Meta Gene 11 (2017) 178–180

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Differential genetic background of primary and secondary tuberculosis in Russians

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ARTICLE INFO

Article history:
Received 8 September 2016
Accepted 18 October 2016
Available online 25 October 2016

Keywords:
Tuberculosis
Genetic susceptibility
Stages of tuberculosis infection

ABSTRACT

Tuberculosis is a global healthcare challenge. Host genetic factors were proven to modify risk of the disease. Genome-wide association studies revealed a number of loci associated with TB in different populations. However, no systematic analysis of genetic bases of susceptibility to different clinical stages, such as primary TB and reactivation, was carried out. We set out to validate the results of GWASs in Russians of West Siberia with a consideration of primary and secondary TB. We chose 45 SNP from five large GWASs and genotyped 445 healthy individuals and 323 TB patients including 74 with primary TB and 249 with reactivation. We found that the rs7821565 and rs40363 SNPs were associated with primary TB in Russians ($p = 0.019$ and 1.4e − 3, respectively), while rs10515787 and rs2837857 were associated with secondary TB ($p = 1.2e-3$ and 0.039, respectively). The results suggest genetic basis of primary and secondary TB differs.

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1. Introduction

Tuberculosis (TB) remains one of the greatest social and healthcare challenges in the developing countries. The WHO estimates 9.6 mln people were affected by the disease in 2015 (World Health Organization, 2015). Despite all efforts of the national healthcare systems and the improvement of prophylactic measures, the TB mortality reaches 2 mln cases annually.

Though *Mycobacteria tuberculosis* infection is a prerequisite for the development of the disease, the only contact between the human and bacteria is not ample. The outcome of the infection is driven by environmental factors, the bacterial strain virulence, and the abundance of the infection as well as individual features of the host immune response. It is estimated that even though about 30% of the world population is infected by *M. tuberculosis*, only in 5%–15% active TB disease develops within two years after the infection either without or after a short latent phase (primary TB) (Ridruechai et al., 2010). Primary TB is common in children, characterised by an acute course and often associated with non-lung lesions due to haematogenous spread of mycobacteria (Cruz and Starke, 2007). In majority of the infected, latent infection develops without clinical and X-ray signs of the disease (Ernst, 2012).

In 90%–95% cases, latent infection never progresses into active phase; however, in the rest 5%–10% clinical disease develops later in life (secondary TB) (Abel et al., 2014). Secondary TB predominantly resides in the lungs, causes damage of lung tissues and is characterised by aerosol dissemination.

Genetic studies in human are carried out to reveal the causes of the high risk of TB in susceptible individuals. Several potentially important genes and loci have been identified using linkage analysis and case-control studies. During the last decade, genome-wide association studies (GWAS) became a standard tool in the analysis of genetic bases of complex human diseases including TB. A number of GWAS for TB was carried out and revealed several chromosomal regions associated with TB including 20q12 (Mahasirimongkol et al., 2012), 18q11.2 (Thye et al., 2010), 11p13 (Thye et al., 2012), 8q24 (Curtis et al., 2015), 3p13 and 7p21.1 (Grant et al., 2016). Importantly, in several cases genomic loci are associated with TB only in specific age groups (Nakauchi et al., 2016), thus suggesting differential susceptibility to TB in younger people as compared with aged patients for which other factors become more important, such as secondary infection and age-related immune system impairment (Alcaïs et al., 2010). This also may suggest differential genetic susceptibility to primary and secondary TB.

We set out to test this hypothesis in Russians of West Siberia via a comparison of the results of the validation of GWAS findings in other populations in primary and secondary TB.

2. Material and methods

2.1. The choice of genetic polymorphisms

The results of the GWAS data carried out for TB were assessed using the following online databases: A Catalogue of Published Genome-Wide Association Studies (http://www.genome.gov/gwastudies/); GWAS

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http://dx.doi.org/10.1016/j.mgene.2016.10.008
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Catalogue (http://www.ebi.ac.uk/gwas/); and GWAS Central (http://www.gwascentral.org/). Five large GWASs were selected for the validation in the current study (Thye et al., 2010; Thye et al., 2012; Curtis et al., 2015; Chimusa et al., 2014; Png et al., 2012). The most statistically significant single nucleotide polymorphisms (SNPs) from these studies were chosen. In case of multiple SNPs in a single chromosomal region, we chose only one. A total of 45 SNPs were chosen for the study (Table 1). All selected SNPs are located in non-coding regions: 20 SNPs in intergenic regions, 17 SNPs in introns, and 1 SNP in 3′UTR of protein-coding genes; 7 SNPs are located in introns of genes with unknown function or non-coding RNA.

2.2. The study group

Tuberculosis patients were recruited in Tomsk Phthisiopulmonology Medical Centre. Diagnosis of tuberculosis was based on the results of comprehensive clinical and laboratory assessment. HIV-positive patients were excluded. The final sample comprised 323 individuals (66.6% males and 33.4% females), mean age was 31.7 ± 16.9 years. The sample was divided into subgroups of primary TB (n = 74) secondary TB (n = 249) depending on the clinical features. Control group included 445 healthy individuals without history of TB (38.7% males, 61.4% females), mean age was 39.5 ± 17.0. All the participants were of Russian ethnicity and resided in the city of Tomsk.

The study was approved by Ethical Committee of the Research Institute of Medical Genetics. All participants or their parents (in case of children) signed informed consent.

2.3. Genotyping and association analysis

DNA was extracted from peripheral blood of the participants using standard phenol-chloroform approach.

Genotyping for the selected SNPs was carried out using MALDI-TOF mass-spectrometry, SNaPshot capillary electrophoresis, and high-resolution melt analysis. Genotyping Assay Design (http://bioscience.sequenom.com) was used to design primers for mass-spectrometry. Primers for SNaPshot were designed using Vector NTI software. Primers for high resolution melt analysis were designed using uDESIGN (https://www.dna.utah.edu/udesign/app.php).

Three multiplex sets were assembled for genotyping, two of which were genotyped using MALDI-TOF mass-spectrometry with MassARRAY Analyzer 4 (Seqeunom) and iPLEX GOLD (Agena Bioscience) kits according to manufacturer protocol. Genotypes call was carried out automatically by MassARRAY Typer 4 software. Another multiplex was genotyped using SNaPShot approach with Applied Biosystems 3730 Genetic Analyzer and SNaPshot® Multiplex Kit (Applied Biosystems) according to manufacturer protocol. Genotypes call was carried out using GenoMapper v.4.1 software. A confirmatory sequencing for selected cases was carried out using Applied Biosystems 3730 Genetic Analyzer with use of BigDye® Terminator v3.1Cycle Sequencing Kit (Applied Biosystems) and Sequencing Analysis v5.4 software.

High-resolution melt analysis was carried out for the rs6575836 SNP which could not be multiplexed along with other SNPs. For this analysis, Aria MX Real-Time PCR System (Agilent Technologies) and Brilliant HRM Ultra-fast loci master mix (Agilent Technologies) were used. Genotypes were revealed by Agilent Aria Mx v.1.0 software.

Association analysis was carried out using PLINK v1.90b3.31. The following filters were applied: Hardy-Weinberg equilibrium cut-off p-value 0.001 ($\alpha = 0.05$ for 45 SNPs); minor allele frequency cut off 3%; missing rate per SNP cut-off 95%; and missing rate per individual cut off 98%. Logistic regression was applied to test for significance of additive model with correction for sex and age. Adaptive permutations were applied to control for multiple testing.

Functional annotation of the SNPs was carried out using RegulomeDB database which allows assigning scores from 1 to 6 to a SNP according to experimental and theoretical data on the regulatory consequence of the genetic variant (Xie et al., 2013).

3. Results and discussion

The study aimed to validate the GWAS findings for TB in Russians of West Siberia stratified by primary and secondary TB. Accordingly, based on the results of five large GWASs we chose 45 SNPs and carried an association analysis between them and primary or secondary TB in Russian citizen of the city of Tomsk.

Using logistic regression and adaptive permutations, two SNPs were found to be associated with primary TB: rs7821565 (OR [95% CI] = 1.65 [1.07–2.6], p = 0.019) and rs40363 (OR = 1.93 [1.25–2.98], p = 1.4e−3). These SNPs were not associated with secondary TB.

The rs7821565 SNP is located on 8q11.22 chromosomal region between *CYCSR22* and *PXDNL* genes. Its functional significance remains unknown. This polymorphism was found to be associated with TB in GWAS carried out in Ghana and Gambia (OR = 1.20 [1.11–1.25], p = 8e−6) (Thye et al., 2010) followed by a replication in Chinese Tibetans (OR = 1.51 [1.12–2.05], p = 0.007) (Hu et al., 2016).

The rs40363 polymorphism is situated in the 6th intron of the *NAA60* gene on the 16p13.3 chromosomal region. In our study, the minor allele A was associated with the increased risk of primary TB, while in the GWAS study in South Africa, the allele appeared to be protective [OR = 0.09 [NR], p = 3.13e−6] (Chimusa et al., 2014). Functional importance of this SNP is also unknown, however, according to the RegulomeDB, the functional score for rs40363 is 1d suggesting high probability of the change of the affinity for transcription factors and, therefore, affected gene expression, due to the polymorphism.

Two other polymorphisms were associated with secondary TB: rs10515787 (OR = 3.61 [1.55–8.39], p = 1.2e−3) and rs2837857 (OR = 1.45 [1.01–2.09], p = 0.039). These SNPs were not associated with primary TB.

The rs10515787 SNP is located on 5q34 in the 8th intron of the *EBF1* gene encoding a B-lymphocyte transcription factor. In close vicinity to the gene, *IL12B* gene resides encoding key cytokine of anti-mycobacterial immunity thus suggesting the linkage disequilibrium between rs10515787 and functionally important variants of *IL12B*. The rs10515787 was found to be associated with TB in Indonesians and Russians, the A allele was reported protective against the disease (joint OR = 0.79 [0.68–0.91], p = 0.001) (Png et al., 2012). On the contrary, in our study, the allele is associated with the higher risk of TB. This situation requires further investigation.

The rs2837857 polymorphism is situated on chromosome 21.q22.2 in the 1st intron of the *DSCAM* gene encoding a member of immunoglobulin superfamily of cell adhesion molecules. The polymorphism was reported to be associated with TB in GWAS in South African coloured populations (Chimusa et al., 2014) and replicated in Chinese Tibetans (Hu et al., 2016). Worth noting, that in Africans, allele C was reported protective, while in the Tibetans an alternative allele T was protective against TB. In accordance with findings in Africans, in our study the rare allele T was associated with the increased risk of TB.

4. Conclusion

Replication of the results of GWAS in independent populations is an important task in the genetics of TB. As with many other complex diseases, population specific environment factors, gene-environment interactions, variable linkage disequilibrium structure and other phenomena can affect the results of the genetic studies of TB in different populations and ethnic groups.

Our study aimed to validate the results of GWASs of TB in Russians of West Siberia with a specific interest in primary and secondary TB. We analyzed 45 SNPs chosen from five large GWAS and showed an association of rs7821565 and rs40363 with primary TB and rs10515787 and rs2837857 with secondary TB. None of the SNPs was found to be

associated with both forms of the disease, suggesting different genetic background for predisposition to primary disease and reactivation.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2016.10.008.

Acknowledgments

The study was supported by Russian Science Foundation (grant # 15-15-00074).

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