



Extent of transmission captured by contact tracing in a tuberculosis high endemic setting

To the Editor:

We have previously documented a high incidence of tuberculosis (TB) (>1000 per 100 000 inhabitants) and considerable *Mycobacterium tuberculosis* transmission in East Greenland despite population-wide screenings implemented by the Greenland health authorities [1–5]. In Greenland, all contacts of TB patients are routinely screened for *M. tuberculosis* infection and TB. Individuals with newly acquired *M. tuberculosis* infection are offered 6 months of preventive isoniazid treatment. Despite the considerable resources spent on clinical examinations and preventive treatment over the years, TB incidence rates have remained high in East Greenland [1].

In a recent study using whole genome sequencing (WGS), we found culture-positive TB cases in East Greenland to be caused by genetically nearly identical *M. tuberculosis* strains [4]. However, for many of the TB cases, results of the WGS suggested direct *M. tuberculosis* transmission from source cases not identified by contact-tracing efforts.

To further explore this, we aimed to estimate the percentage of *M. tuberculosis* culture-positive TB cases with or without a reported contact confirmed by WGS as a potential source, thereby providing evidence of *M. tuberculosis* transmission occurring outside traditional contact tracing circles.

East Greenland is an isolated part of Greenland, inhabited by only 6% (n=3514) of the total Greenland population [6]. Inhabitants live in two towns and five smaller settlements. At birth, all Greenland citizens receive a personal identifier from the civil registration system, which enables a unique linkage of personal data across public registries.

In Greenland, TB is mandatorily notifiable and contact tracing is routinely performed for all TB cases. Information on all notified TB cases from East Greenland (n=134) in the period from 2008 to 2012 and their reported contacts were sought from the TB notification system. Detailed contact tracing data were available for 80% (n=107) of notified cases, amounting to a total of 1241 designated contacts and numbering an average of 12 contacts per TB case. The Committee for Scientific Research in Greenland approved the study (approval no. 2012-071304). The project was reported to and followed all instructions from the Danish Data Protection Agency.

As previously reported [4], isolates from 182 culture-positive cases from 1992 to 2012 (98% of all culture-positive cases) have already been analysed by WGS. In short, *M. tuberculosis* isolates were retrieved from the nationwide strain collection at Statens Serum Institut. After DNA extraction [1], libraries were prepared with Nextera XT kits and run on Illumina next-generation sequencing platforms (Illumina, San Diego, CA, USA). An overall maximum parsimony tree including the 182 culture-positive TB cases was created with Bionumerics®, version 7.5 (Applied Math, Sint-Martens-Latem, Belgium) based on 1385 concatenated single nucleotide polymorphisms (SNPs) [4]. For further details on variant calling, reference mapping and SNP filters, please refer to our previously published paper that also describes how raw data in the form of fastq files were submitted to the EMBL-EBI ENA sequence read archive [4]. In total, 287 TB cases were notified from 1992 to 2012. Of the remaining 105 cases not included in the previous study, 91 had at least one diagnostic sample registered, of which 84 were culture- and microscopy-negative [4].

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In a TB endemic setting >50% of culture-positive TB cases were likely infected via community acquired transmission <http://ow.ly/Rqjd307cf6o>

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The previously conducted WGS study [4] enabled us to compare contact-tracing data obtained from 2008 to 2012 with the overall phylogenetic tree representing cases diagnosed from 1992 to 2012. We evaluated whether isolates linked by contact-tracing data were within a genetic distance suggestive of likely direct transmission. To display this relationship, we compared links identified by contact tracing with WGS-suggested links. We used a maximum distance of five SNPs between isolates as threshold for likely direct transmission, as previously suggested by other authors [7]. We recently showed that *M. tuberculosis* transmission in this setting most likely occurs with strains within an even smaller genetic distance [4], and considered this to be a conservative measure of direct transmission. Sequencing and contact tracing data were compared with NodeXL® (Socialmedia Research Foundation).

Of the 134 TB cases notified from 2008 to 2012, 86 (64%) were culture-positive and therefore included in the WGS analyses. For the remaining culture-negative TB cases, diagnosis was made by clinical criteria only (n=47) and for one culture-negative case by positive microscopy. Of the 86 culture-positive TB cases, 84 had available contact-tracing data, of which 52 (60%) were reported as a contact of another culture-positive case. Of the 86 culture-positive cases included in the WGS analyses, 79 were classified as pulmonary cases. Of the remaining 48 TB cases, 41 were pulmonary TB cases.

However, when contact-tracing data were challenged with the genetic relationship of included isolates, only 40 (47%) of the 86 culture-positive TB cases were within a maximum distance of five SNPs from an isolate from any reported contact. Thus, a likely source of infection among reported contacts was only identified for 47%. Thus in this isolated high TB incidence setting, 53% of the culture-positive TB cases had no reported contact(s) within a genetic distance suggestive of direct transmission (figure 1), despite including all available contact-tracing data and with no regard to smear positivity or the direction of the reported link. Thus, for these cases, traditional contact tracing did not reveal the source of infection despite an average of 12 screened contacts per case.

Our finding from a unique, isolated, high TB incidence setting without HIV or multidrug-resistant TB confirms previous reports using epidemiological data and/or other subtyping methods, which show that community-, rather than reported contact-acquired, transmission is likely in high TB incidence areas [8–12]. For example, a recent study from Malawi using WGS reported a very similar percentage of less than 50% of culture-positive cases; this was explained by transmission from a close contact [13]. Our study is important in order to demonstrate that in high-incidence areas, sources of TB transmission should not only be sought among close contacts.

In areas with a high *M. tuberculosis* infection pressure, contact tracing is challenged by the large number of potential community contacts not included in the screening efforts. Thus, in an area like East Greenland and similar settings worldwide, it seems relevant to include alternative methods to identify TB cases infected through community-acquired transmission. In East Greenland, teenagers, BCG-unvaccinated individuals and inhabitants of previously low endemic areas are particular risk groups [1, 2]. However, the TB incidence in East Greenland is considerably higher than in the rest of Greenland (1000 versus 150 per

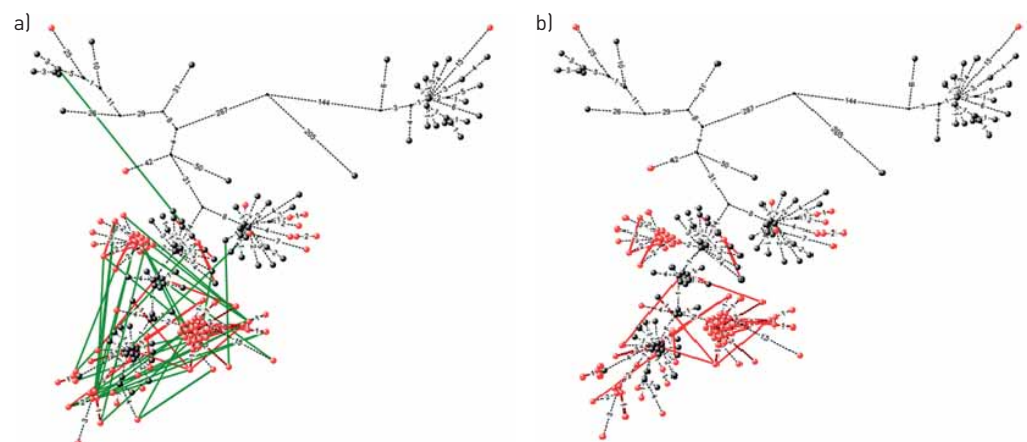


FIGURE 1 An overall maximum parsimony tree based on 1385 concatenated single nucleotide polymorphisms (SNPs) showing the genomic links (black) between 182 culture-positive tuberculosis (TB) cases from East Greenland, 1992 to 2012, compared with contact-tracing links. Only contact-tracing links from culture-positive TB cases from 2008 to 2012 (red) are included in the analyses. a) Any identified link (n=70, green and red) between included TB cases (n=52). b) Only contact tracing links (n=40) between cases (n=40) with a maximum distance of five SNPs (red), suggestive of direct transmission between cases.

100000 inhabitants) and perhaps the entire East Greenland population should be considered at risk. According to World Health Organization guidelines, systematic screenings for active TB of geographically defined subpopulations with a high prevalence of undetected TB are sometimes needed [14].

The major strengths of this study were the completeness of included samples, the isolated geographical setting and the unique data linkage in combination with WGS at a population-based level. The study has two major limitations; contact-tracing data were missing for 20% (n=27) of the 134 cases notified 2008 to 2012 and only culture-positive cases (n=86) were available for WGS analyses. While missing contact-tracing data could lead to underestimation of the true number of possible contacts, only two of the cases with missing data were culture-positive and only one was a smear microscopy-positive pulmonary case. The remainder were therefore less likely to be sources of *M. tuberculosis* transmission and thus we do not believe that our result would have changed greatly if they had been included. In contrast, including all culture-positive cases with no regard to smear positivity could potentially lead to overestimation of the number of possible contacts.

In conclusion, 53% of culture-positive TB cases in East Greenland were most likely infected by a source not identified through traditional contact tracing. Thus, this study illustrates the difficulties in confining *M. tuberculosis* transmission through contact tracing alone in high TB incidence settings. This result has potentially important implications for future TB control measures in TB high endemic regions, as it leads to considerations on further actions needed for TB control and how best to use the resources available; e.g. in small, high TB incidence communities, population-wide TB screenings could be considered as an alternative to traditional contact tracing.

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