# POPULATION STRUCTURE ANALYSIS OF *MYCOBACTERIUM TUBERCULOSIS* BEIJING FAMILY IN JAPAN

Tomotada IWAMOTO

**Abstract** The Beijing family is a genotypic lineage of *M. tuberculosis* that reportedly predominates throughout eastern Asia and in parts of Russia yet dispersed worldwide. About 80% of clinical isolates in Japan are belonging to this family. The predominance of a narrow range of genotypes, in this case predominance by Beijing family strains, hypothesized that these strains may have a selective advantage either with virulence or transmissibility that led to clonal expansion.

The strains are monophyletic within the *M. tuberculosis* species and also reveal further sublineages within this family. It is believed that the genetic and evolutionary background of *M. tuberculosis* strains influence on the propensity to gain drug resistance as well as the pathogen's transmissibility. Thus, understanding the population genetic structure and its dynamics of Beijing family strains will undoubtedly help to unravel the basis for the considerable success and spread of this genotype in Japan.

During the past few years, we intensively studied this notorious clade, *M. tuberculosis* Beijing family, to reconstruct their evolutionary events and phylogeny and to elucidate their epidemiological characteristics at the sublineage levels through the population structure analysis. Here, I summarized the findings of our research in the past few years.

*Singularity of the genetic diversity of Beijing family strains in Japan*

We reconstructed the phylogenetic trees of Beijing family strains by the 15-MIRU-VNTR genotyping method and validated them through profiling of the NTF region, large sequence polymorphisms (LSP), and single-nucleotide polymorphisms (SNPs). Interestingly, we could demonstrate that the ancient Beijing sublineage has remained endemic to Japan, in contrast to the worldwide spread of the modern Beijing sublineage. Moreover, we found that the ancient sublineage strains corre-

sponding to four monophyletic subgroups. The singularity of the genetic diversity of Beijing family strains in Japan, i.e., its high diversity and dominance of the ancient sublineage in contrast to the modern sublineage found worldwide, suggested that they became endemic independently from the evolutionary stream that led to the dominant modern Beijing sublineage in outside Japan.

*Population structure dynamics of Beijing family strains in Japan*

We inferred the population structure dynamics of Beijing family strains during the past decades in Japan by comparing the isolates from elderly TB patients (these strains represent the population structure that existed decades ago) and young TB patients (these strains reflect the population structure of currently prevalent strains). The comparison between the cohorts born in different years suggested that the population structure of the *M. tuberculosis* Beijing family strains in Japan before World War II — when TB was highly prevalent — was significantly different from that of the currently prevalent strains. The results revealed that the spread of a modern sublineage that has high transmissibility is currently increasing, while the spread of an ancient sublineage, STK, has significantly decreased in younger generations. It is interesting to assume that the observed trends in the case of the modern and STK strains may be associated with the *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccination.

*Association between sublineages of Beijing family and multidrug resistance*

*Mycobacterium tuberculosis* Beijing family strains are suspected to be an evolving lineage of *M. tuberculosis* that has acquired the advantage of drug resistance. However, the association between this genotype and drug resistance varies in different countries. This may be due to heterogeneity in the fitness of the sublineages of the Beijing family and different proportions of these sublineages in the local population. To determine whether certain sublineages are associated with multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis, the population structure of the Beijing strains based on 10 synonymous single nucleotide polymorphisms (sSNPs) was analyzed in pan drug-sensitive (DS), MDR, and XDR strains.

The results revealed that two evolutionary subgroups (ST 26 and ST3), which are belonging to an ancient sublineage, occurred with a significantly higher frequency in the MDR/XDR population than in the DS population. This suggests that different sublineages of the Beijing family may differ in their mechanism of adaptation to drug-selective pressure. The greater vigilance in monitoring the occurrence of these strains is indispensable for achieving better TB control in this region.

In the combination of molecular epidemiological data with recent advances in mycobacterial genomics and population genetics, we could provide novel insights into genetic diversity and phylogeny of *M. tuberculosis* Beijing family strains circulating in Japan. This would be a good start to approaching the genetic determinants causing variations in virulence and transmissibility of *M. tuberculosis*. We are currently applying the next-generation sequencing technology to get the whole genome sequencing of the representative strains from each monophyletic subgroup within *M. tuberculosis* Beijing family.

**Key words**: *M. tuberculosis* Beijing family, Population structure, VNTR, Molecular epidemiology, Phylogeny, Genotype, Multidrug-resistant tuberculosis

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The 84th Annual Meeting Educational Lecture

# DEVELOPMENT OF TB TREATMENT SYSTEM BY LIAISON CRITICAL PATH

Eriko SHIGETO

**Abstract** Liaison critical path (LCP) for various diseases is useful to strengthen community medical cooperation and to provide better service for patients. LCP for tuberculosis is not yet spread except for a few trials, while cooperation of health center and hospitals with tuberculosis ward through community DOTS is now going to be established.

We started cooperation between Higashihiroshima Medical Center and Onomichi Medical Association to provide better medical care for tuberculosis patients through LCP. The request of health care workers for LCP by questionnaire study were i) schedule of laboratory examination to check adverse reaction of medication, ii) schedule of chemotherapy, iii) report system of tuberculosis, iv) infection control and so on. LCP was made up based on these requests and the guidelines of standard chemotherapy. We made LCP to be concise and 3 parts; i) standard treatment with PZA, ii) standard treatment without PZA and iii) treatment other than standard regimen. In addition to these LCP for TB treatment, we made information sheets for i) when to suspect and how to diagnose, ii) flow chart for deciding regimen, iii) explanation of standard treatment and DOTS, iv) information for patients and their families about treatment, admission and infection control. These sheets were offered to member of medical association with referral letter on discharge from TB ward or by health center nurse just after notification of TB.

Though the results of these LCP are not yet fully analyzed, following effects are expected and obtained; i) diffusion of standard chemotherapy and DOTS to medical practitioner, ii) confidence that the treatment is appropriate, iii) reduction of burden on TB hospital through early referral to general practitioner or beginning treatment without referral if admission is not necessary. LCP with community DOTS will help TB patients and healthcare workers around the patients. The most important effect of LCP is establishment of human relationship and network of health care workers molded in the process of development itself.

The problems to be faced from now are diffusion of LCP to other TB hospitals and areas, participation of pharmacy and patient care system at home or nursing home. Any part of members may take initiative to develop LCP, but the role of health center nurse is important.

**Key words**: Community medicine cooperation, Critical path, Medical Association

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# RESEARCH AND CONTROL OF RELAPSE TUBERCULOSIS CASES

Chairpersons: [1]Fumio YAMAGISHI and [2]Makoto TOYOTA

**Abstract** With this symposium, we focused on the relapse of tuberculosis in Japan. Out of 19,893 tuberculosis patients registered in 2007 in Japan, 7.48% were classified as relapse cases. Relapse cases have the risk of acquired drug resistance. But we have few analyses of the proportion of relapse tuberculosis cases with standard short course regimens for six months, factors contributing to tuberculosis relapse and the proportion of drug resistance among relapse TB cases in Japan. Therefore we analyzed the relapse tuberculosis cases in two rural areas and three urban areas. We also analyzed the proportion of drug resistance among relapse cases with the data of drug susceptibility survey of Ryoken.

1. Research of relapse tuberculosis cases: Makoto TOYOTA (Kochi City Public Health Center)

To clarify the relapse rate and factors contributing to tuberculosis relapse, we investigated the relapse tuberculosis cases in the municipality where the proportion of elderly tuberculosis patients was high. Out of 902 tuberculosis patients registered in Kochi City Public Health Center during 10 years, 20 pulmonary tuberculosis patients were confirmed relapse cases with initial registered records. Pretreatment cavitations, sputum culture positivity at 2 months, medical miss-management (e.g. number of doses, duration of therapy) and poor adherence were considered to be factors contributing to tuberculosis relapse. Out of 20 relapse cases, 12 cases were detected with symptoms, while only 3 cases were detected by examination in law.

2. A clinical study on relapse cases of pulmonary tuberculosis: Shuichi TAKIKAWA (National Hospital Organization Nishibeppu National Hospital)

The relapse of pulmonary tuberculosis was investigated. In the cases with a treatment history before short course chemotherapy, drug resistance rate was high, and thus it needs to be cautious of drug resistance at the time of the retreatment. In the cases with a treatment history of short course chemotherapy, relapse cases were recognized more significantly in male cases aged 70's. In the cases that deviated from the standard treatment and that became impossible to use rifampicin, it should be careful to emergence of isoniazid resistance.

3. The current status of the recurrence tuberculosis cases in Tokyo: Michiko NAGAMINE (Specific Disease Control Section, Tokyo Metropolitan Government Bureau of Social Welfare and Public Health)

As for the background of the patient whose disease has relapsed, unstable elements are observed. After any symptom, more patients are diagnosed as a relapse case rather than finding by a medical check up. And more than half are related to homeless or life without fixed address. Their status of insurance is the livelihood protection, no insurance or the national health insurance. By RFLP analysis in Shinjuku city, some clusters have recurrent cases, one of clusters has both a relapse and exogenous reinfection. This is able to elucidate an infectious state. Like this, the analysis of each cluster can help effective countermeasures.

4. Recurrence of tuberculosis in the City of Yokohama between 2004 and 2008: Michihiko YOSHIDA (Shinagawa Public Health Center), Takahiro TOYOZAWA (Yokohama Public Health Center)

To identify the TB recurrence rate, we studied a cohort of 40 cases (treatment completion 36 cases, interruption 4 cases) of whom had a previous history of TB treatment including isoniazid and rifampicin. The time for relapse was 7.9±8.6 years and the overall relapse rate was 0.6% (0.47−0.7%). Our study suggested the relapse was almost equal to the low incident countries but the long term follow-up and surveillance data should be carefully evaluated.

5. Comparison of the retreatment cases of pulmonary tuberculosis: Yuka SASAKI (National Hospital Organization Chiba-East National Hospital)

To investigate the factors of the retreatment of pulmonary tuberculosis, 134 retreatment cases were studied. The factors leading to retreatment were cavitary and large lesions in chest X-p, sputum smear positive and heavy alcohol-drinkers. The factors leading to defaulting of the treatment were lack in understanding of the treatment and their economic problems. Reexamination of the treatment and support of the patients are important to prevent the retreatment of the pulmonary tuberculosis.

6. Proportion of drug resistance among relapse tuberculosis cases, summary of Ryoken studies 1977−2002: Takashi YOSHIYAMA (Fukujuji Hospital)

Background and objective: We have no historical analysis of the proportion of drug resistance among relapse TB cases. Therefore we would like to analyze the proportion of drug resistance among relapse cases in Japan.

Method: Re-analysis of the data of drug susceptibility survey of Ryoken from 1977 to 2002.

Result: The proportion of relapse cases among Ryoken has decreased in 1982−1987 and that proportion was 10% in 2002. The average age of relapse cases was 5 years older than the new cases and it was 66 years in 2002. The proportion of drug resistance among relapse cases has decreased form 39% (in 1977) to 16% (in 2002) for isoniazid, was stable and around 10% for rifampicin with 7.5% in 2002. The risk factors for drug resistance were younger age, foreigners and part time job. The proportion of drug resistance was higher among cases that were failure with previous treatment, then default with previous treatment and lower among cases with cure/completion at the previous treatment but this tendency was without significance.

**Key words**: Tuberculosis, Relapse, Factors contributing to tuberculosis relapse, Defaulter, Drug resistance

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**The 84th Annual Meeting Mini-Symposium**

# ADVANCES IN MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS OPEN THE DOOR TO THE NEXT STAGE

Chairpersons: [1]Tomoshige MATSUMOTO and [2]Tomotada IWAMOTO

**Abstract** Before the availability of high-resolution genotyping tools in 1990s, there was a prevailing dogma of little genomic sequence diversity in *Mycobacterium tuberculosis*. Due to the low levels of genetic variation, it was assumed that *M. tuberculosis* exhibit very little phenotypic variation in immunologic and virulence factors. The fingerprinting method based on restriction fragment length polymorphisms (RFLP) of IS*6110* insertion sequences had unveiled the underestimation of the sequence variation in *M. tuberculosis* and the importance of strain-to-strain variation for understanding pathogenesis, immune mechanisms, bacterial evolution, and host adaptation. This method became a gold standard for strain differentiation in the molecular epidemiological study. It had lead to a profusion of studies in molecular epidemiology such as the detection of unsuspected transmission, the estimation of the extent of recent transmission, the identification of laboratory cross-contamination, the identification of outbreaks, and distinction between reinfection and relapse. This, in 1990s, is the opening of the molecular epidemiology of tuberculosis.

After the completion of genome project of the *M. tuberculosis* laboratory strain H37Rv, some of the clinical isolates were completely sequenced. This prompted the in silico genome comparison and identified various genomic markers which can give a unifying framework for both epidemiology and evolutionary analysis of *M. tuberculosis* population. Of them, variable numbers of tandem repeats (VNTR) was found as the most promising PCR-based method which can provide adequate discrimination of *M. tuberculosis* strains in many cases, including the estimation of *M. tuberculosis* transmission and the identification of genetic lineages. PCR-based VNTR analysis is easy, rapid, and highly specific and can generate portable digit-based data, unlike the analog information obtained from IS*6110* RFLP which is labor intensive. In this regards, investigators can easily compare the genotypic data of independent studies between different laboratories. With the advantages, VNTR surpassed IS*6110* RFLP and became the first line genotyping method in molecular epidemiology. One of the most attractive potentials on this method is its applicability for establishment of the database of *M. tuberculosis* genotype which covers not only local area but also world wide scale. This would open the door to "in silico epidemiology" which brings a breakthrough on the current TB control program. The optimization and standardization of the combination of VNTR loci for strain genotyping is the only but hard issue for the development of global database system. Road to the global Mtb genotype database is hard, but we believe, "Yes, We Can!". Another attractive potential of VNTR is its use for phylogenetic analysis, although more intensive research on this with using comprehensive marker sets, such as large sequence polymorphisms and single-nucleotide polymorphisms are required.

Again, with the advantages of VNTR analysis, i.e., easy, rapid, specific, and digit-based data, VNTR became the first line method in molecular epidemiology. Molecular epidemiology of tuberculosis is expanding its research field from the investigation of TB transmission to more basic science such as evolution and phylogeographic distribution.

In this symposium, we have invited four opinion leaders in molecular epidemiology of TB in Japan who are talking about each title as followed.

1. Establishment of the standard VNTR analysis systems for Tuberculosis (TB) and preparation of databases for TB genotyping: Shinji MAEDA and Yoshiro MURASE (Department of Mycobacterium Reference and Research, Research Institute of Tuberculosis, JATA)

We have already reported the JATA (12)-VNTR system for TB genotyping in Japan. However, by comparison of cluster formation rate, the discrimination power of JATA (12)-VNTR was lower than that of IS*6110* RFLP analysis. Therefore, we improved the JATA (12)-VNTR system for developing discrimination power. By addition of 3 loci (ETR-A, VNTR-1982 and VNTR-2163 a) to JATA (12)-VNTR, we established new JATA (15)-VNTR. We found that the discrimination power of JATA (15)-VNTR was almost the same as that of RFLP analysis.

2. Molecular epidemiology of *Mycobacterium tuberculosis* reviewed by molecular epidemiology of other pathogenic bacteria: Eiji YOKOYAMA (Division of Bacteriology, Chiba Prefectural Institute of Public Health)

Molecular epidemiology of *M. tuberculosis* should be progressed to two goals. First is the short-term goal that intends to elucidate the unapparent route of transmission of the organism. Second is the long-term goal that intends to ascertain the phylogeny of the organism. The combination of VNTR loci should be changed according to the goals of molecular epidemiology of the organism.

3. Progress of the research in molecular epidemiology of *Mycobacterium tuberculosis*: Tomotada IWAMOTO (Department of Microbiology, Kobe Institute of Health)

In the past decade, molecular epidemiology of tuberculosis brought significant insights into the transmission of tuberculo-

sis, genetic diversity of *M. tuberculosis*, population structure and geographical distribution of *M. tuberculosis*, etc. In the advanced stage of the molecular epidemiological study, we expect to change the current geno-typing based molecular epidemiology to whole genome-typing based molecular epidemiology on the basis of the rapid innovation of next-generation sequencing technology.

4. Clinical application of molecular epidemiology of tuberculosis: Tomoshige MATSUMOTO (Department of Clinical Research and Development, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases)

The molecular epidemiology can be applied in clinical practice. We showed some examples about usefulness of the clinical application of molecular epidemiology, especially using variable number of tandem repeats (VNTR) analysis. One example we showed: using VNTR, we can know whether two tuberculosis bacilli which developed from the patients, who have close contact, are the same or not in a few days; Especially, when one patient suffers from multidrug-resistant (MDR) strain of or extensively drug resistant (XDR) of tuberculosis, we can easily know whether the other suffers from MDR/XDR tuberculosis or not. The other example we showed: we can know relapse, reinfection, or laboratory contamination by using VNTR in a few days when a patient shows bacteriological relapse during the treatment. By introducing VNTR to clinical practice, we can diminish days of inappropriate hospitalization. Because VNTR data are numerical, we can easily construct VNTR database, compare data, and survey emergence of MDR/XDR-tuberculosis.

**Key words**: *Mycobacterium tuberculosis*, Molecular epidemiology, Genotyping, VNTR, RFLP, Database

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Information

# TUBERCULOSIS ANNUAL REPORT 2008
## — Series 3. Childhood TB —

Tuberculosis Surveillance Center, RIT, JATA

**Abstract** The number of newly notified childhood tuberculosis (TB) patients aged 0−14 years fell dramatically from the 1970s, and newly notified childhood TB cases in 2008 was 95, corresponding to 0.55 per 100,000. Among 95 childhood TB patients, 41 (43.2%) were aged 0−4 years, 5 (5.3%) were foreigners and 36 (37.9%) were extra-pulmonary TB. Meningeal TB among those aged 0−14 years has not been notified since 2006.

The trends of incidence rates of childhood TB differed by age group. Till the early 1970s, the 5−9 age group showed the highest rate among childhood TB, but then showed the lowest rate from the late 1980s. On the other hand, the 0−4 age group has consistently shown the highest rate since the late 1970s.

Concerning the mode of detection in 2008, 43 patients (45.3%) were detected by family contact examination and 8 (8.4%) were detected by other contact examination. Thirty-five patients (36.8%) were detected at medical institutions with some symptoms, and only 3 (3.2%) patients were detected by mass-screening at school.

There are 47 prefectures in Japan, of which 14 had no childhood TB case in 2008. Most childhood TB cases were in Tokyo with 12 patients, followed by Osaka and Kanagawa with 8 patients each.

**Key words**: Tuberculosis, Childhood TB, Age, Trend, Prefectures, Mode of detection

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