Original article

Differences in the population of genetics of *Mycobacterium tuberculosis* between urban migrants and local residents in Beijing, China

DAI Guang-ming, ZHANG Zhi-guo, DING Peng-ju, ZHANG Qian, WANG Li, WANG Li-xia, Dick van Soolingen, HUANG Hai-rong, LI Wei-min and LI Chuan-you

Keywords: *Beijing lineage; Mycobacterium tuberculosis; urban migrants; phylogeny; population of genetics*

Background Currently, migration has become one of the risk factors of high burden of tuberculosis in China. This study was to explore the influence of mass migration on the dynamics of *Mycobacterium (M.) tuberculosis* in Beijing, the capital and an urban area of China.

Methods Three hundred and thirty-six *M. tuberculosis* strains from the Changping district, where the problem of urban migrants was more pronounced than in other Beijing regions, were genotyped by Spoligotyping, large sequence polymorphisms (LSPs 105 and 181), and variable number tandem repeat (VNTR) typing. Based on the genotype data, the phylogeny of the isolates was studied.

Results In Changping district, the proportion of Beijing lineage *M. tuberculosis* isolates amounted to 89.0% (299/336), among which 86.6 % (252) belonged to the modern lineage. The frequency of modern Beijing lineage strains is so high (around 75% (252/336)) that associated risk factors affecting the tuberculosis epidemic cannot be determined. The time to the most recent common ancestor (TMRCA) of the Beijing lineage strains was estimated to be 5073 (95% CI: 4000–6200) years. There was no significant difference in the genetic variation of Beijing isolates from urban migrants and local residents.

Conclusions The clone of modern Beijing lineage *M. tuberculosis*, which is dominant in the Beijing area, most likely started to expand with the five thousand-year-old Chinese civilization. In the future, with the urbanization in the whole of China, modern Beijing lineage *M. tuberculosis* may gain the larger geographical spread.

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Beijing is the capital city and urban area of China. From the 1950s to the 1990s, the tuberculosis (TB) morbidity and mortality in Beijing area declined significantly. However, after the 1990s, the decrease in the TB incidence rate slowed down. There may be specific reasons for this unfavorable situation;[1] however, the high prevalence of the Beijing genotype *Mycobacterium (M.) tuberculosis* and urban migrants may be factors in this area not sufficiently taken into consideration.[2]

In recent years, more solid phylogenic markers, such as large sequence polymorphisms (LSPs) also indicated as Regions of Difference (RDs) and single nucleotide polymorphisms (SNPs), have been used to analyze the population structure of the *M. tuberculosis* complex.[3] It revealed that the global population structure of *M. tuberculosis* complex (MTBC) is defined by six large phylogeny lineages. The East-Asian lineage actually redefined the Beijing genotype strains, as this lineage for the largest part consists of Beijing genotype strains.[4] Beijing lineage *M. tuberculosis*, as a clone, was expanding all over the world and was associated with drug-resistance on some countries.[5] In addition, Beijing lineage *M. tuberculosis* had the hypervirulence and negative immunomodulatory capability, and may partially depend on its production – a

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National Tuberculosis Clinical Lab of China, Beijing Tuberculosis and Thoracic Tumor Research Institute; Beijing 101149, China (Dai GM and Huang HR)
Changping Tuberculosis Prevent and Control Institute of Beijing, Beijing 102206, China (Zhang ZG)
College of Bioengineering, Henan University of Technology, Zhengzhou, Henan 450001, China (Ding PJ)
Institute of Genetics and Development Biology, China Academy of Sciences, Beijing 100101, China (Zhang Q)
School of Medicine, Hangzhou Normal University, Hanzhou, Zhejiang 3110036, China (Wang L)
National Center for Tuberculosis Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China (Wang LX)
National Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment (RIVM), 3720 BA Bilthoven, the Netherlands (van Soolingen D)
Beijing Key Laboratory on Drug-resistant Tuberculosis Research, Beijing Chest Hospital, Capital Medical University, Beijing 101149, China (Li WM)
Department of Bacteriology and Immunology, Tuberculosis and Thoracic Tumor Research Institute, Beijing 101149, China (Li CY)
Correspondence to: Dr. HUANG Hai-rong, National Tuberculosis Clinical Lab of China, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing 101149, China (Tel: 86-10-89509159. Fax: 86-10-89509359. Email: hairong.huangcn@gmail.com); Dr. LI Wei-min, Beijing Key laboratory on Drug-resistant Tuberculosis Research, Beijing Chest Hospital, Capital Medical University, Beijing 101149, China (Tel: 86-10-89509159. Fax: 86-10-89509359. Email: lwm_18@hotmail.com); Dr. LI Chuan-you, Department of Bacteriology and Immunology, No. Ten Clinical Medical College, Capital Medical University, Beijing, 101149, China (Tel: 86-10-89509366. Fax: 86-10-69549819. Email: lichuanyou6688@hotmail.com)
Drs. DAI Guang-ming, ZHANG Zhi-guo, DING Peng-ju contributed equally to this work.
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specific phenolic glycolipid.[6] Tsolaki et al[7] suggested that the Beijing lineage can be defined by the RD105 deletion. Furthermore, the Beijing lineage of *M. tuberculosis* was divided into two sub-groups by presence or absence of RD181. Modern Beijing strains, defined by the absence of RD181, can again be further divided by RD150 and RD142.[7] Previous studies reported that Beijing strains are highly prevalent in China,[8] including Beijing area.

Variable number of tandem repeat (VNTR) typing is considered the current gold standard in genotyping, especially in studies on the molecular epidemiology of TB at population level.[9] This method was also found useful to study the phylogeny of the *M. tuberculosis* complex.[10] Shriver et al[11] built an online web (MIRU-VNTR plus) to analyze the genotype data by neighbor-joining and minimal spanning method, and they[10] also estimated the most recent common ancestor (MRCA) of the *M. tuberculosis* complex in terms of VNTR genotyping data and phylogenetic theory.

China is confronted with a rapid urbanization process, led by the nationwide economic reforms, and this creates large-scale domestic migrations. It is estimated that there will be 240–260 million urban migrants in China by the year 2030. Urban migrants leave their rural hometowns to seek jobs in large cities such as Beijing, Shanghai, Guangzhou, etc. In fact, it is well known that there is a strong correlation between human population migration and the prevalence of TB. At present it was the urban migrants that make the burden of tuberculosis heavier in Shanghai.[12] Beijing was not only the capital but also an urban area of China; hence, we specially want to know the migration dynamics of *M. tuberculosis*. Another important investigation was from San Francisco in 2006.[13] The authors reported that although San Francisco was an urban cosmopolitan environment, *M. tuberculosis* lineages were much more likely to spread in sympatric than in allopatric patient populations. Beijing is an urban metropolitan environment; we, therefore, wondered whether there might be variable host–pathogen compatibilities, such as Beijing sub-lineages of *M. tuberculosis*—Chinese sub-population in terms of their place of birth. The results are all useful to devise local and national optimal TB control strategies. Therefore, in this study, we compared the population genetics of *M. tuberculosis* between local residents and urban migrants, by analyzing the genotype data of the strains from the Changping district, where the problem of urban migrants was very serious in Beijing regions, and hope to find positive results.

METHODS

Study setting

Beijing had approximately 24 million inhabitants in 2004. We decided to study the Changping district, one of the 16 administrative districts in the Beijing region, because it has a most rapid rate of urbanization and the highest increase in the proportion of urban migrants amongst human population in Beijing since the nationwide economic reforms started in 1980s. In 2004, the Changping district had a population of approximately 2.08 million, while 49% were local residents and 51% were urban migrants.[14]

In the Changping district, all suspected cases of pulmonary TB detected in general hospitals or community health centers were referred to the Changping Tuberculosis Prevention and Control Institute of Beijing, where the diagnosis was made by sputum smear microscopy, culture, and chest radiography, according to the "Diagnostic criteria for pulmonary tuberculosis" issued by the Ministry of Health.[15] All the patients confirmed with active pulmonary TB must be reported to the Tuberculosis Registry at the Beijing Research Institute for Tuberculosis Control through the mandatory notification system. In this retrospective study, we covered all confirmed TB patients, who lived in Changping district from 1 June 2004 to 31 December 2006.

Sputum smear examination by Ziehl-Neelsen staining and culture on Lowenstein-Jensen medium was performed for samples from all TB cases. The culture positive isolates further were identified by PNB and TCH.[16] The demographic data of the involved culture positive TB patients was collected, including age, sex, diagnosis (pulmonary TB, pleurisy, peritonitis, disseminated TB, and lymph nodes TB), clinical history (productive coughing ≥3 weeks or <3 months, hemoptysis, chest pain, fever, fatigue, loss of appetite, night sweats, etc), complication, radiology data, BCG vaccination status, drug susceptibility pattern, treatment history, and geographic birth region (local, east, middle, and west area).[8] *M. tuberculosis* H37Rv (ATCC 27294) strain was used as a control in each experiment.

Genotyping

Spoligotyping

The *M. tuberculosis* isolates were typed by the standard spoligotyping method.[17,18]

Identification of genomic LSPs using real time-PCR

Rapid identification of genomic deletions (large sequence polymorphisms) in *M. tuberculosis* strains was performed using the real-time PCR based on Tsolaki's methods.[7] Beijing lineage *M. tuberculosis* can be defined by the absence of RD105. Modern Beijing strains are devoid of RD181, while more ancient ones still possess RD181.

VNTR

M. tuberculosis isolates from Changping were genotyped using the standard 24 loci VNTR method.[9]

Discriminatory power of VNTR

The discriminatory power of VNTR typing method was calculated using the Hunter-Gaston discriminatory index (HGI).[19] The allelic diversity (h) was determined according to Selander's method.[20]

Phylogenetic inference

Identification and phylogenetic tree**:** The VNTR, spoligotyping and RD patterns of *M. tuberculosis* strains

from Changping were all transformed into a digital type, and then uploaded to the MIRU-VNTRplus web-application (*http://www.miru-vntrplus.org/*).[11] Using the "similarity search" module based on the genetic distance from the different lineage reference strains in the VNTR plus database, the lineages of *M. tuberculosis* were defined.

The phylogenetic tree of *M. tuberculosis* was analyzed using the Neighbor-Joining (N-J)[11] program at MIRU-VNTRplus. *Mycobacterium canetii* was used as an outgroup. In addition, a minimum spanning tree was also defined in order to simulate the genetic structure.[11]

Time estimation of Beijing lineage strains from Changping**:** We grouped individual isolates into populations defined by their belonged lineages, which were all from the MIRU-VNTR plus reference strain database. Beijing lineage strains were grouped according to the population of Beijing in the MIRU-VNTR plus database; however, the Beijing strains from the Changping were grouped by the BJ population. Based on 24-loci VNTR patterns, the Nei et al's D_A distance[21] was computed and used to construct neighbor-joining trees with 100 bootstraps on individuals using the software Populations version 1.2.32.

The Ytime method was used to calculate the time to the most recent common ancestor (TMRCA) of the Beijing lineage strains from Changping. The TMRCA was inferred under the assumption of the Simple Stepwise Mutation Model (S-SMM).[22] Using all available loci (N=24) as input, the strains were grouped according to their lineages, which were obtained from the MIRU-VNTR plus database and our results. The ancestral genotype for each group was calculated as the mean of every single locus in the group. In agreement with Shriver et al,[11] the mutation rate of 10^{-4} per year per locus and the assumed growth rate of 10^3 were used. The assumption of demography only affected the confidence intervals and did not affect the mean estimates of TMRCA.

Statistical analysis

We assessed that the modern and ancient Beijing lineage *M. tuberculosis* correlated with factors, such as age, BCG vaccination status, drug susceptibility pattern and geographic birth region, and stratified results of the Beijing lineage clade data by the four human populations from four different birth areas. All analysis was completed in SPSS18.0 (Chicago, IL, USA).

Genetic differentiation

Based on the 24 loci VNTR data of *M. tuberculosis* from four human populations, analysis of Molecular Variance (AMOVA) was used to evaluate the genetic differentiation within Beijing,[23] especially the modern Beijing lineage *M. tuberculosis* between urban migrants and local residents in the Changping district.

RESULTS

Totally, 1065 cases of active pulmonary tuberculosis were reported to the Tuberculosis Registry at the Beijing Research Institute for Tuberculosis Control during the period of June 1, 2004 to December 31, 2006. Sputum samples from these 1065 cases were subjected to mycobacterial culture. The culture of 340 of these isolates showed *Mycobacterium* growth. Thirteen of the isolates belonged to *NTM* species, and the remaining 327 were *M. tuberculosis*, as determined by biochemical tests. Spoligotyping on all 340 mycobacterial samples demonstrated that nine of the 13 NTM samples were in fact mixed *M. tuberculosis* and NTM infections. Thus, 336 samples contained solidity *M. tuberculosis*.

Correlation of Beijing sub-lineages *M. tuberculosis* and patient's birth area

Among the 336 *M. tuberculosis* isolates from the Changping district, 89.0% (299/336) of the isolates revealed the RD105 deletion and Beijing genotype spoligotyping pattern, while the other 37 (11.0%) had an intact RD105, and were of other lineages. Among the 299 Beijing strains, 252 had the RD181 deletion and were considered modern, while the other 47 Beijing strains did not and were assigned to the ancient lineage. Among the 252 modern Beijing strains, 168 strains also exhibited the RD150 deletion, but 84 strains did not; 173 strains had deleted RD142, while 79 strains had not. On the basis of this information, we produced a phylogenetic tree of the *M. tuberculosis* strains isolated from the Changping district, in Beijing (Figure 1). The result indicated that in the Changping district, the modern Beijing lineage of *M. tuberculosis* strains was prevailing at a high frequency (84.3%, 252/299).

No association was observed between one of the two sub-lineages (ancient vs. modern) of the Beijing lineage strains and patient characteristics such as age (<25, 25–49, 50–75, and >75), BCG vaccination status, drug resistance (SM, INH, RFP, and EMB), treatment history (new case and retreatment), and area of host origin (local, east, middle, and west area, P>0.05). Furthermore, not only local residents, but also urban migrants in Changping were mainly (around 75%) infected by modern Beijing lineage *M. tuberculosis*.

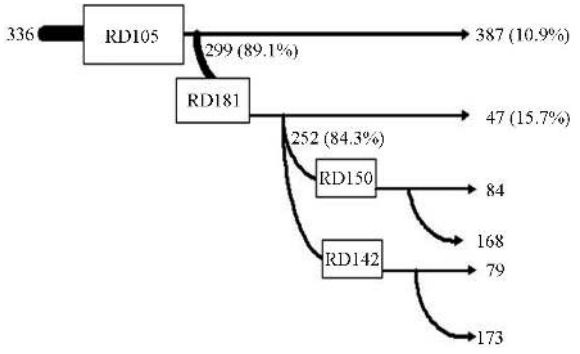

Figure 1. The phylogeny of Beijing/W lineage *M. tuberculosis* in Changping. RD105 defines the Beijing/W family and is further subdivided by RD181, RD150, and RD142. All of the *M. tuberculosis* strains were from Changping, Beijing.

Discriminatory power of 24 loci VNTR typing for Beijing lineage *M. tuberculosis*

Although the 336 *M. tuberculosis* isolates from the Changping district were all subjected to 24 loci VNTR typing, only 318 strains had complete VNTR patterns. The result of allelic diversity showed that VNTR2163b revealed the highest *h* (0.9740) and MIRU27 the lowest (0.2965). There were no identical fingerprints among the VNTR patterns of the 318 isolates, so the HIG amounted to 1. As 24 loci VNTR had sufficient discriminatory power for *M. tuberculosis* strains from Changping, we can use this technique in the next investigation.

Clonal expansion of modern Beijing strains

The 318 VNTR patterns of *M. tuberculosis* isolates from Changping were analyzed using the MIRU-VNTR plus program. The strains were categorized into a phylogenetic N-J tree using *M. canetii* as an outgroup (the figure unpublished). There was a very limited genetic distance between 281 Beijing lineage strains from Changping, which had been determined by spoligotyping and RD typing and had complete VNTR data, and strains of the same lineage in the MIRU-VNTR plus database. The result again confirmed the 281 *M. tuberculosis* belonged to Beijing lineage.

To more robustly define the position of Beijing strains in MTBC, we grouped individual isolates into the populations defined by their lineages. We established an N-J tree on basis of the VNTR allelic frequencies of these populations. The outgroup for this tree was *M. canettii*, which has been recently reported to represent the MTBC progenitor. Our Figure 2 was congruent with the earlier one described by Supply et al[9] The entire MTBC was also clearly distinguishable into two major lineages: clade one and clade two. A very limited genetic distance was observed between the two Beijing lineage groups from the reference database (Beijing) and Changping (BJ). Because of the strong bootstrap value (100), the conclusions for the phylogeny of the Beijing lineage were highly valid.

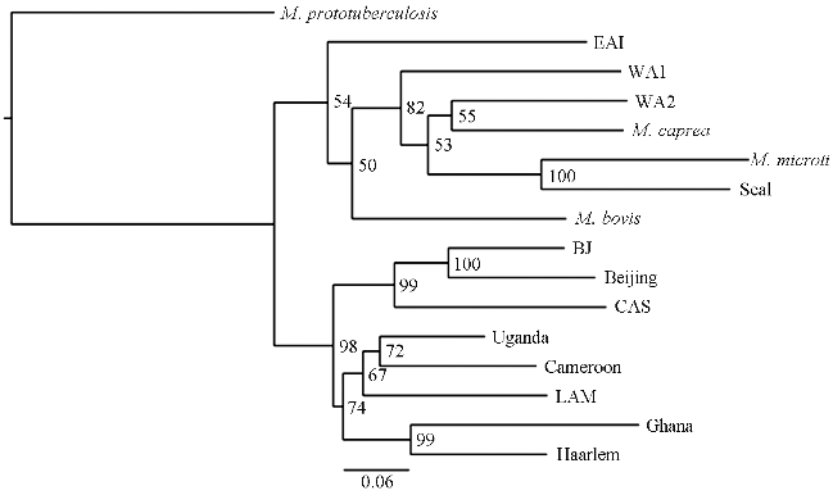

Figure 2. Evolutionary relationship of the *M. tuberculosis* complex. Rooted VNTR population N-J tree based on genetic distance. *Mycobacterium prototuberculosis* was used as an outgroup. Value on the nodes represent the percentage of bootstrap replicates over individuals (*N*=1000) showing the particular nodes. Branch lengths are proportional to the genetic distance between the tubercle lineages.

Table 1. Estimated times (in years) since the most recent common ancestor (TMRCA)

Items	BJ–BJ	Beijing–BJ	BJ–CAS	EAI–BJ
Time (years)	5073	8800	18 500	36 000
95% *CI*	4000–6200	7300–10 400	16 000–21 200	31 400–41 000

We calculated the Ytime value of the Beijing lineage in terms of supply's paper. The age of the Beijing lineage from Changping (BJ) was estimated approximately 5073 years (95% *CI:* 4000–6200); however, the age of the reference Beijing strain (Beijing) was approximately 8800 years (95% *CI*: 7300–10 400, Table 1). This result facilitated us to estimate when the modern Beijing lineage *M. tuberculosis* from Beijing area, as a clone, started to expand.

No variability of genetic differentiation within Beijing, especially modern Beijing lineage *M. tuberculosis* between urban migrants and local residents

In order to discriminate the phylogenetic sub-clades, a N-J tree was constructed in terms of 24 loci VNTR patterns of 281 Beijing lineage *M. tuberculosis* from Changping district (Figure 2). The outgroup for this tree was also *M. canettii*. Four clades were defined: clade 1 (green), clade 2 (yellow), clade 3 (blue), and clade 4 (purple). The four clades of *M. tuberculosis*, comprising in total of 263 isolates, corresponded to 78.3% (263/336) of the culture positive TB cases in Changping between 1 June 2004 and 31 December 2006, who were born in Beijing (local), east, middle, and west area of China. Thirty-six percent of 336 isolates belonged to clade 1, 15% to clade 2, 18% to clade 3, and 9% to clade 4. When we stratified the Beijing lineage *M. tuberculosis* clade data by the four patient populations, no association was evident ($P > 0.05$).

Reflected in a star-like network topology of minimum spanning tree of 24 loci VNTR of *M. tuberculosis* isolates within the Beijing lineage, it underwent rapid population expansion (the Figure unpublished).

From the result of AMOVA in terms of 24 loci VNTR data of 281 Beijing strains (Table 2), we found that there was not variable genetic differentiation within the Beijing, especially modern Beijing lineage of *M. tuberculosis* between urban migrants and local residents in Changping.

DISCUSSION

In the Beijing area, the enormous migration is a potential threat to TB control, as shown in Shanghai.[12] In order to set optimal TB control strategies, we should take into account the population genetics of *M. tuberculosis* and its migration dynamics. Hence, we selected Changping district, as a study area, in which the problem of urban migrants was more serious than that of other areas in Beijing.

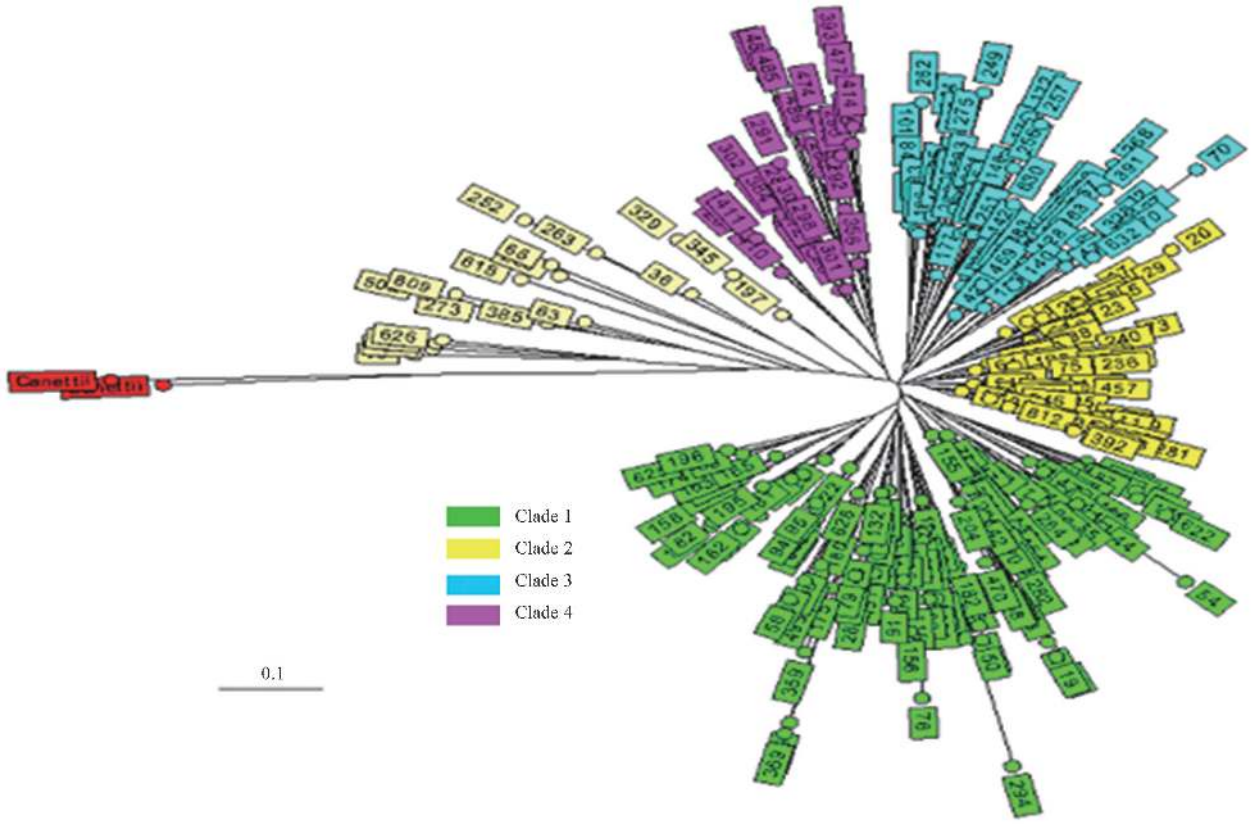

Figure 3. The analysis of 281 *M. tuberculosis* strains by a phylogenetic tree of 24 loci VNTR.

Table 2. The AMOVA of strains of four areas

Location	Local			West			East		
	Fst	*P* values	α<0.05	Fst	*P* values	α<0.05	Fst	*P* values	α<0.05
West	0.009	0.681	–						
East	0.004	0.946	–	0.010	0.829	–			
Middle	0.008	0.292	–	0.014	0.551	–	0.020	0.123	–

Although Beijing strains are genetically highly conserved and reveal a clonal population structure on basis of the markers tested, the 24 loci VNTR, which Supply et al[9] recommended in 2006, had sufficient resolution at strain level. Hence, we decided to construct a database, including background data and VNTR typing results of all *M. tuberculosis* isolated in Changping from 2004 to present, in order to temporarily and spatially monitor the difference in genetic structure of the *M. tuberculosis* population between urban migrants and local residents in Beijing, capital, and an urban area of China.

For two and half years, we collected 336 *M. tuberculosis* strains. Based on their 24 loci VNTR patterns, it was found that in the Changping district, the Beijing lineage strains were predominant (89.0% (299/336)), and the "modern" Beijing lineage was by far more prevalent than the ancient one (84.3% vs 15.7%). In addition, it was evident that the frequency of modern Beijing lineage is so high (around 75%; 252/336) in this region that the birth area of patients cannot be identified as risk factor influence on the TB epidemic.

The remaining scientific question was whether we could estimate the evolutionary age of Beijing lineage strains from Changping. The findings presented in this study indicate that the whole Beijing strain population in Changping emerged from approximately 5100 years ago. This estimate is strikingly close to the proposed time for the start of Chinese 5000-year-old civilization. Our results again supported the assumption that evolutionary history of TB accompanied with that of human.[24]

In Beijing, it was imagined whether there was variable compatibility between host and pathogen, as in San Francisco, even though Beijing sub-lineages *M. tuberculosis* and Chinese sub-population in terms of their born in area. From the investigation of Changping, the answer was no. The reasons may be that Beijing, especially modern Beijing lineage *M. tuberculosis* were dominant in this area, and host population were all Chinese, while they were born in different area of China. From the topology of minimum spanning tree of the *M. tuberculosis*, it was estimated that modern Beijing lineage, as a clone, rapidly expanded, and was possibly introduced into new host populations. Furthermore, based on invariable genetic differentiation, it was suggested that there were gene flows within Beijing, especially modern Beijing lineage *M. tuberculosis* between urban migrants and local residents.

Through the window of the Chanping district, we can look into Beijing and consider that modern Beijing lineage will become dominant form, and furthermore outcompete ancient and other lineages, with continued urban migrant population.[24] It is also apprehended that the mordern

Beijing lineage of *M. tuberculosis* would again expend as it did five thousand years ago, but may be coupled with rapid urbanization in the future. Although government has devised an ambitious plan to eliminate TB in China, some of questions discussed in this study, while primarily of academic interest, should be take into account.

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REFERENCES

1. Center for TB control and prevention, CDC, China. China tuberculosis surveillance (the First quarter of 2009). Beijing: Chinese Center for Disease Control and Prevention; 2009: 31.
2. Steenwinkel EM, Kate MT, Knegt GJ, Kremer K, Aarnoutse RE, Boeree MJ, et al. Drug susceptibility of *Mycobacterium tuberculosis* Beijing genotype and association with MDR TB. Emerg Infect Dis 2012; 18: 660-663.
3. Tsolaki AG, Hirsh AE, DeRiemer K, Enciso JA, Wong MZ, Hannan M, et al. Functional and evolutionary genomics of *Mycobacterium tuberculosis*: insights from genomic deletions in 100 strains. Proc Natl Acad Sci U S A 2004; 101: 4865-4870.
4. Gagneux S, Small PM. Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. Lancet Infect Dis 2007; 7: 328-337.
5. Glynn JR, Whiteley J, Bifani PJ, Kremer K, van Soolingen D. Worldwide occurrence of Beijing/W strains of *Mycobacterium tuberculosis*: a systematic review. Emerg Infect Dis 2002;8: 843-849.
6. Reed MB, Domenech P, Manca C, Su H, Barczak AK, Kreiswirth BN, et al. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature 2004; 431: 84-87.
7. Tsolaki AG, Gagneux S, Pym AS, Salmoniere YLG, Kreiswirth BN, Sooligen D, et al. Genomic deletions classify the Beijing/W strains as a distinct genetic lineage of *Mycobacterium tuberculosis*. J Clin Microbiol 2005; 43: 3185-3191.
8. Li WM, Wang SM, Li CY, Liu YH, Shen GM, Zhang XX, et al. Molecular epidemiology of *Mycobacterium tuberculosis* in China: a nationwide random survey in 2000. Int J Tuberc Lung Dis 2005; 9: 1314-1319.
9. Supply P, Allix C, Lesjean S, Mara CO, Sabine RG, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit: variable-number tandem repeat typing of *Mycobacterium tuberculosis*. J Clin Microbiol 2006; 44: 4498-4510.
10. Wirth T, Hildebrand F, Caroline AB, Wolbeling F, Kubica T, Kristin K, et al. Origin, spread and demography of the *Mycobacterium tuberculosis* complex. PLoS Pathog 2008; 19: e1000160.
11. Shriver MD, Jin L, Boerwinkle E, Deka R, Ferrel RE, Chakraborty R. A novel measure of genetic distance for highly polymorphic tandem repeat loci. Mol Bio Evol 1995; 12: 914-920.
12. Shen X, Xia Z, Li X, Wu J, Wang L, Li J, et al. Tuberculosis in an urban area in China: differences between urban migrants and local residents. PLoS One 2012; 7: e51133.
13. Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, Narayanan S, et al. Variable host-pathogen compatibility in *Mycobacterium tuberculosis*. Proc Natl Acad Sci U S A 2006; 103: 2869-2873.
14. Bureau of Statistics of Changping, Beijing. The bulletin of population of Changping district, Beijing from China population census in 2010. 2011.
15. Ministry of Health. Diagnostic criteria for pulmonary tuberculosis. (Accessed on August 30, 2012 at *http://www.moh.gov.cn/publicfiles/business/cmsresources/zwgkzt/cmsrsdocument/doc3242.pdf*.)
16. Chinese *Medicine* Association. The rule of clinical technique operation tuberculosis branch. Beijing: People Military Medicinal Press; 2003: 36-44.
17. Van Soolingen D, Qian L, Haas PE, Douglas JT, Traore H, Portaels F, et al. Predominance of a single genotype of *Mycobacterium tuberculosis* in countries of East Asia. J Clin Microbiol 1995; 33: 3234-3238.
18. Kamerbeek J, Schouls L, Kolk A, Agterveid MV, Sooligen DV, Kuijper S, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 1997; 35: 907-914.
19. Kremer K, Yanau BK, Waiyip PC, Skuce R, Supply P, Kam KM, et al. Use of variable-number tandem-repeat typing to differentiate *Mycobacterium tuberculosis* Beijing family isolates from Hong Kong and comparison with IS6110 restriction fragment length polymorphism typing and spoligotyping. J Clin Microbiol 2005; 43: 314-320.
20. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Method of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 1988; 51: 873-884.
21. Masatoshi N, Fumio T, Yoshio T. Accuracy of estimated phylogenetic trees from molecular data II. Gene frequency data. J Mol Evol 1983; 19: 153-170.
22. Behar DM, Thomas MG, Skorecki K, Hammer MF, Bulygina E, Rosengarten D, et al. Multiple origins of Ashkenazi Levites: Y chromosome evidence for both near eastern and European Ancestries. Am J Hum Genet 2003; 73: 768-779.
23. Pepperell CS, Grankab JM, Alexander DC, Behr MA, Chui L, Gordonf J, et al. Dispersal of *Mycobacterium tuberculosis* via the Canadian fur trade. Proc Natl Acad Sci U S A 2011; 108: 6526-6531.
24. Gagneux S. Host-pathogen coevolution in human tuberculosis. Philos Trans R Soc Lond B Biol Sci 2012; 367: 850-859.

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