TITLE PAGE: *J Clin Microbiol, full report – Revision 1*

Standard genotyping overestimates transmission of *Mycobacterium tuberculosis* among immigrants in a low incidence country

David Stucki [1,2,3], Marie Ballif [1,2,3], Matthias Egger [3], Hansjakob Furrer [4], Ekkehardt Altpeter [5], Manuel Battegay [6], Sara Droz [7], Thomas Bruderer [8], Mireia Coscolla [1,2], Sonia Borrell [1,2], Kathrin Zürcher [3], Jean-Paul Janssens [9], Alexandra Calmy [10], Jesica Mazza Stalder [11], Katia Jaton [12], Hans L. Rieder [13], Gaby E. Pfyffer [14], Hans H. Siegrist [15], Matthias Hoffmann [16], Jan Fehr [17], Marisa Dolina [18], Reno Frei [19], Jacques Schrenzel [20], Erik C. Böttger [21], Sebastien Gagneux [1,2], Lukas Fenner [1,2,3,#]; for the Swiss HIV Cohort and Molecular Epidemiology of Tuberculosis Study Groups [*]

1 Swiss Tropical and Public Health Institute, Basel, Switzerland
2 University of Basel, Basel, Switzerland
3 Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland
4 Clinic for Infectious Diseases, Bern University Hospital and University of Bern, Bern, Switzerland
5 Division of Communicable Diseases, Federal Office of Public Health, Bern, Switzerland
6 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland
7 Mycobacteriology Unit, Institute for Infectious Diseases, University of Bern, Bern, Switzerland
8 Centre for Laboratory Medicine, St.Gallen, Switzerland
9 Division of Pneumology, University Hospital Geneva, Geneva, Switzerland
10 Division of Infectious Diseases, University Hospital Geneva, Geneva, Switzerland
11 Division of Pneumology, University Hospital Lausanne, Lausanne, Switzerland
12 Institute of Microbiology, University Hospital of Lausanne, Lausanne, Switzerland
13 Epidemiology, Biostatistics and Prevention Institute, University of Zürich, Switzerland
14 Department of Medical Microbiology, Luzerner Kantonsspital, Lucerne, Switzerland
15 ADMed Microbiology, La Chaux-de-Fonds, Switzerland
16 Division of Infectious Diseases, Kantonsspital St. Gallen, St. Gallen, Switzerland
17 University Hospital Zurich, and University of Zurich, Zurich, Switzerland
18 Clinical Microbiology, EOLAB, Bellinzona, Switzerland
19 Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland
20 Laboratory of Bacteriology, University Hospitals of Geneva, Geneva, Switzerland
21 Institute of Medical Microbiology, National Center for Mycobacteria, University of Zurich, Zurich, Switzerland

[*] All members of the study groups are listed in the Acknowledgements

Running title:

Tuberculosis transmission among immigrants

Word count and inserts:

250 words (abstract), 2945 words (main text), 2 tables, 3 figures, 47 references, supplementary information (2 figures, 1 table)

Keywords:

Tuberculosis; transmission; immigrant; MIRU-VNTR; whole genome sequencing; molecular cluster; Swiss HIV Cohort; Switzerland; low TB incidence

 # Corresponding author:

Lukas Fenner, Swiss Tropical and Public Health Institute, Basel; and Institute of Social and Preventive Medicine, University of Bern, Switzerland

Email: lukas.fenner@unibas.ch; lukas.fenner@ispm.unibe.ch

ABSTRACT

Immigrants from high tuberculosis (TB) incidence regions are a risk group for TB in low-incidence countries such as Switzerland. In a previous analysis of a nationwide collection of 520 *Mycobacterium tuberculosis* isolates from 2000-2008, we identified 35 clusters comprising 90 patients based on standard genotyping (24-loci MIRU-VNTR and spoligotyping). Here, we used whole genome sequencing (WGS) to revisit these transmission clusters. Genome-based transmission clusters were defined as isolate pairs separated by ≤12 single nucleotide polymorphisms (SNPs). WGS confirmed 17/35 (49%) MIRU-VNTR clusters; the other 18 clusters contained pairs separated by >12 SNPs. Most transmission clusters (3/4) of Swiss-born patients were confirmed by WGS, as opposed to 25% (4/16) of clusters involving only foreign-born patients. The overall clustering proportion using standard genotyping was 17% (90 patients, 95% confidence interval [CI]: 14-21%), but only 8% (43 patients, 95% CI: 6-11%) using WGS. The clustering proportion was 17% (67/401, 95% CI: 13-21%) using standard genotyping and 7% (26/401, 95% CI: 4-9%) using WGS among foreign-born patients, and 19% (23/119, 95% CI: 13-28%) and 14% (17/119, 95% CI: 9-22%), respectively, among Swiss-born patients. Using weighted logistic regression, we found weak evidence for an association between birth origin and transmission (aOR 2.2, 95% CI: 0.9-5.5, comparing Swiss-born patients to others). In conclusion, standard genotyping overestimated recent TB transmission in Switzerland when compared to WGS, particularly among immigrants from high TB incidence regions, where genetically closely related strains often predominate. We recommend the use of WGS to identify transmission clusters in low TB incidence settings.

INTRODUCTION

Tuberculosis (TB) remains an important public health concern in European countries (1–3). Immigrants from high TB-incidence countries and HIV-infected populations are common risk groups in Switzerland, as in other European countries (4–8). We and others have previously shown that transmission of *Mycobacterium tuberculosis* occurs, but is not more common among immigrants than in the native population (9–12).

Mycobacterial interspersed repetitive-unit–variable-number tandem-repeat (MIRU-VNTR), combined with spoligotyping (13, 14), remains the most commonly used genotyping method in molecular epidemiology of TB (15). However, MIRU-VNTR may not distinguish between genetically closely related strains despite the absence of close epidemiological links between patients (16–18). MIRU-VNTR may be suboptimal to study transmission among immigrants from high TB incidence countries, where genetically closely related strains circulate over extended periods of time (19). Hence, recent transmission among immigrants is potentially overestimated, but the extent of this phenomenon is largely unknown. In contrast to standard genotyping methods, whole genome sequencing (WGS) provides an increased resolution and has been used to study *M. tuberculosis* transmission (20–24). In this study, we re-analyzed transmission clusters previously defined by MIRU-VNTR, using WGS to assess transmission of *M. tuberculosis* among Swiss- and foreign-born TB patients (9).

Journal of Clinical Microbiology

MATERIALS AND METHODS

Study setting and study population

In 2012, we conducted a nationwide study of the molecular epidemiology of TB in Switzerland as a collaborative project between the Swiss HIV Cohort Study (SHCS), the National Center for Mycobacteria, diagnostic microbiology laboratories, departments of respiratory medicine and public health, and the Federal Office of Public Health (www.tb-network.ch) (9, 25–28). The study setting was previously described in detail (9). Briefly, all patients in the SHCS diagnosed with TB between 2000 and 2008 were enrolled (n=93). In addition, we included a random sample of 288 TB cases from the 4,221 culture-confirmed TB patients reported to the National TB Surveillance Registry, and all drug-resistant TB cases reported in Switzerland (n=167) during the same period (categories not mutually exclusive). 24-loci MIRU-VNTR and spoligotyping were used for the molecular detection of transmission clusters (9). In this follow-up study, we performed WGS on the 90 *M. tuberculosis* isolates belonging to one of the 35 MIRU-VNTR/spoligotyping clusters.

Clinical data collection and definitions

The clinical data collection was previously described in detail (9). The clustering proportion was determined by the “n” method expressed as the number of patients in clusters divided by the total number of individuals (29). MIRU-VNTR clusters were defined as a group of isolates with identical MIRU-VNTR and spoligotyping patterns (9). In addition, we used IS6110-Restriction Fragment Length Polymorphism (RFLP) patterns, when available from the National Center for Mycobacteria (30). RFLP has a higher resolution than MIRU-VNTR, particularly in strains of the “Beijing” genotype.

Journal of Clinical Microbiology

Isolates with identical MIRU-VNTR and spoligotype patterns, but different IS6110 patterns, were considered as non-clustered. “Mixed” molecular clusters were defined as clusters with Swiss-born and foreign-born individuals, or foreign-born individuals from different continents.

Whole genome sequencing and phylogenetic analyses

We generated whole genome sequences for all 90 patient isolates identified as part of MIRU-VNTR clusters (9). We used Illumina Nextera XT or TruSeq library preparation kits and Illumina HiSeq, MiSeq or NextSeq devices (Illumina, San Diego, CA) for WGS according to manufacturer’s instructions. Isolates were re-sequenced when the mean read depth was below 20x. FastQ files from multiple sequencing runs of the same isolate were merged. We used KvarQ for initial quality check, determination of *M. tuberculosis* phylogenetic lineages and *in silico* spoligotyping pattern, as previously described (31). We then mapped short sequencing reads to a hypothetical *M. tuberculosis* ancestral genome (identical with H37Rv in structure, but with maximum likelihood-inferred ancestral bases (32), with BWA 0.6.2 (33)). Samtools 0.1.19 was used to call variants (SNPs). We only retained positions with a read depth of ≥10% and ≤200% of the average read depth for the whole genome, and a phred-scaled quality score of ≥30. We excluded positions in known repetitive regions (23), as well as SNPs in genes in which we have previously identified 50 bp sequences with homologous sequences elsewhere on the genome (Supplementary Table 1). We also excluded positions associated with drug resistance (31). For the analyses of read/allele counts at particular genomic loci, we extracted the number of high quality bases from Variant Call Format (VCF)-files with SNPeff/SNPsift (34).

Transmission networks based on SNP distances

We generated an alignment of all variable positions across the 90 isolates. We then calculated the raw genetic distances (number of SNPs) for each isolate pair in each MIRU-VNTR cluster with the "Compute Pairwise Distances" function (using the "Pairwise deletion" option) in MEGA 5.2.2 (35). We defined a MIRU-VNTR cluster as a "true" transmission cluster if all isolate pairs in the cluster were separated by ≤12 SNPs. In a sensitivity analysis, we opted for a stricter definition, whereby a MIRU-VNTR transmission cluster was considered as confirmed if at least one of its isolate pairs was separated by ≤5 SNPs. These thresholds of 12 and 5 SNPs were previously established by Walker *et al*. (17). We imported an alignment of the variable genomic positions into popart (http://popart.otago.ac.nz) to generate median joining networks. Networks were generated for all 35 transmission clusters identified by standard genotyping (MIRU-VNTR and spoligotyping) (9).

Statistical analysis

We re-analyzed risk factors for transmission using the WGS-based ("true") cluster definition. We used weighted logistic regression models to obtain age- and sex-adjusted odds ratios (aOR) for the probability of belonging to a true molecular cluster. We used the Kruskal-Wallis rank sum test to assess differences between mean genetic distances of Swiss-born, foreign-born and mixed clusters. As our study sample included, by design, more HIV-infected and patients with drug-resistant TB (9), we calculated weights to take sampling proportions into account. As a sensitivity analysis, we restricted the analysis of clustering proportion to the patients in the random sample from the TB registry (n=288) (9). All statistical analyses were performed in Stata version 14 (Stata Corporation, College Station, TX) and R 3.1.2 (36).

In addition, we plotted the mean genetic distances (in SNPs) versus the mean geographical distances (in km) of all patient pairs in a molecular cluster (distance between the birth countries' capital cities). Plots were generated with the ggplot2 library in R (37).

Ethics approval

The study was approved by the Ethics Committee of the Canton of Bern, Switzerland (9). Informed consent was obtained from all patients enrolled in the SHCS. For patients outside the SHCS, informed consent was obtained by the treating physicians. In some cases, informed consent could not be obtained from the patient, because he or she could not be located or was known to have died. For these cases, we obtained permission from the Federal Expert Commission on Confidentiality in Medical Research to use the data provided by the treating physician.

RESULTS

Study population

The study population consisted of 520 TB patients from the nationwide study in Switzerland (9, 25, 27, 28, 38). The patient characteristics are described in Table 1. A total of 119 (22.9%) patients were born in Switzerland and 401 (77.1%) abroad. Median age was 36.5 years (Interquartile Range 28–51). Overall, 113 (21.7%) patients were HIV positive. Pulmonary TB accounted for 387 (74.4%) of all cases and extrapulmonary TB for 133 (25.6%) (Table 1).

Transmission clusters

Whole genome sequencing

Isolates were sequenced with a median sequencing depth of 130x (range 22-274x). For quality assurance, we compared laboratory-assay-based phylogenetic lineage classification and spoligotyping pattern with the results generated from the WGS data using KvarQ (9). We found 100% agreement between the two methods for lineage identification and up to two discordant spacers in the spoligotyping patterns.

Identification of molecular clusters based on WGS

In the 35 previously defined MIRU-VNTR clusters, we found pairwise genetic distances of 0 to 224 SNPs (median: 21.5 SNPs) (Figure 1 and Supplementary Figure 1). In the largest cluster (eight isolates), genetic distances were ≥54 SNPs. Seventeen of 35 (48.6%) MIRU-VNTR clusters consisted of pairs separated by ≤12 SNPs, i.e. were confirmed as true transmission clusters, corresponding to 43 of 90 patients (47.8%) (Table 2). The remaining 18 clusters harbored isolate pairs separated by >12 SNPs (47 patients).

The overall clustering proportion decreased from 17.3% (95% confidence interval [95% CI]: 14.2-20.8%) based on standard genotyping (spoligotyping and MIRU-VNTR), to 8.3% (95% CI: 6.0-11.0%) based on WGS. When restricting the analysis to the 288 randomly selected patients, we found 27 clustered patients in 11 genome-based clusters, resulting in a clustering proportion of 9.4% (95% CI: 6.3-13.3%). When using a more stringent WGS definition for transmission clusters (at least one isolate pair in a cluster ≤5 SNPs distance), 13 of 35 (37.1%, CI: 21.5-55.1%) MIRU-VNTR clusters were confirmed. These 13 transmission clusters included 35/520 patients, corresponding to a clustering proportion of 6.7% (95% CI: 4.7-9.2%).

Infection with multiple M. tuberculosis strains

In five isolates that were part of transmission clusters defined by MIRU-VNTR, we detected multiple alleles at several MIRU-VNTR loci, potentially indicating infection with multiple *M. tuberculosis* strains. We therefore conducted an allele frequency analysis based on sequencing reads for each SNP call. Despite the presence of multi-allelic variant calls in all isolates (potential microevolutionary events), none of the five isolates with multiple MIRU-VNTR bands showed evidence of lineage- or sublineage-specific markers with multiple alleles in the sequencing reads.

Molecular clustering in Swiss-born, foreign-born and HIV-positive patients

Four MIRU-VNTR clusters involved Swiss-born patients only, 16 clusters foreign-born only and 15 clusters were of mixed birth group origin. Three of four clusters (75.0%) involving only Swiss-born patients were confirmed by WGS as true clusters (8/10 [80.0%] clustered Swiss-born patients). On the other hand, only 4/16

(25.0%) immigrant clusters (born on the same continent) were true clusters (8/37 [21.6%] patients). Of the 15 mixed clusters, 10 (66.7%) were true clusters (27/43 [62.8%] clustered patients) (Table 2). We assessed whether foreign-born patients were overrepresented in MIRU-VNTR clusters not confirmed by WGS. Among all 90 patients from the MIRU-VNTR clusters, foreign-born patients were more likely in clusters not confirmed by WGS compared to true clusters (aOR 4.5, CI: 1.5-13.6 p=0.008) (Table 2).

We then calculated the true (genome-based) clustering proportion for both Swiss- and foreign-born patients. The clustering proportion among Swiss-born cases decreased only slightly, from 19.3% (23/119, 95% CI: 12.7-27.6%) using MIRU-VNTR to 14.3% (17/119, 95% CI: 8.5-21.9) using WGS data. In contrast, the clustering proportion among immigrants was more than halved, from 16.7% (67/401, 95% CI: 13.2-20.7) to 6.5% (26/401, 95% CI: 4.3-9.4). Figure 2 summarizes the possible factors leading to an overestimation of *M. tuberculosis* transmission based on standard genotyping, among foreign-born and native TB patients in low TB incidence settings.

The median genetic distance differed significantly between the three groups: 9 SNPs (range 8-15) in clusters with Swiss-born individuals only, 2 SNPs (range 0-162) in mixed clusters, and 24 SNPs (2-224) in clusters with foreign-born individuals only (p=0.030, Figure 3).

Geographical and genetic distances within molecular clusters

Plotting the mean genetic distance (in SNPs) versus the mean geographical distance between patient origins (capital cities) for each molecular cluster further supported the different patterns of clustering by birth origin of the patients (Supplementary Figure 2). Among foreign-born patients, a majority of MIRU-VNTR

clusters (11/16, 68.8%) consisted of patients born within 3,500 km of geographic distance, but harboring genetic distances of >12 SNPs. This indicates that genetically closely related strains, circulating in a geographically restricted area in the region of origin, were imported to Switzerland. Among the mixed MIRU-VNTR clusters, for which transmission is expected to have happened in Switzerland, a majority of clusters (9/15, 60%) had mean geographic distances above 3,500 km, but genetic distances of ≤12 SNPs, indicating recent transmission in Switzerland (Supplementary Figure 2).

Risk factors for transmission

Female (aOR 0.39, CI: 0.18-0.85) and HIV-positive patients (aOR 0.39, CI: 0.16-0.93) were significantly less likely to be involved in true transmission clusters (Table 1). In contrast, patients with cavitary disease were more likely to be associated with transmission (aOR 2.31, CI: 1.05-5.10). There was a weak evidence for an association between being born in Switzerland and being involved in a true transmission cluster (aOR 2.21, 95% CI: 0.88-5.52 for Swiss-born compared to foreign-born patients). Overall, the risk factors for transmission remained similar to those previously reported when using standard genotyping methods (9).

DISCUSSION

In this nationwide study on 520 TB patients, 18 of 35 transmission clusters identified by standard molecular genotyping (spoligotyping and MIRU-VNTR) were refuted by WGS. This suggests that transmission of *M. tuberculosis* is generally overestimated in low TB incidence countries such as Switzerland. Furthermore, we found a striking difference between transmission clusters involving Swiss-born cases and clusters involving foreign-born patients. WGS confirmed three quarters of the clusters involving Swiss-born individuals only, but only one quarter of clusters involving foreign-born patients only, hence indicating that transmission was especially overestimated among the immigrant population.

M. tuberculosis strains from immigrants, which were defined as clustered by MIRU-VNTR, but not by WGS, are likely genetically closely related genotypes, imported independently from a high-incidence region, where they are highly prevalent (19). Such strains accumulate genetic mutations over time (often leading to pairwise SNP distances of >12 SNPs), but the MIRU-VNTR pattern may not change. In such a situation, identical MIRU-VNTR will be wrongly interpreted as recent transmission in the country of immigration (9). Similar observations were made in the UK, where immigrant TB cases were identified in transmission clusters based on standard MIRU-VNTR genotyping, although no epidemiological link could be found during contact investigations (16).

The clustering proportion (indicating recent transmission) among Swiss-born individuals was similar using standard genotyping and WGS, but more than two-fold lower among foreign-born individuals when using WGS. In reality, the clustering proportion among foreign-born individuals might even be lower, as we cannot exclude that WGS-confirmed clusters (≤12 SNPs) involving immigrants might partly

Journal of Clinical Microbiology

represent transmission that happened in the country of origin and not in Switzerland. Only social contact tracing could provide further insights into transmission dynamics, but such investigations are notoriously difficult to perform, particularly among immigrants (23, 39). The low proportion of true transmission clusters among immigrants in our study was further supported by the weighted analysis of predictors for transmission, which showed that foreign-born TB cases tended to be less likely involved in true clusters compared to Swiss-born cases. Of note, the clustering proportion among immigrants is remarkably similar to previous observations among immigrant MDR cases diagnosed in Switzerland, which showed a clustering proportion of 8% (compared to 7% in our study) based on standard genotyping and contact tracing (30).

The majority of mixed molecular clusters as defined by MIRU-VNTR (i.e. involving Swiss- and foreign-born individuals) showed small SNP distances (≤12 SNPs), confirming the intuitive explanation that transmission between Swiss-born and foreign-born likely occurred in Switzerland. This was further supported by the analysis of geographical distances between patient birth countries, which indicated that most isolate pairs in the mixed clusters were from patients born far away from each other, despite small genetic distances. The five mixed clusters harboring larger SNP distances may reflect TB cases due to infections by circulating global or European *M. tuberculosis* genotypes, such as the recently described large cluster in Eastern Europe (40).

We found no evidence of infection with multiple strains among clustered TB cases using the WGS data, despite the presence of double alleles in the MIRU-VNTR patterns of five clustered isolates. Infections with multiple strains could potentially also influence the identification of molecular clusters, as individual strains in an infection with multiple strains cannot be resolved by MIRU-VNTR typing. The

prevalence and relevance of such multiple infections need to be further studied (41–43).

A potential limitation of our study is the definition of transmission clusters by WGS. The threshold of 12 SNPs, which we used to exclude transmission, has been established by Walker *et al.* (17) and is in the range of what other studies have used (21, 44, 45). However, an adequate cluster definition may be adapted according to the setting (low versus high TB incidence), the study population and the technical specifications of the WGS analysis pipeline (i.e. whether particular genomic regions such as the PE/PGRS genes are excluded from the analysis, which was the case here). For comparison, we repeated the analyses with a stricter definition of transmission clusters also proposed by Walker *et al.* (MIRU-VNTR clusters in which at least one pairwise distance was ≤5 SNPs (17)), which further reduced the number of true clusters to 13, but did not change the overall clustering proportion significantly. A further limitation may be our sample size: we included 12.3% of all TB cases diagnosed between 2000 and 2008 in Switzerland, which potentially underestimates the overall clustering proportion (29). Indeed, SNP distances could in fact become shorter upon inclusion of additional patient isolates with intermediate genotypes, hence increasing the proportion of true clusters.

In conclusion, only one quarter of foreign-born transmission clusters previously identified by MIRU-VNTR were confirmed as true transmission clusters by WGS. We therefore recommend the use of WGS for a more accurate identification of recent transmission of *M. tuberculosis* among immigrants in low TB incidence countries, but also in high TB incidence countries, where genetically closely related strains circulate. Although analyzing WGS remains resource-intensive, the strategy adopted in the UK documents that implementing WGS in the routine public laboratory surveillance system is feasible (21, 46), and allows the prompt identification of

Journal of Clinical Microbiology

transmission clusters as well as information about the drug resistance genotype (46, 47). Our results also indicate that the native population in Switzerland may also play a role in spreading TB, particularly individuals belonging to high risk populations (22, 23). Additional prospective studies using WGS are needed, possibly complemented with social network analyses (20), to evaluate the usefulness of real-time analyses of TB transmission dynamics in low TB incidence countries.

ACKNOWLEDGEMENTS

We thank all tuberculosis patients for participating in this study, the treating physicians for providing clinical information and the microbiology laboratories for providing strains. We are grateful to the National TB Surveillance Registry at the Federal Office of Public Health.

This work was supported by the Swiss National Science Foundation (grant no. 324730-12544 and PP00P3_150750), the Swiss HIV Cohort Study (grant no. 588 and 740), the Federal Office of Public Health (grant no. 09.007368), the National Center for Mycobacteria, and the European Research Council (grant no. 309540-EVODRTB). The Swiss HIV Cohort Study is supported by the Swiss National Science Foundation (grant no. 33CS30-134277). Sequencing calculations were performed at sciCORE (http://scicore.unibas.ch/) scientific computing core facility at the University of Basel. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The members of the Molecular Epidemiology of Tuberculosis Study Group

Central coordinating team:

Lukas Fenner and Matthias Egger, Institute of Social and Preventive Medicine, Bern; Sebastien Gagneux and Marcel Tanner, Swiss Tropical and Public Health Institute, Basel; Hansjakob Furrer, Inselspital Bern.

National Center for Mycobacteria:

Erik C. Böttger, Institute of Medical Microbiology, University of Zurich, Switzerland.

Microbiology laboratories:

Reno Frei, Clinical Microbiology, University Hospital Basel; Thomas Bodmer and Sara Droz, Institute for Infectious Diseases, University of Bern; Jacques Schrenzel, Laboratory of Bacteriology, University Hospitals of Geneva; Katia Jaton, Institute of

Journal of Clinical Microbiology

JCM

Journal of Clinical Microbiology

406 Microbiology, University Hospital of Lausanne; Hans Siegrist, ADMed Microbiology,
407 La Chaux-de-Fonds; Gaby E. Pfyffer, Department of Medical Microbiology, Luzerner
408 Kantonsspital, Luzern; Thomas Bruderer, Detlev Schultze, Centre for Laboratory
409 Medicine, St.Gallen; Marisa Dolina, Clinical Microbiology, EOLAB, Bellinzona,
410 Switzerland; Olivier Dubuis, Viollier AG Switzerland, Allschwil.
411 Swiss HIV Cohort Study:
412 Manuel Battegay, University Hospital Basel; Enos Bernasconi, Andrea Parini
413 Lugano; Matthias Hoffmann, St.Gallen; Hansjakob Furrer, Inselspital Bern; Matthias
414 Cavassini, University Hospital of Lausanne; Bernard Hirschel, Alexandra Calmy,
415 University Hospital of Geneva; Jan Fehr, University Hospital of Zurich.
416 Respiratory clinics:
417 Jean-Paul Janssens, University Hospital of Geneva; Jesica Mazza Stalder,
418 University Hospital of Lausanne.
419 Federal Office of Public Health:
420 Peter Helbling and Ekkehardt Altpeter, Division of Communicable Diseases.
421 Other:
422 Hans L. Rieder, Epidemiology, Biostatistics and Prevention Institute, University of
423 Zürich, Switzerland.

424 **The members of the Swiss HIV Cohort Study**

425 Barth J, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy
426 A, Cavassini M, Cellerai C, Egger M, Elzi L, Fehr J, Fellay J, Flepp M, Francioli P
427 (President of the SHCS), Furrer H (Chairman of the Clinical and Laboratory
428 Committee), Fux CA, Gorgievski M, Günthard H (Chairman of the Scientific Board),
429 Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hirschel B, Hösli I,
430 Kahlert C, Kaiser L, Keiser O, Kind C, Klimkait T, Kovari H, Ledergerber B,
431 Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Pantaleo G,

Journal of Clinical Microbiology

JCM

Journal of Clinical Microbiology

Rauch A, Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schmid P, Schultze D, Schöni-Affolter F, Schüpbach J, Speck R, Taffé P, Tarr P, Telenti A, Trkola A, Vernazza P, Weber R, Yerly S.

ACCESSION NUMBERS

Raw sequencing data were submitted to the European Nucleotide Archive (ENA) under project accession number PRJEB12179.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. **World Health Organization**. 2014. Global tuberculosis report 2014. WHO, Geneva, Switzerland.
2. **Falzon D**, **Kudjawu Y**, **Desenclos JC**, **Fernandez de la Hoz K**, **Dadu A**, **Zaleskis R**. 2008. Stopping TB in Europe: some progress but still not there. Euro Surveill **13**.
3. **Zellweger JP**. 2013. Current issues in the management of tuberculosis in Europe. Panminerva Med **55**:145–55.
4. **Federal Office of Public Health**. 2011. Tuberkulose in der Schweiz 2005-2009. Bull BAG 205–213.
5. **Abgrall S**, **del Giudice P**, **Melica G**, **Costagliola D**, **on behalf of FHDH-ANRS CO4 1**. 2010. HIV-associated tuberculosis and immigration in a high-income country: incidence trends and risk factors in recent years. AIDS **24**:763–771.
6. **Dahle UR**, **Eldholm V**, **Winje BA**, **Mannsåker T**, **Heldal E**. 2007. Impact of immigration on the molecular epidemiology of *Mycobacterium tuberculosis* in a low-incidence country. Am J Respir Crit Care Med **176**:930–935.
7. **Wolff H**, **Janssens JP**, **Bodenmann P**, **Meynard A**, **Delhumeau C**, **Rochat T**, **Sudre P**, **Costanza MC**, **Gaspoz JM**, **Morabia A**. 2010. Undocumented migrants in Switzerland: geographical origin versus legal status as risk factor for tuberculosis. J Immigr Minor Health **12**:18–23.
8. **Lillebaek T**, **Andersen AB**, **Bauer J**, **Dirksen A**, **Glismann S**, **de Haas P**, **Kok-Jensen A**. 2001. Risk of *Mycobacterium tuberculosis* transmission in a low-incidence country due to immigration from high-incidence areas. J Clin Microbiol **39**:855–61.
9. **Fenner L**, **Gagneux S**, **Helbling P**, **Battegay M**, **Rieder HL**, **Pfyffer GE**, **Zwahlen M**, **Furrer H**, **Siegrist HH**, **Fehr J**, **Dolina M**, **Calmy A**, **Stucki D**, **Jaton K**, **Janssens J-P**, **Stalder JM**, **Bodmer T**, **Ninet B**, **Böttger EC**, **Egger M**. 2012. *Mycobacterium tuberculosis* transmission in a country with low tuberculosis incidence: role of immigration and HIV infection. J Clin Microbiol **50**:388–395.
10. **Barniol J**, **Niemann S**, **Louis VR**, **Brodhun B**, **Dreweck C**, **Richter E**, **Becher H**, **Haas W**, **Junghanss T**. 2009. Transmission dynamics of pulmonary tuberculosis between autochthonous and immigrant sub-populations. BMC Infect Dis **9**:197.
11. **Kamper-Jorgensen Z**, **Andersen AB**, **Kok-Jensen A**, **Bygbjerg IC**, **Andersen PH**, **Thomsen VO**, **Kamper-Jorgensen M**, **Lillebaek T**. 2012. Clustered tuberculosis in a low-burden country: nationwide genotyping through 15 years. J Clin Microbiol **50**:2660–2667.
12. **Guzman Herrador BR**, **Rønning K**, **Borgen K**, **Mannsåker T**, **Dahle UR**. 2015. Description of the largest cluster of tuberculosis notified in Norway 1997-2011: is the Norwegian tuberculosis control programme serving its purpose for high risk groups? BMC Public Health **15**:367.
13. **Supply P**, **Mazars E**, **Lesjean S**, **Vincent V**, **Gicquel B**, **Locht C**. 2000.

Journal of Clinical Microbiology

Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. Mol Microbiol **36**:762–771.

14. **Kamerbeek J**, **Schouls L**, **Kolk A**, **van Agterveld M**, **van Soolingen D**, **Kuijper S**, **Bunschoten A**, **Molhuizen H**, **Shaw R**, **Goyal M**, **van Embden J**. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol **35**:907–914.

15. **Kato-Maeda M**, **Metcalfe JZ**, **Flores L**. 2011. Genotyping of *Mycobacterium tuberculosis*: application in epidemiologic studies. Future Microbiol **6**:203–216.

16. **Munang ML**, **Browne C**, **Khanom S**, **Evans JT**, **Smith EG**, **Hawkey PM**, **Kunst H**, **Welch SB**, **Dedicoat MJ**. 2015. Tuberculosis microepidemics among dispersed migrants, Birmingham, UK, 2004-2013. Emerg Infect Dis **21**:524–7.

17. **Walker TM**, **Ip CLC**, **Harrell RH**, **Evans JT**, **Kapatai G**, **Dedicoat MJ**, **Eyre DW**, **Wilson DJ**, **Hawkey PM**, **Crook DW**, **Parkhill J**, **Harris D**, **Walker AS**, **Bowden R**, **Monk P**, **Smith EG**, **Peto TEA**. 2013. Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study. Lancet Infect Dis **13**:137–146.

18. **Glynn JR**, **Bauer J**, **de Boer AS**, **Borgdorff MW**, **Fine PEM**, **Godfrey-Faussett P**, **Vynnycky E**, **European Concerted Action on Molecular Epidemiology and Control of Tuberculosis**. 1999. Interpreting DNA fingerprint clusters of *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis **3**:1055–1060(6).

19. **Wampande EM**, **Mupere E**, **Debanne SM**, **Asiimwe BB**, **Nsereko M**, **Mayanja H**, **Eisenach K**, **Kaplan G**, **Boom HW**, **Sebastien G**, **Joloba ML**. 2013. Long-term dominance of *Mycobacterium tuberculosis* Uganda family in peri-urban Kampala-Uganda is not associated with cavitary disease. BMC Infect Dis **13**:484.

20. **Gardy JL**, **Johnston JC**, **Ho Sui SJ**, **Cook VJ**, **Shah L**, **Brodkin E**, **Rempel S**, **Moore R**, **Zhao Y**, **Holt R**, **Varhol R**, **Birol I**, **Lem M**, **Sharma MK**, **Elwood K**, **Jones SJM**, **Brinkman FSL**, **Brunham RC**, **Tang P**. 2011. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. N Engl J Med **364**:730–739.

21. **Walker TM**, **Lalor MK**, **Broda A**, **Ortega LS**, **Morgan M**, **Parker L**, **Churchill S**, **Bennett K**, **Golubchik T**, **Giess AP**, **Del Ojo Elias C**, **Jeffery KJ**, **Bowler ICJW**, **Laurenson IF**, **Barrett A**, **Drobniewski F**, **McCarthy ND**, **Anderson LF**, **Abubakar I**, **Thomas HL**, **Monk P**, **Smith EG**, **Walker AS**, **Crook DW**, **Peto TEA**, **Conlon CP**. 2014. Assessment of *Mycobacterium tuberculosis* transmission in Oxfordshire, UK, 2007–12, with whole pathogen genome sequences: an observational study. Lancet Respir Med **2**:285–292.

22. **Roetzer A**, **Diel R**, **Kohl TA**, **Rückert C**, **Nübel U**, **Blom J**, **Wirth T**, **Jaenicke S**, **Schuback S**, **Rüsch-Gerdes S**, **Supply P**, **Kalinowski J**, **Niemann S**. 2013. Whole genome sequencing versus traditional genotyping for investigation of a *Mycobacterium tuberculosis* outbreak: A longitudinal molecular epidemiological study. PLoS Med **10**:e1001387.

23. **Stucki D**, **Ballif M**, **Bodmer T**, **Coscolla M**, **Maurer A-M**, **Droz S**, **Butz C**, **Borrell S**, **Längle C**, **Feldmann J**, **Furrer H**, **Mordasini C**, **Helbling P**, **Rieder HL**, **Egger M**, **Gagneux S**, **Fenner L**. 2015. Tracking a tuberculosis outbreak

over 21 years: strain-specific single-nucleotide polymorphism typing combined with targeted whole-genome sequencing. J Infect Dis **211**:1306–1316.

24. **Coscolla M**, **Barry PM**, **Oeltmann JE**, **Koshinsky H**, **Shaw T**, **Cilnis M**, **Posey J**, **Rose J**, **Weber T**, **Fofanov VY**, **Gagneux S**, **Kato-Maeda M**, **Metcalfe JZ**. 2015. Genomic epidemiology of multidrug-resistant *Mycobacterium tuberculosis* during transcontinental spread. J Infect Dis **212**:302–310.

25. **Fenner L**, **Egger M**, **Bodmer T**, **Altpeter E**, **Zwahlen M**, **Jaton K**, **Pfyffer GE**, **Borrell S**, **Dubuis O**, **Bruderer T**, **Siegrist HH**, **Furrer H**, **Calmy A**, **Fehr J**, **Stalder JM**, **Ninet B**, **Böttger EC**, **Gagneux S**. 2012. Effect of mutation and genetic background on drug resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother **56**:3047–53.

26. **Fenner L**, **Gagneux S**, **Janssens J-P**, **Fehr J**, **Cavassini M**, **Hoffmann M**, **Bernasconi E**, **Schrenzel J**, **Bodmer T**, **Böttger EC**, **Helbling P**, **Egger M**, **for the Swiss HIV Cohort and Molecular Epidemiology of Tuberculosis Study Groups**. 2012. Tuberculosis in HIV-negative and HIV-infected patients in a low-incidence country: clinical characteristics and treatment outcomes. PLoS One **7**:e34186.

27. **Fenner L**, **Malla B**, **Ninet B**, **Dubuis O**, **Stucki D**, **Borrell S**, **Huna T**, **Bodmer T**, **Egger M**, **Gagneux S**. 2011. “Pseudo-Beijing”: Evidence for convergent evolution in the direct repeat region of *Mycobacterium tuberculosis*. PLoS One **6**:e24737.

28. **Fenner L**, **Egger M**, **Bodmer T**, **Furrer H**, **Ballif M**, **Battegay M**, **Helbling P**, **Fehr J**, **Gsponer T**, **Rieder HL**, **Zwahlen M**, **Hoffmann M**, **Bernasconi E**, **Cavassini M**, **Calmy A**, **Dolina M**, **Frei R**, **Janssens J-P**, **Borrell S**, **Stucki D**, **Schrenzel J**, **Böttger EC**, **Gagneux S**, **for the Swiss HIV Cohort and Molecular Epidemiology of Tuberculosis Study Groups**. 2013. HIV infection disrupts the sympatric host–pathogen relationship in human tuberculosis. PLoS Genet **9**:e1003318.

29. **Glynn JR**, **Vynnycky E**, **Fine PE**. 1999. Influence of sampling on estimates of clustering and recent transmission of *Mycobacterium tuberculosis* derived from DNA fingerprinting techniques. Am J Epidemiol **149**:366–71.

30. **Somoskovi A**, **Helbling P**, **Deggim V**, **Hömke R**, **Ritter C**, **Böttger EC**. 2014. Transmission of multidrug-resistant tuberculosis in a low-incidence setting, Switzerland, 2006 to 2012. Eurosurveillance **19**.

31. **Steiner A**, **Stucki D**, **Coscolla M**, **Borrell S**, **Gagneux S**. 2014. KvarQ: targeted and direct variant calling from fastq reads of bacterial genomes. BMC Genomics **15**:881.

32. **Comas I**, **Chakravartti J**, **Small PM**, **Galagan J**, **Niemann S**, **Kremer K**, **Ernst JD**, **Gagneux S**. 2010. Human T cell epitopes of *Mycobacterium tuberculosis* are evolutionarily hyperconserved. Nat Genet **42**:498–503.

33. **Li H**, **Durbin R**. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics **25**:1754–1760.

34. **Cingolani P**, **Patel VM**, **Coon M**, **Nguyen T**, **Land SJ**, **Ruden DM**, **Lu X**. 2012. Using *Drosophila melanogaster* as a model for genotoxic chemical mutational studies with a new program, SnpSift. Front Genet **3**.

35. **Tamura K**, **Peterson D**, **Peterson N**, **Stecher G**, **Nei M**, **Kumar S**. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol **28**:2731–2739.

36. **R Core Team**. 2014. R: a language and environment for statistical computing. Vienna, Austria.

37. **Wickham H**. 2009. ggplot2: elegant graphics for data analysis. Springer-Verlag New York.

38. **Fenner L**, **Gagneux S**, **Janssens J-P**, **Fehr J**, **Cavassini M**, **Hoffmann M**, **Bernasconi E**, **Schrenzel J**, **Bodmer T**, **Böttger EC**, **Helbling P**, **Egger M**. 2012. Tuberculosis in HIV-negative and HIV-infected patients in a low-incidence country: clinical characteristics and treatment outcomes. PLoS One **7**:e34186.

39. **Cook VJ**, **Shah L**, **Gardy J**, **Bourgeois A-C**. 2012. Recommendations on modern contact investigation methods for enhancing tuberculosis control. Int J Tuberc Lung Dis **16**:297–305.

40. **De Beer JL**, **Kodmon C**, **van der Werf MJ**, **van Ingen J**, **van Soolingen D**, **ECDC MDR-TB Molecular Surveillance Project Participants**. 2014. Molecular surveillance of multi- and extensively drug-resistant tuberculosis transmission in the European Union from 2003 to 2011. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull **19**.

41. **Huyen MNT**, **Kremer K**, **Lan NTN**, **Cobelens FGJ**, **Buu TN**, **Dung NH**, **Caws M**, **Tiemersma EW**, **van Soolingen D**. 2012. Mixed tuberculosis infections in rural South Vietnam. J Clin Microbiol **50**:1586–92.

42. **Cohen T**, **Wilson D**, **Wallengren K**, **Samuel EY**, **Murray M**. 2011. Mixed-strain *Mycobacterium tuberculosis* infections among patients dying in a hospital in KwaZulu-Natal, South Africa. J Clin Microbiol **49**:385–8.

43. **Mallard K**, **McNerney R**, **Crampin AC**, **Houben R**, **Ndlovu R**, **Munthali L**, **Warren RM**, **French N**, **Glynn JR**. 2010. Molecular detection of mixed infections of *Mycobacterium tuberculosis* strains in sputum samples from patients in Karonga District, Malawi. J Clin Microbiol **48**:4512–8.

44. **Cohen KA**, **Abeel T**, **Manson McGuire A**, **Desjardins CA**, **Munsamy V**, **Shea TP**, **Walker BJ**, **Bantubani N**, **Almeida D V**, **Alvarado L**, **Chapman SB**, **Mvelase NR**, **Duffy EY**, **Fitzgerald MG**, **Govender P**, **Gujja S**, **Hamilton S**, **Howarth C**, **Larimer JD**, **Maharaj K**, **Pearson MD**, **Priest ME**, **Zeng Q**, **Padayatchi N**, **Grosset J**, **Young SK**, **Wortman J**, **Mlisana KP**, **O'Donnell MR**, **Birren BW**, **Bishai WR**, **Pym AS**, **Earl AM**. 2015. Evolution of extensively drug-resistant tuberculosis over four decades: whole genome sequencing and dating analysis of *Mycobacterium tuberculosis* isolates from KwaZulu-Natal. PLoS Med **12**:e1001880.

45. **Bryant JM**, **Harris SR**, **Parkhill J**, **Dawson R**, **Diacon AH**, **van Helden P**, **Pym A**, **Mahayiddin AA**, **Chuchottaworn C**, **Sanne IM**, **Louw C**, **Boeree MJ**, **Hoelscher M**, **McHugh TD**, **Bateson ALC**, **Hunt RD**, **Mwaigwisya S**, **Wright L**, **Gillespie SH**, **Bentley SD**. 2013. Whole-genome sequencing to establish relapse or re-infection with *Mycobacterium tuberculosis*: a retrospective observational study. Lancet Respir Med **1**:786–792.

46. **Pankhurst LJ**, **del Ojo Elias C**, **Votintseva AA**, **Walker TM**, **Cole K**, **Davies J**, **Fermont JM**, **Gascoyne-Binzi DM**, **Kohl TA**, **Kong C**, **Lemaitre N**, **Niemann S**, **Paul J**, **Rogers TR**, **Roycroft E**, **Smith EG**, **Supply P**, **Tang P**, **Wilcox MH**, **Wordsworth S**, **Wyllie D**, **Xu L**, **Crook DW**. 2015. Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. Lancet Respir Med.

47. **Walker TM**, **Kohl TA**, **Omar S V**, **Hedge J**, **Del Ojo Elias C**, **Bradley P**, **Iqbal Z**, **Feuerriegel S**, **Niehaus KE**, **Wilson DJ**, **Clifton DA**, **Kapatai G**, **Ip CLC**, **Bowden R**, **Drobniewski FA**, **Allix-Béguec C**, **Gaudin C**, **Parkhill J**, **Diel R**, **Supply P**, **Crook DW**, **Smith EG**, **Walker AS**, **Ismail N**, **Niemann S**, **Peto TEA**. 2015. Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study. Lancet Infect Dis **15**:1193–1202.

TABLES

Table 1. Patient characteristics of tuberculosis (TB) cases diagnosed in Switzerland between 2000 and 2008, overall and comparing clustered and unclustered TB cases (unweighted), as well as risk factors for transmission (weighted analysis) as defined by genome-based molecular clustering.

Characteristic	Unweighted (n=520)						Weighted (n=4,221)	
	All cases n=520		Clustered n=43		Unclustered n=477		Adjusted Odds Ratio (95% CI) *	P-value
	n	(%)	n	(%)	n	(%)		
Age at TB diagnosis, years								0.46
16-29	154	(29.6)	14	(32.6)	140	(29.4)	1	
30-49	226	(43.5)	16	(37.2)	210	(44.0)	0.56 (0.22-1.41)	
≥50	140	(26.9)	13	(30.2)	127	(26.6)	0.71 (0.29-1.73)	
Sex								0.018
Male	254	(48.8)	26	(60.5)	228	(47.8)	1	
Female	266	(51.2)	17	(39.5)	249	(52.2)	0.4 (0.18-0.85)	
Country of birth								0.026
Switzerland	119	(22.9)	17	(39.5)	102	(21.4)	1	
Europe, without Switzerland	106	(20.4)	2	(4.7)	104	(21.8)	0.16 (0.03-0.83)	
Asia	120	(23.1)	3	(7.0)	117	(24.5)	0.24 (0.05-1.08)	
Sub-Saharan Africa	132	(25.4)	16	(37.2)	116	(24.3)	1.11 (0.36-3.45)	
Central and South America	20	(3.8)	3	(7.0)	17	(3.6)	1.99 (0.40-9.85)	
Other regions/Unknown	23	(4.4)	2	(4.7)	21	(4.4)	0.80 (0.13-5.02)	
Born in Switzerland								0.091
No	401	(77.1)	26	(60.5)	375	(78.6)	1	
Yes	119	(22.9)	17	(39.5)	102	(21.4)	2.21 (0.88-5.52)	
Residence status								0.41
Swiss nationals	130	(25.0)	14	(32.6)	116	(24.3)	1	
Foreigner/Residents	199	(38.3)	11	(25.6)	188	(39.4)	0.45 (0.17-1.23)	
Asylum seeker/Refugee	101	(19.4)	8	(18.6)	93	(19.5)	0.42 (0.11-1.56)	
Unknown	90	(17.3)	10	(23.3)	80	(16.8)	0.80 (0.22-2.81)	
HIV status								0.033
Negative	407	(78.3)	34	(79.1)	373	(78.2)	1	
Positive	113	(21.7)	9	(20.9)	104	(21.8)	0.39 (0.16-0.93)	
Site of disease								0.054
Extra-pulmonary TB	133	(25.6)	5	(11.6)	128	(26.8)	1	
Pulmonary TB	387	(74.4)	38	(88.4)	349	(73.2)	3.14 (0.98-10.07)	
Cavitary disease								0.039
No	407	(78.3)	28	(65.1)	379	(79.5)	1	
Yes	113	(21.7)	15	(34.9)	98	(20.5)	2.31 (1.05-5.10)	
Positive smear microscopy								0.049
No	352	(67.7)	21	(48.8)	331	(69.4)	1	
Yes	168	(32.3)	22	(51.2)	146	(30.6)	2.18 (1.00-4.73)	
TB in family or social surroundings in last 2 years								0.068
No	482	(92.7)	38	(88.4)	444	(93.1)	1	
Yes	38	(7.3)	5	(11.6)	33	(6.9)	2.95 (0.92-9.41)	

* Adjusted for age and sex, and weighted for sampling proportions

95% CI, 95% confidence iInterval; TB, tuberculosis

Table 2. Number of MIRU-VNTR clusters and number of patients in MIRU-VNTR clusters confirmed by whole genome sequencing. MIRU-VNTR clusters not confirmed by WGS ("false positive" clusters) and clusters confirmed by WGS ("true" clusters) are presented, according to the countries of birth of cases involved: clusters involving Swiss-born TB cases only, foreign-born cases only, or mixed clusters (e.g., involving both Swiss-born and foreign-born TB cases, or foreign-born cases from different continents).

	MIRU-VNTR clusters confirmed by WGS n (%)	MIRU-VNTR clusters not confirmed by WGS n (%)	Total number of clusters n
Molecular clusters *			
Swiss-born only	3 (75.0)	1 (25.0)	4
Mixed	10 (66.7)	5 (33.3)	15
Foreign-born only	4 (25.0)	12 (75.0)	16
Total	17 (48.6)	18 (51.4)	**35**
Patients in molecular clusters **			
Swiss-born only	8 (80.0)	2 (20.0)	10
Mixed	27 (62.8)	16 (37.2)	43
Foreign-born only	8 (21.6)	29 (78.4)	37
Total	43 (47.8)	47 (52.2)	**90**

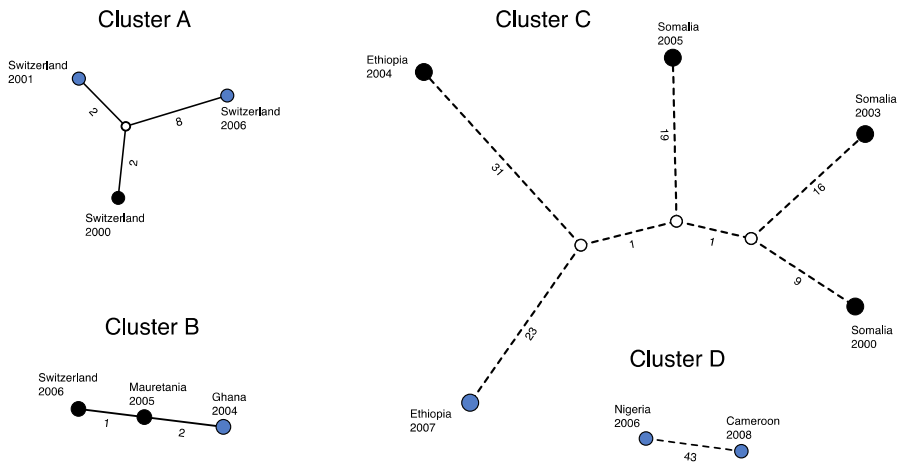
MIRU-VNTR, mycobacterial interspersed repetitive-unit–variable-number tandem-repeat; WGS, whole genome sequencing

* Fisher's exact test: p-value=0.031
** Fisher's exact test: p-value <0.0001

FIGURES

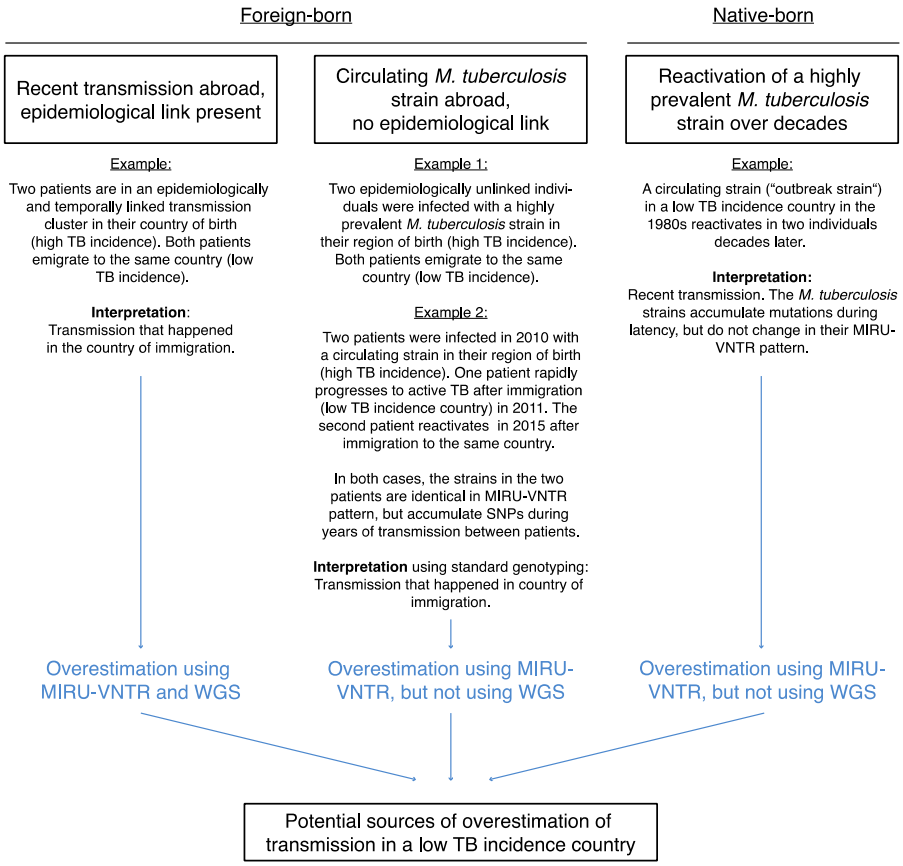
Figure 1. *M. tuberculosis* transmission network using SNP data from whole genome sequencing. Representative examples of different types of transmission clusters that were previously identified by MIRU-VNTR: cluster A (all patients from Switzerland) was confirmed as a true transmission cluster by WGS, with distances of ≤12 SNPs between all isolates. Cluster B is a mixed cluster (one patient from Switzerland and two patients from West Africa), which was confirmed to be a true transmission cluster with one and two SNPs between patient isolates. Clusters C and D were identified by MIRU-VNTR as transmission clusters, but WGS did not confirm these clusters as true clusters (genetic distances of >12 SNPs). Filled circles represent patient isolates, white circles „median vectors“, i.e. hypothetical isolates inferred from the sequencing data. Blue circles indicate HIV-positive patients. Numbers next to lines indicate SNP distances. Countries of birth and years of tuberculosis diagnosis are indicated next to circles. Clusters with solid lines are “true” clusters, i.e. clusters confirmed by WGS (≤12 SNPs), whereas clusters with dashed lines are clusters not confirmed by WGS (>12 SNPs). All other transmission clusters are shown in Supplementary Figure 1.

Journal of Clinical Microbiology



679
680

Figure 2. Possible situations leading to an overestimation of *M. tuberculosis* transmission in a low tuberculosis incidence country.



MIRU-VNTR, mycobacterial interspersed repetitive-unit–variable-number tandem-repeat; TB, tuberculosis; WGS, whole genome sequencing

Figure 3. Median genetic distance in MIRU-VNTR-defined transmission clusters. Each data point shows the mean genetic distance (as number of single nucleotide polymorphisms [SNPs]) in one of the 35 MIRU-VNTR-defined transmission clusters. Solid black lines indicate median values of mean pairwise distances within molecular clusters. The distribution of clusters with patients born in Switzerland, mixed clusters and immigrant patients born on the same continent (except Switzerland) was significantly different (Kruskal-Wallis non-parametric test, p=0.030). The dashed line represents the cut-off for the definition of genome-based molecular clusters (≥12 SNPs).