

HOST GENETICS OF SUSCEPTIBILITY

Mutations in genes underlying atypical familial mycobacteriosis are not found in tuberculosis patients from Siberian populations



Alexey A. Rudko^{a,*}, Anna F. Garaeva^a, Elena Yu. Bragina^a, Nadezda P. Babushkina^a, Olga V. Kolokolova^b, Olga N. Lipaenkova^b, Valery P. Puzyrev^{a,b}, Maxim B. Freidin^{a,*}

^a Research Institute for Medical Genetics, Siberian Branch of Russian Academy of Medical Sciences, Tomsk, Russia

^b Siberian State Medical University, Tomsk, Russia

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SUMMARY

Objectives: Atypical familial mycobacteriosis (AFM, OMIM #209950) is caused by mutations in genes regulating IL12/IFNG pathway. Some of the mutations exhibit incomplete penetrance, and they have been proposed to be involved in the common (polygenic) predisposition to tuberculosis (TB). We set out to test this hypothesis in two populations from Siberian region of Russia with high prevalence of TB.

Material and methods: The prevalence of twelve mutations in IL12/IFNG pathway genes were analysed in 331 Russians and 238 Tuvinians TB patients and in 279 healthy Russians and 265 healthy Tuvinians. A screening for new mutations and rare polymorphisms was carried out in 10 children with severe generalized TB and severe BCG-vaccine complications using Sanger's bidirectional sequencing.

Results: Twelve mutations most commonly identified in AFM patients appeared to be "wild-type" monomorphic in the studied groups. No new mutations or rare polymorphisms were identified by sequencing. However, 15 common single nucleotide polymorphisms were found, none of which was associated with TB after correction for multiple testing.

Conclusion: The results of the study contradict with a hypothesis that mutations underlying AFM syndrome are involved in the predisposition to TB.

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1. Introduction

Tuberculosis (TB) remains one of the most common and dangerous infection diseases worldwide, accounting for more than 1.5 million deaths annually [1]. Host genetic factors are known to be important modifiers of the TB risk as approved by epidemiologic, twins, and molecular genetic studies [2,3]. In contemporary genetics, there are two main directions in the study of inherited determinants of TB risk: the analysis of common (polygenic) predisposition in endemic areas and the analysis of families with rare monogenic (Mendelian) forms of susceptibility to mycobacterial infection designated as "atypical familial mycobacteriosis" (AFM) or "Mendelian susceptibility to mycobacterial disease" in the OMIM database (MIM #209950) [4,5].

AFM is defined as a severe clinical disease, disseminated or localized, recurrent, caused by weakly virulent bacteria species, such as *Mycobacterium bovis* BCG, *M. marinum*, *M. smegmatis*, *M. avium*, *Salmonella enterica* and others in otherwise healthy individuals. The disease is a rare inherited syndrome, clinically described for the first time in 1951 as a disseminated disease caused by BCG vaccination [6]. It is now established that AFM is caused by mutations in 9 genes: *IL12B* (p40 subunit of IL-12), *IL12RB1* (β 1 subunit of the receptor to IL-12), *IFNGR1*, *IFNGR2* (first and second domains of the receptor to IFN- γ), *STAT1* (IFN- γ -associated signal transducer and activator of transcription), *ISG15* (interferon-induced protein 15), *IRF8* (interferon regulatory factor 8), *IKBKG* (*NEMO*; main modulator of NF- κ -B), and *CYBB* (cytochrome b(558), beta subunit) [7,8]. These mutations have common pathogenetic effect based of the impairment of the IFN- γ signalling, which is the main activator of the macrophages anti-mycobacteria defence [4].

This syndrome was described in more than 500 patients from 44 countries. The age of the patients with AFM is less than 14 years; most of them originate from non-endemic areas of countries and

* Corresponding authors. Research Institute for Medical Genetics, 10 Nabereznaya Ushaiki Street, Tomsk, 634050, Russia. Tel.: +7 3822 513146.

E-mail addresses: aleksey.rudko@medgenetics.ru (A.A. Rudko), mfreidin@medgenetics.ru (M.B. Freidin).

groups with high frequency of inbreeding [9,10]. Mutations in the *IL12RB1* and *IFNGR1* genes account for about 80% of all recorded cases of AFM syndrome. Most of the mutations are autosomal, except for X-linked defects in *IKBK*G and *CYBB*, and exhibit complete or incomplete penetrance.

Incomplete penetrance of some mutations allowed hypothesising that these genetic abnormalities can be distributed in populations with high frequency (>1%). This, in turn, makes it possible that the rare polymorphisms with major effect to the development of TB and low penetrance can account for susceptibility to the disease in common population and not only in separate families. The results of theoretical estimates of Mendelian susceptibility among children affected by disseminated TB showed that the frequency of AFM can vary between 3% and 45% [11]. This means that almost a half cases of children TB can be caused by Mendelian susceptibility. Also, these mutations can play a significant role in susceptibility to TB among adults. At least one study revealed *IL12RB1* loss-of-function homozygous mutations in two out of 50 children with severe TB, thus confirming that some of AFM causing mutations may be responsible for predisposition to TB [12]. To the best of our knowledge, no systematic screening for mutations in AFM genes was carried out in Russia. Therefore, we set out to perform such the screening of the AFM causing mutations in the *IL12B*, *IL12RB1*, *IFNGR1*, *IFNGR2*, *STAT1*, *IKBK*G gene in Tuvinians and Russians, ethnically divergent populations from Siberian region of Russia with high prevalence of TB.

2. Material and methods

The study protocol was approved by the Ethics Committee of the Research Institute for Medical Genetics of the Siberian Branch of Russian Academy of Medical Sciences. Signed informed consent was obtained from each participant or his/her parents (in case of children).

The study was carried out in three stages (Figure 1). First, we screened for known AFM-causing mutations in patients with most severe forms of primary TB. Then, we carried out a bidirectional Sanger's sequencing to seek novel mutations in exons and exon–intron junctions of the *IL12RB1*, *IFNGR1*, *IFNGR2*, *STAT1*, and *IKBK*G genes. Finally, we performed a case-control study for association between TB and polymorphisms revealed by sequencing.

Genotyping for known mutations and polymorphisms was done using restriction fragments length polymorphism assays (details are available on request). The screening for novel mutations and

rare polymorphisms possibly underlying AFM was done by bidirectional Sanger's sequencing of DNA samples of 10 children with generalized forms of TB and severe complications of BCG-vaccination using 3130xl Genetic Analyzer (Applied Biosystems, USA). The following exons were sequenced: *STAT1*, exons 3–25; *IL12RB1*, exons 1–18; *IL12B*, exons 2–7; *IFNGR1*, exons 1–7 (approximately 75% of exon 7 was covered); *IFNGR2*, exons 1–7; *IKBK*G, exon 2. The results of the sequencing were analysed using BioEdit [<http://bioedit.software.informer.com>] and Sequence Scanner v.1.0 [<http://www.appliedbiosystems.com/absite/us/en/home/support/software-community/free-ab-software.html>] software.

The case-control study was carried out using 331 DNA samples of Russians from Tomsk region (113 women, 218 men; mean age \pm S.D. of 31.7 ± 15.4) and 238 DNA samples of Tuvinians from Tuva Republic (116 women, 122 men; mean age of 33.5 ± 12.9) with the diagnoses of primary lung TB and severe secondary TB (Table 1). Control samples included 279 Russians (208 women, 71 men, mean age of 45.4 ± 21.7) and 265 Tuvinians (63 women, 202 men; mean age 33.1 ± 8.5) never suffered from TB.

A screening for new mutations and rare polymorphisms of AFM-genes was carried out in 10 children including 8 girls and 2 boys. The age ranged from 2 to 18 years with mean \pm S.D. of 5.68 ± 5.65 . Eight children were diagnosed with generalized TB further subdivided into lymphadenopathy complicated disseminated pulmonary TB ($n = 5$), TB meningitis ($n = 2$), and renal TB ($n = 1$). Also, two children were diagnosed with BCG osteitis.

All the study participants were unrelated to each other and HIV-negative.

Logistic regression models were fitted to analyse association between gene polymorphisms and TB using SNPAssoc package for R [13]. Codominant, dominant, recessive and log-additive genetic models were tested. Bonferroni correction was applied to control for multiple testing.

3. Results and discussion

The study was designed to test a hypothesis of an impact of rare mutations and polymorphisms causing AFM syndrome into common (polygenic) predisposition to TB in endemic populations.

On the first stage of the study, in sub-samples of 76 Russians and 38 Tuvinians with most severe forms of TB, we performed a screening for most common AFM causing mutations in the *IL12RB1*, *IFNGR1*, *IFNGR2*, and *STAT1* genes. Twelve mutations described at

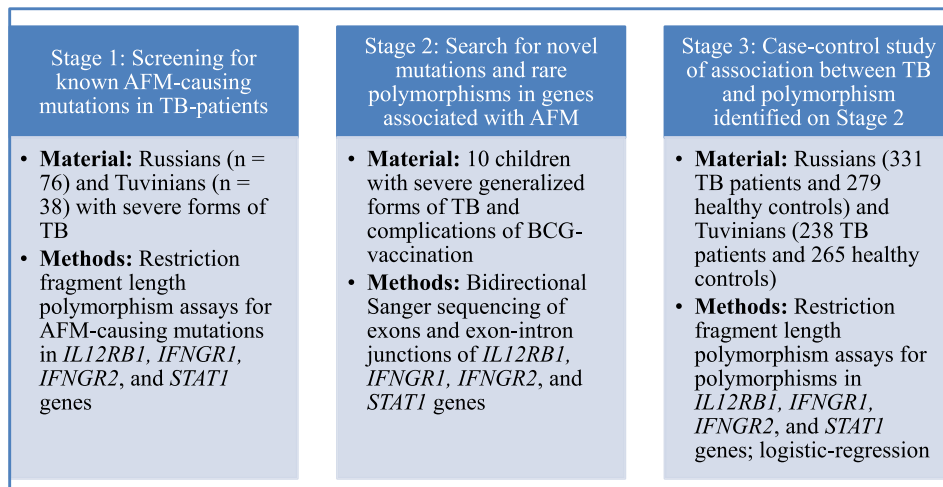


Figure 1. The study pipeline.

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