Characterization of Finnish *Mycobacterium tuberculosis* Isolates by Spoligotyping

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The molecular epidemiology of tuberculosis (TB) in Finland was studied by spoligotyping 380 *Mycobacterium tuberculosis* isolates. The isolates were obtained during a 1-year study period from July 2000 to June 2001 and represented 90% of new *M. tuberculosis* findings by culture in the whole country during the study period. The spoligotyping results were compared to the World Spoligotyping Database of the Institut Pasteur de Guadeloupe, which contains data from >14,000 *M. tuberculosis* isolates obtained worldwide. A total of 138 different spoligotypes were identified among the 380 *M. tuberculosis* isolates. Thirty-eight (10%) isolates had unique spoligotypes, while 342 (90%) isolates belonged to 100 shared types. The four most common spoligotypes caused approximately one-third of the Finnish TB cases. Forty-seven of the 138 (34.1%) spoligotypes and 61 (16.1%) of the 380 *M. tuberculosis* isolates had spoligotypes that had not been previously reported. Only four (1.1%) patients were infected with an isolate belonging to the Beijing genotype. The characterization of Finnish *M. tuberculosis* isolates by spoligotyping shows that ubiquitous spoligotypes were common, but many spoligotypes specific to Finland were also found. However, Beijing family isolates were rarely encountered, although this spoligotype is predominant in our eastern and southern neighbors.

In Finland there has been a dramatic fall in the incidence of tuberculosis (TB). While in 1930, ~10,000 Finnish patients died of TB (22a), the incidence dropped to <10 cases per 100,000 population in 2001, with 494 new cases (1). Most patients were elderly, and only six pediatric cases were reported in 2001. Only 14% of the cases were among foreign-born patients in 2001, and the number of cases involving multidrug-resistant (MDR) TB ranged between 0 and 4 from 1995 to 2001 (1).

Geographically, Finland is located between countries with contrasting epidemiological situations for TB. In the neighboring western countries, Sweden and Norway, reported TB rates have gradually decreased, and in 2000, the incidence was 5/100,000 in both countries (3). The reported TB rates of Finland's eastern and southern neighbors, Russia and Estonia, are significantly higher and have been increasing. In 2000, the incidence of TB was 98/100,000 in Russia and 57/100,000 in Estonia (3). In addition, both countries have high rates of MDR TB (2).

Since the discovery of polymorphic DNA in *Mycobacterium tuberculosis*, strain differentiation has become a valuable tool in the study of the epidemiology of TB. Various genetic elements contribute to DNA polymorphism in *M. tuberculosis*. The chromosomal locus, containing a large number of 36-bp direct repeats interspersed with unique spacer sequences varying in size from 35 to 41 bp, is the target of the spoligotyping (spacer

oligonucleotide typing) technique (11). This rapid method has been applied to the study of molecular epidemiology and the evolutionary genetics of TB (7, 18, 21). Since the technique is PCR based, it requires less DNA than conventional molecular typing, particularly IS6110 restriction fragment length polymorphism. This also allows typing directly from primary specimens (11). However, spoligotyping is not as discriminative as the restriction fragment length polymorphism method for isolates with high IS6110 copy numbers, whereas spoligotyping is more discriminative for isolates containing five or fewer IS6110 copies (4, 10–12, 19, 26).

Particular *M. tuberculosis* strains may have enhanced capacity to spread within a community. One group of strains, the Beijing family, has been associated with outbreaks in a number of communities throughout the world. All strains belonging to this family have identical spoligotypes containing only the last 9 of the 43 possible spacers (25). *M. tuberculosis* strains of the Beijing genotype were first identified in TB patients in the Beijing area of China, and it has been dominant in countries of East Asia since the mid-1950s (17, 24, 25). Recently, the Beijing genotype has been associated with several outbreaks in the United States, South Africa, Germany, the Canary Islands, Russia, and Estonia (5, 6, 13–15, 23), and it is the most prevalent spoligotype in the world (21).

The aim of this work was to study the molecular epidemiology and evolution of TB in Finland by spoligotyping. We wanted to determine which Finnish *M. tuberculosis* isolates have spoligotypes that are common in the world and which spoligotypes are specific to Finland. In addition, we wanted to investigate whether the Beijing family of *M. tuberculosis* isolates had spread to Finland.

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TABLE 1. Spoligotypes specific to Finnish *M. tuberculosis* isolates

[illegible]

^a Shared type, designation of the spoligotype in the World Spoligotype Database.

^b Spoligotype, arbitrary spoligotype designation.

^c Numbers 1 to 43, spoligotype probes. Solid square, hybridization; open squares, lack of hybridization.

^d *n*, number of patients infected by an isolate with this spoligotype.

^e Origin, country of birth of the patients infected by an isolate with this spoligotype.

MATERIALS AND METHODS

Bacterial strains. A total of 380 *M. tuberculosis* isolates were collected during a 1-year study period, from July 2000 to June 2001, from 380 TB patients living in Finland. The isolates represented 90% of new *M. tuberculosis* findings by culture in the whole country during the study period.

DNA methods. Mycobacterial isolates were cultivated on Löwenstein-Jensen medium. A loopful of bacterial colonies was suspended in 1 ml of 0.9% NaCl, inactivated at 80°C for 10 min, and pelleted by centrifugation. The pellet was resuspended in 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) and mixed by vortexing it for 2 min with glass beads, and 10 µl of the supernatant was used in PCR amplification. Spoligotyping was performed with a commercially available kit (Isogen Bioscience BV, Maarssen, The Netherlands) according to the instructions of the manufacturer.

Comparison of results. The spoligotyping results were entered in a binary format as Excel spreadsheets and were compared to the World Spoligotype Database of the Institut Pasteur de Guadeloupe (21), which contains 14,651 isolates and 935 shared types, including these data. An isolate was assigned a shared type if the same spoligotype was found for isolates obtained from two or more patients in the world. If no matching spoligotype was identified in the database, the isolate was defined as unique. The shared types formed by a unique isolate in the database and a Finnish isolate, or two or more Finnish isolates alone, were defined as new shared types.

Geographic origins of cases. The geographic origin of each case was defined primarily as the country of birth of the patient and secondarily as the most recent nationality, both acquired from the national population register using a unique person identifier. For the few cases without the unique person identifier, the current nationality was used for defining origin, as reported by the attending physician.

TABLE 2. Major spoligotypes in Finland

Spoligotype ^a	Shared type ^b	n (%)		Geographic distribution ^d
		Finland	Database ^c	
S29	53	49 (12.9)	972 (6.6)	Ubiquitous
S13	47	29 (7.6)	294 (2.0)	Ubiquitous
S28	50	28 (7.4)	609 (4.2)	Ubiquitous
S260	42	25 (6.6)	390 (2.7)	Ubiquitous
F30	49	23 (6.1)	52 (0.36)	AFR, EUR, OCE, CAM, NAM
S133	46	9 (2.4)	24 (0.16)	AFR, EUR, OCE, SAM, CAM, NAM
F55	262	8 (2.1)	23 (0.16)	EUR, NAM
F6	928	8 (2.1)	9 (0.06)	EUR, NAM
S254	280	7 (1.8)	19 (0.13)	EUR, OCE, NAM
F20	914	7 (1.8)	8 (0.05)	EUR, NAM
S104	2	6 (1.6)	117 (0.8)	ASI, EUR, SAM, CAM, NAM
S77	37	5 (1.3)	81 (0.55)	Ubiquitous
F41	921	5 (1.3)	5 (0.03)	EUR (Finland)

^a Spoligotype, arbitrary spoligotype designation.

^b Shared type, designation of the spoligotype in the World Spoligotype Database.

^c Database, number of isolates in the World Spoligotype Database.

^d Ubiquitous, spoligotype present in all seven geographic areas; AFR, Africa; ASI, Asia; EUR, Europe; OCE, Oceania; SAM, South America; CAM, Central America; NAM, North America.

RESULTS

A total of 138 different spoligotypes were identified among the 380 *M. tuberculosis* isolates. Thirty-eight (10%) isolates had unique spoligotypes, while 342 (90%) isolates belonged to 100 shared types. Of the spoligotypes belonging to the shared types, 23 were new and 77 were already defined in the World Spoligotype Database. One isolate from Finland and another isolate obtained elsewhere in the world created 14 new shared types, while 9 new shared types were created by Finnish isolates alone. Thus, 47 (34.1%) of the 138 spoligotypes and 61 (16.1%) of the 380 *M. tuberculosis* isolates had spoligotypes that had not been previously reported. The 47 spoligotypes specific to Finland are shown in Table 1. Patients with 3 of the 47 spoligotypes were foreign born (F39, Cambodia; F86, Zambia; and F74, Somalia), and a Finnish patient and a Somali patient shared one spoligotype (F43; shared type, 922). All 47 of the spoligotypes lacked spacers 33 to 36, suggesting that the isolates belong to genetic group 2 or 3 as defined by Soini et al. and Sreevatsan et al. (18, 22).

Major spoligotypes were defined as types that contained five or more isolates each in the Finnish data set. There were 13 major spoligotypes consisting of 209 (55%) isolates. The comparison of the major Finnish spoligotypes with the World Spoligotype Database is shown in Table 2. A total of 87 minor spoligotypes, containing less than five isolates each, were identified; 133 isolates (35%) belonged to the minor types. Only 4 patients (1.1%) out of the 380 were infected with an isolate belonging to the Beijing genotype (S1; shared type, 1), and 3 of the 4 patients were foreign born (Afghanistan, China, and Russia).

DISCUSSION

This is the first report describing the molecular epidemiology of TB in Finland. We analyzed 380 *M. tuberculosis* isolates by spoligotyping and obtained a total of 138 spoligotypes. Thirty-four percent of the spoligotypes were found to be specific to Finland, and thus, 16.1% of the patients were infected with an *M. tuberculosis* isolate having a spoligotype that had not been previously submitted to the global database.

We believe that due to Finland's historical isolation a pool of unique *M. tuberculosis* isolates has emerged and remained in the country. Relatively small founder populations have slowly spread over a large country, and small isolated clusters have developed within Finland. This hypothesis is analogous to, and is supported by, the Finnish disease heritage, comprising some 30 unique genetic diseases (16). The history of unique Finnish *M. tuberculosis* isolates may be comparable to the enrichment of the hereditary diseases. Since spoligotyping is a relatively new typing method, the presence of unique spoligotypes may also be explained by lack of data from other countries. However, our data were compared to the spoligotyping data from >14,000 *M. tuberculosis* isolates obtained worldwide (21), making this alternative less likely.

Two-thirds of the spoligotypes obtained from the Finnish *M. tuberculosis* isolates belonged to previously identified shared spoligotypes. The four most common spoligotypes caused approximately one-third of the Finnish TB cases, and these spoligotypes are also among the most common in the world (8, 18, 20, 21). In particular, spoligotypes S29 (shared type, 53) and S28 (shared type, 50), currently representing 11.8% of *M. tuberculosis* isolates in the World Spoligotype Database, seem to have been prevalent in Europe for several centuries (H. D. Donoghue, H. Fletcher, I. Pap, and M. Spigelman, presented at the 22nd Annual Congress of the European Society of Mycobacteriology, Berlin, Germany, 2001). The spoligotypes S28 and S29 differ from each other by only one spacer, and it has been reported that the absence of spacer 31 can be caused by a lack of its amplification due to an asymmetric split of the target sequence because of the insertion of a copy of IS6110 (9). It is thus possible that the S28 and S29 isolates actually share the same direct-repeat locus. The finding of identical spoligotypes in widely separated countries may be explained either by recent or past transmission events or by phylogenetic convergence (21). In the case of the ubiquitous spoligotypes found in Finland, it seems more likely that they have spread to Finland from abroad.

M. tuberculosis isolates belonging to the Beijing genotype are very common in Finland's eastern and southern neighbors,

Russia and Estonia, and the isolates are often associated with MDR TB. In a study based on the pulmonary TB patients in the Archangel Oblast, in the northwestern part of Russia, 44.5% of the isolates belonged to the Beijing genotype and 43.4% of the Beijing genotype isolates were MDR (23). In Estonia, the proportion of Beijing *M. tuberculosis* isolates was 29%, while 87.5% of all MDR isolates belonged to this genotype (13). The Beijing genotype may possess particular phenotypic properties, such as antigens and virulence factors, which are expressed as distinct manifestations in the pathology and epidemiology of TB (12). Three of the four Beijing genotype isolates identified in our study were obtained from patients born in countries where the genotype is prevalent. Two of the isolates were fully drug sensitive, while two isolates were resistant to isoniazid alone and to a combination of isoniazid, rifampin, streptomycin, and ethambutol, respectively. As the prevalence of the Beijing genotype was only 1.1% in our study, it appears that the Beijing family is not yet spreading extensively in Finland. This is interesting, considering that Finland was part of imperial Russia between 1808 and 1917, which intensified immigration and travel. However, both before this period and after until the 1990s, population interaction and movement between the countries were very limited. Increasing travel to and from Estonia and Russia, however, may have an impact on the epidemiological situation of TB in Finland in the future.

In conclusion, *M. tuberculosis* isolates with ubiquitous spoligotypes were found to be common in Finland, but many spoligotypes specific to Finland were also detected. Nevertheless, Beijing family isolates were rarely encountered, although this spoligotype is predominant in Finland's eastern and southern neighbors.

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