Tuberculosis xxx (2015) 1–4

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

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

ARTICLE INFO

Article history:
Received 23 September 2014
Received in revised form
6 January 2015
Accepted 8 January 2015

Keywords:
Tuberculosis
Common susceptibility
Mendelian susceptibility
Genetic polymorphism
Atypical mycobacteriosis

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 of 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.

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* (β1 subunit of the receptor to IL-12), *IFNGR1*, *IFNGR2* (first and second domains of the receptor to IFN-γ), *STAT1* (IFN-γ-associated signal transductor 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).

http://dx.doi.org/10.1016/j.tube.2015.01.002
1472-9792/

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 *IKBKG* 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, IKBKG* 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 *IKBKG* 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 BGC-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; *IKBKG*, 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 ±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 ± 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

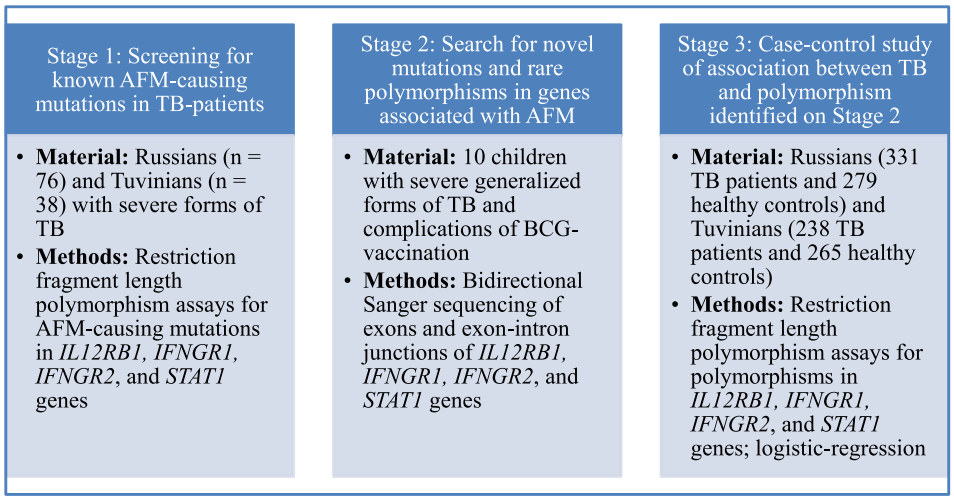

Figure 1. The study pipeline.

Please cite this article in press as: Rudko AA, et al., Mutations in genes underlying atypical familial mycobacteriosis are not found in tuberculosis patients from Siberian populations, Tuberculosis (2015), http://dx.doi.org/10.1016/j.tube.2015.01.002

Table 1
Clinical forms of tuberculosis (TB) in the studied groups.

Clinical forms of TB	Number of cases, (%)	
	Russians	Tuvinians
Infiltrative pulmonary TB	155 (46.8)	148 (62.2)
Fibrotic-cavernous pulmonary TB	4 (1.2)	38 (15.9)
Disseminated pulmonary TB	68 (20.5)	28 (11.8)
Focal pulmonary TB	28 (8.5)	9 (3.8)
TB of the intra thoracic lymph nodes	47 (14.2)	2 (0.8)
Primary tuberculosis complex	3 (0.9)	4 (1.7)
Generalized TB	8 (2.4)	1 (0.5)
Another rare forms*	18 (5.5)	8 (3.3)
Total	331	238

* Pulmonary tuberculoma, caseous pneumonia, miliary pulmonary TB, cirrhotic pulmonary TB, tuberculous pleurisy, TB of bronchus.

least in two independent cases were chosen for this purpose: *IL12RB1* Gln32Ter, Gln376Ter, Arg213Trp; *IFNGR1* Ile87Thr, 4-bp Del NT818, 1-bp Del NT818; *IFNGR2* 2-bp Del 278AG, Thr168Asn, 663Del27; *STAT1* Leu706Ser, Gln463His, Glu320Gln.

The results of the genotyping revealed no pathogenic variant alleles in the studied samples (only wild-type alleles were identified), thus rejecting an idea of the presence of these mutations in common population. However, this cannot exclude a possibility of the presence of other AFM causing mutations not tested in our study. This is consistent with the observation that genetic bases of AFM remain unknown in approximately 50% of cases [9]. This issue can be addressed by the systematic direct sequencing of genes underlying AFM in patients with rare forms of TB. Such study was carried out for *IL12RB1* gene and revealed two cases of homozygote loss-of function mutations (K305X and R173W) among 50 children with severe TB from Iran, Morocco and Turkey [12]. Another study claimed the discovery of autosomal-dominant IL12Rβ2 deficiency in individuals with disseminated TB; however, the detailed results were not provided [14].

Taking this into account, on the second stage, we performed bidirectional Sanger sequencing of the translating exons and exon–intron junctions of the studied genes in 10 children suffered from severe generalized forms of TB and complications of BCG-vaccination. No known or novel mutations causing AFM were found using this approach; however, 15 known single nucleotide polymorphisms (SNPs) were identified in different studied genes (Table 2). Seven discovered SNPs are located in introns, 3 SNPs in regulatory regions, 3 SNPs are synonymous, and 2 SNPs in the *IL12RB1* gene are missense-mutations.

Table 2
Polymorphisms in the genes causing atypical familiar mycobacteriosis found by bidirectional Sanger sequencing in children with generalized tuberculosis and BCG-vaccine complications.

Gene	Polymorphism	mRNA exchange	Protein exchange	Number of mutated alleles detected
IL12RB1	rs11086087	c.387G > A	p.Val129=	4
	rs11575934	c.641A > G	p.Gln214Arg	7
	rs17852635	c.684C > T	p.Pro228=	3
	rs401502	c.1132G > C	p.Gly378Arg	7
	rs12461312	c.1983 + 47G > T	–	5
	rs17882555	c.1983 + 24C > T	–	6
	rs3746190	c.*34C > T	–	5
IL12B	rs919766	c.483-36T > G	–	2
IFNGR1	rs2234711	c.-56T > C	–	6
	rs17181457	c.-72C > T	–	2
	rs7749390	c.85 + 10T > C	–	6
	rs11754268	c.85 + 45G > A	–	2
	rs11914	c.1050T > G	p.Ser350=	3
IFNGR2	rs17883129	c.879 + 19C > T	–	8
STAT1	rs2066797	c.1038-23A > G	–	1

On the third stage of the study, we tested SNPs found in 10 children with severe TB and BCG-vaccine complications for an association with TB in Russians and Tuvinians using all available DNA samples. To increase the power of the study 3 synonymous variants were excluded. Also we excluded rs919766 polymorphism of the *IL12B* gene as it was monomorphic in Tuvinians and too rare in Russians.

In control groups, the prevalence of the genotypes was concordant with Hardy-Weinberg expectations for all of the studied SNPs except for the rs2066797 polymorphism of the *STAT1* gene in Tuvinians ($p = 0.018$).

In Russians, a nominally statistically significant association between the rs2066797 polymorphism of the *STAT1* gene and TB was identified ($p = 0.007$ and 0.010 for dominant and log-additive models, respectively) (Supplementary Table). Common allele A and genotype A/A of this polymorphism appeared to be risk factors for TB in Russians (OR [95% CI] = 2.00 [1.16–3.45] and 2.13 [1.22–3.70], respectively). However, this association becomes non-significant after Bonferroni correction for multiple testing using a multiplier of 24 (3 genes × 2 populations × 4 genetic models). This multiplier was applied because of high linkage disequilibrium between all tested SNPs in *IL12RB1* and *IFNGR1* genes, so the number of independent tests equals to the number of tested genes, not the polymorphisms.

Overall, our study provides no evidence in support of the importance of AFM causing mutations, and tested genes, in predisposition to TB. However, the impact of mutations that we did not analyse and also mutations in other genes causing AFM syndrome cannot be excluded. Recently, three novel immune response genes (*IRF8*, *ISG15*, and *CYBB*) have been found to underlie the development of AFM [8,15–17]. We did not analyse them in our study as it was implemented before their importance for AFM was revealed. Potentially, their study by our or similar methodology can be fruitful. From the other hand, a number of genome-wide association studies carried out so far did not identify AFM causing genes as associated with TB [18–22], thus testifying against their impact on common predisposition to TB. Nevertheless, further analysis of associations between AFM genes variation and TB in different world populations may be required taking into account essential population specificity of genetic predisposition to the disease [23,24].

4. Conclusion

The results of our study do not support a hypothesis about the impact of rare mutations in IFNG/IL12 pathway genes on common susceptibility to TB in endemic populations.

Acknowledgements

The study was supported in part by the grants of Ministry of Education and Science of the Russian Federation N8042 and N8156.

Funding: None.

Conflict of interests: None to disclose.

Ethical approval: 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.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tube.2015.01.002.

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