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The role of molecular epidemiology in contact investigations: a US perspective

C. L. Daley,*† L. M. Kawamura*†‡

*** Department of Medicine, University of California, San Francisco, † F J Curry National Tuberculosis Center, San Francisco, ‡ Tuberculosis Control Section, San Francisco Department of Public Health, San Francisco, California, USA**

SUMMARY

Preventing tuberculosis (TB) transmission through treatment of active cases and contact investigation is the highest priority of TB control programs in the United States. The role of contact investigation is becoming increasingly important as the number of TB cases declines nationally. However, the effectiveness of contact investigation has been difficult to assess because, prior to the availability of molecular genotyping techniques, levels of transmission were crudely measurable. Epidemiological links within and outside the traditional concentric circle approach are limited by the quality of the contact investigation, the skill and knowledge of the investigator and the information provided by the patient. Molecular epidemiology has added a new dimension by enabling the recognition of unsuspected transmission, likely locations of transmission, and quantification of the extent of transmission that is occurring within a given population. In the future, as real-time genotyping becomes more available, the role of molecular epidemiology is likely to expand.

KEY WORDS: tuberculosis; *Mycobacterium tuberculosis*; tuberculosis control; genotyping; contacts

PREVENTING TUBERCULOSIS (TB) transmission, through treatment of active cases and contact investigation, is the highest priority of TB control programs in the United States. Evaluating contacts of infectious TB cases is highly cost-effective,[1] and is one of the most productive methods of identifying persons with TB disease (and infection).[2–4] Among medically evaluated contacts in 78 areas of the US during 1990, the rate of clinically active TB was 700 per 100 000 population.[2] More recent studies found that approximately 1–2% of contacts are found to have clinically active TB.[3,4] Although not all cases found through contact investigations are the result of transmission from the index case, particularly among foreign-born populations,[5] early identification and treatment of these patients will reduce transmission in the community.

Contact investigations also identify preventable cases of TB. Recently infected contacts carry an eight fold risk of developing TB disease compared with persons infected more remotely, and are considered a high priority for treatment of latent tuberculosis infection (LTBI).[6] The Centers for Disease Control and Prevention (CDC) estimates that an average investigation of a case of smear-positive pulmonary TB in the US results in a median of four contacts (average of six),[3] of whom at least 36% are found to have tuberculous infection. However, among certain high-risk groups, the expected frequency of a positive tuberculin skin test (TST) may be much higher. Contact investigations among high-risk groups such as the foreign-born will often identify a large number of individuals who have a positive TST, not all of whom have been recently infected but many of whom will benefit from treatment of LTBI. The examination of contacts or persons exposed to a case of TB is, therefore, one of the most important methods of case finding for either tuberculosis infection or disease. Modeling studies have demonstrated that identification and treatment of persons recently infected, such as contacts, can have a profound impact on a TB epidemic.[7]

Despite the importance of contact investigations in the prevention and control of TB in the US, the currently available recommendations and guidelines are based on expert opinion, with few studies available to guide the approach to contact investigations.[8] The concentric circle approach is the method most commonly used in the US to perform contact investigations. However, the concentric circle method may not address all contact investigation needs, and there are considerable limitations with this technique.[9] Molecular epidemiology has helped to identify some of these limitations.

Correspondence to: Charles L Daley, Department of Medicine, University of California, San Francisco, UCSF Box 0841, San Francisco, CA 94143, USA. Tel: (+1) 415-206-4694. Fax: (+1) 415-695-1551. e-mail: cdaley@itsa.ucsf.edu

Lessons learned from molecular epidemiology

Molecular genotyping has revolutionized our ability to track strains and validate suspected transmission of *Mycobacterium tuberculosis* in the community. Prior to the availability of molecular genotyping methods, the only biomarkers that were available to track strains of *M. tuberculosis* were drug susceptibility patterns and phage typing, both of which have significant limitations.[10] Studies of the molecular epidemiology of TB have elucidated both suspected and unsuspected transmission. Use of genotyping has identified contacts that were not found via traditional contact investigations, as well as networks and places of transmission that were not originally suspected.[11–25] In addition, genotyping has distinguished between true secondary cases and coincidental, active TB among contacts.[5,21]

In lower incidence areas, such as Zurich,[12] Amsterdam,[13] and San Francisco,[14] only 5–10% of cases that had identical IS*6110*-based genotyping patterns were identified as contacts by the source case. For the majority of TB cases, contact investigations failed to identify transmission links between the source cases and secondary cases. Investigators in Baltimore identified epidemiologic links in only 24% of cases with matching DNA genotype patterns despite extensive contact investigations.[15] In a well-documented study of TB transmission in the San Francisco Bay Area, 73 (33%) of 221 TB cases in the community were the result of a single strain of *M. tuberculosis* as documented by IS*6110*-based restriction fragment length polymorphism (RFLP) analysis.[22] Remarkably, 39 (53%) of the 73 cases occurred because they were never identified as contacts of source cases. In this study, as well as those described previously, the cases identified through genotyping demonstrated the extent to which transmission could occur in a community, transmission that may otherwise have gone unrecognized. These findings provide valuable lessons for contact investigation by distinguishing areas that can or cannot be improved and in developing alternative strategies that can be used to identify contacts.

Identification of unsuspected transmission: the importance of social networks

The identification of secondary cases of TB among those previously unsuspected demonstrates the limitations of traditional contact investigation. In one of the first genotyping studies, investigators in Switzerland described the transmission of TB among members of a defined social group (drug addicts, homeless persons, and alcoholics) and documented its spread to the general population.[11] Subsequently, several studies have shown that the influence of social networking may account for some of the 'missed' cases in genotyping studies.[6–24] In an investigation of TB transmission among members of a church choir, all TB cases were reported separately by each town in very diverse communities.[23] Traditional contact investigation methods did not identify workplace or 'out-of-home' family contacts; it was not until molecular genotyping results were available that the epidemiologic connections were made. In a floating card game through a rural southern county reported by Bock et al., the failure to identify the existence of numerous social networks propagated an outbreak of TB.[18] Re-interviewing the contacts 19 months later, after genotyping results became available, revealed previously unrecognized epidemiologic links. These studies demonstrate that once a connection is suspected or identified, valuable information can be obtained by re-evaluating contacts; they also demonstrate the importance of congregate activities outside of work and outside of the scope of the typical contact investigation.

Outbreak investigations: the importance of locations

Molecular genotyping of *M. tuberculosis* has been particularly useful in defining the presence and scope of TB outbreaks. Traditionally, identification of outbreaks depended on contact tracing alone. Traditional contact investigations are based on screening the index patient's family and associates for TB infection and disease. This strategy is ineffective in many TB outbreaks because TB patients often do not live in stable family settings, and either do not know or are unwilling to reveal the names of their contacts.[19] With genotyping, strains of *M. tuberculosis* can be linked and personal or geographic connections between individuals harboring the strains can then be sought.

There are numerous examples in the literature that illustrate the value of genotyping in the setting of outbreak investigations and the ability of genotyping to identify sites of transmission.[11–25] In almost all of these reports, routine contact investigations either failed to detect an outbreak or underestimated its size and scope. In one report, a highly infectious individual transmitted TB directly or indirectly to 6% of the cases of TB in San Francisco over a 2-year period.[14] The largest number of cases in the cluster occurred at a human immunodeficiency virus (HIV) residential care facility.[25] In this outbreak, 50% (15/30) of the HIV-seropositive contacts became infected with *M. tuberculosis*, of whom 73% developed TB disease within a 106-day period. Genotyping provided the 'hard' evidence needed to perform urgent contact investigations in order to halt the rapid progression to disease associated with HIV infection. Prioritizing HIV-infected contacts, ensuring rapid evaluation and treatment, became the San Francisco tuberculosis control policy following this outbreak. It appears that in some circumstances, measures to reduce TB transmission should be location-specific rather than concentric circle-based.[15]

Identification of source cases: it is not always who you think

When a second case is recognized as part of a contact investigation, it is generally assumed that the two

cases harbor the same strain of *M. tuberculosis*. Molecular epidemiology studies have established that this is not always the case. Of 11 211 contacts evaluated in San Francisco, there were 66 pairs of culture-positive index and contact cases.[5] Molecular genotyping was available in 54 instances. The index and contact cases were infected with the same strain of *M. tuberculosis* 70% of the time, but had unrelated strains 30% of the time. Unrelated infections were more common among foreign-born, particularly Asian, contacts. Of note, the drug susceptibility pattern was more likely to be discordant in those pairs harboring different strains of *M. tuberculosis*. Similarly, in a study from Denmark, among 22 cases in contact with one or more persons with TB, six (27%) cases among the contacts had different genotype patterns from the presumed source case; in two instances the case and contact lived in the same household.[21] Therefore, in populations with high rates of drug-resistant TB, providers should exercise caution when assuming that the drug susceptibility patterns between the source case and contact are the same.

Use of genotyping in low prevalence areas

In regions where the prevalence of TB is low and the population is geographically stable, clustering is less likely to represent recent transmission than in areas where the incidence of TB is high and *M. tuberculosis* strains are continually introduced by a mobile population.[26] However, even in low-prevalence areas, genotyping can be helpful. For example, use of RFLP-based genotyping has allowed the state of Alabama to detect 'micro-epidemics' where transmission occurred over many years and to identify sites and behaviors associated with transmission.[27] After identifying transmission among the homeless population, specific interventions[28] were put into place that resulted in a decrease in TB among the homeless over the ensuing 3 years. In the floating card game cited previously, there had been only seven cases of TB in the previous 10 years. Thus, recognition of a possible outbreak allowed investigators to use genotyping to establish epidemiologic connections that ultimately aided the contact investigations and eventual control of the outbreak.

Use of genotyping in tuberculosis control

Most studies describing the use of molecular genotyping do so in the context of specific studies and generally do not describe how the availability of genotyping data can be used to alter contact investigations or improve TB control. In the Netherlands, the results of genotyping are given to public health nurses throughout the country to assist with contact investigations. Among 1753 cases reported between 1995 and 1997, epidemiologic links were established in 45% (784) of the cases: 31% of the contacts were detected in the first circle, 29% in the second circle, and 40% were in the third circle or beyond.[29] Thus, genotyping was most helpful in identifying contacts that were more remote than the usual contact investigation, which tends to focus on the inner circles. In San Francisco, the results of genotyping are entered into a computer that is available for investigators to use in order to identify clustered patients, previously unrecognized transmission or verify a contact as a secondary case. Although this system has not been studied formally, it is considered an important part of the San Francisco TB control program.

Molecular epidemiology has also been used to evaluate the performance of the San Francisco TB control program. After intensifying a number of TB control activities, including contact investigations, Jasmer et al. in San Francisco noted a decline in the rate of clustering among US-born individuals consistent with a decrease in transmission.[30] This study demonstrated that molecular genotyping can be used as a tool to evaluate TB control interventions aimed at decreasing transmission, not just as an adjunct to contact and outbreak investigations.

Limitations of molecular genotyping

The current widely available technique of IS*6110*-based RFLP is limited by a number of factors. First, identical patterns in strains of *M. tuberculosis* do not prove that transmission has occurred between individuals. There are endemic strains in some communities that make it difficult to establish definitive transmission links between individuals based solely on matching genotype patterns. Braden et al. studied the molecular epidemiology of TB in Arkansas and noted examples of elderly patients with matching genotype patterns that had no identifiable contact history and that were geographically separated.[26] In the study reported by Chin et al., the isolates of 33% of the patients had the same genotype pattern.[22] This particular strain had thus become endemic in the area, limiting the ability of IS*6110*-based genotyping to detect new transmission in the community.

Second, some strains of *M. tuberculosis* contain few copies of the IS*6110* element. In these cases, a secondary typing method is needed to better differentiate strains. Spoligotyping or detection of the polymorphic guanine/cytosine-rich repetitive sequences in *M. tuberculosis* are the most commonly used secondary typing methods.[31]

Another important limitation is that cultures are needed to perform the RFLP analysis. Because of this delay, valuable epidemiologic information can be lost. However, with newer amplification-based genotyping tools, this is likely to change. Several polymerase chain reaction (PCR) based genotyping assays have been developed, such as spoligotyping, mixed-linker PCR, and variable number tandem repeat typing.[32] These newer methods, and others in development, will provide 'real time' genotyping information that will allow contact investigations to be more fluid, changing

focus as the genotyping results identify unsuspected transmission. With such tools, the role of molecular genotyping will have to be revisited, although standard epidemiological investigations will still be required to establish the presence of epidemiologic links.

Finally, several methodological issues must be addressed when conducting and interpreting molecular epidemiology studies. For example, the amount of transmission represented by clustering using genotyping techniques will depend on the sampling strategy and duration of the study.[33,34] Undersampling can bias the estimates of the proportion of TB cases caused by recent transmission of *M. tuberculosis*, and thus bias the estimates of risk factors associated with clustering. Similarly, studies of short duration are less likely to identify the source and secondary cases in a chain of transmission.

Current and future role of molecular genotyping in contact investigations

The significant improvements that molecular genotyping has provided in assessing transmission has greatly enhanced our understanding of the successes and limitations of current contact investigation practices. Many programs have altered their approach and strategies as a result of genotyping data to control transmission beyond contact investigations. The shift towards a social network approach to contact investigation in populations with high transmission rates is strongly supported by genotyping research, as are site-specific screening practices. Genotyping also validates the need to prioritize immunocompromised contacts for rapid evaluation and treatment.

A recent Institute of Medicine report recommends enhanced contact investigation techniques.[9] Molecular genotyping is one method that can be used to improve contact and outbreak investigations. The development of real-time molecular genotyping techniques will likely improve our ability to conduct effective investigations. However, it is only through the combination of genotyping and directed contact investigations that we can identify specific patient behaviors, sites, or circumstances that facilitate TB transmission in order for appropriate TB control measures to be undertaken. As molecular typing techniques improve, it is likely that genotyping will become an increasingly important tool in the prevention, control, and eventual elimination of TB in the US.

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