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NOTES

Molecular Characteristics of the *Mycobacterium tuberculosis* LAM-RUS Family Prevalent in Central Russia▽†

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We analyzed IS*6110*-associated polymorphisms in the phospholipase C genes of 107 isolates of *Mycobacterium tuberculosis* selected to be representative of isolates circulating in central Russia. We found that the majority of Latin American-Mediterranean family strains contained an insertion in a unique position in the *plcA* gene, suggesting a common ancestor. This insertion can serve as a specific genetic marker for this group, which we designate the LAM-RUS family.

Russia is a country with a high prevalence of tuberculosis (TB), with an estimated 119 new cases per population of 100,000 in 2005 (18). Although the incidence rate has begun to decrease slowly during the past few years, the number of TB-related deaths in Russia remains substantial. It is generally accepted that different *Mycobacterium tuberculosis* strains have distinctive epidemiological and clinical characteristics: virulence, clinical presentation, and behavior in animals appear to be strain dependent (7). Some *M. tuberculosis* strains are noted for their wide dissemination and acquisition of drug resistance (4). As a result, there has been increased attention paid to *M. tuberculosis* strain identification recently.

Phospholipase C proteins have been shown to be involved in *M. tuberculosis* virulence (9). Four genes, *plcA*, *plcB*, *plcC*, and *plcD*, encode phospholipase C proteins. It has been shown previously that *plc* genes in clinical isolates are often interrupted with IS*6110* insertion elements (15, 17). The *plcD* gene was previously suggested to be a hot spot for IS*6110* integration; insertions into *plcABC* are less common (15). In this study, we analyzed the frequency of IS*6110* insertions into the phospholipase C locus (*plcABC* genes) among clinical isolates of *M. tuberculosis* from central Russia.

A set of strains was selected from a collection of *M. tuberculosis* clinical isolates maintained at the State Research Center for Applied Microbiology and Biotechnology, Obolensk, Russia. The collection contains more than 1,200 isolates obtained during the period from 1998 to 2006 from patients in Moscow city and in the Moscow, Tula, and Kaluga regions. All strains with a unique spoligotype (accounting for 57 of a total of 72 spoligotypes) were included in the sample. Among the strains with shared spoligotypes, two to eight epidemiologically unlinked strains were chosen for analysis. The resulting panel of 107 strains should be representative of the diversity of *M. tuberculosis* strains circulating in the region.

IS*6110* insertions into the *plcABC* locus were detected by measuring the sizes of six overlapping PCR amplicons that cover the entire locus. Of the 107 isolates analyzed, 47 contained an IS*6110* insertion. In 45 of the 47 isolates, the IS*6110* element was inserted at the same position and in the same orientation within the *plcA* gene (insertion type 2 [Ins2]), as determined by sequencing. One of the remaining two isolates contained an insertion at a different position in *plcA* (Ins1), and the other contained an insertion in the *plcC* gene (Ins3) (Table 1).

The genetic relatedness of the isolates in the sample was assessed using five genotyping methods: IS*6110*-restriction fragment length polymorphism (16), spoligotyping (6), mycobacterial interspersed repetitive unit-variable-number tandem repeat (MIRU-VNTR) typing (14), and single-nucleotide polymorphism (SNP)-based identification of principal genetic groups (PGGs) (13) and SNP clustered groups (SCGs) (3). Genetic distance calculations and phylogenetic tree construction were performed using MathLab 6.5 statistics and bioinformatics toolboxes. The genetic characteristics of the strains are listed in Fig. S1 in the supplemental material.

The distribution of isolates with *plcABC*::IS*6110* insertions between the PGGs and SCGs was uneven (Table 1). Both of the isolates with IS*6110* Ins1 or Ins3 were members of SCG6/PGG3. All but two isolates from SCG5/PGG2 contained Ins2 in *plcA*.

A dendrogram of the 105 unique IS*6110* fingerprint patterns found in the 107 isolates was constructed (see Fig. S1 in the supplemental material). Ins2-containing strains showed highly similar IS*6110* fingerprint patterns and were grouped together on the dendrogram. An unrooted phylogenetic tree of 58 MIRU-VNTR genotypes was constructed using the neighbor-joining method. The tree is shown in Fig. 1, and the groups are colored according to their SCG/PGG genotype. The Ins2-containing isolates form a compact, discrete group on the dendro-

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TABLE 1. Prevalence of *plcABC*::IS*6110* genotype in different phylogenetic groups

Phylogenetic group	Total no. of isolates	No. of isolates with *plcABC*::IS*6110* genotype (insertion type)	Position of insertion (nt)[a]	Adjacent sequence[b]
PGG1/SCG2	10	0		
PGG2/SCG3	30	0		
PGG2/SCG5	47	45 (Ins2, *plcA*)	2630571	CGGGT**GTG-GTG**GTTTC
PGG3/SCG6	20	1 (Ins1, *plcA*)	2630703	GCAACG**GG-GG**TGGCTC
		1 (Ins3, *plcC*)	2628680	TTAG**CCAG-CCAG**GAAT

[a] Positions are numbered according to the *M. tuberculosis* H37*Rv* genome. nt, nucleotide.
[b] Duplicated nucleotides are shown in bold.

gram. In addition, the SCG5 isolates with an uninterrupted *plcA* gene showed a high degree of similarity to some of the SCG6 strains.

The small genetic distance across the Ins2-containing group and the presence of an IS*6110* element at a unique site of integration suggest that the strains are derived from a common progenitor. The combination of IS*6110*-restriction fragment length polymorphism, spoligotyping, and SCG/PGG genotyping methods allowed the recognition of this group as being related to the Latin American-Mediterranean (LAM) family (1). The *M. tuberculosis* LAM family was initially described as a clade on a neighbor-joining tree of spoligotypes (12). However, as data accumulated, the genetic heterogeneity of this family became evident and suggested the presence of a number of distinct LAM groups in various geographical regions (2, 8, 10). To reflect the high prevalence of this type of strain in central Russia as well as its epidemiological importance, we propose naming the group of Ins2-containing strains the LAM-RUS family and using Ins2 as a specific genetic marker for the group.

To identify members of the LAM-RUS family, a multiplex PCR assay was developed. In brief, two pairs of primers were used in the PCR: U (universal *plcA*-specific forward primer, 5′GAAGTTGATTCGCGCCCGGTT) and R (universal *plcA*-specific reverse primer, 5′GCTGGGAGTCCCGCGGACG) and H (hybrid forward primer specific to the IS*6110* Ins2 site in the *plcA* gene, 5′CCAACTCAGGAAACCA**CAC**TGAACC [boldface indicates the site of insertion]) and I (reverse IS*6110*-specific primer, 5′CTCCGAATCGTGCTGACCGC). The amplification reaction was performed in a total volume of 30 μl containing ~1 ng of DNA template, 200 μM (each) deoxynucleoside triphosphates, 0.3 μM (each) primers, and 2 U of *Taq* DNA polymerase (Fermentas, Lithuania) in the buffer recommended by the manufacturer. LAM-RUS strains produced two amplicons (163 and 410 bp), while strains without an insertion produced a product of 268 bp. Strains with other insertions and deletions in the analyzed region produced PCR products of various lengths.

Although further study is needed to determine the actual prevalence of the LAM-RUS family, the high degree of clustering of these strains, the high frequency of multidrug resistance in this family, and the increased risk of outbreaks associated with these strains have already been reported. About 45 and 30% of *M. tuberculosis* isolates collected in prison hospitals in Tula and Moscow regions, respectively, were members of the newly defined LAM-RUS family (5, 11). More than 80% of

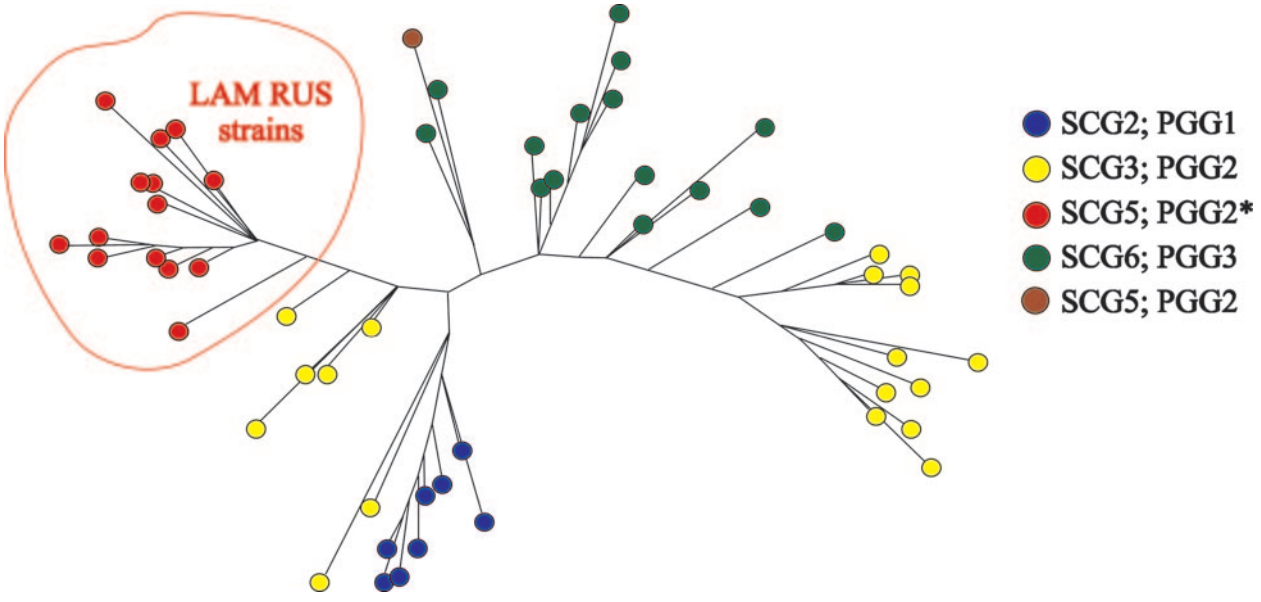

FIG. 1. Distribution of Ins2-containing strains on a phylogenetic tree based on their MIRU-VNTR patterns. PGGs and SCGs are color coded. LAM-RUS strains are circled. *, strains containing IS*6110* Ins2.

these isolates were multidrug resistant, and 68% of them were resistant to four or five of the drugs tested (rifampin, isoniazid, streptomycin, and ethambutol or kanamycin). Wide dissemination of these strains in two remote penitentiaries implies that we may face an outbreak of extensively drug-resistant TB in the region before long.

Molecular methods are gradually establishing their role in studies of TB epidemiology. The availability of convenient strain- and lineage-specific PCR markers simplifies strain classification, speeds up the identification of clinically relevant strains, and may give a hint about the nature of the disease before microbiological and drug resistance tests can be completed. Epidemiological studies benefit from efficient tools for unambiguous strain differentiation and tracking. We believe that the description of the specific genetic characteristics of the LAM-RUS *M. tuberculosis* family will contribute to the TB control programs in the region and worldwide.

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