The molecular epidemiology of tuberculosis

Recent trends in a low burden country

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1 INTRODUCTION

"One cannot condemn the past for not following the standards of the present."

John P. Moore et al. 1999 (8).

However, it is only reasonable to expect us to do so.

1.1 GLOBAL BURDEN OF TUBERCULOSIS

After several years of neglect by the international health community, the 1990s have been characterised by a new understanding of the global tuberculosis burden and acceleration in tuberculosis control efforts worldwide (9). Before that it was felt, particularly in the low incidence countries, that the advances in tuberculosis chemotherapy had determined a quick disappearance of tuberculosis as a public health problem (9, 10). However, tuberculosis has killed more than 100 million people during the past 100 years, despite access to tuberculosis chemotherapy for half a century (11, 12). Tuberculosis is still the world's second most common cause of death from an infectious disease, after HIV/AIDS, killing nearly 2 million people each year (11). One third of the world's population has been infected with *M. tuberculosis* (13). Every year, 50-100 million persons are infected with *M. tuberculosis*, and there are 8-9 million new cases of tuberculosis (11, 13).

The burden of tuberculosis falls principally on the developing nations where 95% of cases, and 98% of deaths due to the disease occur (14). Sub-Saharan Africa has the highest incidence rate of tuberculosis, but the most populous countries of Asia hold the largest number of cases: India, China, Indonesia, Bangladesh, and Pakistan together account for more than half the global burden, and 80% of all new cases occur in 22 high incidence countries (11). In addition, the global tuberculosis caseload appears to be growing slowly (11). Case numbers have declined more or less steadily in western and central Europe, North and South America, and the Middle East, but there have been striking increases in countries of the former Soviet Union and in sub-Saharan Africa (11, 15-17). E.g. tuberculosis notification rates have doubled or tripled in some African countries during the 1990s in a period as short as 10 years (9).

Apart from the growing global population, HIV infection accounts for much of the increase in the global tuberculosis caseload (9, 18-20). In 2000, an estimated 11% of new adult tuberculosis cases worldwide were infected with HIV. In sub-Saharan Africa, the association with HIV was even stronger with an estimated 38% of new adult tuberculosis cases being HIV co-infected, and in Botswana, South Africa, Zambia, and Zimbabwe, the rates of HIV infection among tuberculosis patients exceed 60% (11, 15).

In 1993, WHO declared tuberculosis a "global emergency", an unprecedented step in public health (9). One year later, WHO launched a new tuberculosis control policy "framework for effective tuberculosis control" (21). This policy defined five essential elements of tuberculosis control, later referred to under the name "DOTS" (9). During the 1990s, the DOTS-policy was universally accepted, and it has now been introduced in 148 out of 210 countries and territories worldwide. The DOTS-policy has been shown to be effective in reducing tuberculosis mortality and incidence (9), and one of the five elements in the policy, "assessment of treatments results", inspired the writing of the first article in this thesis (1).

1.2 BURDEN IN LOW INCIDENCE COUNTRIES

Killing at its height perhaps 1% of the population annually in several European metropolitan areas, the annual number of cases and deaths caused by tuberculosis have declined dramatically over the past century (22-25). Tuberculosis low incidence countries have been defined as countries where the incidence of all forms of active tuberculosis is below 10-20 per 100,000 population (22, 23). During the 1980s and 1990s, the downward trend ceased in some of the low incidence countries, where the number of cases levelled of, was halted, or even reversed (9, 23, 25, 26). Today, tuberculosis is the leading infectious killer of young people and adults in the WHO European region (26). In Denmark, tuberculosis morbidity and mortality rates have been among the lowest in the world for decades, and the goal of eradication has been closer than in most other countries (10). In 1986, 299 cases of tuberculosis were notified, the lowest number ever registered (1). In 2000, however, the number was 548, an increase of 83% (2). This increase was mainly due to immigration from high incidence countries (see section 3), but recently, a specific increase has also been observed among socially marginalised Danish men (see section 4) (7, 27-29). In other industrialised countries, the reasons for the resurgence of tuberculosis during the last two decades are complex and have not been entirely elucidated (9, 25, 30). Socioeconomic factors such as war, civil conflict, poverty and poor living conditions with overcrowding and micro epidemics are important, and so are inconsistent treatment policies with poorly managed, under funded and incorrectly conceptualised tuberculosis control programmes (18, 22, 26, 30-33). Risk groups include people with HIV infection and chronic alcohol and/or drug abuse (13, 25, 32).

In USA, tuberculosis notifications increased from 1986 as in Denmark, and it is believed that the renewed effort in tuberculosis control during the 1990s was fuelled by this development (9, 14, 34). As observed in Denmark, a growing proportion of tuberculosis cases in USA occurred among recent immigrants from high incidence countries generating an awareness that no sustainable tuberculosis control in low incidence countries can be reached without addressing the epidemic in the high incidence countries (4, 9, 35).

1.3 MOLECULAR EPIDEMIOLOGY

Molecular subtyping methods are increasingly applied in the control of infectious diseases. The new technologies have revolutionised our understanding of the transmission of *Mycobacterium tuberculosis* and enabled us to clarify fundamental questions about the epidemiology and pathogenesis of tuberculosis that were previously obscure (36-40). Although the *M. tuberculosis* genome is genetically highly conserved, insertion sequences, repetitive elements, genomic deletions and single nucleotide polymorphisms cause genetic polymorphisms. These polymorphisms can be visualised by various subtyping techniques, often referred to as DNA fingerprinting. Hereby, specific strains of the *M. tuberculosis* complex can be characterised on the basis of their DNA patterns allowing a high degree of discrimination between different strains (41).

The most widely applied DNA fingerprinting technique for the

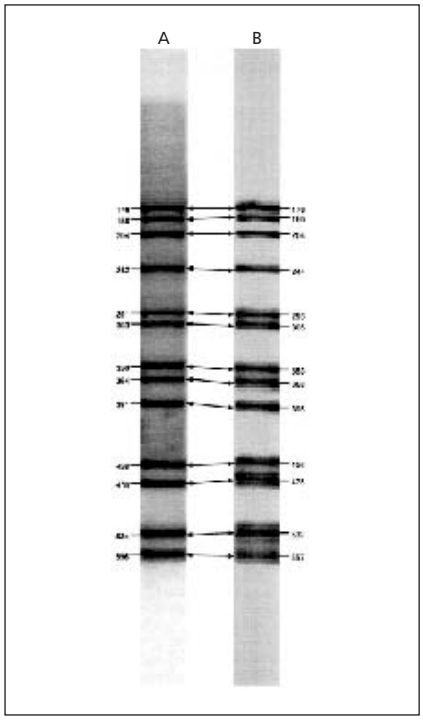

Figure 1. Identical DNA RFLP patterns of *M. tuberculosis* isolates from a father (A), in1961, and his son (B), in 1994. Nos. indicate band positions; identical bands are marked by arrows. Source and details (3).

M. tuberculosis complex is Restriction Fragment Length Polymorphism (RFLP) typing using the insertion sequence IS*6110* as a probe for strain differentiation (**Figure 1** and **Figure 2**) (38). IS*6110* is a member of the IS3 family of transposable elements, which is specific to the *M. tuberculosis* complex (41). The RFLP-method is based on differences in the IS*6110* copy numbers per strain, ranging from 0 to about 25, and variability in the chromosomal positions of these IS*6110* insertion sequences (38). The numbers and positions can be visualised and compared using internationally standardised protocols (42-44). In brief, *M. tuberculosis* complex DNA is extracted and digested with the restriction enzyme *Pvu*II. The digested DNA is separated by electrophoresis in agarose gel, and the DNA fragments are transferred to a nylon membrane and probed with a chemiluminiscence-labelled 245-bp sequence of IS*6110*. The resulting band pattern, the so called fingerprint, is characteristic of a specific *M. tuberculosis* complex strain. It is possible to compare the fingerprint with fingerprints from other strains using a computer program (36). Thus, it is potentially possible to determine whether strains from different patients could have a common origin, a property which has wide implications for the surveillance and control of tuberculosis (37, 39, 45).

During the last decade, RFLP-typing has been used both in outbreaks of tuberculosis and in large population-based studies, including studies across national boundaries. E.g. RFLP-typing has been used to: confirm/rule out suspected transmission and, perhaps more importantly, to detect unsuspected transmission; to distinguish endogenous reactivation from exogenous reinfection and thus to document recent transmission; to discover the emergence of specific strains, or families of strains, in different regions worldwide; and to monitor transmission of strains with different patterns of drug susceptibility and transmission among specific risk groups, e.g. HIV patients. RFLP-typing has also been used to detect laboratory cross-contamination and to identify individual strains of the *M. tuberculosis* complex. Therefore, the term "molecular epidemiology" (38, 39).

Since 1922, all microbiological analyses of mycobacteria in Denmark have been carried out at the International Reference Laboratory of Mycobacteriology at Statens Serum Institut, Copenhagen. This is the only laboratory which performs culture-based tuberculosis diagnosis for Denmark, Greenland, and the Faroe islands, processing approx. 20,000 specimens on an annual basis. It also serves as an international reference laboratory for Iceland and Lithuania. Because all specimens are processed in a single laboratory, and because of the long-standing mandatory centralised tuberculosis notification system, the data are believed to be nearly complete and highly representative of culture-positive tuberculosis from the areas covered. This is a factor of major importance for interpreting DNA fingerprint clustering (39, 45-47). In 1992, the International Reference Laboratory of Mycobacteriology introduced RFLP-typing of strains of the *M. tuberculosis* complex on a nationwide scale as the first country in the world (36, 48). Since then, more than 5,300 isolates, representing 97% of all culture positive patients in the country, have been analysed by the RFLP method (27).

1.4 PURPOSE OF REVIEW

The purpose of this review is to describe some of the recent trends in the tuberculosis epidemic in a low incidence country. It is based on studies of the resurgence of tuberculosis in Denmark during the last one to two decades, incorporating molecular subtyping as a research tool (1-7).

2 LATENT INFECTION AND TUBERCULOSIS

2.1 NATURAL HISTORY

The *Mycobacterium tuberculosis* complex consists of *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canetii*, and *M. microti*, which are closely related organisms sharing >99% identity at the nucleotide level (49). The pathology and course of *M. tuberculosis*, *M. africanum*, and *M. bovis* disease in humans is similar, but *M. tuberculosis* is the major infectious agent of tuberculosis (49). *M. tuberculosis* is spread in airborne droplet nuclei, which are particles of 1-5 μm in diameter (50). Because of their small size, the particles can remain airborne for minutes to hours after "expectoration" by people with pulmonary or laryngeal tuberculosis during coughing, sneezing, singing, talking and other similar activities (11, 50). After inhalation, the infectious droplet nuclei passes down the bronchial tree and lodge in the alveoli in the peripheral airways (11, 50). The bacilli are taken up by alveolar macrophages, which play a central role in tuberculosis, initiating a cascade of events that result in either successful clearing or containment of the initial infection or in progression to active disease (11, 49, 51). Subsequently, *M. tuberculosis* spread through lymphatic channels to regional lymph nodes and through the blood stream to more distant sites (50, 52). At the cellular level, alveolar macrophages infected with *M. tuberculosis* interact with T lymphocytes via several cytokines (11, 53). The infected macrophages release interleukins, which stimulate predominantly CD4-positive T lymphocytes. This, in turn, stimulates the phagocytosis and intracellular killing of *M. tuberculosis* in the macrophage (11, 53). Activated T lymphocytes and macrophages are attracted and form granulomas that limit further replication and spread of the organism (11, 49, 54).

Most infected individuals are able to mount an effective cell-mediated immune response, which limits proliferation of the bacteria and produces a long-lasting partial immunity, both against reactivation of latent bacteria, a process often referred to as "endogenous reactivation", and against new infections, a process often referred to as "exogenous reinfection" (55, 56). Thus, latent infection has been defined as a sub clinical *M. tuberculosis* infection without any clinical, bacteriological or radiological signs or symptoms of manifest disease, and it is most often recognised in an individual who has a positive TST and normal chest radiography (23).

2.2 ENDOGENOUS REACTIVATION

In an immune competent individual, the latent infection remains contained and active disease may never occur (11). However, one of the cardinal features of *M. tuberculosis* is, that in some individuals, it is capable of surviving and multiplying within the hostile environment of the macrophage and other phagocytic cells (49, 57). In approximately 5% of all infected individuals, control of the initial in-

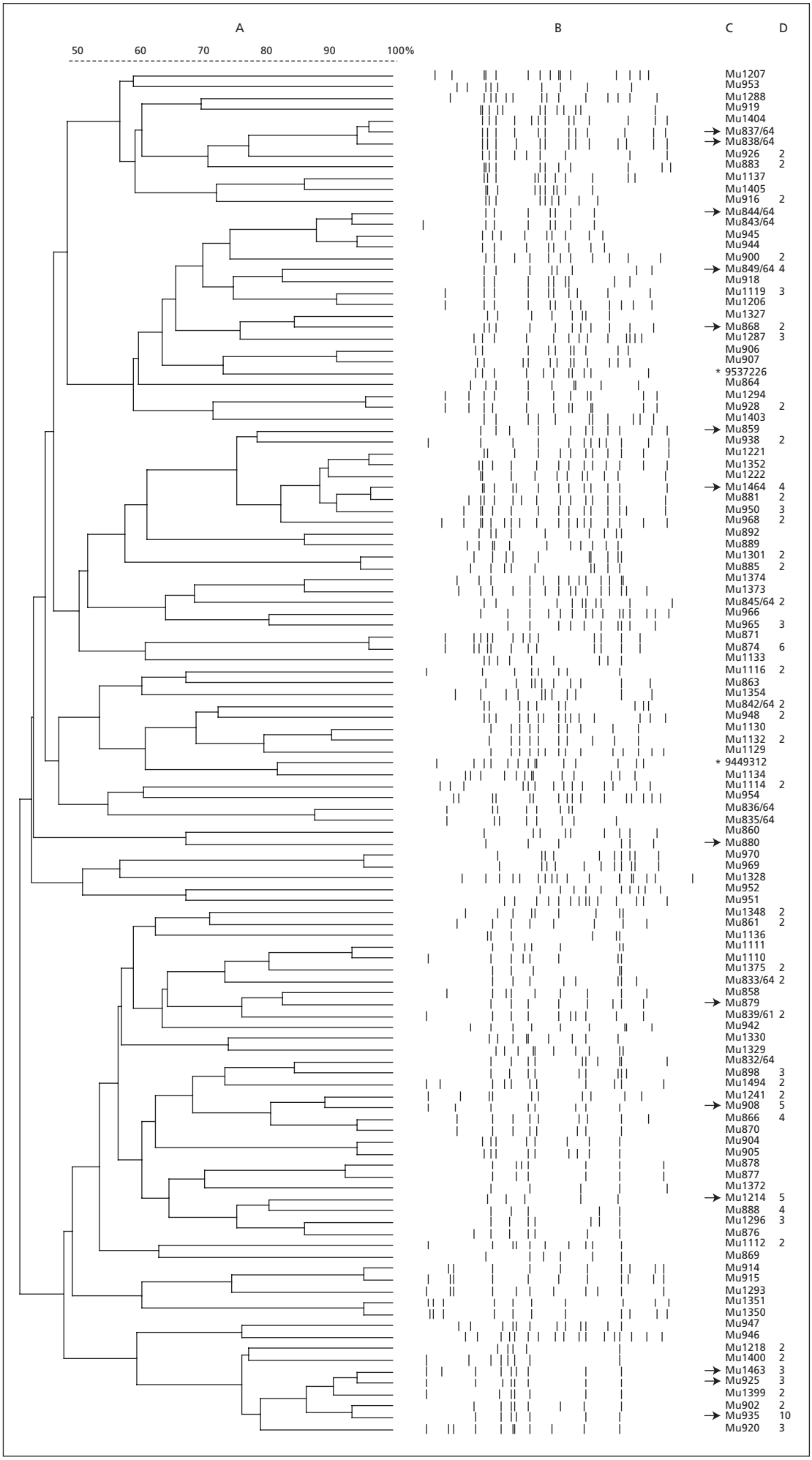

Figure 2. Dendrogram with similarity values in percent (A) and 1 branch for each computerised IS*6110* RFLP pattern (B) of the historical strains of *M. tuberculosis*. Also included are the 2 most-frequent Danish clusters in the 1990s, from the top cluster 2 and cluster 1 (stars); the strain numbers (C); and the number of strains with each pattern (D). The arrows pinpoint the 14 100%-identical patterns described in section 2.2. Source and details (5).

fection is inadequate, and "early" progressive disease occurs within 2-5 years of infection (50, 56, 58). In another 5%, containment of *M. tuberculosis* fails more than 2-5 years after the initial infection, resulting in "late" reactivation disease. Overall, approximately 10% of all persons infected with *M. tuberculosis* develop active disease sometime during their life (50, 56, 58). The risk of development of active disease varies depending on time since infection, age at infection, and host immunity (11, 59, 60). E.g. the risk of developing early progressive disease is much greater in the first few months after PPD conversion and seems to decrease over time (49).

Several factors can trigger subsequent development of active disease by endogenous reactivation of the latent infection, but HIV infection, which selectively eliminates the CD4-positive T-lymphocyte population, is the greatest single risk factor for progression to active disease in adults worldwide (11, 13, 18). Other conditions compromising the immune system predisposing to development of active tuberculosis include poorly controlled diabetes mellitus, renal failure, underlying malignant disease, cancer chemotherapy, corticosteroids and other immunosuppressive drugs, malnutrition, and deficiency of vitamin D or A (11, 50).

Although the possibility of endogenous reactivation after longer periods of time is a generally accepted dogma, the existence of reactivation in humans after a latency of more than a few years, and its contribution to the total incidence of tuberculosis, has not previously been demonstrated directly (55). Indirect evidence derives from models based on trends in historical epidemiological data, often tuberculin surveys, which have sometimes been analysed in combination with more recent data (55, 56, 60-62). Using molecular subtyping methods, endogenous reactivation over a span of up to 8 years has been demonstrated by others (63-66).

By retrieval of freeze-dried *M. tuberculosis* strains from the 1960s, we were able to demonstrate directly endogenous reactivation after several decades of latent infection (3, 5). In Denmark, between 1961 and 1967, a total of 205 *M. tuberculosis* strains were collected and freeze-dried. The majority came from persons living in the capital city (Copenhagen) and its surroundings where the highest rate of new tuberculosis cases were, and still are, found (67). The strains, all retrieved from patients suspected to be part of various chains of local transmission, were divided in two groups by a bacteriophage BK1 and the results were compared against data on epidemiological linkage (67-69). They were stored as freeze-dried samples for 33 to 39 years, until 203 of the 205 strains were successfully recultured. The DNA fingerprints of these strains were compared with those from 4,102 strains collected from 1992 through 2001. During the analysis of the freeze-dried *M. tuberculosis* isolates, on the very first membrane analysed, a 13-band DNA pattern in a specimen collected in 1961 was detected that was identical with the DNA pattern of a specimen collected in 1994 (3). They proved to be from a father (1961) and son (1994) (Figure 1). The father had highly infectious pulmonary TB with cavitation for one year before he was diagnosed in 1961. It was assumed that the father infected his son, who was living in the same household, during this period, and that the son subsequently developed TB in 1994 due to reactivation of the dormant bacilli. This theory was supported by the fact that the DNA pattern for the isolates from the father and son was not found in any of the other more than 4,000 isolates examined in the 1960s and 1990s, reducing the likelihood of a large number of other potential sources. RFLP-typing cannot directly reveal the occurrence of an actual transmission event, and other scenarios are possible (3), but this finding is believed to be the first instance of molecular evidence of endogenous reactivation of *M. tuberculosis* in man spanning decades (33 years) of latent infection (12, 49, 70-75).

Subsequently, all freeze-dried *M. tuberculosis* isolates from Denmark from the 1960s were analysed, and additional molecular epidemiologic evidence of endogenous reactivation decades after the initial infection was found (Figure 2) (5). Fourteen DNA patterns identified in the strains from the 1960s were 100% identical with DNA fingerprint patterns identified in strains from the 1990s. The identical patterns spanned a period of up to 39 years, and accounted for 41 and 40 patients during the 1960s and 1990s, repectively. Seven of the 40 patients from the 1990s were not in Denmark and/or were too young to be alive during the 1960s, but 5 of these 7 patients were clustered with at least one AFB smear positive possible source case from the 1990s. In addition to the 7 patients that could not physically have been infected during the 1960s, one 62 year old Danish-born woman who had onset of tuberculosis during 1998 was most likely infected by her 66 years old Danish-born partner who had onset of sputum smear positive tuberculosis in 1997. This left 32 of the 40 patients who fulfilled the criteria for possible reactivation, that is they were both alive and in the country during the 1960s and were probably not infected during the 1990s. The mean ages of the 32 patients from the 1990s and the patients with clustered strains from the 1960s were 62.9 years [SD 16.6] and 28.7 years [SD 21.5], respectively, also pointing in the direction of reactivation. In addition to the 14 DNA fingerprint patterns from the 1990s that were 100% identical with patterns identified in strains from the 1960s, 17 and 7 patterns were found that were only 1 and 2 band different respectively. The 1 and 2 band different patterns accounted for 24 and 7 patients respectively among whom 20 and 5 were alive and in Denmark during the 1960s and had probably not been infected during the 1990s. Thus, in total, $32 + 20 + 5 = 57$ patients with identical or 1 or 2 band different strains probably developed tuberculosis as a result of endogenous reactivation of an infection acquired during the 1960s. This additional molecular epidemiological evidence of endogenous reactivation spanning up to 39 years was based on several criteria, and supported by additional epidemiological evidence, discussed in further details in the papers (3, 5).

2.3 EXOGENOUS REINFECTION

Another fundamental question regarding the natural history of tuberculosis concerns recurrent tuberculosis and the role of exogenous reinfection versus relapse tuberculosis (76). Recurrent tuberculosis is a subsequent episode of tuberculosis occurring after a previous episode has been considered cured. Exogenous reinfection tuberculosis is a recurrent episode of tuberculosis, caused by a new strain of *M. tuberculosis*, whereas relapse tuberculosis is a recurrent episode of tuberculosis caused by the original strain of *M. tuberculosis*. The possibility that a person previously infected with *M. tuberculosis* can be exogenously reinfected has been debated for decades (60, 62, 63, 77, 78). Thus, often in the tuberculosis literature, a recurrent episode of tuberculosis is referred to as a relapse, not taking into account the possibility of exogenous reinfection (79, 80). However, the origin of infection has potentially important implications for tuberculosis control. Relapse tuberculosis can often be related to problems with treatment compliance (1), whereas exogenous reinfection, among others, can be related to the risk of infection, and thus transmission control efforts in a given area (38, 60, 77, 81). E.g. in the Netherlands, where the risk of infection is low, reinfection has been reported to play a minor role (82). In South Africa, where the risk of infection is high, an extremely high percentage of reinfection has been reported (79). In the past, in England and Wales, where the risk of infection was high, reinfection made an important contribution to tuberculosis morbidity according to mathematical models (60). Still, the importance of exogenous reinfection as a cause for recurrence of tuberculosis is not fully understood (12, 76). Few studies have addressed this issue and included a sufficient number of observations, and the reported proportion of recurrences due to exogenous reinfection has varied from 0% to 100% (63, 76, 79, 81, 83, 84). The relative contribution of exogenous reinfection versus endogenous reactivation still needs to be determined (55, 56, 61-63). This will provide a firmer scientific basis for efforts aimed at global tuberculosis control. E.g. it will determine whether preventive therapy will play a role in diminishing transmission (see section 2.5) (49).

The debate whether a first episode of tuberculosis is due to endogenous reactivation of a remote infection or due to a more recent infection applies, to some extent, to a recurrent episode (76). In a normal host, the immunologic response to the initial infection provides some degree of protection against exogenous reinfection (50). Thus, the likelihood of exogenous reinfection must be a function of the risk of reexposure, the intensity of such reexposure, and the integrity of the host's immune system (50). In patients previously treated and cured, a recurrent episode of tuberculosis would traditionally be considered a case of endogenous reactivation. However, recently, in South Africa exogenous reinfection causing tuberculosis has been found to be predominant in previously treated and cured patients (79), and by now, a handful of molecular subtyping studies have documented tuberculosis caused by exogenous reinfection, even in immunocompetent individuals, from mainly high and moderate incidence areas (49, 79-81, 85). Thus, endogenous reactivation may not contribute as much to the global disease burden as previously thought (49). Reactivation most likely accounts for the great majority of active cases in areas of low *M. tuberculosis* transmission (49, 86). However, in areas of high *M. tuberculosis* transmission, exogenous reinfection probably plays a greater role than was previously appreciated (49, 77, 79). This is in agreement with models developed in the 1970s (78, 87) and 1990s (60) which, based on the annual risk of infection and incidence of tuberculosis, suggest that the relative contribution of exogenous reinfection changes in parallel with the incidence of disease (79).

In Denmark, there are no published studies analysing the importance of exogenous reinfection. It has been documented that 3% (n=11) of all patients notified with tuberculosis in 1992 had recurrent tuberculosis during a 4 year follow-up period (1). Three of these eleven recurrent cases had a history of alcohol and/or drug abuse, another three cases were HIV positive, and one case had non-insulin-dependent diabetes mellitus (1). Also, seven of the recurrent cases had their initial episode of tuberculosis culture verified in 1992. Among these patients, none of the recurrent episodes occurred among those that were culture positive at 5 months or later during treatment of the initial episode (the failure cases), or those that interrupted treatment of the initial episode for 2 months or more (the defaulter cases) (1). The original study did not include molecular subtyping data, but later IS*6110* RFLP patterns were added and showed that all were infected with the same strain of *M. tuberculosis* during the initial and subsequent episode of tuberculosis (own data, unpublished). Thus, the recurrent episodes were "true relapses", which is consistent with the low risk of re-exposure to an infectious case in Denmark. In Denmark, there has also been examples of tuberculosis caused by exogenous reinfection. Among 58 cases of culture verified recurrent tuberculosis from 1992 through 1998, eight were caused by exogenous reinfection on the basis of IS*6110* RFLP patterns (Lillebaek T et al; in preparation).

2.4 RECENT TRANSMISSION

Molecular subtyping allows specific strains of *M. tuberculosis* to be characterised on the basis of their DNA fingerprint pattern. Furthermore, many studies have shown that epidemiologically unrelated strains have different patterns, whereas those of related strains generally have identical patterns (88). Therefore, the majority of such studies assume that the strains with identical patterns are connected by recent transmission (45). This recognition has led to an increasing number of studies measuring the proportion of tuberculosis cases which are "clustered" (share identical DNA fingerprint patterns), and many of these studies have concluded that the extent of recent transmission is more common than previously thought (38, 63, 70, 89, 90), distracting attention from endogenous reactivation to such an extent that some investigators have even doubted its existence (61). E.g. 57% of all Danish tuberculosis patients with onset during the period 1992-1995 were infected with strains of *M. tuberculosis* that were part of a cluster, and disease in the majority of these cases has been attributed to recent transmission (91). However, in order to assume that clustering equals recent transmission, it is essential to know the rate at which DNA fingerprint patterns change. If the pace of this so-called "molecular clock" is too rapid for the time window of an epidemiological investigation, linked cases will be missed. Conversely, the long-term stability of patterns may lead to an overestimation of recent transmission (92). Some studies have determined the "half-life" of IS*6110* patterns by measuring the rate at which DNA fingerprint patterns change (93, 94). The half-life has been defined as the average time required for 50% of IS*6110* patterns to gain or lose one band (5).

The assumption that identical DNA fingerprint patterns represent recent transmission is consistent with a short half-life for DNA fingerprint patterns, as implied by studies of serial isolates collected from patients during relatively short time periods. In these studies, it has been estimated that the half-life of fingerprint patterns of strains involved in active disease can be as short as 3 years (93, 94). However, the half-life of DNA fingerprint patterns of strains of *M. tuberculosis* involved in latent infection has never been examined (92). Provided the half-life of DNA fingerprint patterns under certain circumstances may be very long, and given the fact that tertiary, fourth and even fifth generation cases in the same chain of transmission may share identical DNA fingerprint patterns with the primary case, clustering cannot be assumed in all cases to reflect recent transmission.

In Denmark, the comparison of freeze-dried strains of *M. tuberculosis* from the 1960s and 1990s provided an unique opportunity, to analyse the rate of change and half-life of DNA fingerprint patterns during latent infection (5): Assuming that the average time interval between initial infection and reactivation was 30 years, it was possible to express the numbers of identical patterns and patterns which differed by 1 and 2 bands from the historical strains as a function of the rate of change of DNA fingerprint patterns, and by "maximum likelihood" to compare this to the observed numbers among reactivated cases. Thus the rate of change of DNA fingerprint patterns was estimated to be 1.94%/year (95% CI: 1.29%-2.82%). This rate of change corresponds to a half-life for DNA fingerprint patterns of 36 years (95% CI: 25-54 years), indicating that the half-life of DNA fingerprint patterns of strains involved in latent infection may be much longer than those of strains involved in active disease (36 years vs. 3 years respectively). The observation supports the suggestion that the rate of change is composed of a high rate during the early disease phase with active replication and a low rate of change during latency (92). DNA fingerprint patterns remain very stable during latency, and strains of *M. tuberculosis* harboured in vivo for up to 39 years were genetically almost as stable as the freeze-dried samples.

The findings have important implications for the interpretation of clustering studies which are currently being carried out in many populations. As the time window increases, the proportion of clustering which involves reactivation increases and this phenomenon appears to be important. The 57 patients in the study with tuberculosis due to reactivation during the 1990s had no epidemiological links to recent cases, but they all had "partners" with identical or almost identical patterns and clinical disease during the 1960s, that is tuberculosis up to 39 years previously. Thus, the findings emphasise that clustering should be interpreted cautiously with respect to recent transmission. A long half-life has important implications for the interpretation of clustering among the indigenous elderly population in many western countries. Some of these cases thus may be clustered as a result of simultaneous reactivation of a strain which was prevalent many years ago, rather than as a result of recent transmission (95). Also, in a rural area in Arkansas epidemiological links could be found only for 42% of clustered cases during the early 1990s, suggesting that clustering was attributable to simultaneous reactivation of identical strains which were prevalent in the past (95). Likewise, many years from now, we will observe endogenous

reactivation and clustering of strains of *M. tuberculosis* with the two IS*6110* RFLP patterns which are currently the most prevalent in Denmark, the Danish cluster 1 and cluster 2, as a result of current transmission events (7, 27, 91, 96). Indeed, groups of strains may be identical for reasons other than recent transmission. The amount of clustering will depend on the duration of the study, the incidence of tuberculosis in the population and the prevalence of dominant strains of *M. tuberculosis*. In some studies, it has been possible to establish epidemiological links between clustered cases, and in these circumstances it is reasonable to accept that they are part of the same chain of transmission. In other cases, epidemiological links have not been found, partly because clustering does not necessarily represent recent transmission, as discussed above, and partly because it is not always possible to obtain information about connections between people (45). Thus, the lack of epidemiological links for clustered cases may also reflect the importance of casual contact in the transmission of *M. tuberculosis*. On the other hand, cases actually due to recent transmission may not be recognised as clustered if they are new immigrants to the population or if all cases in the population are not included in the study (45).

2.5 SIGNIFICANCE OF LATENT INFECTION

Since Robert Koch described *M. tuberculosis* in 1882 (97), the natural history following primary *M. tuberculosis* infection has been subject of debate. The capability of *M. tuberculosis* to remain latent within the tissue of humans has allowed *M. tuberculosis* to remain one of the world's great killers into the 21st. century (12). In 1997, it was estimated that one third of the worlds population were infected with *M. tuberculosis*, with 16.2 million cases of active disease, while the rest were asymptomatic but infected (98). Thus, latent infection is a frequent outcome of untreated or incompletely treated *M. tuberculosis* infection, creating a long-standing reservoir of future disease (12). Latent infection and endogenous reactivation is not only of fundamental biological interest, but a central issue for tuberculosis control programmes (49).

Today's tuberculosis control programmes are not fully efficient. They are based on passive case-finding and treatment of active cases combined with contact tracing (99). They are not designed to find and treat cases with latent infection and prevent endogenous reactivation. In low incidence countries with declining infection rates, however, tuberculosis patients are becoming older year after year, and a significant proportion of cases are found in the population segment aged 65 yrs and older (22, 100). The majority of these cases are attributable to endogenous reactivation of an infection acquired in the past (22, 86). Endogenous reactivation also plays an important role for the increasing number of tuberculosis cases among foreign-born persons in low incidence countries, as discussed in details in section 3 (4, 101).

M. tuberculosis is a resilient organism that can adapt to a wide variety of environmental conditions, making it a successful human pathogen (55). Better understanding of the molecular pathogenesis of latency, may point to ways of preventing endogenous reactivation, which is a prerequisite for eliminating the disease (12, 55). Primary *M. tuberculosis* infection leads to a substantial risk of developing early progressive disease, but it should also be taken into account that *M. tuberculosis* may remain latent for years and thereby constitute a persistent risk of late reactivation disease (3-5). Preventing new cases of tuberculosis caused by such reactivation may contribute significantly to the goal of elimination of tuberculosis (102). As case rates of active disease caused by recent transmission decreases in low incidence countries, the tuberculosis programs should be expanded to focus on finding and treatment of latent infection (102). Conversely, in most high incidence countries, where the tuberculosis burden is several fold greater, case finding and treatment of patients with active disease will continue to be the main components of tuberculosis control programs (102).

Preventive therapy in selected patients will become a key element in low-incidence countries approaching the elimination phase, at best based on evidence from clinical trials (23, 103). Preventive therapy is the treatment of persons with latent *M. tuberculosis* infection to reduce the risk of endogenous reactivation (23). It has generally consisted of daily administration of Isoniazid for 6-12 months (11, 103), and such treatment is 60-90% effective in reducing the lifelong risk of endogenous reactivation (11, 104). It is, however, an inefficient tool if used indiscriminately, i.e. a large number of infected individuals has to be treated to prevent the occurrence of a single case (22). Thus, it is necessary to clearly define groups at particularly high risk of developing tuberculosis, in whom preventive therapy provides individual and public health benefit, such as the Somalis in Denmark (see section 3.2) (4, 22). A blood test specific for *M. tuberculosis* infection has recently been introduced, which overcomes many of the drawbacks of TST and this test may prove valuable in finding latent cases (105). Until now, a policy of preventive therapy has not been implemented in Denmark (4).

3 IMMIGRATION AND TUBERCULOSIS

3.1 RECENT TRENDS

By the 1980s, the incidence of tuberculosis in many high income countries was very low and, consequently, clinical and research interests were diverted away from this disease to other health issues (14). At the same time, however, it was becoming clear that the long awaited eradication of tuberculosis was not imminent (14, 106). The century long decline in the annual number of cases and deaths due to tuberculosis was levelling off in these countries and more cases were being described in immigrant and ethnic minority communities (14, 26). Actually, some of the high income countries with substantial levels of immigration from areas in the world with a high prevalence of the disease, experienced increasing number of cases in immigrants that reversed the downward trend (23, 107-109).

Today, the proportion of immigrants among patients notified with tuberculosis exceeds 50% in Denmark, Israel, the Netherlands, Norway, Sweden, Switzerland, United Kingdom, and USA (17, 101, 110-114). In USA, three-quarters of all tuberculosis cases were among US-born in 1990 (115). A decade later, there were less cases among US-born than foreign-born (101). Also, from 1990 to 2000, there was a marked fall in the number of cases among US-born, but during the same period of time, the absolute number of cases among foreign-born rose despite increased investment in tuberculosis control (12). In Denmark, the total number of cases nearly doubled from 1986 through 2000 (4). While the overall incidence remained fairly stable among Danish-born cases (see section 4), most of the increase was explained by immigration from areas in the world with a high prevalence of tuberculosis, especially immigration from Somalia (2, 4, 28, 116). In 2000, two thirds of all tuberculosis cases in Denmark were immigrants, and nearly half of these were Somalis (4). Although during the last years (2001-2003), notifications declined again, probably due to the political determined decrease in immigration, this thesis will document that there is no room for complacency yet (27).

In other European countries and in North America, immigrants from Somalia also accounts for a noticeable proportion of tuberculosis cases (117-124). When a civil war, which began in the late 1970s, was intensified in the late 1980s, nearly half of all Somalis were forced from their homes, and by 1992, starvation threatened about one-fourth of the population (118). Many Somalis escaped to refugee camps in the bordering countries of Kenya and Ethiopia (118), and in the beginning of the 1990s, some of the Somalian refugees began to arrive in tuberculosis low incidence countries including Denmark (4).

Somalia has been known for an exceptionally high incidence and prevalence of tuberculosis for decades (125), and it has been documented that immigrants from Somalia have an incidence of up to 2000 per 100,000, which is among the highest reported in the world (4, 126, 127). The two main articles in this section focus on the

Somalian immigrants who arrived in Denmark during the 1990s (2, 4).

3.2 INCIDENCE AFTER ARRIVAL

The epidemiological importance of migration for tuberculosis low-incidence countries has been recognised for several years, and the main countermeasure has been the implementation of screening programmes for immigrants at the time of arrival (109, 128). The practice of screening on arrival was reported to be current in 20 of 23 European countries in 1994 (109). This measure is based on the fundamental assumption of a prompt decline in the incidence of tuberculosis in immigrants from high incidence areas during their first few years of residence in low incidence countries. Surprisingly, only a limited number of studies have addressed this question (116, 129-132). In Denmark, a study was undertaken to explore the changes after arrival in the incidence of tuberculosis in immigrants from high-incidence areas, and to evaluate the implications for the customary practice of screening on arrival (4). The study focussed on 13,535 Somalis who arrived in Denmark during the 1990s, 901 of whom were subsequently notified as having tuberculosis. Over the study period, there was a considerable increase in the number of Somali immigrants in Denmark, from 743 in 1991 to 14,856 in 2000. Because of this sustained rise, the annual increase in the number of Somali immigrants was classified as "net arrival" in Denmark, not taking into account the, in this context, small numbers who were born, died or left the country during the study period. Calculating incidences of tuberculosis in relation to duration of residence in Denmark, special attention was paid to Somalis notified as having tuberculosis during the period 1995 through 1999 (**Table 1**). Each incidence was calculated from the number of Somalis notified as having tuberculosis after a given number of years of residence in Denmark, divided by the total number of Somali immigrants who had resided in Denmark for the same number of years, as explained in details in the article (4).

The overall annual incidence of tuberculosis for Somalis remained fairly steady within 1.1-2.0%, but when the duration of residence in Denmark was taken into account, the incidence gradually decreased from 2.0% [CI 1.7-2.5%] during the year of arrival to 0.7% [CI 0.4-1.2%] during the 6th year of residence. Thus, the study documented that there was a high initial incidence of tuberculosis in Somalis entering Denmark, and perhaps more importantly, that this high initial incidence declined only gradually over seven observation years following arrival. The incidence of tuberculosis in Somalis in Denmark was higher than in any other foreign-born population group in the country (133, 134), and was comparable to, or even higher than, the estimated incidence in Somalia (125). During their first two years of residence in Denmark, no less than 3.9% of all Somalis developed tuberculosis, and it was calculated that during their first 7 years of residence in Denmark, 9.5% would develop tuberculosis.

Table 1. Risk for tuberculosis related to duration of residence in Denmark for Somali immigrants, 1995-1999. Source and details (4).

		Reported TB cases		
Average residence[a] (yrs)	Person-observation years	No.	Incidence % (no. in % of yrs) (95% CI)[b]	Cumulated incidence (%)
1/2[b]	4,788[b]	97	2.0 (1.7-2.5)	1.0[b]
1	9,746	158	1.6 (1.4-1.9)	2.6
2	9,876	131	1.3 (1.1-1.6)	3.9
3	8,490	95	1.1 (0.9-1.4)	5.0
4	5,696	63	1.1 (0.9-1.4)	6.1
5	3,885	45	1.2 (0.9-1.6)	7.3
6	2,394	17	0.7 (0.4-1.2)	8.0
7	842	13	1.5 (0.9-2.7)	9.5

a) Average duration of residence in Denmark
b) Person-observation years and cumulated incidence only "counts half" in year after arrival (see Methods); 95% CI,95% confidence interval.

The precise reasons for the extraordinarily high and only slowly declining incidence of tuberculosis in Somalis in Denmark remain unknown, but there are some theoretical possibilities such as active *M. tuberculosis* transmission and/or endogenous reactivation of latent *M. tuberculosis* infection. From a tuberculosis control point of view, an important consequence of excess tuberculosis cases among immigrants from high-incidence areas, could be an increase in the rate of active *M. tuberculosis* transmission in the recipient low-incidence country (135). As explained in section 3.3, however, active *M. tuberculosis* transmission among the Somalis in Denmark is limited, and the Somalis in Denmark have not substantially increased the risk of *M. tuberculosis* infection among Danes and immigrants from other countries (2). Therefore, active transmission cannot explain the high and only slowly declining incidence of tuberculosis in Somalis in Denmark. Regarding endogenous reactivation of latent *M. tuberculosis* infection, there is a "pool" of latent *M. tuberculosis* infection in immigrants from high-incidence areas, from which active disease could develop. Without taking BCG vaccination-status into account, TST of 300 Somalis in Copenhagen have indicated that 80-90% of all adults (16-49 years) and 25% of all children were infected at the time of arrival (A. Kok-Jensen, personal communication). Latent infection in combination with impairment of the immune system could cause endogenous reactivation in these Somalis, e.g. due to HIV infection, (3, 61). But only few Somalian tuberculosis patients in Denmark are found to be HIV positive, and four studies in Somalia from the early 1990s reported a very low prevalence of HIV infection, even among prostitutes attending a clinic for sexually transmitted diseases in the capital Mogadishu (125, 136-138). Thus, it is not the impression that HIV infection plays a major role for endogenous reactivation of latent infection in Somalis in Denmark. The precise mechanisms behind the initially high and only gradually declining incidence of tuberculosis in Somalis remain unknown, but the principal hypotheses is still that the immigrant population contains many cases of latent infection that later produce reactivation disease (139). Factors, other than HIV, that can promote this reactivation need to be identified, such as vitamin deficiencies, genetic constitution and immune defects (11, 140, 141). E.g. D-vitamin deficiency appears to be frequent among the Somalis (A. Kok-Jensen, personal communication). Also, part of the initial incidence in Denmark may be due to a high prevalence at arrival. Many cases of tuberculosis may not have been identified in the refugee camps, or even if they were diagnosed, may not have received proper treatment due to lack of resources (131). The discovery of such "ignored" cases during screening on arrival in Denmark could explain the extraordinary high initial incidence and partly explain why this could be higher than the estimated rate in Somalia, where such cases may remain unrecorded (125).

Only a limited number of studies in the world have described the trend in the incidence of tuberculosis in immigrants over the years following their arrival from an area of high incidence (116, 129-132, 142, 143). Three of these studies were restricted to immigrants arriving from Asia (116, 130, 131), and two covered only a short period of observation (131, 132). The main finding was a prompt decline in the incidence during the first few years of residence in the receiving country, although especially later studies have reported an increased tuberculosis risk many years after arrival, like that observed for the Somalis in Denmark (129, 131, 142, 143). The observation of a prompt decline in incidence has had major influence on the countermeasures taken to prevent and control the disease in the low incidence countries. Nearly all low-incidence countries have implemented programmes in which immigrants are screened only at the time of arrival (109, 128). In Denmark, all refugees and asylum seekers are encouraged to attend a medical evaluation not specific for tuberculosis only at the time of arrival in the country. Those who do not arrive as refugees or asylum seekers, e.g. for family reunion, do not undergo systematic medical evaluation but are entitled to contact the free of charge public health care system on their own ini-

tiative. After the medical evaluation on arrival for those who accept it, the immigrants, refugees and asylum seekers in Denmark, as in most other low-incidence countries, follow the national tuberculosis programme based on passive case-finding and treatment of active cases combined with contact tracing (99). However, the slow gradual decline in incidence in the years following arrival observed for Somalis in Denmark and different nationalities in USA, the Netherlands and Canada (129, 131, 142, 143), seriously challenges the adequacy of the present policy of screening only on arrival. National tuberculosis programmes in low-incidence countries should be expanded to include surveillance of trends in the incidence of tuberculosis in the specific immigrant populations during subsequent years as well. If a similar only gradual decline can be observed as in the Somalis in Denmark and in different nationalities elsewhere, the present policy of screening only on arrival needs to be revised and refocused. Such revision would probably include as an important feature the inclusion of voluntary regular health examinations, at reasonable intervals after arrival, for specifically identified high-risk immigrant groups, as the risk may persist for many years. Intervention needs to be an ongoing process that includes both latent *M. tuberculosis* infection as well as active tuberculosis. Therefore, an important way of preventing tuberculosis in high-risk groups like the Somalis in Denmark could be preventive chemotherapy, as discussed in section 2.5. On an individual basis, several controlled studies have documented the effectiveness of such a strategy in preventing reactivation of disease (144), but the effectiveness of preventive chemotherapy on a population scale basis needs further evaluation (22, 135). The cover and compliance of participants is crucial for obtaining satisfactory results (144). E.g. a large meta-analysis showed that only 60,5% of 1,084,760 persons completed preventive chemotherapy (145). If used indiscriminately, a large number of infected individuals would have to be treated to prevent the occurrence of a single case of tuberculosis (144), and all those treated would be at risk of side effects from the medication (145). However, preventive chemotherapy could indeed decrease suffering for the 9.5% of Somalis who develop tuberculosis during the first 7 years of residence in Denmark, if the medication could be efficiently distributed to the Somalis with latent infection. It is striking, that practically no preventive therapy is given in Denmark, which house the immigrant group with perhaps the highest documented risk worldwide of reactivation disease (2, 4, 134).

3.3 TRANSMISSION AFTER ARRIVAL

It still remains to be fully clarified to what extent increased immigration from tuberculosis high incidence countries contributes to an increased risk of tuberculosis among low incidence resident populations (114, 122). Recently, such a study was undertaken in Denmark combining conventional epidemiology and molecular subtyping (2). The aim was to analyse the importance of *M. tuberculosis* transmission among Somalis in Denmark, and between Somalis and Danes. Thus, to determine if *M. tuberculosis* was transmitted significantly to the Danish population, and whether part of the high Somalian tuberculosis incidence in the years after arrival in the country could be explained by active *M. tuberculosis* transmission occurring after arrival (4). If so, such factors could indicate a poor quality of the national tuberculosis control programme. The Danish study was a retrospective cohort analysis of all *M. tuberculosis* culture positive patients notified in Denmark from 1992 through 1999 (n=3320). The magnitude of *M. tuberculosis* transmission was analysed by three different approaches. The most important approach was a detailed analysis of each of the clustered Somalian immigrants with *M. tuberculosis* culture positive tuberculosis during 1996 through 1998 (n=391) (**Table 2**), as explained in details in the article (2).

A total of 35 different clusters were identified in the study. Only 9 minor clusters were "mixed" i.e. included both Somalian and Danish tuberculosis patients. In these 9 clusters, the vast majority of patients (>75%) had the same nationality, and actually 5 out of the 9 clusters included only one patient with "the other" nationality. Based on the criteria mentioned above, possible routes of *M. tuberculosis* transmission among and between the Somalian immigrants and Danes were analysed (Table 2): 75% (293/391) of all Somalian immigrants appeared to have been infected before arrival in Denmark, either because they were harbouring unique DNA fingerprints, or because they belonged to clusters containing no AFB smear positive "source" patients. Further 23% were most likely infected by other Somalian immigrants, because they belonged to clusters where all, or the vast majority of patients (>75%), were Somalian immigrants. The last 2% (n=7) were most likely infected by Danes, because they belonged to clusters where all other, or the vast majority of patients (>75%), were Danes. In the same period, 1% (n=4) of all Danish tuberculosis patients belonged to clusters where all other, or the vast majority of patients (>75%), were Somalian immigrants.

Table 2. Analysis of *M. tuberculosis* transmission among and between 391 Somalian immigrants with tuberculosis from 1996 through 1998[a] and the resident population. Source and details (2).

	No. cluster/total[c] (%)			
Age (yrs)	**1996**	**1997**	**1998**	**1996-1998**
≤15	1/13 (7.7)	5/20 (25.0)	5/13 (38.5)	11/46 (23.9)
16-30	13/56 (23.2)	22/86 (25.6)	25/85 (29.4)	60/227 (26.4)
31-45	6/21 (28.6)	8/38 (21.1)	11/42 (26.2)	25/101 (24.8)
46-60	0	0/5 (0.0)	1/3 (33.3)	1/3 (33.3)
61-75	0	1/3 (33.3)	0/1 (0.0)	1/3 (33.3)
>75	0	0	0	0
No. cluster/ total[c] (%)	20/95 (21.1)	36/152 (23.7)	42/144 (29.2)	98/391 (25.1)

a) More than 97% of all reported *M. tuberculosis* culture-positive Somalian patients in Denmark.
b) Assumptions are specified in Materials and Methods; in brief, the cluster should include one or more AFB smear positive source patients.
c) Number of patients in a cluster divided by total number.

Another approach to analyse the magnitude of recent transmission in Denmark was also used: The IS*6110* RFLP patterns from all clusters with possible *M. tuberculosis* transmission among Somalian immigrants in Denmark, were compared to the Dutch IS*6110* RFLP pattern database held in the Mycobacterium Reference Laboratory in Bilthoven, the Netherlands. This comparison was done to determine whether these Somalian IS*6110* RFLP patterns were "internationally" frequent and provided this was the case it would be less likely to represent recent transmission in Denmark (45). The Dutch database included 9387 DNA fingerprint patterns from all culture positive tuberculosis patients in the Netherlands collected since 1993. DNA fingerprint patterns from 18 out of the 35 Somalian clusters with possible *M. tuberculosis* transmission in Denmark were also found in the Dutch DNA fingerprint database, representing 60% (59/98) of the clustered Somalian tuberculosis patients in Denmark. Altogether 578 Dutch matches were found distributed on 17 clusters and one unique strain. The 17 clusters included mainly Somalian patients, of which one cluster is the previously reported 4th. largest cluster in the Netherlands (146). Seven of the clusters included ≥ 10 patients.

It is generally assumed that the proportion of clustered patients in a population reflects the extent of recent *M. tuberculosis* transmission. However, as discussed in section 2.4, the inference of the proportion of disease due to recent transmission from a crude proportion of clustered patients is debatable and should be interpreted with care (45). Until recently, no predominating Somalian *M. tuberculosis* strains had been reported. However, in the Danish study, nearly 90% of all the clustered Somalian immigrants could be included in just 16 different clusters, of which the 4 most frequent clusters accounted for 45% of all clustered patients. Furthermore, 17 out of the 35 clusters identified with possible active transmission in Denmark, were also identified as clusters in the Netherlands. These 17 clusters accounted for 60% of all the clustered patients. Based on these findings

the existence of several predominant Somalian strains was proposed, explaining the high overall figure of clustered Somalis in Denmark, which therefore do not necessarily reflect the actual extent of recent transmission in Denmark. This is furthermore likely to be true because some of the Somalis clustered in Denmark could very well have acquired the infection in Somalia. On the other hand, some of the unique strains in the Somalian immigrants in Denmark may have arisen from recent transmission outside the country, like for instance in refugee camps, but this would of cause not influence the magnitude of recent transmission in Denmark. Some of the unique strains could be clustered with unknown source cases in Denmark, and such an event would increase the "true" extent of recent transmission, but due to the nationwide fingerprinting, the centralised notification and treatment system, and significant number of Somalian immigrants in Denmark, this is believed to be the case in only a minority of cases. In conclusion, *M. tuberculosis* transmission leading to tuberculosis among Somalis in Denmark is limited, and similar transmission between Somalis and Danes is almost non existing. The majority of all Somalian immigrants developing tuberculosis in Denmark has been infected before their arrival.

By now, several studies have documented limited *M. tuberculosis* transmission between immigrants from high incidence countries and the resident population in low incidence countries. In San Francisco USA, US-born tuberculosis cases generated many more secondary cases than immigrants, especially young black US-born cases (147). In a later study, also from San Francisco, none of 252 foreign-born cases were recently infected in the city. Conversely, 19 of 115 US-born cases occurred after recent infection in the city, and comparable to the situation in Denmark, only two US-born cases were infected by a foreign-born patient (148). In Maryland USA, most recent transmission leading to tuberculosis occurred between US-born persons (149). In contrast, transmission between foreign-born persons occurred exclusively in households, and there were no instances of transmission between US-born and foreign-born persons (149). In Somalian immigrants in London, the risk of reactivation was found to be much greater than the risk of recent transmission (123) Also in Norway, tuberculosis among immigrants was mainly due to reactivation of a previous infection, and transmission between Norwegian-born and immigrants was limited, despite many Somalian immigrants in Norway with a high incidence of tuberculosis (111, 150). In contrast to studies mentioned above, a Dutch study found 17% of the tuberculosis cases among the resident Dutch population to be attributable to active transmission from non-Dutch source cases (122). Also, it has been predicted that by 2030 60% of the tuberculosis cases among the Dutch population will be attributed to infection from a non-Dutch source case (114). In the Danish study, only 1% of all Danish tuberculosis patients appeared to be infected by Somalian immigrants, although 39% of all the immigrants with tuberculosis in Denmark were from Somalia. Some of the disparity between the Danish and the Dutch figures might be explained by differences in the methods used. In the Dutch study, patients in any clusters with mixed nationalities were regarded as cases of possible transmission from a non-Dutch source case, and the presence or absence of an AFB smear positive possible source case within each cluster was not taken into account. Some of the disparity between the Dutch and Danish study, and some of the other studies, may be explained by differences in the social integration of immigrants, and it may also reflect social differences between the groups of immigrants. E.g. in Denmark, the Somalian immigrants tend to live with only limited social contact with the Danish population. With time and integration, more *M. tuberculosis* transmission is likely to occur as observed for Greenlanders and Danes (48).

3.4 SIGNIFICANCE OF IMMIGRATION

In the previous sections of this chapter it is described how the increasing rates of tuberculosis in Denmark, as well as in many other low incidence countries, are associated with immigration from areas in the world with a high prevalence of the disease, especially during the past decade (2, 4). Furthermore, how tuberculosis among these immigrants can be largely attributed to endogenous reactivation of latent *M. tuberculosis* infection (2, 4). It was documented that the majority of immigrants were infected prior to arrival (2), and their prevalence of infection largely mirrored that of their country of origin (4, 22). Conversely, there was only limited active *M. tuberculosis* transmission after arrival (2).

As the rates of tuberculosis declines in low incidence countries, transmission of *M. tuberculosis* is markedly reduced and an increasing number of cases can be attributed to endogenous reactivation in selected populations who have previously been infected (108). In most cases, the initial infection has occurred in the same country, as it has been observed in the older segments of the Danish-born tuberculosis patients (5), or the infection has occurred in another country and has subsequently been "imported", as it has been observed in the Somalis in Denmark (2, 4). Thus, tuberculosis has increasingly come to be a disease of specific subgroups of the population in low-incidence countries (135). This development provides an opportunity for targeted intervention, the success of which will depend on correctly identifying the population groups at risk. Because of considerable geographic variations in the prevalence of tuberculosis in immigrants from different countries and different trends in incidence following arrival in different host countries, approaches to controlling and preventing tuberculosis should be tailored to the specific foreign-born populations at risk (17). Control and elimination strategies need to aim at diminishing the incidence and prevalence of latent infection to reduce the pool of tuberculosis infection from which future cases of tuberculosis will emanate. Our control measures are, however, primarily based on screening of immigrants on arrival, passive case-finding, treatment of active cases, and contact tracing. These measures will not be effective among the immigrants and Danish-born cases with reactivation disease, emphasizing the potential importance of preventive therapy discussed in section 2.5 and section 3.2.

In Denmark, the influence of immigration on the rates of tuberculosis may be changing once more. For the first time in more than a decade, there has been a notable decrease in the total number of cases in 2001 through 2003, because of a politically determined decrease in the immigration from areas in the world with a high prevalence of the disease. Furthermore, the relative share of Somalian immigrants fell from 47% in year 2000 to 31% in year 2002. But the pool of latent infection is still there, and endogenous reactivation will continue to generate new cases in the years to come. Furthermore, tuberculosis will continue to become a relatively more important problem among immigrants because of the overall decreasing rates of tuberculosis among the majority of the indigenous population in Denmark and in other low incidence countries (22). In year 2002, two thirds of all tuberculosis cases in Denmark were found among immigrants despite the notable decrease in the total number of cases.

Over the past 1-2 decades, an increasing part of tuberculosis in low incidence countries can be associated with immigrants arriving from high incidence areas. Little evidence exist, however, to show that early detection of tuberculosis in immigrants conveys appreciable public health benefit to those born in the host country (151). This is not to say that people who live in close proximity are not at any risk, but if the health of immigrant populations and those who live in close proximity is the cause of concern, then this should be the explicit rationale, and these individuals should be offered adequate prevention, treatment and care (151). In connection with this, culturally-sensitive services are essential if the elimination strategy is to succeed (23). However, the global perspective of tuberculosis should be borne in mind. Because of human migrations, the elimination of tuberculosis in low incidence countries cannot be envisaged without concomitant improvements in its control in high incidence resource poor countries (25).

4 DOMINANT STRAINS AND TUBERCULOSIS

4.1 DOMINANT STRAINS CONCEPT

Advances in our understanding of the molecular biology of *M. tuberculosis* have proven invaluable in constructing the phylogenetic structure of the species (41). As described in section 1.3, the *M. tuberculosis* genome changes over time. This gives rise to strain variants that can be detected on the basis of subtle genetic changes (41). Especially two classes of repetitive DNA elements have been used to visualise such changes: insertion sequence elements and small repetitive DNAs (152). The insertion sequence elements, such as the gold standard marker IS*6110*, have the capacity to move within the genome of *M. tuberculosis*, whereas the small repetitive DNAs predominantly rearrange themselves by homologous recombination (152). Subsequently, the genetic changes can be visualised by RFLP- and spoligotyping, that appear as additions or deletions of band(s) and spacer(s), respectively (41, 42, 153).

Together, a collection of closely related strains derived from a common ancestor can be classified as a group and, over time, progeny of such a group can disseminate into new populations (clonal expansion) giving rise to new groups of related strains (41, 154). The identification of independent genetic markers common to members of multiple groups provides a framework to classify such groups more broadly into a family structure (41, 152, 155). Thus, individual members of a family are closely related on the molecular level. They are believed to be the progeny of a single ancestral *M. tuberculosis* cell that has undergone clonal expansion, and the members form a large branch in the *M. tuberculosis* phylogenetic lineage (156). Because the changes in the genome of *M. tuberculosis* are time-dependent, the degree of polymorphism among progeny of a particular strain is a reflection of the time that has elapsed since their divergence (152). Analysis of the population structure of strains of *M. tuberculosis* may therefore provide information about the evolutionary history and dissemination of particular strain(s) in the population, or information about the evolutionary history and dissemination of particular strain(s) in populations in different geographical regions in the world (41, 157). So far, the largest family of *M. tuberculosis* strain recognised is the Beijing family (41, 156, 157). These strains carry a large number of IS*6110* insertion elements, and their IS*6110* patterns are highly similar (152, 155).

By now, several population-based genotyping studies have demonstrated that a small percentage of strains can cause a disproportionately large number of cases (39). These strains are sometimes referred to as "dominant strains". The next two sections describe the most dominant family of strains characterised worldwide, and the most dominant strain characterised in Denmark, respectively.

4.2 THE BEIJING FAMILY

The groups of *M. tuberculosis* strains collectively known as "the Beijing family" is the most dominant family of *M. tuberculosis* strains characterised worldwide. This family has attracted special attention and has been reported extensively in the tuberculosis literature (41, 157). It was discovered in the Beijing area in China in 89% of all isolates from tuberculosis patients hospitalised in 1992 and 1993 (152). The "W-strain family", concurrently identified on the American (156) and Asian continents (152), represent the same genotype family (155).

The traditional view is that different strains of *M. tuberculosis* are equally virulent (39). The first Beijing report suggested, however, that the Beijing strains may possess selective advantages compared to other strains, and that they may be expanding aggressively to other countries and continents (152). Subsequently, Beijing strains have been associated with (multi)drug resistance (156, 158-162), and with specific pathogenic properties and increased virulence (158, 159, 163, 164). There have been many reports that Beijing strains are increasing in frequency and spreading to new geographical areas (156, 160, 165, 166), and they have been reported to be highly prevalent throughout Asia and in the countries of the former Soviet Union (152, 158, 159, 167-169). It has also been suggested that the transmission potential of Beijing strains is likely to be enhanced, as compared with that of other strains, because Beijing strains are more readily aerosolised, can establish infection more effectively, and/or progress more frequently from infection to disease (39).

Until recently, the significance of Beijing strains was unknown in Denmark. Therefore, an investigation was undertaken inspired by a European Union Concerted Action (CA) project (6, 155). Within the framework of the European Union CA project "New Generation Genetic Markers and Techniques for the Epidemiology and Control of Tuberculosis" the worldwide occurrence of Beijing strains is being investigated. In connection with this, the Beijing definition has been validated and the correlation between different DNA fingerprinting methods used to recognise Beijing strains among strains of *M. tuberculosis* determined (155). Spoligotyping was taken as the gold standard (153). Presence of at least three of the last nine spacers (no. 35-43) and none of the spacers 1 to 34 is considered to be 100% specific for the Beijing family (155). However, worldwide, the RFLP IS*6110* method is the most widely applied DNA fingerprinting technique for strains of *M. tuberculosis*, and by 2001, estimated 50,000 strains had been genotyped by this method (41). Therefore, the CA project group also explored how to identify Beijing strains on the basis of their IS*6110* RFLP patterns enabling the search for Beijing strains in large IS*6110* RFLP pattern collections, such as the Danish (155): 19 "IS*6110* Beijing reference strains" were defined using a representative sample of strains of *M. tuberculosis*. The IS*6110* RFLP patterns of these 19 Beijing reference strains were found to be highly specific and sensitive in retrieving Beijing strains from large IS*6110* RFLP databases, when compared with the "Beijing gold standard" of spoligotyping.

In the Danish study (6), strains of *M. tuberculosis* from 3844 tuberculosis patients were searched for the Beijing genotype. This represents 97% of all culture positive tuberculosis patients in Denmark from 1992 through 2001. In addition, 201 "historical strains" of *M. tuberculosis*, retrieved from culture positive tuberculosis patients in Denmark from 1961 through 1967, were analysed (3, 5, 67, 68). Among the historical strains, no Beijing strains were identified, but among the more recent strains, 96 Beijing strains were retrieved from different patients. Overall, 56% of the 3844 tuberculosis patients were foreign-born, originating from 90 different countries. Among Danish-born patients, 1.0% had Beijing strains compared to 3.5% among the foreign-born patients. The highest prevalence of Beijing strains was found among patients from Asia. By country of origin the prevalence of the Beijing strain varied: 24.2% from Vietnam, 33.3% from Thailand, 9.7% from Indonesia, 8.8% from Sri Lanka, 0% from the Philippines and 0% from Pakistan. Beijing strains were also found in 1.7% of patients from Somalia and in patients from the Middle East, including 7.5% from Iraq, 11.1% from Iran, and 3.9% from Afghanistan. None were found in patients from Eastern Europe: most of these (149) were from the former Yugoslavia, and only 6 from the former Soviet Union. From 1992 through 2001, there was no evidence of an increase in the prevalence of Beijing strains among the Danish- or foreign-born patients, even though it was documented that Beijing strains had been present in Denmark for at least 10 years. Among Danish-born patients, but not among immigrants, those with Beijing strains were more likely to be drug resistant. Although this association was formally statistically significant, it was based on only 2 resistant cases among 16 Danish born patients with Beijing strains. Thus, the Danish study found only weak evidence of an association with drug resistance and low levels of Beijing strains. The study included an estimated 8% of all strains of *M. tuberculosis* IS*6110* RFLP typed worldwide during the study period, of which 57% were retrieved from foreign-born patients from 90 different countries.

In section 3.2 and 3.3, the two studies analysing the significance of *M. tuberculosis* transmission in Denmark due to immigration from a high incidence country and the persistent high incidence of tuber-

Table 3. Proportion of tuberculosis patients with Beijing family strains. Source and details (6).

	Denmark-born NN (%)	Non-Denmark-born NN (%)	Total NN (%)
All	17/1,659 (1.0)	79/2,183 (3.6)	96/3,844 (2.5)
Male	9/1,057 (0.85)	49/1,163 (4.2)	58/2,220 (2.6)
Female	8/602 (1.3)	30/1,018 (3.0)	38/1,620 (2.4)
Age group (yrs)*			
<25	2/118 (1.7)	21/655 (3.2)	23/773 (3.0)
25-44	7/553 (1.3)	48/1,159 (4.1)	55/1,712 (3.2)
45-64	4/522 (0.77)	6/247 (2.4)	10/769 (1.3)
65+	4/466 (0.86)	4/121 (3.3)	8/587 (1.4)
Year			
1992-1993	4/335 (1.2)	12/249 (4.8)	16/584 (2.7)
1994-1995	2/371 (0.54)	19/418 (4.6)	21/789 (2.7)
1996-1997	2/330 (0.61)	16/506 (3.2)	18/836 (2.2)
1998-1999	4/316 (1.3)	15/555 (2.7)	19/871 (2.2)
2000-2001	5/307 (1.6)	17/455 (3.7)	17/764 (2.9)
Area of origin*			
Western Europe	17/1.659 (1.0)	0/71 (0.0)	17/1,730 (0.98)
Eastern Europe		0/174 (0.0)	
Indian subcontinent		8/290 (2.8)	
South East Asia		37/183 (20.2)	
East Asia and Pacific		3/10 (30.0)	
Middle East		6/211 (2.8)	
North Africa		1/38 (2.6)	
Sub-Saharan Africa		20/1,111 (1.8)	
Americas and Caribbean		0/16 (0.0)	
Previous TB*			
No	17/1,550 (1.1)	78/2,164 (3.6)	95/3,716 (2.6)
Yes	0/109 (0.0)	1/19 (5.3)	1/128 (0.79)
Site of TB			
Pulmonary	16/1,394 (1.2)	56/1,248 (4.5)	72/2,642 (2.7)
Extrapulmonary	1/263 (0.38)	23/930 (2.2)	24/1,193 (2.0)

*) Information on immigration status missing for three patients; on region of origin for 81; on age for 3; on sex for 4; and on site of tuberculosis (TB) for 9.

culosis among the immigrants in the years after arrival were described (2, 4). These studies concluded that the majority (>75%) were infected before their arrival and reactivated their latent infection (4), and that nearly all of those that could have been infected after arrival (<23%) were most likely infected by a source from the country of origin (2). Therefore, it was relevant to compare the observed prevalence of Beijing strains in the Danish study with that in the country of origin (**Table 3**). For example, 24% of immigrants from Vietnam had Beijing strains compared with 54% in Hanoi and Ho Chi Minh City (159). In the Vietnamese study, 563 samples from the late 1990s were included, whereas the majority of Vietnamese-born immigrants arrived in Denmark during the early 1980s (116). This could indicate that Beijing strains emerged in Vietnam primarily after the early 1980s, which fits with the observed higher prevalence of Beijing strains in younger age patients observed in the Vietnamese study. Regarding strains from patients born in Eastern Europe, none of the 174 immigrants had Beijing strains compared with reports of 22% to 71% (157, 162, 166, 170). However, the majority of strains analysed were from immigrants from the Former Yugoslavia, where the prevalence of Beijing strains is unknown. These immigrants arrived in Denmark during the 1990s, so the Danish data suggest that the prevalence of Beijing strains was very low in this area at least at that period of time. There are few reports from Africa (171-174). In the Danish study, only 1.7% out of the 984 Somalian-born immigrants, nearly all arriving in Denmark during the 1990s (4), had Beijing strains. Among the remaining 122 immigrants born in 22 other African countries, only 3 additional Beijing strains were retrieved, from patients born in Zimbabwe, Kenya and Angola. Beijing strains seem to be rare on the African continent, but local studies are needed to prove this. Immigrants do not represent a random sample from the country of origin and some immigrants may even have acquired their *M. tuberculosis* infection during the process of escaping from one region to another.

The Danish study included one of the largest samples of strains of *M. tuberculosis* searched for Beijing strains worldwide until now. Although it was regarded as highly representative for the Danish population in the 1990s, and partly for the Danish-born population in the 1960s, the IS*6110* RFLP patterns of the strains from the foreign-born patients is unlikely to be an accurate reflection of the distribution of patterns in their country of origin. Also, identified patterns were a mixture of "recent" *M. tuberculosis* transmission and reactivation of latent infections and thus also represented patterns circulating decades ago (3, 5). However, the low prevalence of Beijing strains found in the Danish study contrasts some reports, but there is limited information available from most areas of the world making definite conclusions about the extent of spread of Beijing strains and their associations with drug resistance premature (157). Furthermore, publication biases may distort the picture. Studies in which Beijing strains have been looked for but not found may not have been published. Recently, two studies from Delhi and Bombay in India reported very few Beijing strains (175, 176). Similarly, both in this study and in a previous study, the prevalence of Beijing strains in the Philippines was found to be very low, 0% and 2%, respectively (177). This indicates, that even in Asia there may be great variation in prevalence. In Finland, only 1% of all tuberculosis patients were infected with Beijing strains (178), whereas in the Netherlands, Beijing strains accounted for 6% of all cases and were significantly associated with diagnosis in recent years, young age, nationality, and multi-drug resistance (179). More unbiased studies, also reporting negative findings, are needed.

4.3 THE DANISH CLUSTER 2

In order to look for other predominant strains of *M. tuberculosis*, and additional search was performed among RFLP-patterns from 4102 "recent strains" from tuberculosis patients from 1992 through 2001, and 201 "historical strains" from tuberculosis patients from

1961 through 1967 (7, 27). 43% of all the recent strains were retrieved from Danish-born patients, and 9% of these strains were resistant to at least one of the five drugs Isoniazid, Rifampicin, Ethambutol, Pyrazinamide and Streptomycin, as well as 9% of the recent strains retrieved from foreign-born patients. Only 0.1% and 0.6%, respectively, were multi-drug resistant. All the historical strains were retrieved from Danish-born patients, and 7% of these strains were resistant to at least one of the three drugs streptomycin, Para-amino salicylic acid and Isoniazid, which were used in standard regimens in Denmark during the 1960s (further details in the article) (7).

The two most frequent strains of *M. tuberculosis* found in Denmark were the "Danish Cluster 1" and "Danish Cluster 2" strains (Figure 2) comprising 5% and 7%, respectively, of all recent strains examined. These strains were almost exclusively retrieved among Danish-born patients, among whom Cluster 1 and Cluster 2 accounted for 10% and 15%, respectively, as compared to 0.6% and 0.6% for foreign-born patients. Among the Danish-born patients, the proportion of Cluster 1 strains from 1992 through 2001 decreased gradually from 13% to 7% ($p<0.15$), whereas the proportion of Cluster 2 strains increased sharply from 6% to 29% ($p<0.001$) (**Figure 3**). The Danish-born Cluster 2 patients were on average younger (42 versus 51 years), more likely to be males (81% versus 64%, $p<0.02$), and more likely to have pulmonary involvement only (90% versus 65%, $p<0.12$) than the other Danish-born tuberculosis patients. During the first four observation years, the Danish-born Cluster 2 patients were mainly found in the capital city (Copenhagen) and its surroundings, but later on also increasingly in the north-western and south-eastern parts of Denmark. In total, Cluster 1 and Cluster 2 accounted for 184 and 272 strains of *M. tuberculosis*, respectively. Thus, they comprised without comparison the two largest clusters in Denmark, and they are among the largest clusters reported worldwide (165). Conversely, none of the historical strains were Cluster 1 or Cluster 2 strains, with the highest similarities between the IS*6110* patterns at only 81.5% and 77.8% respectively. For Cluster 1 this is in agreement with a theory that this strain was imported from Greenland to Copenhagen in the 1980s (48), and for Cluster 2 it might indicate that the strain arrived in Denmark after 1967, as one would also expect from linear backward extrapolation of the increasing Cluster 2 curve.

The search for potentially dominant strains of *M. tuberculosis* in Denmark documented a remarkable and previously unknown increase in the relative frequency of Cluster 2 strains among the Danish-born tuberculosis patients during the last few years, and that this genotype is increasing and spreading to new parts of the country, although not spreading to the foreign-born population (7, 27). A new dominant strain of *M. tuberculosis* is emerging in Denmark. It is most likely an outbreak caused by active transmission, although the specific reasons for the increasing dominance and the change in geographical distribution of the Cluster 2 strain still needs to be explored. It is worrying, however, and perhaps partly explained by the fact that Cluster 2 is associated with younger males with pulmonary disease manifestation. During the last decade, the decrease in incidence of tuberculosis among Danish-born cases has stagnated, masking a falling incidence among elderly Danes, and increasing incidence among younger to middle-aged Danes (7, 27, 180). This increasing incidence among the younger to middle-aged Danes has been associated with active *M. tuberculosis* transmission in the inner Copenhagen area, especially among 25-54 years old males with records of social problems such as homelessness and alcohol and/or drug abuse, and records of sputum smear positive pulmonary tuberculosis (28). The strain of *M. tuberculosis* isolated from this group of patients is typically the Cluster 2 strain. The change in geographical distribution might be associated with the increasing living costs in the capital area of Copenhagen, causing socially marginalised groups to migrate to the cheaper areas in the country "carrying along" the Cluster 2 strain. Cluster 2 has also been reported in the southern part of Sweden, a geographical area neighbouring Denmark, but it has not been found in the "international database" of IS*6110* RFLP patterns (91).

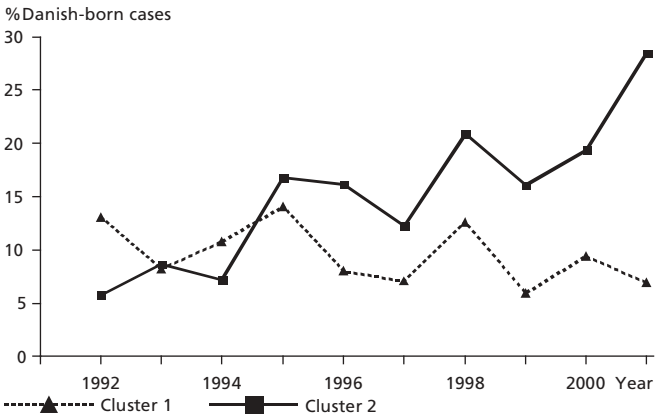

Figure 3. Trends in Danish Cluster 1 and Danish Cluster 2 strains of *M. tuberculosis* among all culture-positive Danish-born tuberculosis patients reported in Denmark from 1992 to 2001. Source (7).

In Denmark, as in most other low incidence countries, the national tuberculosis programme is based on passive case-finding and treatment of active cases combined with contact tracing (99). This program involves: chest x-ray examination if pulmonary symptoms persist for more than 6 weeks, examination for *M. tuberculosis* if chest X-ray shows infiltration compatible with tuberculosis, examination by chest x-ray every 6 months for about 3 years in tuberculin positive subjects with recent close exposure to a smear positive tuberculosis patient, examination for *M. tuberculosis* from extra pulmonary sites if symptoms indicate tuberculosis, free of charge four-drug short-course treatment regimens for tuberculosis cases, and preventive chemotherapy only for tuberculin converters below 7 years of age (99). However, the national tuberculosis programme is not always followed (or known), especially in areas with very few cases and lack of experience. The Danish study documented that Cluster 2 patients were more often sputum smear positive, as compared to other Danish-born tuberculosis patients, pointing in the direction of late diagnosis and treatment. The key to diagnosing tuberculosis is to suspect tuberculosis in any person with signs or symptoms of the disease, and to obtain appropriate specimens for bacteriological examination (181). However, patients with social problems such as poor living conditions, alcohol consumption and drug abuse might be less likely to seek medical attention causing patients delay. Furthermore, the awareness of tuberculosis has been weakened in Denmark due to the low prevalence of the disease, especially among the Danish-born part of the population. This sometimes results in long "doctor's delay" and thus prolonged active transmission (182).

Another important issue in connection with Cluster 2 is contact investigations. Contacts of persons with infectious tuberculosis are at risk of infection and disease. This risk is dependent on the infectiousness of the source case, the characteristics of the contact, and the environment they share (181). As soon as the diagnosis of tuberculosis in the source case is strongly suspected on laboratory and/or clinical basis, investigations of contacts should begin. Such investigations are mandatory in Denmark (183), but they are not systematically performed (27). When the contact investigations are not performed, or if they are performed inefficiently, active transmission can continue, as documented in the Danish Cluster 2 outbreak.

The present situation justifies an increased focus on early tuberculosis diagnosis and control of transmission in Denmark, and the use of extra resources in order to change the development. A plan for active intervention needs to be designed and implemented. Our performance in the next years may have great influence on the tuberculosis situation in Denmark during the next decades and thus whether tuberculosis will be eradicated in Danes by the year 2040 as previously predicted (184).

4.4 SIGNIFICANCE OF DOMINANT STRAINS

Chance would predict that, eventually, a few strains would emerge as dominant in any given population, but this cannot explain why one genotype family of *M. tuberculosis* has become predominant in many different populations worldwide, and implies, that some genotypes may have an advantage over others in their ability to be transmitted and cause disease (152, 163, 165). Such strains of *M. tuberculosis* are sometimes referred to as "virulent strains" (158, 159, 163, 164), but the definition of virulence is controversial (49). From the pathogens point of view, virulence may be measured by the number of organisms required to establish an infection, rate of bacterial growth, dissemination, steady-state bacterial load, long-term persistence, and even transmissibility (49). From the host point of view, virulence may be measured by susceptibility to infection, tissue pathology, severity of disease symptoms, number of susceptible individuals in a population, rate of disease progression, and mortality (49). All these factors should be taken into account in a comprehensive assessment of virulence, but many factors have been inadequately examined only (49).

Also, the fact that a strain of *M. tuberculosis* is dominant does not necessarily mean it is more virulent. You may ask what came first, the chicken or the egg? Are strains frequent because they are virulent or are they believed to be virulent because they happen to be frequent at the moment? Beijing strains have been associated with specific pathogenic properties and increased virulence (156, 158-164), but as discussed in details in section 4.3, the increasing number of Cluster 2 strains in Denmark is most likely an outbreak caused by active transmission, preferentially in the poorest segments of the population (7, 27). Even if the Danish Cluster 2 strain were to be more virulent than other strains, this would only emphasise the need of an increased focus on early tuberculosis diagnosis and control of transmission in Denmark.

As the incidence of tuberculosis decreases in low incidence countries it is likely that outbreaks will become more commonly recognised (22). Thus an appropriate plan to handle such outbreaks need to be implemented, and this plan should take into account that tuberculosis preferentially strikes the poorest segments of the population.(23). Emphasis must shift away from *M. tuberculosis* as the "cause" of tuberculosis towards focus on the underlying social and political factors (106). The surveillance system should be able to provide detailed epidemiological information, which allows the identification of specific population groups that have an incidence of tuberculosis that is in excess of that in the general population, because these groups may gain particular benefit from preventive interventions (22). General notification figures on an annual basis are not enough as emphasized by the Danish Cluster 2 outbreak. The significant increase of cluster 2 patients in Denmark, despite warnings (28, 184) and the potential availability of effective control measures (99, 185, 186), is a disgrace to the Danish health care system.

5 CONCLUSIONS AND FUTURE ASPECTS

Like other tuberculosis low-incidence countries (23), Denmark is confronted with specific problems and challenges as a result of the successful shift from high to low incidence during the last decades. These problems and challenges are a consequence of: The overall steady declining incidence in the national-born part of the population, the gradually increasing relative and absolute importance of latent *M. tuberculosis* infection and tuberculosis from other countries and the emergence of tuberculosis in groups at particular high risk (1-7). An adaptation of available intervention strategies to the ever changing situation is required if elimination of tuberculosis is to be achieved as previously predicted (184). Although passive case-finding and treatment of active cases combined with contact tracing, commonly considered the most cost-effective approaches, remain the basis of the case-detection policy, a more aggressive approach seems to be justified.

A spectrum of interventions is available (23). First, early detection and treatment of all cases to reduce active transmission, human suffering and avoidable deaths. Second, reducing the incidence of infection by identifying the sources/risk groups of infection in the community at the earliest possible time and interrupting the chain of transmission. Third, reducing the prevalence of latent *M. tuberculosis* infection among those at high risk of progression to manifest disease.

The Danish tuberculosis control interventions have not been fully successful during the last decade (4, 7, 27, 182). The remarkable and previously unknown increase in the relative frequency of cluster 2 strains among the Danish-born tuberculosis patients emphasizes that there is a need of an increased focus on early tuberculosis diagnosis and control of transmission in Denmark (7, 27). The extraordinary high and only slowly declining incidence of tuberculosis in Somalis in Denmark and elsewhere emphasizes that there is a need of an increased focus on prevention of endogenous reactivation of latent *M. tuberculosis* infection (4).

In the future, in low incidence countries, it will become increasingly important to reduce the prevalence of latent *M. tuberculosis* infection among those at high risk of progression to active disease, to diminish the pool of latent infection from which future cases of tuberculosis will emanate. Thus, tuberculosis control programmes should be expanded to focus on finding and treatment of latent *M. tuberculosis* infection (23).

The global perspective of tuberculosis should also be borne in mind: the disease burden exists principally in the developing nations where 95% of all cases and 98% of deaths due to tuberculosis occur (14). Intervention in such high-incidence areas is also crucial for the elimination of tuberculosis in low incidence areas. Tuberculosis has been resurrected as a major public health problem worldwide during the 1990s, and there is no room for complacency. A lot remains to be done, and the global burden of tuberculosis despite the availability of effective control measures is a public eyesore for the conscience of humankind (106).

ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
BCG	bacille Calmette-Guèrin
DNA	deoxyribonucleic acid
HIV	human immunodeficiency virus
IUATLD	the International Union Against Tuberculosis and Lung Disease
KNCV	the Royal Netherlands Tuberculosis Association
PPD	purified protein derivate
RFLP	restriction fragment length polymorphism
Spoligotyping	spacer oligotyping
TST	tuberculin skin test
USA	the United States of America
WHO	World Health Organisation

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