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Rapid Assay for Detection of the Epidemiologically Important Central Asian/Russian Strain of the *Mycobacterium tuberculosis* Beijing Genotype

Igor Mokrousov,[a] Ekaterina Chernyaeva,[b] Anna Vyazovaya,[a] Yuriy Skiba,[c] Natalia Solovieva,[d] Violeta Valcheva,[e,f] Klavdia Levina,[g] Natalya Malakhova,[c] Wei-Wei Jiao,[h,i,j,k] Lia Lima Gomes,[l] Philip N. Suffys,[l] Marge Kütt,[g] Nagima Aitkhozhina,[c] A-Dong Shen,[h,i,j,k] Olga Narvskaya,[a,d] Viacheslav Zhuravlev[d]

[a]Laboratory of Molecular Epidemiology and Evolutionary Genetics, St. Petersburg Pasteur Institute, St. Petersburg, Russia
[b]Dobzhansky Center for Genome Bioinformatics, St. Petersburg State University, St. Petersburg, Russia
[c]Laboratory of the Genome, Aitkhozhin Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan
[d]St. Petersburg Research Institute of Phthisiopulmonology, St. Petersburg, Russia
[e]Department of Infectious Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[f]"In vitro" Laboratory for Evaluation of Biological Activity and Toxicity, Biopharmaceutical Laboratory Complex, Sofia Tech Park, Sofia, Bulgaria
[g]North Estonian Medical Centre Foundation, Tallinn, Estonia
[h]Beijing Key Laboratory of Pediatric Respiratory Infection Diseases, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China
[i]National Clinical Research Center for Respiratory Diseases, Beijing, China
[j]National Key Discipline of Pediatrics, Capital Medical University, Beijing, China
[k]Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing, China
[l]Fundação Oswaldo Cruz-Fiocruz, Laboratório de Biologia Molecular Aplicada a Micobactérias, Rio de Janeiro, RJ, Brazil

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Mycobacterium tuberculosis isolates of the Beijing 94-32 cluster (also named the Central Asian/Russian Beijing strain) constitute an important component of the population structure of the pathogen in the countries of the Former Soviet Union (1–4). A variable-number tandem-repeat (VNTR)-based analysis suggested that this genotype could speculatively trace its origins to the northwestern regions of China (5).

Beijing 94-32 is the largest type within the VNTR-defined CC1 group (6) and falls within the East Europe 1 group as defined by whole-genome sequencing (WGS) (7). Our analysis of all CC1 isolates compiled in the work of Merker et al. (6) demonstrated that type 94-32 presents the largest node in the central position in the phylogenetic network (see Fig. S1 in the supplemental material), and we therefore suggest naming this clonal complex the Beijing 94-32 cluster.

The 94-32 cluster isolates were associated with multidrug-resistant/extremely drug-resistant tuberculosis in Russia (8) and in Uzbekistan (termed the Central Asia outbreak strain [2]), and in immigrants in Western Europe (9, 10). This justifies the interest in having a simple tool to rapidly detect this clinically and epidemiologically relevant strain.

In this study, DNA of 19 Russian *M. tuberculosis* isolates of the Beijing genotype was subjected to WGS on the MiSeq platform (Illumina). The next-generation sequencing (NGS) data were deposited in the NCBI Sequence Read Archive (project number PRJNA305488). The fastq and vcf files were subjected to comprehensive bioinformatics

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Address correspondence to Igor Mokrousov, imokrousov@mail.ru.

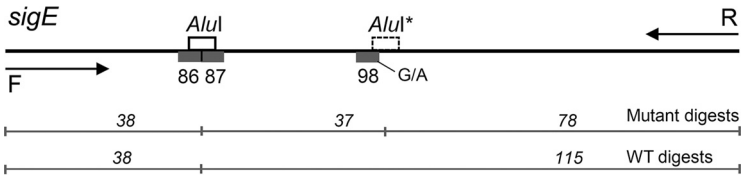

FIG 1 Schematic view of the *sigE* gene fragment targeted by the PCR-RFLP assay. Arrows show primers. The asterisk shows the AluI site created in the case of the codon 98 CTG→CTA mutation in a Beijing 94-32 cluster isolate. Gray boxes show particular codons. The permanent AluI site (codons 86 to 87) was observed in all genomes in the GMTV database and thus does not interfere with PCR-RFLP analysis of codon 98. F, forward; R, reverse; WT, wild type.

analysis (see the legend of Fig. S2 in the supplemental material for details). In total, 24 single-nucleotide polymorphisms (SNPs) specific of the Beijing 94-32 cluster were found. We looked more closely at these SNPs in order to find one that could be a synonymous mutation in a coding gene sequence and would be practically suitable for PCR-restriction fragment length polymorphism (RFLP) analysis with a common and inexpensive restriction enzyme. We chose a mutation in the *sigE* gene at codon 98, CTG→CTA (position 1364706, G→A in the genome of reference strain H37Rv [RefSeq accession number NC_000962.3]), as a candidate SNP for subsequent analysis and assay development. This mutation creates an additional AluI site (AGCT) and thus can be detected by this restriction endonuclease (Fig. 1). The PCR-RFLP profile consists of two bands in the case of the wild-type allele (38 plus 115 bp) and three bands in the case of the mutant allele (being visualized as single strong band of two fragments of 38 plus 37 bp and one band of 78 bp) (Fig. 2).

A portion of the *sigE* gene was amplified with primers SigF (5′-CCACCGGGGAC AAGGCCAC) and SigR (5′-GGACCGACCGGAACACCCTG) under the following conditions: initial denaturation at 95°C for 4 min; 35 cycles of 94°C for 20 s, 66°C for 20 s, and 72°C for 15 s; and a final elongation at 72°C for 4 min. The 153-bp PCR product was digested by AluI (Roche) according to the manufacturer's instructions, and the digests were separated in a short-run agarose gel (either 2% MetaPhor agarose [Cambrex Bio Science Rockland, Inc.] plus standard agarose [Quantum Biotechnologies]) at 1:1 or 1.8% standard agarose) and photographed in UV light (Fig. 2A and B, respectively).

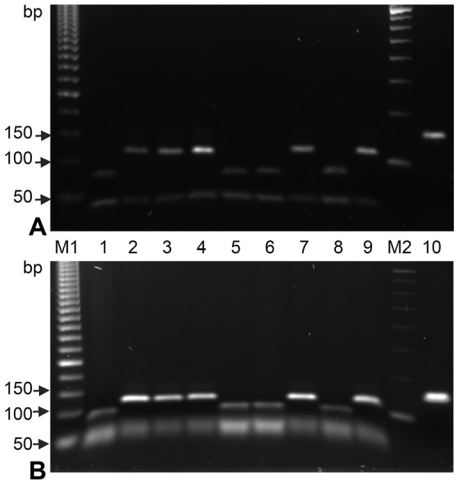

FIG 2 Gel electrophoresis of the amplified *sigE* fragment and the products of its digestion by AluI separated in a 2% high-resolution 1:1 MetaPhor agarose-standard agarose gel (A) and 1.8% standard agarose gel (B). Lanes: M1, 50-bp DNA ladder; M2, 100-bp DNA ladder; 1, 5, 6, and 8, Beijing 94-32 cluster isolates with a *sigE98* CTA mutant codon (37/38 plus 78 bp); 2 to 4, 7, and 9, other genotypes with a *sigE98* CTG wild-type codon (38 plus 115 bp); 10, undigested PCR product (153 bp).

The specificity of the *sigE98* CTG→CTA mutation was verified through a search of the GMTV database (http://mtb.dobzhanskycenter.org/) and analysis of the vcf files with the PhyTB tool (http://pathogenseq.lshtm.ac.uk/phytblive/index.php) (see the legend of Fig. S2 in the supplemental material for details).

Initial assessment and optimization of the PCR-RFLP assay were done with DNA samples of isolates with available NGS data. Experimental validation of the assay was performed with the *M. tuberculosis* DNA collections (342 isolates in total [Table S1]). DNA was extracted from bacterial cultures with a GenoLyse kit (Hain Lifescience) (Estonia collection), by the Qiagen protocol for DNA purification using the QIAamp spin procedure (Kazakhstan collection), or by the recommended cetyltrimethylammonium bromide (CTAB), phenol-chloroform extraction, and isopropanol precipitation-based procedure as described in reference 11 (all other collections). The Beijing collections came from distinct and partly contrasting settings: (i) Russia, where the 94-32 type is detected in half of the Beijing isolates, i.e., one-fourth of all circulating isolates (4, 8); (ii) Kazakhstan, where this type constitutes 70% of the population (1); (iii) China, where the Beijing family is dominant but type 94-32 is negligibly rare or absent (2, 5); and (iv) Brazil, where Beijing isolates have been imported recently from diverse sources and remain sporadic (12). Collections of the non-Beijing genotypes represented all major phylogenetic lineages of human *M. tuberculosis*. Our results demonstrate that this CTG→CTA *sigE*98 mutation is present only in the Beijing 94-32 cluster isolates.

The assay was performed successfully irrespective of the different protocols used for DNA extraction. In addition, we preliminarily tested the assay with cell lysates obtained by boiling/centrifugation of a small amount of wet bacterial mass. The PCR conditions were the same as those described above except for the number of PCR cycles, which was increased to 40 cycles, and dimethyl sulfoxide was added to the PCR mix, and good results were achieved in terms of both PCR yield and subsequent restriction endonuclease analysis. We are currently evaluating the assay directly on clinical samples as a part of a large-scale, long-term prospective study.

In conclusion, we developed an assay for rapid detection of isolates of the epidemiologically important Central Asian/Russian Beijing strain (94-32 cluster) that may serve for prospective screening and for retrospective assessment of large collections when comprehensive and time-consuming tools, such as multilocus VNTR and WGS, are not available or practical.

The test to detect Central Asian/Russian Beijing 94-32 cluster isolates developed presently may be used in parallel with a previously developed test to detect isolates of the Beijing B0/W148 cluster termed "successful Russian clone" (13) on strains identified as of the Beijing genotype. The combined use of these PCR-based assays will permit a rapid (1-day) detection of both the major and the epidemic clonal clusters of *M. tuberculosis* circulating in the countries of the Former Soviet Union and being spread by immigrants to different parts of the world.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01551-17.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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We declare that we have no conflicts of interest.

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