ANALYSIS OF *TYK2* P1104A VARIANT IN A COHORT OF COLOMBIAN PATIENTS WITH PULMONARY TUBERCULOSIS.

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ABSTRACT

Tuberculosis continues to be one of the main causes of death worldwide with around 10 million people infected annually, which is a direct problem for public health. Data that has accumulated over decades suggests that variability in human susceptibility to tuberculosis disease has a strong genetic component. Both primary and acquired immunodeficiencies (PID) are strongly related to broad infectious phenotypes that include the full range of diseases caused by the infectious agent Mycobacterium tuberculosis; Recently it was found that a PID found in up to 1/600 individuals of European descent, in fact a variant was found that affects the catalytic activity of TYK2 therefore selectively alters the immunity to IFN-g mediated by IL-23 while the responses to IL-10, IL-12, and IFN-a / b are intact. These PIDs are only expressed symptomatically after exposure to *Mycobacterium tuberculosis*. However, we hypothesize that homozygous for the *P1104A* variant or compound heterozygosity in this same TYK2 gene can confer a selective response, thus predisposing to TB. This study intends to 1. Identify the variant *P1104A* of the *TYK2* gene in Colombian patients with pulmonary tuberculosis, 2. Identify other rare or common variants in the *TYK2*

gene in patients with pulmonary tuberculosis, 3. Correlating data from the Colombian population with what has been found in other populations

Key words: *Mycobacterium Tuberculosis*; *TYK2* Gene; Primary Immunodeficiencies, P1104A; Genotypes; Susceptibility to tuberculosis; Immune response.

INTRODUCTION

Tuberculosis (TB) is one of the world's major public health problems, infecting around a third of its population. According to the annual report on global tuberculosis control of the World Health Organization, in 2018, it is estimated that the active prevalence of tuberculosis is around 10 million people, and that of infection (latent TB) is approximately 1.8 billion people in a number that has remained relatively stable in recent years. All these figures place TB within the top 10 causes of mortality in the world infectious diseases, occupying the top positions worldwide. In the same year, there were 1.2 million deaths from tuberculosis among HIV-negative people, and another 251,000 deaths among HIV-positive people. Geographically, the majority of tuberculosis cases in 2018 were in the WHO Regions of South-East Asia (44%), Africa (24%), and the Western Pacific (18%), with lower percentages in the Eastern Mediterranean (8%), the Americas (3%) and Europe (3%). (1).

Tuberculosis is a disease in which social, economic, environmental (overcrowding, large families, poor asepsis, darkness, and humidity) (2), and mainly genetic factors (2–4) profoundly influence host susceptibility. In Colombia, as in other regions of the world, it is one of the leading public health problems. In the last 43 years, nearly 500,000 cases of tuberculosis have been reported. Although the incidence rate has decreased from 58.62 cases per 100,000 inhabitants in 1970 to 33 cases per 100,000 inhabitants in 2013, the number of cases detected annually has remained stable (1).

In 2018, more than 16 thousand cases of active TB with an incidence of 33 per 100,000 inhabitants (1), and around 65% of the cases were concentrated in the most

productive segment of the population (20-59 years) (5–7). The department of Antioquia reports the highest number of cases, and Medellín, the capital of this department, is the city of Colombia that reports the highest number of cases with an incidence of 53 per 100,000 inhabitants, but some localities have a high incidence (100-200 per 100,000 population). However, at the population level, there are no clear reports on the number of infections that result from non-tuberculous *mycobacteria* (NTM), therefore it is reasonable that the prevalence of mycobacterial disease could be higher (8).

Mycobacterium tuberculosis (Mtb) is the specific agent of human TB. The most frequent form of the disease is pulmonary TB (86% approximately). TB (9, 10). The majority (90-95%) of primary infections do not produce clinical symptoms and eventually enter a latent phase in a condition presently known as latent TB disease (11–14). A variable percentage of latent infections (5-10%) is reactivated with the disease's signs and symptoms (active TB). The infection is not usually transmitted during the primary stage and does not spread in the latent phase. When someone develops active tuberculosis, symptoms (cough, fever, night sweats, weight loss, hemoptysis, among other symptoms) may be mild for many months (14). This can delay the affected person to seek medical attention, with the consequent risk of the bacteria being transmitted to other subjects. A person with active TB can infect between 10 and 15 people by direct contact over a year. Without adequate treatment, over 45% of HIV-negative people with TB and nearly all people with TB/HIV co-infection will die (1).

In recent years, a large number of clinical and epidemiological studies have suggested that Mtb infection and TB per se have a strong genetic basis (15, 16). Candidate gene studies have shown evidence of an association between host genetic polymorphisms and TB susceptibility in different human populations. Genes such as chemokine (C-C motif) ligand-2 (*CCL-2*)/monocyte chemoattractant protein 1 (*MCP-1*), natural resistance-associated macrophage protein 1 (*NRAMP-1*)/solute carrier protein 11A1 (*SLC11A1*), *IRGMI*, interleukin (IL) 8, several Toll-like receptors (TLR), and nucleotide-binding oligomerization domain-containing

protein 2 (*NOD 2*) genes, among others (17–22). Accordingly, TB is an infectious disease caused by *M. tuberculosis*, prepared and determined by genetic polymorphism. These genetic factors play a fundamental role in pathogenesis and directly influence and modulate the immune response, which is why not everyone develops the disease.

Both primary (PID) and acquired immunodeficiencies are strongly related to broad infectious phenotypes that include the full range of diseases caused by the infectious agent *Mycobacterium tuberculosis* (Chronic Granulomatous Disease (CGD), defects in NF-kB-mediated signaling, hereditary defects in the nicotinamide adenine dinucleotide phosphatase (NADPH) oxidase complex, among others), which supports the hypothesis that some defects in the immune system could be associated with the pathogenesis of TB (23). In this context, Mendelian Susceptibility to Mycobacterial Diseases (MSMD) is a rare group of PIDs characterized by the presence of selective susceptibility to clinical disease caused by BCG vaccines and environmental mycobacteria (NTM), and may even present tuberculosis caused by *Mycobacterium tuberculosis* (24–28), in otherwise healthy patients, and who are normally resistant to other organisms. So far, 15 genes have been associated with the MSMD condition with allelic heterogeneity explaining 30 genetic disorders, mainly associated with the interferon gamma (IFN-γ) immunity (29).From those genes, autosomal recessive (AR) interleukin-12 receptor deficiencies (*IL12RB1*) and tyrosine kinase 2 (*TYK2* variant *P1104A*) are the only two inborn errors of immunity reported to date underlying primary tuberculosis in patients (30, 31). Together, these studies demonstrate the first common monogenic cause of TB in humans.

TYK2 is a Janus kinase involved in four cytokine signaling pathways (those mediated by IL-12, IL-23, IFN-α, and IL-10). The consequences of complete deficiency in the TYK2 gene differ according to the pathway that is affected, when responses are affected to IL-12 and IL-23 the result is mycobacterial susceptibility (MSMD and TB) (32, 33), impaired responses to IFN-α leading to viral infections (34), impaired responses to IL-10 are clinically silent (35, 36). Despite

this, only half of the patients with this complete deficiency have developed BCG disease even when they have been vaccinated with BCG, which shows incomplete penetrance for MSMD. This can explain or provide proof that tuberculosis may be monogenetic but does not explain the public health problem.

Based on the Boisson-Dupuis (3) study, it was estimated that the penetrance of TB is high (at least 50%) in endemic disease areas, while that of MSMD is much lower, no more than 0.5%, which agrees that M. tuberculosis is much more virulent than BCG and EM.

The minor allele frequency (MAF) for *P1104A* is 4.2% in the European population (from gnomAD) The MAF for *P1104A* is approximately 1 to 2% in other parts of the world except in East Asia and Sub-Saharan Africa, where it is much rarer, if not completely absent. Based on ancient DNA studies, it is estimated that the frequency of *P1104A* has decreased notably in European populations, which is consistent with the purging of TB cases throughout history. It is also estimated that the homozygosity of this variant may underlie a considerable proportion of TB cases, perhaps representing ~ 1% of TB cases in Europeans and ~ 0.33% of cases in most other regions of the world. This considerable proportion is based on figures that at least in Europe tuberculosis may have killed around one billion people (37) suggesting that around ten million people may have died due to homozygosity of *TYK2* (*P1104A*).

In Colombia specifically, it should be clarified that the *P1104A* variant, contrary to the previously expressed frequency, is a common issue in the study populations, but to date, there have not been enough studies to decipher the association between the *P1104A* variant in *TYK2* and the TB in the Colombia, a TB endemic country. In a preliminary study carried out by doctoral student Carlos Andres Franco with a cohort of 722 patients diagnosed with pulmonary tuberculosis, no homozygous individuals were found, but some were heterozygous for the variant. This result would suggest the possibility of compound heterozygotes.

HYPOTHESIS

In heterozygous patients for the *TYK2* P1104A variant, there may be a second

variant that contributes to susceptibility to pulmonary tuberculosis.

OBJECTIVES

GENERAL

Determine the presence of variants in the *TYK2* gene exons as possible triggers associated with active pulmonary TB in Colombian patients

SPECIFIC

I. Identify the *TYK2 P1104A* variant in a cohort of Colombian patients with pulmonary TB.
II. Sequence the exons of the *TYK2* gene by the Sanger method.
III. Identify heterozygous compound genotypes in Colombian patients with pulmonary TB.
IV. Correlating data from the Colombian population with what has been found in other populations

MATERIALS AND METHODS

Ethics statement

This study was conducted following the Helsinki Declaration, with written informed consent obtained from the patients, their parents (for minors), and other family members. Approval for this study was obtained from the Regional Ethics Committee of the Universidad de Antioquia, Medellin, Colombia.

Studied population

The genomic DNA (gDNA) samples (n=722) used in this study were obtained from the Cellular Immunology and Immunogenetics Group (GICIG), Faculty of Medicine, University of Antioquia. These samples were collected from grants 11150416335, 34261817270, and 111540820482 (Colciencias, Bogotá, Colombia,), in which a cohort of active pulmonary TB patients (n=433) was followed-up between March 2005 and December 2009. More of 90% of the recruited TB patients were HIV negative and positive for *M. tuberculosis infection*, as assessed by sputum microscopy and culture. Under the same criteria, a parallel cohort was established in the cities of Cali (Departamento del Valle, Colombia; n=106), and Popayan (Departamento del Cauca; n=26) with active pulmonary TB (median age = 33 years, range 18-95),

between the years 2005-2006. Eighty percent were classified as positive for baciloscopy (38, 39) (Figure 1).

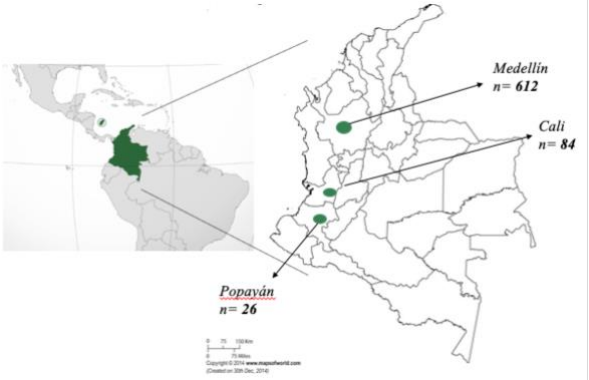

Figure 1: Geographical description of the study patients

gDNA extraction, qPCR and Sanger sequencing

Genomic DNA was extracted from whole blood using the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN). A qPCR method was used to identify the TYK2 SNPs rs34536443 (C/G) (P1104A) allele frequency. For this, a TaqMan® SNP Genotyping Assay (Applied Biosystems) was used for discrimination of alleles encoding TYK2 Pro-1104 and Ala-1104). The TaqMan genotyping assay was validated using controls of each genotype and the results were compare to the reference human genome GRCh37.

Conventional Polymerase chain reaction (PCR) was used in order to confirm the P1104A presence and to sequence the *TYK2* exons from 3 to 23 using gDNA as template. The oligonucleotides and cycles used in each of the PCRs are described in supplementary Tables 1, and 3, and supplementary Figure 1. The commercial Taq polymerase (Thermo Fisher Scientific, USA) was used in a reaction with a final volume of 40 µL containing 150 ng of gDNA and 0.2 µM of each of the oligonucleotides (supplementary Table 2). All the amplicons were analyzed using 2% agarose gels.

All the PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) using the sense and antisense oligonucleotides used for each of the PCR reactions. Sequencing products were purified using Sephadex G-50 Superfine Resin (GE Healthcare). Sequences were determined by using ABI 3730 DNA Analyzer (Applied Biosystems). Sequencing spectrum data were analyzed using the SnapGene software. The chromatograms obtained were analyzed with the SnapGene program using as reference the sequence of *TYK2* reported

in the National Center for Biotechnology Information (NCBI) database (NG_0078721). The position of the variants was determined using the Alamut Visual ™ program (Interactive Biosoftware, France), version 2.7.2, using the GRCh37 reference genome, while the analysis of the pathogenesis of the mutations found was done with the gnomAD, SIFT and PolyPhen programs. The nomenclature used to name the variants and mutations was based on the nomenclature guidelines of the Human Genome Variation Society (HGVS).

RESULTS

Identifying the *TYK2 P1104A* variant in Colombian patients

In the present study, the TYK2 p.Pro1104Ala (P1104A) variant (rs34536443) was genotyped by qPCR in 722 Colombian patients with TB. After the analysis was carried out, 19 individuals with the *TYK2 P1104A* variant were found in a heterozygous condition (15 from Medellin, and 4 from Cali) and no individual carrying the variant was found in a homozygous condition (Table 2).

Number of individuals studied with TB	Number of **homozygous** individuals for the P1104A variant	Number of individuals **heterozygous** for the P1104A variant	**MAF** (Minor allele frequency**) %**
722	0	19	1,3

Table 2: Study population, individuals homozygous and heterozygous for TYK2 P1104A, and MAF.

No homozygous for the P1104A variant of the *TYK2* gene were found in the 722 TB patients. However, 19 heterozygous individuals were found, suggesting the allelic frequency (MAF) of the TYK2 P1104A variant was determined, resulting in 1.3% (19/1444) in the studied population. The MAF of P1104A. The possibility that other genetic variants within the same gene may create a predisposition to the development of active TB in Colombian patients. The presence of P1104A was confirmed by Sanger sequencing as shown as an example for patient 16 (P16) (Figure 2).

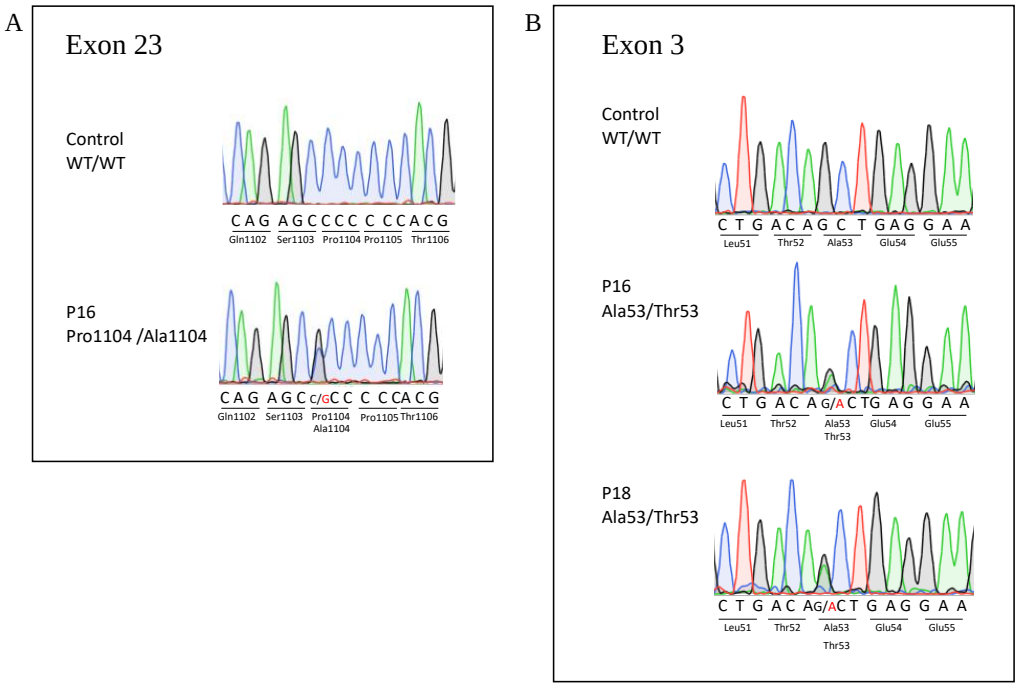

Figure 2. Analysis of the TYK2 gene by chromatogram of the sequences corresponding to the healthy control and to the patients P16, and P18. **A.** Chromatograms of exon 23 corresponding to control and patient P16. The heterozygous change *P1104A* is indicated in red by the affected nucleotide (it was the same image and result for the 19 heterozygous patients). **B.** Chromatograms of exon 3 of the control, patient P16 and P18. The heterozygous change *p.Ala53Thr* in both patients are indicated in red. The nucleotide position is based on the gDNA sequence reported in the National Center for Biotechnology Information (NCBI)

Searching for *TYK2* gene variants in a Colombian population of TB patients

Therefore, all the coding exons were amplified in search of a possible heterozygous variant that can explain a possible compound heterozygous condition with P1104A. The 23 coding exons were amplified by conventional PCR and confirmed by agarose electrophoresis as shown as an example for exon 8-9, and 23 (Figure 3) .Some of the exons were amplified together since they were very small; in total, 304 amplicons were obtained for analysis by the Sanger sequencing. In this search, we found that two patients (P16 and P18) as the variant g.10488926C>T in *TYK2* exon 3 in an heterozygous condition, resulting in a p.Ala53Thr (A53T) previously reported in gnomaAD (dbSNP, rs55762744) (Figure 2)

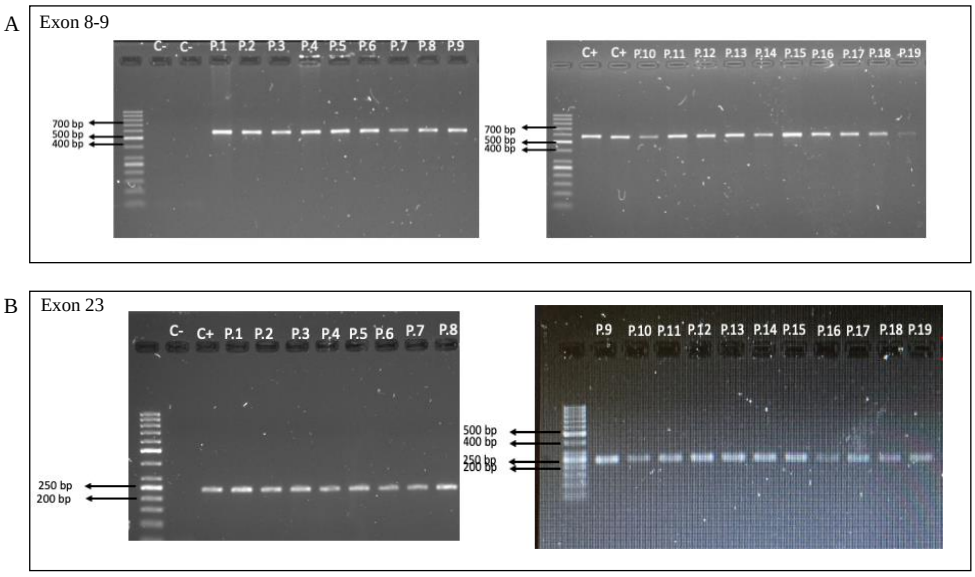

Figure 3. Agarose Gel Electrophoresis for PCR Products **A.** Electrophoresis of PCR products from exon 8-9 of the TYK2 gene. This exon was amplified by PCR from genomic DNA in the 19 patients studied and in the healthy individual that served as a positive control (+). Subsequently, a 2% agarose gel electrophoresis was performed using each of the PCR products and the presence of a band equivalent to 572 bp was determined by means of GelRed. For the negative PCR control (-), all PCR reagents except gDNA were used. **B.** Electrophoresis of PCR products of exon 23 of the TYK2 gene with a band equivalent to 236 bp; (The same protocol was used for all exons).

DISCUSSION

Two recently published studies (3, 4) delve into this genetic question of TB disease and reveal, firstly, that the immune response to mycobacteria is driven by the interleukins IL-12 and IL-23 and, secondly, that those people with two copies of a variant of the TYK2 gene has a high predisposition to develop tuberculosis.

In the first of the studies, the researchers analyze the role of the interleukins IL-12 and IL-23 in the immune response against *M. tuberculosis*. Lack of receptors for either of these two immune systems signaling molecules leads to high susceptibility to mycobacterial infection. These experiments of nature show that human IL-12 and IL-23 are both required for optimal IFN-γ–dependent immunity to mycobacteria (4). The second study works precisely on this question. The team of researchers identified a P1104A genetic

variant in the *TYK2* gene whose presence in homozygosis (in both copies of the gene) increases the risk of TB. This genetic variant interferes with the induction of IFN-γ mediated by IL-23. Researchers have found that the frequency of the variant has decreased in European populations over the past 4,000 years, suggesting that natural selection is acting to eliminate the alleles responsible for increased susceptibility to tuberculosis (3).

A very interesting aspect this is the estimate that about one in every 600 people of European descent carries both copies of the *TYK2* gene for TB susceptibility (the frequency varies between 1 / 1,000 and 1 / 10,000 in other populations). This result may be especially relevant for some people. The frequency of TB and the risk of exposure to the infection is low in countries that show a higher frequency of carriers of variants of susceptibility to the disease. However, if these people travel to high-risk regions with high exposure, they would have a much higher chance of becoming infected (3). Therefore, this is the greatest relevance of the study.

Specifically, in this study, no patients were found homozygous for the P1104A variant. However, 19 individuals were heterozygous (15 from Medellin, and 4 from Cali). Assuming that these patients may have another variant in a different exon, it was then decided to sequence each of the coding exons. In this search, we found that two of the patients had the p.Ala53Thr variant revealing a possible compound heterozygous condition with P1104A in these patients. The p.Ala53Thr (rs55762744) is reported in genomeAD as a variant encoding a missense mutation in exon 3 of the *TYK2* and in silico analysis by SIFT and PolyPhen predicted this variant to be deleterious and probably damaging, respectively. It has an allelic frequency of 0.7% in total populations and 0.3% in Latino populations, and has been previously associated with susceptibility to multiple sclerosis (40). Unfortunately, in our case the sequencing method used does not allow us to identify the particular allele carrying the variant. In this sense, the compound heterozygosity is hypothetical and further studies are necessary. Furthermore, it will be necessary to experimentally determine the phenotypic effect of this variant to define a potential effect on TB susceptibility, and the induction of IFN-γ mediated by IL-23

in tests similar to those performed by Boisson-Dupuis S in 2018 (3).

Comparing *TYK2 P1104A* with other populations worldwide

The modern human species - *Homo sapiens sapiens* - originated in sub-Saharan Africa ~ 200,000 years ago, this species began migrating from Africa ~ 70,000 years ago; Humans spread east and west populating Asia and Europe and just ~ 20,000 years ago populated America (41). Thousands of years of geographic isolation were accompanied by a population genetic diversification, giving rise to the diverse human population groups that can be seen throughout the world to this day. The colonization of America and the slave trade brought the different populations into close and sustained contact and as a consequence, new mixed populations were created (42, 43). This is particularly true for Latin America, where populations are characterized by high levels of genetic mixing between populations of African, European, and Native American origin (42, 44).

The *TYK2 P1104A* variant is believed to have a European origin, having a current frequency of approximately 4.2%, the lowest frequency of P1104A is recorded in populations of non-European descent, including in particular its very low frequency in East Asia and Sub-Saharan Africa (37). Taking into account the above, specifically the Colombian population has an ancestral European component, but also African and indigenous; This study has been focused mainly on the studied sample of the population of Medellin, therefore it would not represent the ethnic complexity of the Colombian population. According to the study conducted by King Jordan (45) of Genetic Ancestry in Latin America, "Several genes regulated by ancestry-specific SNPs were found to play specific roles in the immune system and in responses to infectious diseases. In particular, it was discovered that the genes of the immune system innate and adaptive are regulated by SNPs enriched by ancestry that exert specific regulatory effects on the population ". Furthermore, it was identified that the population of Medellín has a mostly European genetic ancestry, which would explain that we have patients with the P1104A polymorphism in a heterozygous state; This ancestral genetics varies at the level

of all Latin American populations, therefore, it cannot be certain or confirmed until more studies are carried out, how this variant is distributed at the level of the entire Colombian population or in general Latin American.

CONCLUSIONS

It is already certain that a large number of tuberculosis cases are related to P1104A in a homozygous state in European individuals, specifically in Latin American or Colombian individuals, no study has been carried out based on susceptibility to tuberculosis by this same variant. In this study, we did not find the variant in a homozygous state; however, we found the *TYK2 P1104A* variant in 19 Colombian patients in a heterozygous state, which is why it was assumed that other genetic variants within the same gene could create a predisposition to the development of TB active in these Colombian patients. This hypothesis was confirmed by sequencing and the *p.Ala53Thr* variant was found in two of the patients. This variant has not been reported in studies related to tuberculosis; however, studies are necessary to experimentally determine the phenotypic effect of this variant and define a potential effect on susceptibility to tuberculosis. However, we cannot rule out the possibility that a possible lack of sufficient statistical power may be limiting our results. In the future, the ideal would be to carry out a study at the Latin American level with Colombian, Chilean, Argentine and Brazilian populations, since they all have genetic components of ancestry. European, African and Indigenous in different proportions. This study is of great importance for public health and opens a great path for diverse investigations of clinical and genetic understanding for the management of tuberculosis infection depending on the population.

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SUPPLEMENTARY MATERIAL

Table 1: Oligonucleotides used for TYK2 PCR and sequencing.

Exon	Oligonucleotides name	Oligonucleotides sequence (5´-3´)
ex3	TYK2-1F	AGATGGGAGCCCAGAATCTT
	TYK2-1R	CAGCTGCTGCTTGCACAC
ex4-5	TYK2-2F	GTCTTTGGGGCTGACGGTAG
	TYK2-3R	AAATGGGGGCTCCTCAACT
ex6	TYK2-4F	TGTAGGGCCCAAGACACTTC
	TYK2-4R	GTCTACCCTGGCTCCCAGAT
ex7	TYK2-5F	ACCTGGCTAGTGTGCCTGTT
	TYK2-5R	TCAGAGGCTAGGGGTCAAGGA
ex8-9	TYK2-6F	GGGGAAAGTGCAGGAGGTAT
	TYK2-7R	CCCCTAGGGCTCACAGTCTA
ex10-12	TYK2-8F	GCTGTGTGTGTGCCCTCTAA
	TYK2-10R	GAATACCGCCATGGTGAAAG
ex13-14	TYK2-11F	CGGGTTGACCAGAAGGAGAT
	TYK2-12R	GAGGGTTGGGGTACAGATCA
ex15	TYK2-13F	CTGCATTCCGCATTTCTTCT
	TYK2-13R	AGGGCGAAACTCCACCTAAA
ex16-17	TYK2-14F	TCACATTGGCTGTCCCTATG
	TYK2-15R	AGAAGGGATGCAGCTTTGAG
ex18	TYK2-16F	CTCTGGGGACTTGACTCTGC
	TYK2-16R	GCTTATGAATGCCACTGCAA
ex19-20	TYK2-17F	AGAATCGGATCCTGGGGTAG
	TYK2-18R	CTCACCCAGATGCCAAGAAC
ex21	TYK2-19F	CTCTGCTGGGCTCAAGGTAG
	TYK2-19R	AACAGTTCGGAGGTCAGTGC
ex22	TYK2-20F	CTTTCTGGCTCAGCCTCCTT
	TYK2-20R	GGGTGATATGCTCATTGGCTA
ex23	TYK2-21F	AAGGGGCGGTGTATAGAGTG
	TYK2-21R	TCTCGATCTCCTGACCTCGT
ex24-25	TYK2-22F	TGGGATTACAGGCATGAGC
	TYK2-23R	ATCCCCCTCTTGGTTTCATC

Table 2: Description of the standardization for the PCR master mix

			$MgCl_2$ concentration		
	Stock	**Final concentration**	**1,5 mM**	**2 mM**	**2,5 mM**
(NH4)2SO4	10X	1X	4	4	4
dNTPs	10 mM	2 mM	4	4	4
Primer F	10 µM	0,2 µM	0,8	0,8	0,8
Primer R	10 µM	0,2 µM	0,8	0,8	0,8
MgCl2	25 mM	1,5 2 2,5	2,4	3,2	4
TaqPol	5U	1U	0,34	0,34	0,34
H2O			27,66	26,86	26,06
DNA			1	1	1
Final volume			40	40	40

Table 3: Description of the individual conditions for each of the amplified exons

EXON	ANNELING TEMPERATURE	$MgCl_2$ CONCENTRATION
3	61,8°C	2,5 mM
4 and 5	61,8°C	1,5 mM
6	60,1°C	2 mM
7	62,3°C	2,5 mM
8 and 9	61,8°C	2,5 mM
10, 11 and 12	58°C	2,5 mM
13 and 14	61,8°C	2,5 mM
15	60,3°C	2,5 mM
16 and 17	52,5°C	2,5 mM
18	61,2°C	2 mM
19 and 20	55°C	2 mM
21	69,8°C	2 mM
22	64°C	2 mM
23L	67,9°C	1,5 mM
23S	56,7°C	2,5 mM

Table 4: Example of the machine and samples for qPCR

Real time PCR Calculations and Concentrations								
PCR:	qPCR TYK2 P1104A							
Genes:	TYK2							
Final Volume per well (uL):	**15**							
Reactions and master mix	1						**105**	
Reagent	**Initial concentration**		**Final concentration**		**Volume**		**Volume**	
	Value	**Unit**	**Value**	**Unit**	**Value**	**Unit**	**Value**	**Unit**
PROBE TYK2	40	X	1	X	0.4	µL	39	µL
Master Mix Genotyping	2	X	1	X	7.5	µL	788	µL
gDNA	25	ng/uL	3.33	ng/uL	2.0	µL	210	µL
Water					5.1	µL	538	µL
Final volume					**15**	**µL**	**1575**	**µL**

Image form: Carlos Andres Arango

Figure 1: Description of the thermocycler conditions