined with 2 x 2 contingency tables using the EPISTAT statistical software (Version 5.0; USD Incorporated 1990, Stone Mountain, Georgia). Fisher's exact test was used if the quantity in any cell of the contingence table was lower than five. Statistical significance was accepted at an alpha level of less than or equal to 0.05.

Ethics approval

Informed consent was given by our Institution through the Ethical Committee because patient's samples were obtained by autopsy. Other studies in these samples have been previously published [11]. In the present study, we presented the letter to the committee before the beginning of the study.

Results

We analyzed 63 paraffin embedded tissues that included samples from patients with Takayasu's arteritis (33), and a group the tuberculosis patients (30).

Tuberculosis site

The proportion of men was 48.4% in TA and 66.6% in TB group. The mean age was 22.8 ± 13.1 years for TA and 40.7 ± 18.8 years for the TB group.

The clinical demographic data were obtained from the autopsy reports and clinical history from the files (Table 1). The causes of death of patients with TA were highly variable including terminal congestive cardiac failure in 7 (21.2%), chronic renal failure with uremic syndrome, acute myocardial infarct in 6 (18.1%), acute pulmonary edema in 5 (15.1%), stroke in 3 (9.1%), hypertensive crisis 3 (9.1%), pulmonary artery hypertension in 3 (9.1%), pulmonary thromboembolism in 3 (9.1%), a tearing of the aorta and hypovolemic shock, cardiogenic shock in 1 (3%), tuberculosis meningitis in 1(3%), myocarditis and aortic contraction in 1 (3%). Both groups were residents of endemic tuberculosis sites, and therefore no significant differences were found.

Variable	Takayasu Arteritis N=33	Tuberculosis control N=30	p
Clinical Characteristics			
Age at diagnosis (years)	22 ± 13	41 ± 19	0.0001
Age at the time of death	29 ± 14	49 ± 18	0.0001
With tuberculosis disease well determined previously to death n (%)	0	30 (100%)	0.0001
Exposed at tuberculosis (clinical history) n (%)	7 (21.2)	30 (100)	0.0001
Native from residents of tuberculosis endemic zone (clinical history) n (%)	17 (51.5)	19 (63.3)	NS

Table 1: Clinical and demographic data by groups.

According to the sites of infection observed during autopsy (Table 2), a high percentage of lung injury in patients with tuberculosis was found (p=0.001, OR= 6.4, 95% CI=1.94-21.67). An increase in extra pulmonary lesion was found in patients with TA; however, there was no statistical difference.

Site of infection	Tuberculosis Group	Takayasu´s Group
	N (%)	N (%)
Without lesion	0 (0)	14 (42.4)
Pulmonary	16 (53.3)*	5 (15.1)
Extrapulmonary	9 (30)	12 (36.3)
Both	2 (6.75)	2 (6.1)
Miliar	3 (10)	0 (0)
Total	30	33

Table 2: Localization of tuberculosis lesion by groups (* p= 0.001 vs. type of lesion in the same group).

A1513C and -762 polymorphisms

Conformity with the Hardy Weinberg law of genetic equilibrium (HWE) was assessed by a non- significant Chi square test comparing the observed vs. the expected genotypes among cases and controls. (*P2X7 -762:* X^2=0.001, p=0.09 and X^2= 1.13, p=0.28; *P2X7 1513:* X^2= 1.2 p=0.27; X^2=0.28, p=0.59, for TB and TK, respectively).

The distribution of the *P2X7 -762* (rs660339) and *P2X7 A1513C* (rs1800849) polymorphisms was similar between the two groups studied in all of the models analyzed (Table 3).

Genotype frequency (%)								
					MAF	Model	OR (95% CI)	p value
$P2X_7$ -762		T/T	T/C	C/C				
Tuberculosis (N= 30)	N (%)	17 (56.7)	11 (36.7)	2 (6.7)	0.250	Recessive Codominant	1.34 (0.56-3.22) 1.17 (0.50-2.74)	0.504 0.710
Takayasu´s (N=33)	N (%)	23 (69.7)	8 (24.2)	2 (6.1)	0.18.1	Dominant	0.70 (0.11-4.43)	0.522
$P2X_7$ 1513		A/A	A/C	C/C				
Tuberculosis (N= 30)	N (%)	20 (66.7)	10 (33.3)	0 (0)	0.166	Recessive Codominant	0.57 (0.23-1.41) 1.46 (0.66-3.24)	0.227 0.339
Takayasu´s (N=33)	N (%)	21 (63.7)	10 (30.3)	2 (6.1)	0.212	Dominant	2.93 (0.61-14.1)	0.138

Table 3: Frequencies of UCP´s polymorphisms (MAF: Minor Allele Frequency)

With the purpose of knowing whether the presence of the mycobacterium gene present in our study groups according to genotype *$P2X_7$* could be involved in the disease, patients were divided according to their allele frequencies (Table 4). The percentage of patients with the gene *IS6110* was 73.3% (n=22) for tuberculosis followed by Takayasu's group with 63.6% (n=21).

-762	Total population			In presence of *IS6110*		
	ALLELE T	ALLELE C	p	ALLELE T	ALLELE C	p
	N (%)	N (%)		N (%)	N (%)	
Tuberculosis	45 (75.0%)	15 (25.0%)	0.351	35 (79.5%)	9 (20.5%)	0.283
Takayasu´s	54 (81.8%)	12 (18.2%)		37 (88%)	5 (12%)	
1513	ALLELE A	ALLELE C		ALLELE A	ALLELE C	
Tuberculosis	50 (83.3%)	10 (16.7%)	0.516	38 (86.3%)	6 (13.6%)	0.0001
Takayasu´s	52 (78.8%)	14 (21.2%)		31 (73.8%)	11 (26.2%)	

Table 4: Alleles frequencies in total population and divided by presence of *IS6110*

The analysis of allelic frequencies in the total population of study showed no significant differences in any of the two polymorphisms studied. However, when performing the analysis taking into account only those subjects that had the *IS6110* gene of the *Mycobacterium*, we found a statistically significant difference in the polymorphisms A1513C (p=0.001, OR=11.6 95%, CI=3.11-43.27) in Takayasu´s versus tuberculosis subjects. In the same context, when we did the same analysis but comparing the frequencies between allele C in the total population versus in the presence of *IS6110* in the Takayasu´s group, despite the fact that there was not a significant difference, there was an increase in the frequency.

Finally, with the purpose of analyzing the presence of *M. tuberculosis* according to the site of the lesion, an analysis was done considering only those subjects positive to *IS6110* and taking into account only subjects with pulmonary or extra pulmonary tuberculosis. From the 22 subjects with tuberculosis and with presence of the *IS6110* gene, half of these subjects (n=11) had pulmonary lesion, 13.6% had the 1513 allele C and the 22.3% had -762 allele C. Also, from the 11 subjects with damage outside the lungs, eight had the gene *IS6110* in the aorta (extra pulmonary) and 18.8% had the 1513 allele C and the 25% had the -762 allele C. In the Takayasu's group, from the 21 subjects with the *IS6110*, 4 had pulmonary and 9 had extra pulmonary disease, 44.5% had the1513 allele C and 22.3% had the -762 allele C (Table 5).

Presence of IS6110					
Pulmonary	**A1513C**	**Genotype AA**	**Genotype AC+CC**	**ALLELE A**	**ALLELE C**
		N (%)	N (%)	N (%)	N (%)
	Tuberculosis	8 (72.3)	3 (27.3)	19 (86.3)	3 (13.6)
	Takayasu´s	4 (100)	0	8 (100)	0
Extra pulmonary					
	Tuberculosis	5 (62.5)	3 (37.5)	13 (81.2)	3 (18.8)
	Takayasu´s	2 (27.3)	7 (77.7)	10 (55.5)	8 (44.5)
Pulmonary	**-762**	**Genotype TT**	**Genotype TC+CC**	**ALLELE T**	**ALLELE C**
	Tuberculosis	6 (54.5)	5 (45.5)	17 (72.3)	5 (22.3)
	Takayasu´s	4 (100)	0	8 (100)	0
Extra pulmonary					
	Tuberculosis	4 (50)	4 (50)	12 (75)	4 (25)
	Takayasu´s	5 (55.5)	4 (44.5)	14 (77.7)	4 (22.3)

Table 5: $P2X_7$ polymorphisms in presences of *IS6110* gene according to the site of injury.

Discussion

The pathophysiology of TA is not completely understood, but it is thought to be multifactorial, involving infectious agents (*Mycobacterium tuberculosis*, different types of viruses), autoimmunity and genetic influences. Tuberculosis may remain undiagnosed for years due to the chronic course of the disease, with potentially life-threatening long-term complications.

Mycobacterium tuberculosis causes infections in immunocompetent and immunocompromised subjects and produces pathology in pulmonary and extra pulmonary sites [21,22]. Most of the studies of *Mycobacterium tuberculosis* have been focused on pulmonary infections, while extra pulmonary infections have been poorly explored, however extra pulmonary tuberculosis accounts for about 10% to 20% of tuberculosis cases. Nevertheless, in both pulmonary and extra pulmonary infections there are other factors determining the outcome of the infection and the type of clinical expressions, including the amount of agent inoculated the type of mycobacteria, the site where it is inoculated and multiple mycobacterium evasion mechanisms. In our study subjects, the highest frequency of extra pulmonary lesions was observed in the group of Takayasu's arteritis, in which it was higher than in the average reported for other populations with tuberculosis [23,24].

One possible explanation is that Takayasu´s arteritis is related to previous infection with tuberculosis and some clinical evidences have shown the presence of the latent bacillus, which can be activated later due to: a) decreased metabolism depending on environmental stress, b) mechanisms of evasion of mycobacteria, c) the loss of tolerance to self-stress induced cell molecules and finally, d) to the initiation of the inflammatory response. This information is consistent with previously proposed hypothesis that suggest a possible association of TA to mycobacterium by a previous infection in earlier stages [9,22].

Furthermore, the $P2X_7$ receptor is highly expressed in macrophages. It participates in the (ATP)-induced killing of phagocytized Mycobacterium tuberculosis and in the subsequent apoptosis of the infected macrophage [12-14]. Polymorphisms in $P2X_7$ such as the -762 in the promoter region or A1513C have been associated to functional defects with decreased ATP-induced apoptosis [16-18]. Thus, these polymorphisms may lead to a change in functionality of the receptor and, as consequence, might be associated with changes in susceptibility to these diseases.

It has been shown that the glutamic acid to alanine substitution at position 496 in exon 13 results in the expression of a non-functional $P2X_7$ receptor in macrophages from subjects homozygous at this locus [25-27]. Most of the reports have shown differences in A1513C of the *$P2X_7$* gene with tuberculosis [13,17,28,29]. Therefore, $P2X_7$ polymorphism could contribute to susceptibility or resistance to infections like tuberculosis in different populations throughout the world.

Our results showed no differences among genotypes studied in any of the three models analyzed in any of two $P2X_7$ polymorphisms analyzed. These results are consistent with those reported in a meta-analysis [30,31]; where the association between $P2X_7$ polymorphisms and susceptibility to pulmonary tuberculosis was determined. Moreover, in the different models studied, they did not find a significant differences in both *$P2X_7$ -762 C/T* and -1513 A/C [32], except in the Indian population [33], where they found a significant association in *$P2X_7$ -1513C* in both allelic and recessive models (p=0.002).

Many reports show discrepancy in the allele frequencies for -1513 C, with a range varying from 7.6% in the Gambian population [18] to 31% in Caucasian population [12]. In these yields, an expected prevalence of the homozygous condition of 1 -2 % in some Caucasian populations would be expected [29]. In both study groups the frequencies were between 18% to 25%.

Despite the existence of these studies none of them has verified the presence of the *M. tuberculosis* at a molecular level. Therefore in this study, we analyzed the association between polymorphisms of $P2X_7$ receptors in patients with Takayasu's arteritis and tuberculosis tested by the presence of a specific gene of the mycobacteria through (*IS6110*) in the aortic tissues.

Our results, through DNA analysis of *M. tuberculosis* (*IS6110 gene*) showed some differences. The frequency for -1513 allele C in the presence of *IS6110* gene in the Takayasu´s group are increased and almost doubled when compared to the group of tuberculosis. This could be relevant since the presence of *IS6110* proves the existence of the mycobacterium directly in the tissue with lesions and this genotype may be associated with host susceptibility to develop injuries. This data could explain the aortic damage in Takayasu's arteritis, since it is hypothesized that this disease might be due to extra pulmonary tuberculosis, and may be highly associated with allele -1513C.

Another possible explanation is that in the Takayasu's group, the tuberculosis lesion was not diagnosed as such, and therefore the lesions observed in the aorta were not studied in the autopsy being described as granulomatous and patches and considered as results of fibrosis or a chronic inflammatory process. One way to prove this hypothesis could be that the tissues obtained from surgery be studied as any other biopsy when extra pulmonary tuberculosis is suspected.

For the moment, studies relating the disease through the confirmation of the *Mycobacterium* by molecular methods have not been reported. Our data show the presence of damage from *Mycobacterium tuberculosis* in Takayasu's arteritis, however, the functional role of this $P2X_7$ SNP is not yet determined. As in other complex diseases that are multifactorial, host genetic factors contributing substantially to the development of TB and /or TA. Factors might include host genes such as HLA, which is associated to susceptibility to the disease and gene modifications in the tubercle bacillus that permits the evasion of the immune response. This leads to the diverse clinical expression detected in this condition. However, our findings suggest that the purinergic receptor gene involved in macrophage activity, plays an important role in the mechanisms of injury. A limiting factor in this work is the number of patients studied; however, it must be taken into account that samples are of aortic tissue and that the prevalence of Takayasu's arteritis is not high. So it is important to perform more studies with larger numbers of subjects

To our knowledge, this is the first work that explores the involvement of $P2X_7$ polymorphisms in patients with both tuberculosis and Takayasu´s arteritis, taking into account the presence of the gene *IS6110* at molecular level which is specific for *M. tuberculosis* in aortic tissues.

In summary, our study shows a high percentage of subjects with *Mycobacterium tuberculosis* presence in both study groups, and in the case of patients with Takayasu's arteritis, it could represent a double risk when the of $P2X_7$ -1513C polymorphisms is present and that this synergism might predispose to the development of this vasculitis. Takayasu´s arteritis could eventually be considered as a manifestation of extra pulmonary tuberculosis.

Competing interests

The authors declare that they have not conflict of interest.

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References

1. Numano F, Okawara M, Inomata H, Kobayashi Y (2000) Takayasu's arteritis. Lancet 356: 1023-1025.
2. Seko Y (2000) Takayasu arteritis: insights into immunopathology. Jpn Heart J 41: 15-26.
3. Johnston SL, Lock RJ, Gompels MM (2002) Takayasu arteritis: a review. J Clin Pathol 55: 481-486.
4. Kinare SG (1970) Aortitis in early life in India and its association with tuberculosis. J Pathol 100: 69-76.
5. Moraes MF, Ordway D, Oliveira L, Costa IL, Badura R, et al. (1999) Cellular immune responses to Mycobacterium tuberculosis in a patient with Takayasu's arteritis. Rev Port Cardiol 18: 359-367.
6. Lupi-Herrera E, Sánchez-Torres G, Marcushamer J, Mispireta J, Horwitz S, et al. (1977) Takayasu's arteritis. Clinical study of 107 cases. Am Heart J 93: 94-103.
7. Lupi Herrera E, Contreras R, Espino Vela J, Sánchez Torres G, Horwitz S (1972) [Nonspecific arteritis in childhood. Clinical and anatomo-pathological observations]. Arch Inst Cardiol Mex 42: 477-493.
8. Arnaud L, Cambau E, Brocheriou I, Koskas F, Kieffer E, et al. (2009) Absence of Mycobacterium tuberculosis in arterial lesions from patients with Takayasu's arteritis. J Rheumatol 36: 1682-1685.
9. Serratrice J, Benyamine A, Vidal V, Talet HB, Amri AB, et al. (2008) Simultaneous occurrence of diffuse Takayasu's arteritis and severe disseminated tuberculosis. Rev Med Interne 29: 520-523.
10. Russell DG (2007) Who puts the tubercle in tuberculosis? Nat Rev Microbiol 5: 39-47.
11. Soto Me, Ãvila-Casado M, Huesca-GÃmez C, AlarcÃn GV, Castrejon V, et al. (2012) Detection of IS6110 and HupB genes sequences of Mycobacterium tuberculosis and bovis in aortic tissues patients with Takayasu´s arteritis. BMC Infectious Disease 12: 194.
12. Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Goldberg H, et al. (2007) A polymorphism in the P2X7 gene increases susceptibility to extrapulmonary tuberculosis. Am J Respir Crit Care Med 175: 360-366.
13. Gu BJ, Zhang W, Worthington RA, Sluyter R, Dao-Ung P, et al. (2001) A Glu-496 to Ala polymorphism leads to loss of function of the human P2X7 receptor. J Biol Chem 276: 11135-11142.
14. Rassendren F, Buell G, Newbolt A, North RA, Surprenant A (1997) Identification of amino acid residues contributing to the pore of a P2X receptor. EMBO J 16: 3446-3454.
15. Tekin D, Kayaalti Z, Dalgic N, Cakir E, Soylemezoglu T, et al. (2010) Polymorphism in the p2x7 gene increases susceptibility to extrapulmonary tuberculosis in Turkish children. Pediatr Infect Dis J 29: 779-782.
16. Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Wiley JS, et al. (2005) Gene dosage determines the negative effects of polymorphic alleles of the P2X7 receptor on adenosine triphosphate-mediated killing of mycobacteria by human macrophages. J Infect Dis 192: 149-155.
17. Ben-Selma W, Ben-Kahla I, Boukadida J, Harizi H (2011) Contribution of the P2X7 1513A/C loss-of-function polymorphism to extrapulmonary tuberculosis susceptibility in Tunisian populations. FEMS Immunol Med Microbiol 63: 65-72.
18. Li CM, Campbell SJ, Kumararatne DS, Bellamy R, Ruwende C, et al. (2002) Association of a polymorphism in the P2X7 gene with tuberculosis in a Gambian population. J Infect Dis 186: 1458-1462.
19. Negi SS, Anand R, Pasha ST, Basir SF, Gupta S, et al. (2006) Detection of M. tuberculosis in clinical samples of diversified nature by IS6110 based PCR. J Commun Dis 38: 325-332.

20. Mokrousov I, Sapozhnikova N, Narvskaya O (2008) Mycobacterium tuberculosis co-existence with humans: making an imprint on the macrophage P2X(7) receptor gene? J Med Microbiol 57: 581-584.
21. Stout JE (2006) Evaluation and management of patients with pulmonary nontuberculous mycobacterial infections. Expert Rev Anti Infect Ther 4: 981-993.
22. Duzova A, Türkmen O, Cinar A, Cekirge S, Saatci U, et al. (2000) Takayasu's arteritis and tuberculosis: a case report. Clin Rheumatol 19: 486-489.
23. Lin JN, Lai CH, Chen YH, Lee SS, Tsai SS, et al. (2009) Risk factors for extra-pulmonary tuberculosis compared to pulmonary tuberculosis. Int J Tuberc Lung Dis 13: 620-625.
24. Sreeramareddy CT, Panduru KV, Verma SC, Joshi HS, Bates MN, et al. (2008) Comparison of pulmonary and extrapulmonary tuberculosis in Nepal- a hospital-based retrospective study. BMC Infect Dis 8: 8.
25. Humphreys BD, Rice J, Kertesy SB, Dubyak GR (2000) Stress-activated protein kinase/JNK activation and apoptotic induction by the macrophage P2X7 nucleotide receptor. J Biol Chem 275: 26792-26798.
26. Kusner DJ, Barton JA (2001) ATP stimulates human macrophages to kill intracellular virulent Mycobacterium tuberculosis via calcium-dependent phagosome-lysosome fusion. J Immunol 167: 3308-3315.
27. Coutinho-Silva R, Stahl L, Raymond MN, Jungas T, Verbeke P, et al. (2003) Inhibition of chlamydial infectious activity due to P2X7R-dependent phospholipase D activation. Immunity 19: 403-412.
28. Sambasivan V, Murthy KJ, Reddy R, Vijayalakshimi V, Hasan Q (2010) P2X7 gene polymorphisms and risk assessment for pulmonary tuberculosis in Asian Indians. Dis Markers 28: 43-48.
29. Xiao J, Sun L, Yan H, Jiao W, Miao Q, et al. (2010) Metaanalysis of P2X7 gene polymorphisms and tuberculosis susceptibility. FEMS Immunol Med Microbiol 60: 165-170.
30. Yi L, Cheng D, Shi H, Huo X, Zhang K, et al. (2014) A meta-analysis of P2X7 gene-762T/C polymorphism and pulmonary tuberculosis susceptibility. PLoS One 9: e96359.
31. Ge HB, Chen S (2016) A meta-analysis of P2X7 gene-1513A/C polymorphism and pulmonary tuberculosis susceptibility. Hum Immunol 77: 126-130.
32. Wang X, Xiao H, Lan H, Mao C, Chen Q (2011) Lack of association between the P2X7 receptor A1513C polymorphism and susceptibility to pulmonary tuberculosis: a meta-analysis. Respirology 16: 790-795.
33. Sharma S, Kumar V, Khosla R, Kajal N, Sarin B, et al. (2010) Association of P2X7 receptor +1513 (A-->C) polymorphism with tuberculosis in a Punjabi population. Int J Tuberc Lung Dis 14: 1159-1163.