



# 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 cavitory 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

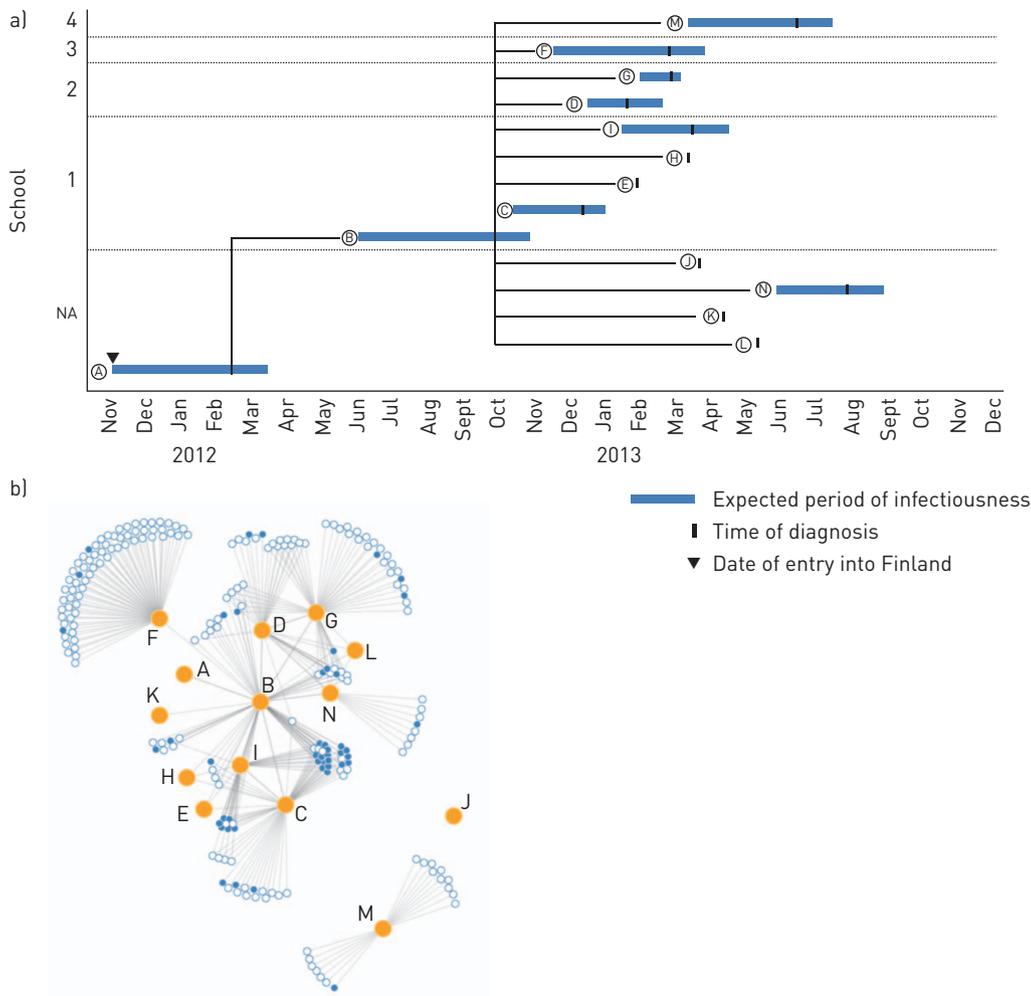


FIGURE 1 a) Transmission dynamics of the 14 tuberculosis (TB) cases in 2012–2013, Turku, Finland. TB cases are designated (A–N) in order of date of diagnosis. b) Social network analysis of school-aged close contacts involved in the TB outbreak in Turku, Finland, 2012–2013. Orange: TB cases; blue: interferon-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

(33 (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.



@ERSpublications

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

Pieter W. Smit<sup>1,2</sup>, Tuula Vasankari<sup>3,4</sup>, Hanna Aaltonen<sup>3</sup>, Marjo Haanperä<sup>2</sup>, Nicola Casali<sup>5,6</sup>, Harri Marttila<sup>7</sup>, Jane Marttila<sup>8</sup>, Päivi Ojanen<sup>3</sup>, Aino Ruohola<sup>9</sup>, Petri Ruutu<sup>2</sup>, Francis Drobniewski<sup>5,6,10</sup>, Outi Lyytikäinen<sup>2</sup> and Hanna Soini<sup>2</sup>

<sup>1</sup>European Public Health Microbiology Training Programme (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden. <sup>2</sup>Dept of Infectious Disease Surveillance and Control, National Institute for Health and Welfare, Helsinki and Turku, Finland. <sup>3</sup>Dept of Respiratory Diseases, Turku University Hospital, Turku, Finland. <sup>4</sup>Finnish Lung Health Association (FILHA), Helsinki, Finland. <sup>5</sup>Dept of Infectious Diseases and Immunity, Imperial College, London, UK. <sup>6</sup>Centre for Immunology and Infectious Disease, Blizard Institute, Queen Mary University of London, London, UK. <sup>7</sup>Dept of Hospital Hygiene and Infection Control, Turku University Hospital, Turku, Finland. <sup>8</sup>Infection Control Unit, Welfare Division, City of Turku, Turku, Finland. <sup>9</sup>Dept of Pediatrics and Adolescent Medicine, Turku University Hospital, Turku, Finland. <sup>10</sup>National Mycobacterium Reference Laboratory, London, UK.

Correspondence: Pieter Smit, Dept of Infectious Disease Surveillance and Control, National Institute for Health and Welfare, THL, Helsinki, FI 00271, Finland. E-mail: Pieter.smit@thl.fi

Received: June 19 2014 | Accepted after revision: Aug 12 2014

Conflict of interest: None declared.

Acknowledgements: We would like to thank Freek de Bruin (Leiden University Medical Centre, Leiden, the Netherlands) for creating the figures, Androulla Efstratiou (Public Health England, Colindale, UK) and Aftab Jasir (European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden) for critically reading the manuscript, and the technicians of THL Mycobacterial Reference Laboratory for excellent technical assistance. We also thank the personnel from the Infection Control Unit, School Health and Occupational Health of the City of Turku, the Chest Clinic and the Hospital Hygiene and Infection Control of Turku University Hospital.

## References

- 1 Walker TM, Ip CL, Harrell RH, *et al.* Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study. *Lancet Infect Dis* 2013; 13: 137–146.
- 2 Kato-Maeda M, Ho C, Passarelli B, *et al.* Use of whole genome sequencing to determine the microevolution of *Mycobacterium tuberculosis* during an outbreak. *PLoS One* 2013; 8: e58235.
- 3 Zhang H, Li D, Zhao L, *et al.* Genome sequencing of 161 *Mycobacterium tuberculosis* isolates from China identifies genes and intergenic regions associated with drug resistance. *Nat Genet* 2013; 45: 1255–1260.
- 4 Gardy JL, Johnston JC, Ho Sui SJ, *et al.* Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. *N Engl J Med* 2011; 364: 730–739.
- 5 Casali N, Nikolayevskyy V, Balabanova Y, *et al.* Microevolution of extensively drug-resistant tuberculosis in Russia. *Genome Res* 2012; 22: 735–745.
- 6 Roetzer A, Diel R, Kohl TA, *et al.* Whole genome sequencing *versus* traditional genotyping for investigation of a *Mycobacterium tuberculosis* outbreak: a longitudinal molecular epidemiological study. *PLoS Med* 2013; 10: e1001387.
- 7 Diel R, Loddenkemper R, Zellweger JP, *et al.* Old ideas to innovate tuberculosis control: preventive treatment to achieve elimination. *Eur Respir J* 2013; 42: 785–801.
- 8 D'Ambrosio L, Dara M, Tadolini M, *et al.* Tuberculosis elimination: theory and practice in Europe. *Eur Respir J* 2014; 43: 1410–1420.
- 9 Esposito S, D'Ambrosio L, Tadolini M, *et al.* ERS/WHO Tuberculosis Consilium assistance with extensively drug-resistant tuberculosis management in a child: case study of compassionate delamanid use. *Eur Respir J* 2014; 44: 811–815.

- 10 Codecasa LR, Ciconali G, Mazzola E, *et al.* Managing an XDR-TB outbreak: the public health face of the medal. *Eur Respir J* 2014 [In press DOI: 10.1183/09031936.00134114].
- 11 Smit PW, Haanperä M, Rantala P, *et al.* Molecular epidemiology of tuberculosis in Finland, 2008–2011. *PLoS One* 2013; 8: e85027.
- 12 Rangaka MX, Wilkinson KA, Glynn JR, *et al.* Predictive value of interferon- $\gamma$  release assays for incident active tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; 12: 45–55.
- 13 Hinks TS, Varsani N, Godsiff DT, *et al.* High background rates of positive tuberculosis-specific interferon- $\gamma$  release assays in a low prevalence region of UK: a surveillance study. *BMC Infect Dis* 2012; 12: 339.
- 14 Bradshaw L, Davies E, Devine M, *et al.* The role of the interferon gamma release assay in assessing recent tuberculosis transmission in a hospital incident. *PLoS One* 2011; 6: e20770.
- 15 Fox GJ, Barry SE, Britton WJ, *et al.* Contact investigation for tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2013; 41: 140–156.

Eur Respir J 2014; In Press | DOI: 10.1183/09031936.00125914 | Copyright ©ERS 2014