St. Petersburg Pasteur Institute, Russia
Laboratory of Molecular Epidemiology and Evolutionary Genetics

Activity report, 2017-2018: Tuberculosis and other mycobacteria

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PROJECTS AND COLLABORATIONS

Funded Projects

– Russian Science Foundation project #14-14-00292 «Evolution of pathogenetic potential of phylogenetic lineages of Mycobacterium tuberculosis», 2014-2018 (PI – I. Mokrousov).
– Russian Foundation for Basic Research project №17-54-30020 «A personalized approach to fight the HIV and drug resistant TB epidemic in Irkutsk, Siberia» (project PI – O. Ogarkov, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia), 2017-2019.
- Russian Foundation for Basic Research project 17-04-00367 "Population of Mycobacterium tuberculosis in the Western Siberia region: current molecular epidemiology in the context of macroevolutionary reconstruction" (PI – I. Mokrousov), 2017-2018.
- Russian Foundation for Basic Research project 18-04-01035 "Investigation of the role of the repeat element IS6110 in the micro- and macroevolution of Mycobacterium tuberculosis phylogenetic lineage 2" (PI - E. Shitikov, Center of Physico-Chemical Medicine, Moscow), 2018-2019
- Russian Foundation for Basic Research, Project #19-04-00263 "Pathogenomic features and epidemic potential of highly resistant strains of ancient sublineage of Mycobacterium tuberculosis Beijing genotype" (PI – I. Mokrousov), 2019-2020

International collaborations

National Institute for Public Health and the Environment, RIVM (2018-2021), Aitkhozhin Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan (2015-2017), Kobe Institute of Health, Japan (2016-2018), Beijing Children's Hospital, China (2017-2021), Hospital General Universitario Gregorio Marañón (Madrid Spain), North Estonian Medical Centre (Tallinn Estonia), Biomedical Research and Study Centre, University of Riga (Latvia).

National collaborations

Central Research Institute for Epidemiology (Moscow), Omsk State Medical University, Scientific Center of Family Health and Reproductive Problems (Irkutsk), Ural Research Institute of Phthisiopulmonology (Ekaterinburg), Anti-tuberculosis dispensaries in Kaliningrad, Petrozavodsk (Karelia), Syktyvkar (Komi), Murmansk, Pskov.

MAJOR RESEARCH RESULTS

Analysis of whole-genome data for phylogenetic analysis and search of molecular markers of significant genovariants of M. tuberculosis

Mycobacterium tuberculosis Beijing 94-32-cluster dominates in Kazakhstan and Central Asia, and is one of two main subtypes of *M. tuberculosis* in Russia, often associated with MDR / XDR and marked by global distribution due to migration flows. As a result of bioinformatics genomic analysis, we identified a single nucleotide polymorphism in the *sigE* gene, specific for Beijing 94-32 cluster strains, and developed a method for its detection in the PCR-RFLP and real-time PCR formats. The methods were validated in silico

(by analyzing the GMTV database) and experimentally, on the global collection of strains of different genotypes (342 isolates from Russia, Kazakhstan, China, Vietnam, Bulgaria, Estonia, Brazil). The developed methods for detecting the Beijing 94-32-cluster genotype can be used (simultaneously with the method of detecting the other significant Russian epidemic strain Beijing B0/W148) in prospective studies or for retrospective evaluation of historical DNA collections .

Mycobacterium tuberculosis population in region of West Siberia: current molecular epidemiology in the context of macroevolutionary reconstruction

New data were obtained on the trends in the prevalence of multi- and extensive-drug resistant (MDR / XDR) M. tuberculosis population in the Omsk region of Western Siberia. Molecular analysis of 425 M. tuberculosis strains isolated from patients with pulmonary tuberculosis in different years in the Omsk region revealed a diversity of the structure of genotypes and the prevalence of strains of the Beijing genotype in different time points (~ 60%) and among TB/HIV-negative and co-infected patients. 80.2% of cases with an unfavorable outcome of the disease (lethal cases) were infected with strains of the Beijing genotype. At the same time, the proportion of Beijing B0/W148 cluster increased 1.4 times (19.2% in 2017 against 13.5% in 2015-2016) and these strains were associated with MDR. The younger age of patients infected with Beijing genotype strains indicates their active circulation in the population. In general, three families make a significant contribution to the spread of drug resistant strains - Beijing, LAM (Latin American Mediterranean) and Ural.

For the first time, two clusters of the early ancient sublineage of the Beijing genotype were identified as emerging and potentially epidemic subtypes (**FIG. 1**). Almost all ancient Beijing strains were MDR, and half of them were XDR/pre-XDR. A comparison with the globally available data has shown that these two clones mainly circulate in the Asian part of Russia and demonstrate a certain phylogenetic affinity with strains from Japan, Korea and northeastern China. Based on phylogenetic, phylogeographic, and historical data, we put forward a hypothesis that these two clones of the early ancient sublineage of the Beijing genotype were probably brought to Russia ~ 70 years ago after World War II with Japanese prisoners of war and until recently, mostly circulated in Siberia and the Far East. Their relatively higher prevalence in Omsk, along with an extremely strong association with not only MDR, but also pre-XDR/XDR, also observed in other regions of the Russian Federation, demonstrates their epidemic potential and requires permanent monitoring.

Clonal complexes of Mycobacterium tuberculosis Beijing genotype in the North-West Russian regions, bordering the countries of the European Union

The high prevalence of the multidrug-resistant tuberculosis in north-west Russia may have a negative impact on tuberculosis control programs not only in Russia but in neighboring the countries of the European Union (EU). 304 isolates of M. tuberculosis (2013-2017) isolated from newly diagnosed tuberculosis patients living in northwestern regions of Russia (Pskov, Kaliningrad and Murmansk regions, the Republic of Karelia) were studied (**FIG. 2**). The prevalence of M. tuberculosis strains of the Beijing genotype differed and amounted to 44.9% in the Pskov region, 55.1% in Karelia, 48.4% in the Murmansk region, 63% in the Kaliningrad region. B0/W148 cluster was detected in 6.7%, 17.9%, 9.4%, and 19.2% M. tuberculosis strains, respectively. The prevalence of the Beijing 94-32-cluster in the four studied territories did not differ: 29.2%, 28.2%, 31.3% and 28.8%, respectively. In the Pskov and Murmansk regions, 62.9% and 60.9% of M. tuberculosis strains were susceptible to anti-TB drugs, and 17.9% and 26.6% of strains were MDR, respectively. MDR strains prevailed in Karelia and the Kaliningrad region - 51.3% and 43.8%; while 41.0% and 36.9% strains, respectively, were susceptible. In total, in four regions, 90.0% of M. tuberculosis Beijing B0/W148 isolates were MDR. The Beijing 94-32 strains had similar proportions of drug sensitive (41.6%) and MDR (34.8%) isolates. Thus, in the territories of the North-West of Russia, bordering the EU countries, the proportion of Beijing strains in the structure of M. tuberculosis genotypes varied from 44.9% to 63.0%. The Beijing strains of cluster 94-32 (28.2% - 31.3%) and cluster B0/W148 (6.7% - 19.2%)

prevailed. However, these M. tuberculosis clones differed significantly in the proportion of MDR. The circulation of MDR strains of the M. tuberculosis Beijing B0/W148 cluster poses a serious problem for local and national tuberculosis control programs.

Dynamics of MDR-TB in Estonia: key role of immigration and National TB control program.
We assessed the genetic structure of the M. tuberculosis population in Estonia with particular reference to the main epidemic/endemic clones and determinants of drug resistance. 39.8% of isolates are attributed to the Beijing genotype; 56.8% of them were MDR. In contrast, all three major other genotypes (LAM, Haarlem, Ural) were mainly drug sensitive. MDR was more common among Beijing B0/W148 isolates (81.8%) compared to other Beijing isolates (20.0%, P = 0.0007). The Pre-XDR phenotype was found in 8 isolates, of which 6 belonged to Beijing B0/W148. All resistant to rifampicin and ofloxacin and 97% of isoniazid-resistant isolates had mutations in rpoB, gyrA, katG. The most common mutations were rpoB S531L, katG S315T and embB M306V. The main pool of isolates of the Beijing genotype was brought to Estonia in 1945-1990 due to massive human immigration from USSR. However, the active circulation of the hazardous MDR-related cluster Beijing B0/W148 started only in the last 20 years, and represents a serious threat to the fight against TB in Estonia.

Kazakhstan: the influence of Russia and China on the structure of the M. tuberculosis population.
Kazakhstan is characterized by the dominance of strains of Central-Asian / Russian Beijing 94-32 cluster (probably originating in northwestern China) and the rarity of strains of the LAM family (which until recently almost completely belonged to the Russian branch of LAM-RUS). In our study, we identified the first genetically confirmed strain of the LAM RD-Rio sublineage in Kazakhstan. Molecular analysis and comparison with proprietary LAM global database showed that it belongs to a phylogenetic branch endemic to the north of European Russia/Eastern Europe. Contrary to the popular (and justified) opinion about the association of RD-Rio strains with MDR, this branch included only drug-sensitive strains. We hypothesize that this strain was brought to southern Kazakhstan not from the primary Ibero-American region of origin of RD-Rio, but from the secondary circulation zone (Russia) (**FIG. 3**).

Evolution and origin of significant Russian subtypes Beijing 94-32 and Beijing B0-W148/100-32 in the light of the Beijing genotype evolution in China
The study included 369 isolates that represented different provinces in all geographic parts of China. All isolates were assigned to the Beijing genotype, according to spoligotyping, and all had a deletion of RD105. All isolates were additionally analyzed by: (i) markers of large sublineages - mutations in the mutT2, mutT4 and deletion RD181 and (ii) in 24-VNTR loci. Geographically closest to China are two Eurasian epidemic strains defined by the 24-MIRU-VNTR loci: the Russian type 100-32 and the “Central Asian-Russian” type 94-32. Their profiles differ in loci MIRU26 and QUB26 (7 and 7 repeats for 100-32 and 5 and 8 repeats for 94-3217). When these profiles were placed in the Chinese tree of the Beijing genotype, they were both associated with the central and “modern” part of the network. Profile 24-MIRU-VNTR type 100-32 (B0/W148) is a single-locus variation relative to strains from Shanxi and Jilin provinces in our study. Both of these provinces represent northeast China, which has historical ties with the Russian Empire/USSR. The "Central Asian-Russian" type of Beijing 94-32 is widely distributed in the former Soviet Central Asia and is one of the two largest and most significant subtypes of Beijing in Russia. This profile is separated by a single-focus polymorphism from a strain from Qinghai province, a neighboring province with Xinjiang Uygur district and Central Asia as a whole. The revealed phylogeographic patterns indicate that large-scale (but not medium/small-scale) migration remains one of the decisive factors in the genetic divergence of M. tuberculosis populations in China. Analysis of the diversity and topology of local collection networks seems to confirm the recent hypothesis about the South China origin of the Beijing genotype. A review of the results obtained in the Eurasian context suggests that two significant Russian epidemic clones of the Beijing genotype could have their origin in the northeast and northwest regions of China, respectively.

Emerging resistant clone of M. tuberculosis in Western Asia

M. tuberculosis strains of the genotype NEW-1 / SIT127, are endemic for Western Asia (Iran, Afghanistan), are found in Central Asia and sporadically in Russia. Phylogenetic analysis of the Lineage 4 isolates confirmed the separate position of the NEW-1 family, which we provisionally designated L4.5.1/Iran. The hypothesis of the evolutionary migration scenario was developed: origin of L4.5 1000-1300 years in China, subsequent emergence of the intermediate genotype pre-NEW-1 in Tibet, further migration to Xinjiang and, finally, to Iran 800 years ago (emergence of NEW-1/SIT127) (**FIG. 4**). After this, SIT127 slowly spread eastward from Iran, possibly as a result of a continuum of Iranian languages, trade exchange and interaction in the Islamic world. Data analysis for the last 20-25 years has shown a sharp increase in the prevalence of NEW-1 strains in Iran, Afghanistan and Pakistan, moreover, accompanied by a significant association with multidrug resistance (**FIG. 5**). Migrations of the population, especially the flows of Afghan refugees, can lead to a wider spread of the resistant subtype NEW-1, which we named an emerging resistant clone of M. tuberculosis in Western Asia.

Conclusions on the role of migrations in the spread of epidemic strains of Mycobacterium tuberculosis.

1. The usual exchange (sporadic contacts, tourism) is insufficient to bring and settle the imported strain in a new population. 2. A new emerging strain becomes epidemic in the region of its origin, where the ancestral strain have been circulating. 3. Massive immigration of the population is a critical factor leading to a change in the local population of the tuberculosis pathogen. 4. The imported strain must be not only epidemic, but also sufficiently prevalent in its country of origin.

Contagiosity, virulence of an *M. tuberculosis* strain is conditional, not absolute. Speculatively, a kind of human resistance is developed in local population through its co-existence with historical local clones, and acting against imported clones: hence role of host genetics.

Even within "uninteresting" *M. tuberculosis* families, such as NEW-1/Iran and Ural, more hazardous clones may emerge. The notorious Beijing and LAM genotypes are not an exception. We do not know where new emerging *M. tuberculosis* strain will emerge. Once it emerges, it is advised to look at their genomics and phenotype and no less closely at relevant migration flows, to predict its spread.

Reasons of success of different emerging clones may be different in each particular case and be related to strain properties.

Single nucleotide polymorphisms in hsp65 and MACPPE12 genes of Mycobacterium avium subsp. hominissuis

Mycobacterium avium subsp. *hominissuis* (*MAH*) is the typical inhabitants of the environment, which are known as opportunistic pathogens of animals and humans. The aim of our study was to analyze single-nucleotide polymorphisms (SNPs) in the *hsp65* and MACPPE12 genes to characterize the Russian population of *MAH* in the context of studying phylogenetic relationships and the evolution of geographically distant populations of *M. avium* subsp. *hominissuis*. The sequence analysis of the *hsp65* and MACPPE12 genes was applied for 40 *MAH* strains isolated from humans (patients with mycobacteriosis) (**FIG. 6**). The nucleotide sequences were aligned to the reference genome of *M. avium* subsp. *hominissuis* 104 (accession no. NC_008595.1.). The mutational profiles of Russian strains were compared with those isolated in other countries. In total, the 40 *MAH* strains were classified into three different *hsp65* sequevars: code 1, code 2 and code 3. The majority of *MAH* strains (72.5%) belonged to code 1, the same sequevar as for *MAH* strain 104. The sequence analysis of the MACPPE12 gene revealed 20 SNPs grouped into nine sequevars at the nucleic acid level: NA01, NA02, NA03, NA06, NA10, NA13, NA14, NA19, and NA_Rus01. Among 20 SNPs eight were nonsynonymous resulting in seven sequevars at the amino acid level: AA01, AA02, AA04, AA07, AA08, AA13, and AA_Rus01. The sequevar AA02 consisted of three different NA variants with synonymous SNPs profiles: NA02, NA03, and NA06. Half of the *MAH* strains

belonged to the sequevar AA02 (type NA02). Presently, the predominant cluster AA02 (type NA02) / code 1 and the unique variant AA_Rus01 (NA_Rus01) were identified among *MAH* strains from Russia. Thus, we confirmed the relative conservativeness of the nucleotide sequence of the *hsp65* gene but the polymorphism of the MACPPE12 gene. At the same time, a comparative analysis of the SNPs profiles of the *hsp65* and MACPPE12 genes allowed to identify differences and similarities between geographically distant populations of *MAH*, which highlighted the variability of the global population of *M. avium* species.

PUBLICATIONS IN PEER-REVIEWED JOURNALS:

IN ENGLISH. 18 articles **(**12 in Q1); cumulative IF = 92.453; mean IF = 5.136

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Jagielski T; Partnership to Fight Against TB in Central and Eastern Europe (Aleksa A, Bachiyska E, Bakos Á, Crudu V, Dziadek J, Homolka J, Homorodean D, Jansone I, Katalinic-Jankovic V, Krenke R, Kuzmic U, **Mokrousov I**, Nikolayevskyy V, Nikolenka A, Osmani GM, Papaventsis D, Pole I, Porvazník I, Savic B, Shubladze N, Solovic I, Szabó N, Tafaj S, Ustamujic A, van Ingen J, Vasiliauskiene E, Yablonsky PK, Zemanova I, Zhuravlev V, Žolnir-Dovc M.). FATE: the new partnership to Fight Against TB in Central and Eastern Europe. Lancet Infect Dis. 2017 Apr;17(4):363. doi: 10.1016/S1473-3099(17)30120-2. Impact factor 19.864. Q1.

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IN RUSSIAN: 1 book, 4 articles

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PATENTS

Patent (Mokrousov I., Vyazovaya A., Zhuravlev V., Solovieva N., Vishnevsky B., Narvskaya O., Method of detection of *Mycobacterium tuberculosis* Beijing genotype B0-cluster by real-time PCR). Priority: №2017123302 of 30.06.2017. Approved on 09.01.2019.

Patent pending (Mokrousov I., Vyazovaya A., Chernyaeva E., Solovieva N., Narvskaya O., Zhuravlev V., Method of detection of *Mycobacterium tuberculosis* Beijing genotype 94-32-cluster by real-time PCR). Priority: №2017142885 of 11.12.2017.

Patent pending (Mokrousov I., Vyazovaya A., Solovieva N., Mushkin A.Y., Vishnevsky B.I., Narvskaya O., Zhuravlev V., Method of detection of *Mycobacterium bovis* BCG strains by real-time PCR). Priority: №2018120630 of 06.06.2018.

SCIENTIFIFC MEETINGS AND CONFERERNCES

2nd St. Petersburg Symposium on Tuberculosis and Mycobacteria: Molecular Approach, St. Petersburg, Russia, 5-6 December 2018 (Symposium chairmen, oral talk – I. Mokrousov; posters – D. Starkova, A. Gerasimova).

14 Conference of molecular epidemiology and evolutionary genetics of infectious diseases (MEEGID XIV), Sitges, Spain, 6-9 November 2018 (I. Mokrousov - invited speaker).

Institut Pasteur International Network Symposium 2018 «Combating resistance: microbes & vectors», Paris 15-16.11.2018 (I. Mokrousov - oral and poster presentations).

International scientific and practical conference Molecular Diagnostics 2018, Minsk, Belarus, 27-28.09.2018. (A. Vyazovaya - oral presentation).

39th Annual Congress of European Society of Mycobacteriology, Dresden, Germany, July 1-4, 2018 (I. Mokrousov - oral and poster presentations; A. Vyazovaya – poster).

All-Russian scientific and practical conference of phthisiatricians with international participation "Topical issues of TB care in the Russian Federation: consolidation of efforts in the fight against tuberculosis". 31.05.2018, Moscow. (I. Mokrousov - oral presentation).

Seminar at School for Life Science at Nazarbaev University, Astana, Kazakhstan, 3rd May 2018 (I. Mokrousov – invited lecture).

International Conference "New Approaches to the Elimination of the Epidemic of Tuberculosis", dedicated to the 85th anniversary of the National Research Center for Phthisiopulmonology of the Ministry of Health of the Republic of Kazakhstan, Almaty, 26-27.04.2018. (I. Mokrousov - oral talk).

10th Balkan Congress of Microbiology / Microbiologia Balkanica'2017, София, Болгария, 16-18.11.2017 (I. Mokrousov – invited speaker).

2nd International conference of human migration "What can Genomic Diversity studies tell us about migration?" Mexico-City, Mexico, 17-21.10.2017 (I. Mokrousov – invited speaker).

7th Congress of European Microbiologists (FEMS 2017), Valencia, Spain, 9-13.07.2017. Poster.

27th ECCMID, 22-25 April 2017, Vienna, Austria. (I. Mokrousov - symposium co-chair).

IX Congress on molecular diagnostics, Moscow, 18-20.04.2017 (I. Mokrousov - oral talk, session co-chair)

Scientific Session dedicated to the 70-th Anniversary of the Institute of Microbiology, Bulgarian Academy of Sciences. Sofia, Bulgaria, 14-15.03.2017. (I. Mokrousov – invited speaker).

2nd Asian African Congress of Mycobacteriology, Isfahan, Iran 25-28.02.2017 (I. Mokrousov - invited speaker, session co-chair).

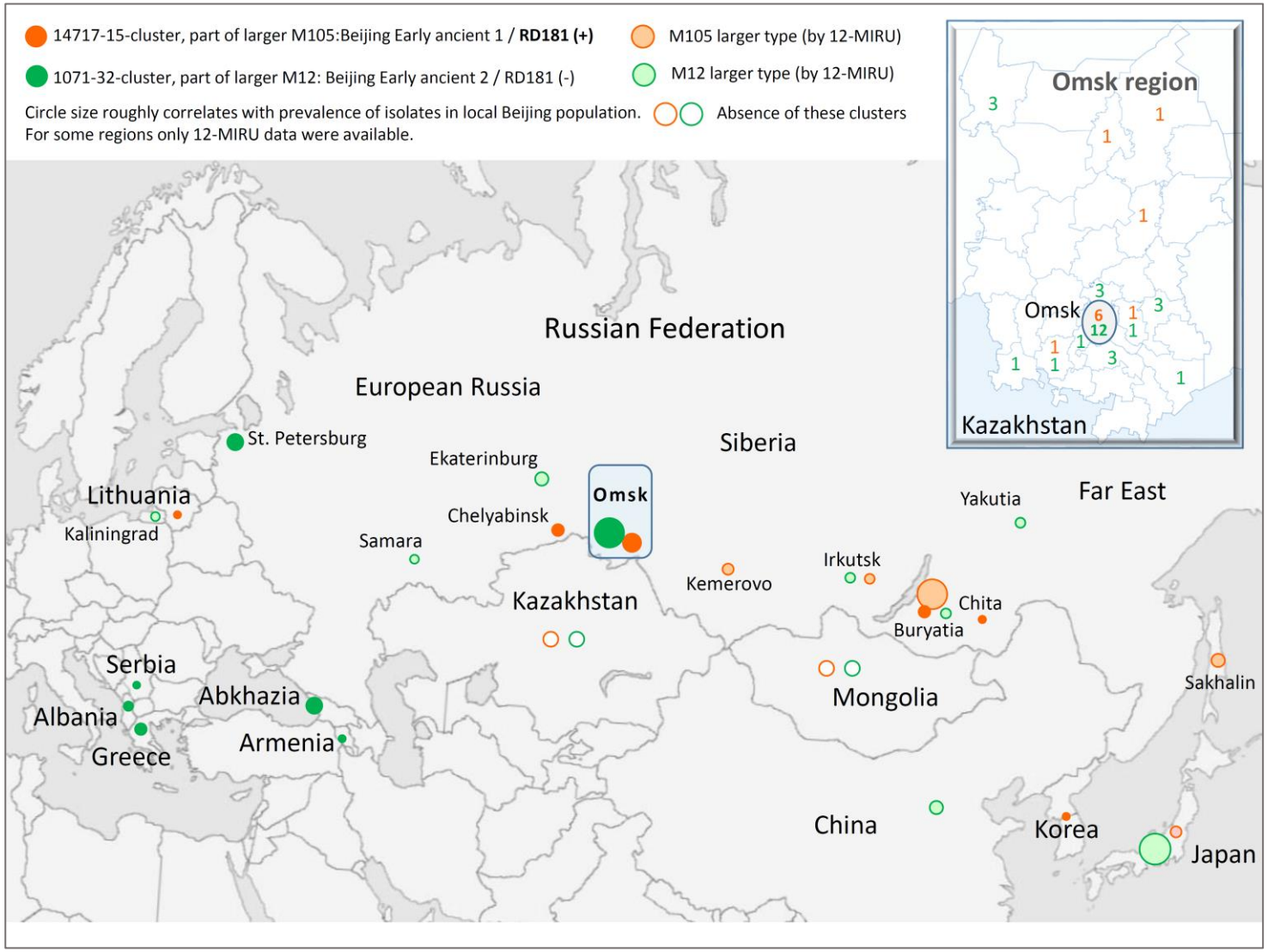

Figure 1. Geographical distribution of the *M. tuberculosis* strains of ancient Beijing subtypes in different locations in northern Eurasia and different districts of the Omsk region.

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Figure 2. Distribution of *M. tuberculosis* Beijing genotype subtypes in northwestern Russia.

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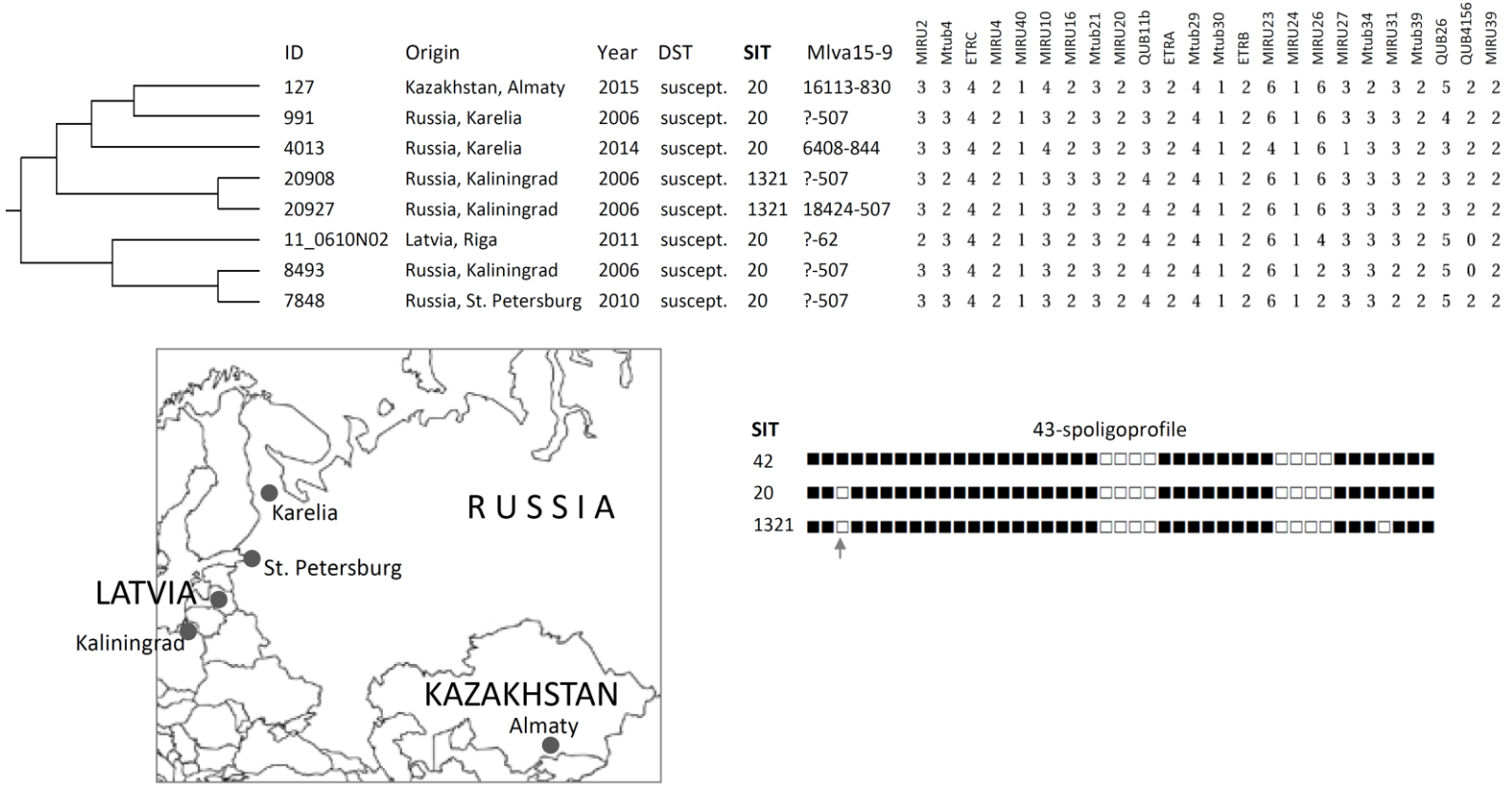

Figure 3. The part of the MIRU-VNTR-based dendrogram of the *M. tuberculosis* LAM family with enlarged branch including SIT20 strain from Kazakhstan, locations of isolation of the studied strains and their binary spoligoprofiles.

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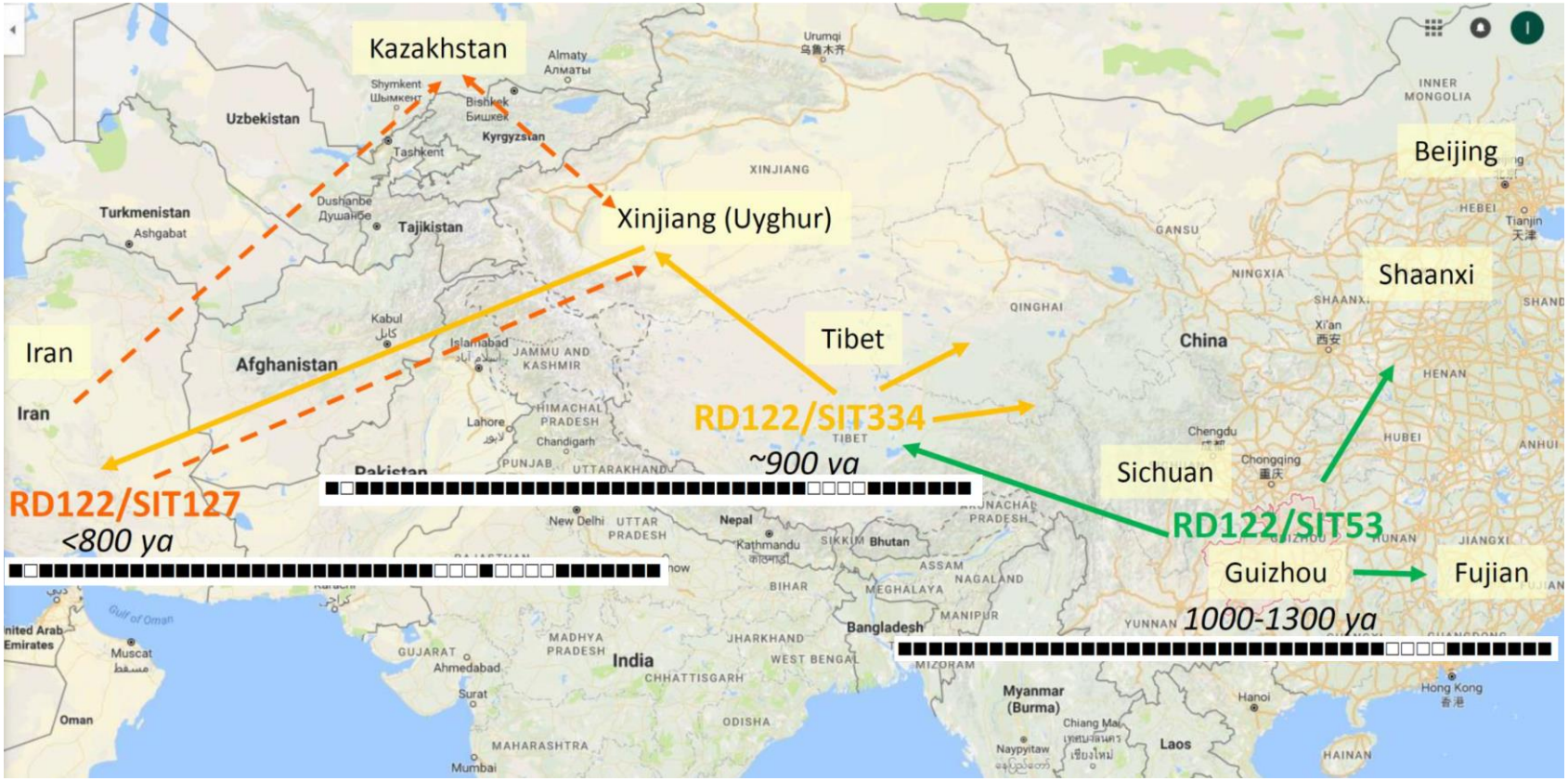

Figure 4. Evolution/migration scenario of RD122/SIT127 lineage of *M. tuberculosis*.

Origin of L4.5/RD122 1000-1300 ya in China. Origin of intermediate SIT334 in Tibet, further migration to Xinjiang/Uyghur, and Iran 800 ya, possibly, via expansion of the Mongol Yuan empire. Origin of NEW-1 founding type SIT127 in south Iran. Dispersal of SIT127 from Iran, eastwards: via Iranian languages, trade exchange, Islamic world. Reproduced from Mokrousov et al. (2017). Copyright Elsevier NV.

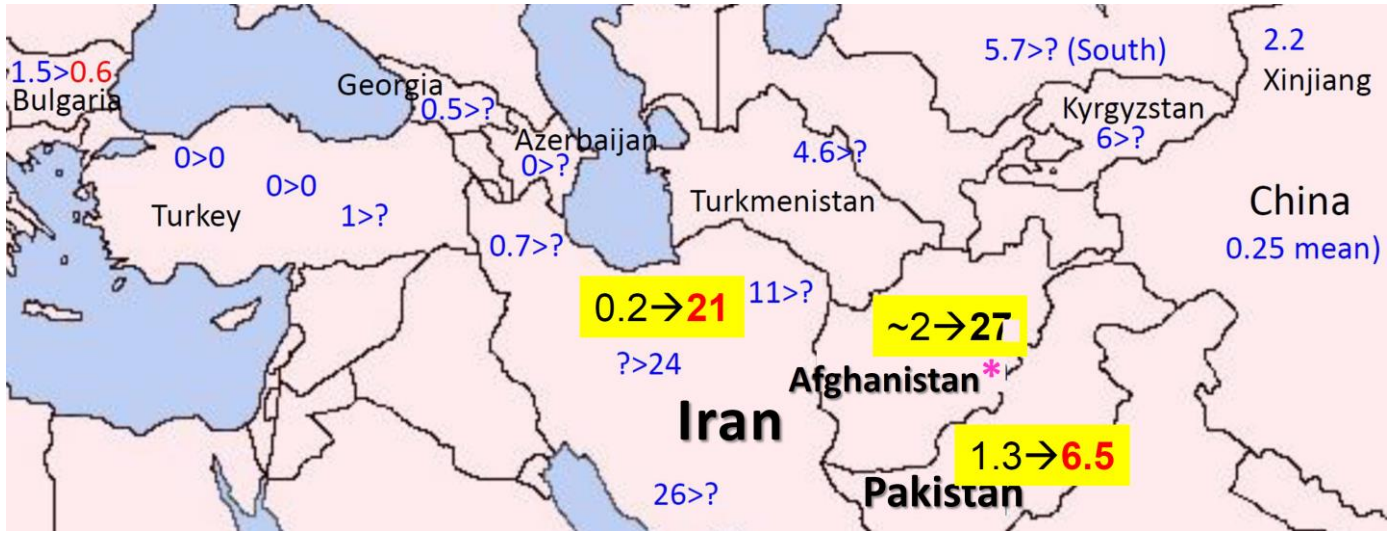

Figure 5. Longitudinal data since 1990s reveal an increase of *M. tuberculosis* SIT127/NEW-1 in Iran and neighbors, along with association with MDR.

Arrows: transition from earlier (2005–08) to recent studies (2014–16). Red: significant association with multidrug resistance. Data on Afghanistan are proxied by Afghan refugees in Iran. Modified from Mokrousov et al. (2017). Copyright Elsevier NV.

Figure 6. Dendrogram of VNTR profiles of 40 *M. avium* subsp. *hominissuis* isolates with information on their SNP types (*MACPPE12, hsp65).*

The following information is provided for each isolates in boxes: strain ID, *MACPPE12*/ *hsp65* sequence types and MATR-VNTR type (IP stands for individual profile). A to C clusters combine *MAH* isolates with identical MATR-VNTR and sequence type profiles. *a* to *f clusters* combined isolates with identical *MACPPE12* and *hsp65* SNP types, but individual MATR-VNTR profiles.