

# Enhanced tuberculosis outbreak investigation using whole genome sequencing and IGRA

To the Editor:

Whole genome sequencing (WGS) is a new powerful technology for characterisation of bacterial genomes and has been used successfully to investigate *Mycobacterium tuberculosis* isolates associated with tuberculosis (TB) outbreaks and to elucidate mutations conferring drug resistance [1–6]. Enhanced contact investigation and improved diagnosis and treatment of latent TB infection (LTBI) are an important strategy for TB control and elimination [7–10].

Between February 2012 and July 2013, 14 TB cases (12 confirmed by culture, two clinically confirmed cases) associated with an outbreak were identified in Turku, Finland. The median age of the TB cases was 19 years (range 16–32 years). Four cases were Finnish born, nine were foreign born with an East-African origin and one was foreign born with a Central European origin. Social network analysis, WGS and interferon- $\gamma$  release assays (IGRAs) were used in an ongoing outbreak investigation to complement the traditional contact investigation and to assess the public health relevance of these methods.

Traditional contact investigation methods were used in accordance with the Finnish guidelines. All the identified contacts were interviewed, clinically assessed and screened for active TB by chest radiography. Additionally, classmates and other close contacts (aged <35 years) of sputum smear-positive TB cases were tested for LTBI by IGRA (QuantIFERON-TB Gold In-Tube; Cellestis, Carnegie, Australia). All *M. tuberculosis* isolates were genotyped with MIRU-VNTR-24 (method of typing using 24 mycobacterial interspersed repetitive units (MIRU) for variable number of tandem repeat (VNTR) analysis) and spoligotyping [11].

The complete genomes of the 12 *M. tuberculosis* outbreak isolates and seven historical isolates from our strain collection with the same genotypic profile (same spoligotype and MIRU-24) were sequenced at the Genome Centre of Queen Mary University of London, London, UK [5]. The mean mapping depth for all 19 isolates was 76 (range 43–144). On average, the coverage of the reference genome (the percentage of bases with a quality score of >15) was 95.2% (range 71.8–99.3%). Single-nucleotide polymorphisms (SNPs) were considered valid if supported by at least two and >70% of mapped reads on each strand with a minimum mapping quality of 45.

The index case (case A) was diagnosed 3 months after arrival in Finland (fig. 1a). His relative refused to be examined and was later diagnosed with TB after an obligatory visit to the school nurse, after he had attended school 1 for 2 weeks (case B). He had sputum smear-positive, pulmonary TB with cavitary lesions at the chest radiography. The contact investigation of case B revealed four additional TB cases in school 1 (cases C, E, H and I) and two cases in school 2 (cases D and G; relatives and friends of case B). Three more TB cases were detected among the contacts of case B: a relative (case K, who is also related to case A) and two friends (cases L and N).

Based on genotyping results, three additional cases were identified. Case F was a Finnish-born female with an autoimmune disease receiving tumour necrosis factor (TNF)- $\alpha$  blocking treatment. She developed TB meningitis and the only link between her and the other outbreak cases was that she had attended the same private party with case B in September 2012. Case M was a Finnish-born student and case J was a 32-year-old male of East-African origin. Evident epidemiological links to the other outbreak cases could not be established for these two cases despite strenuous efforts.

Contact investigation in the city of Turku involved more than 600 close contacts. The social network surrounding the TB cases among school-age contacts is shown in figure 1b. Cases B, C and I were connected to the majority of school-age contacts (73 (87%) out of 84) and case B was located at a central position, connecting cases from different schools and social settings.

A total of 219 close contacts aged <35 years were tested by IGRA and 46 (21%) were positive. Overall, 31 (16%) out of the 190 Finnish-born contacts and 15 (52%) out of the 29 foreign-born contacts were IGRA positive. Additionally, case B was epidemiologically linked to the majority of IGRA-positive contacts (33

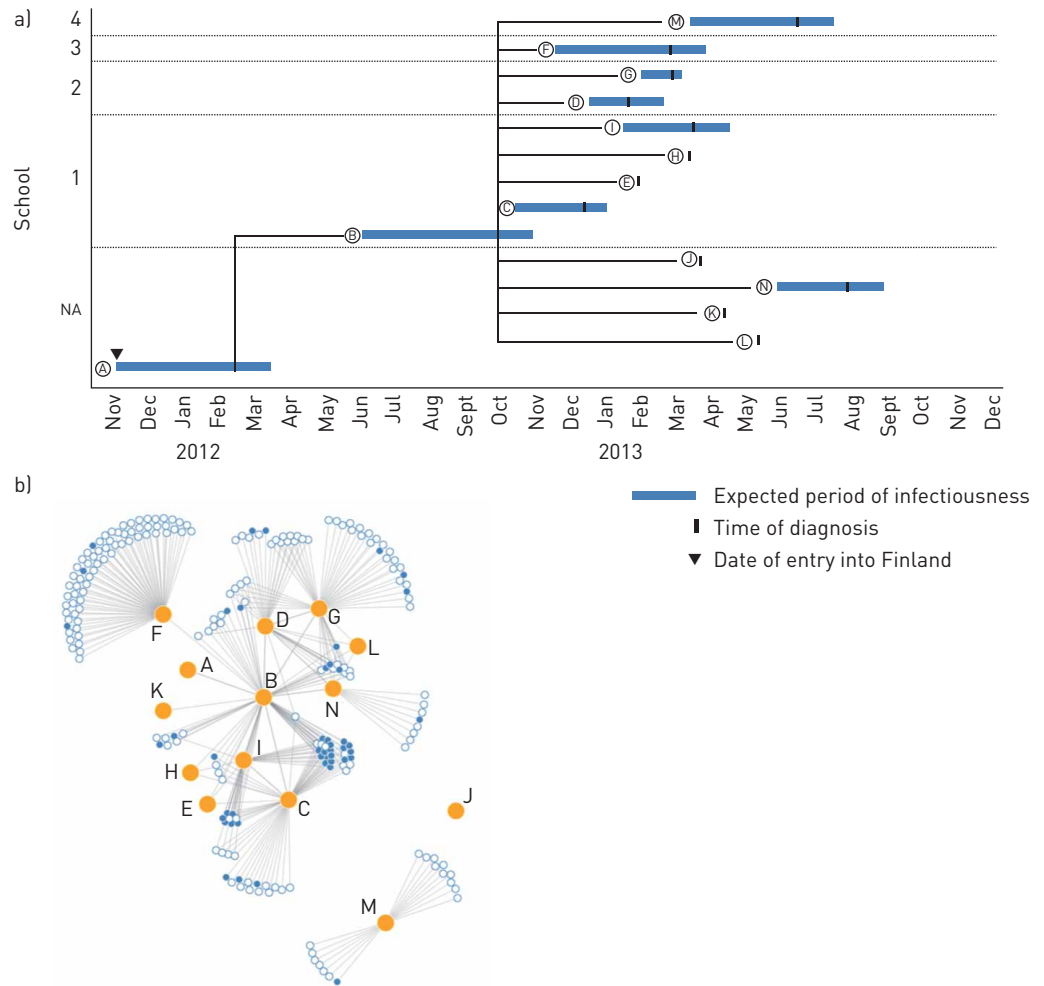


FIGURE 1 a) Transmission dynamics of the 14 tuberculosis (TB) cases in 2012–2013, Turku, Finland. TB cases are designated (A–N) in order of date of diagnosis. b) Social network analysis of school-aged close contacts involved in the TB outbreak in Turku, Finland, 2012–2013. Orange: TB cases; blue: interferon- $\gamma$  release assay (IGRA)-positive close contacts; white: IGRA-negative close contacts.

(72%) out of 46). When medically examined, four (9%) of the IGRA-positive contacts (cases H, I, K and L) appeared to have active TB disease. LTBI treatment was offered to all IGRA-positive contacts.

All the 12 outbreak isolates had identical genotypic profiles (SIT149 and 594-15), suggesting a clonal outbreak. Based on WGS results, one SNP was found in 11 of the outbreak isolates and not found in the index case (suggesting directionality from case A to other cases). The SNP was found in Rv3915, a coding sequence probably involved in cellular metabolism. Moreover, an additional SNP between 10 of the outbreak isolates and case J was noticed (two SNP differences between cases A and J). The SNP was found in Rv2152c, MurC, a region coding for a cell wall biosynthesis enzyme. The seven historical isolates differed from the outbreak isolates by 15–63 SNPs.

In our study, social network analysis, WGS and IGRA data were used together to establish and interpret the transmission dynamics of an ongoing TB outbreak. All culture-positive cases, including the two cases with no epidemiological link, and the seven historical isolates had the same MIRU-24 and spoligotype profile. As genotyping results were not discriminatory enough to understand the transmission dynamics of this outbreak, WGS was used. WGS strongly confirmed the association of the two cases with no evident epidemiological links to the outbreak, whereas the historical isolates were clearly not linked. The interpretation of the WGS data was that transmission to casual contacts had occurred, unfortunately implying that a substantial number of young individuals had potentially been infected by TB in Turku. We were not able to continue the already extensive contact investigation given that no additional groups or locations could be identified. Since TB is such an uncommon disease in Finland, particularly among younger Finnish-born cases, additional training for clinicians in Turku has been given during the

epidemic. In November 2013, a relative of case B living in a different city developed TB, suggesting that the outbreak is still ongoing and spreading outside the city of Turku. Unfortunately, no microbiological confirmation was received for this case.

Several cases were diagnosed at an early stage because of positive IGRA results, limiting the possibility of transmitting the disease further. The strength of association between IGRA positivity and active TB is known to be weak to moderate [12] and complicated by high background rates [13], meaning that it may be difficult to differentiate previous TB infection from recent transmission in individuals originating from countries with high TB incidence [14]. This was also seen in our study, where 52% of foreign-born contacts were IGRA positive. Since contacts exposed to TB patients are at substantial risk of developing LTBI and active TB [15], a notification was made in the medical records of IGRA-positive contacts that refused LTBI treatment.

The delayed diagnosis of one very infectious case resulted in a large TB outbreak and caused a considerable resource burden for the city of Turku. Awareness of the possibility of TB must be heightened, especially in immigrants with congruous clinical presentation. Based on this outbreak and several recent TB transmission incidents in schools in Finland, the Finnish guidelines are being modified to offer TB screening not only for refugees or asylum seekers but for all individuals arriving in Finland from countries with high TB incidence.



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**TB outbreak investigation can be enhanced by using whole genome sequencing, IGRA and social network analysis** <http://ow.ly/AzxfH>

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## False-negative interferon- $\gamma$ release assay results in active tuberculosis: a TBNET study

*To the Editor:*

Tuberculosis is one of the leading causes of morbidity and mortality worldwide [1]. Rapid identification of contagious tuberculosis patients and effective treatment are necessary to prevent the spread of *Mycobacterium tuberculosis*, the causative bacterium of the disease. Although interferon- $\gamma$  release assays (IGRAs) have been developed for the diagnosis of latent infection with *M. tuberculosis*, these assays are sometimes used as adjunctive tests in the diagnostic workup for active tuberculosis, despite poor specificity [2].

A systematic review and meta-analysis [2] found a pooled sensitivity for the diagnosis of culture-proven active tuberculosis of 81% and 92% of the QuantiFERON Gold in-tube test (QFT-GIT) (Qiagen, Dusseldorf, Germany) and the T-SPOT.TB test (Oxford Immunotec, Oxford, UK), respectively. Thus, approximately 8–19% of patients have a negative IGRA result when presenting with active tuberculosis.

Several risk factors were associated with negative IGRA results including immunodeficiency, young or advanced age, a negative tuberculin skin test (TST) result, extrapulmonary tuberculosis, disseminated tuberculosis, concomitant tuberculosis treatment and smoking. However, these studies were limited by an observational design implemented in single centres and most of them did not include large numbers of patients with culture-confirmed tuberculosis.

An international, multicentre, retrospective, cross-sectional study was performed by the Tuberculosis Network European Trials Group (TBNET) ([www.tb-net.org](http://www.tb-net.org)) to identify risk factors associated with false-negative IGRA results in patients with active tuberculosis.

Clinical data and laboratory results from patients enrolled at 25 participating centres with a confirmed diagnosis of active tuberculosis (*i.e.* positive *M. tuberculosis* culture and/or positive *M. tuberculosis*-specific nucleic acid amplification assay) who had a routine IGRA investigation by the T-SPOT.TB test or the QFT-GIT, as part of the diagnostic evaluation between April 2006 and May 2011, were retrospectively recorded on a standardised anonymous questionnaire. For each patient with a negative IGRA test result, two tuberculosis patients with a positive IGRA result admitted directly before and after the patient with a negative IGRA result were included as controls. Immunocompromised patients were defined as patients with at least one of the following medical conditions: HIV infection, treatment with immunosuppressive