Possible underlying mechanisms for successful emergence of the Mycobacterium tuberculosis Beijing genotype strains

Ida Parwati, Reinout van Crevel, Dick van Soolingen

The wide geographic distribution of one clade of Mycobacterium tuberculosis, the Beijing genotype family, and its genetic homogeneity, suggests that strains belonging to this grouping might have a selective advantage over other M tuberculosis strains. This hypothesis was addressed by reviewing molecular-epidemiological, experimental, and clinical studies. Beijing strains represent about 50% of strains in east Asia and at least 13% of strains worldwide. Their emergence might be linked to escape from BCG vaccination, and to multidrug resistance, which is associated with the Beijing genotype in many areas. Different animal models have shown Beijing strains to be more virulent, and to cause more histopathological changes, higher outgrowth, and increased mortality. At a molecular level, Beijing strains have specific properties in terms of protein and lipid structures and their interaction with the human immune system. Finally, the Beijing genotype has been linked to polymorphisms in immune genes, suggesting the possibility of human–mycobacterial co-evolution. The emergence of the Beijing genotype family might represent an evolutionary response of M tuberculosis to vaccination or antibiotic treatment, with an important negative impact on tuberculosis control. More research is needed to further unravel the mechanisms underlying the emergence of M tuberculosis Beijing genotype strains, and examine the implications for future control strategies.

Introduction

The Mycobacterium tuberculosis Beijing genotype, which was first described in 1995,1 is one of the most successful clades in the present worldwide tuberculosis epidemic.2–6 Initially, the Beijing genotype was recognised on the basis of highly conserved spoligotyping (spacer oligotyping) patterns,7 and characteristic IS6110 RFLP8 patterns of M tuberculosis isolates from the Beijing region and Mongolia.1 Many studies have since reported that Beijing genotype strains have spread worldwide, and are emerging in various areas, often in association with multidrug resistance.6–9 Because Beijing strains from different geographic areas show a remarkable degree of genetic conservation compared with other M tuberculosis strains, one might hypothesise that this genetic lineage has selective advantages over other genotypes of M tuberculosis, and have started spreading relatively recently. Previous reviews have already described the spread and phylogeny of Beijing genotype strains,10,11 so we review the molecular-epidemiological, experimental, and clinical studies related to these strains.

Recognition and molecular typing

In 1995, the Beijing genotype family was defined on the basis of their highly conserved spoligotype patterns and characteristic multiband IS6110 RFLP patterns.1 In 2004, in an extended study that used first-generation spoligotyping, M tuberculosis Beijing strains were defined as strains that hybridised to at least three of the spacers 35–43 in the genomic direct-repeat region, and that showed absence of hybridisation to spacers 1–34.11 Second-generation spoligotyping, with the 43 spacers partly redesigned and 51 new spacers added, provided more discriminatory power, which enabled finer discrimination between different subtypes of Beijing strains.12 In addition, a standard reference set of 19 IS6110 RFLP patterns of Beijing strains was made available to help identify Beijing strains. Rapid identification of Beijing strains was also made possible by use of PCR that targets the mycobacterial interspersed repetitive unit (MIRU) locus 26.13

In the USA, a W-Beijing poly-probe assay was developed to target the genomic dnaA–dnaN intergenic region, the noise transfer function (NTF) chromosomal region, and the direct-repeat locus used in spoligotyping. This poly-probe method is highly sensitive and specific in the detection of Beijing strains among other M tuberculosis strains.14 Several methods with more discriminatory power have been proposed for epidemiological studies on the spread of Beijing strains. IS6110 RFLP typing was initially the main method used in the molecular epidemiology of tuberculosis. However, because this method is technically demanding and labour intensive, variable number tandem repeat (VNTR) typing using 15 loci was proposed as the new international standard for typing the M tuberculosis complex.15 Recently, more discriminatory power for Beijing strains has been established by use of 14 particular VNTR loci in addition to the exact tandem repeat loci A to E.16 A 12-locus VNTR typing method used in Japan is reported to be superior to 15-locus and 24-locus typing methods used in the typing of Beijing strains, and therefore seems to be the best tool for genotyping M tuberculosis isolates in areas where Beijing family strains are predominant.17 However, additional studies are needed to determine the stability and reproducibility of the variation associated with particular loci, which is also a crucial factor in molecular typing.

Beijing strains from widespread geographic areas are thus genetically highly conserved compared with other M tuberculosis clades. For example, the Haarlem genotype family, which is also widespread, is genetically much more heterogeneous and more difficult to define.4 On the basis of various genetic markers, we can clearly define
M tuberculosis Beijing strains, and recognise its sublineages and individual strains.

Phylogeny of Beijing genotype strains
Beijing genotype strains represent group 1 of Sreevatsan and colleagues’ original grouping, which was based on a limited number of single-nucleotide polymorphisms. These strains are now thought to be part of the modern lineage of M tuberculosis, based on large-sequence polymorphisms.7 The Beijing clade consists of at least two major groups based on the specific IS6110 insertion in the NTF region: the so-called typical and atypical Beijing strains.11,19–21 This distinction seems relevant, because these two lineages may have different properties,19 as will be discussed below. Beijing strains can also be subdivided into various evolutionary lineages through large-sequence polymorphisms, which divide this family into four monophyletic subgroups on the basis of the regions of difference RD105, RD181, RD150, and RD142.22 Recently, the Beijing genotype of M tuberculosis has also been described as the major part of the east-Asian lineage.23,24 A minor sub-lineage of the Beijing genotype family that caused multiple outbreaks among patients infected with HIV that were in prisons and hospitals in New York, USA, in the 1990s was designated the W strain.25 However, W strains only constitute a small branch on the phylogenetic tree of the Beijing genotype strains,26 and many other phylogenetic branches exist worldwide. Therefore, we will refer to Beijing strains as an indication of the worldwide Beijing genotype family as a whole.

The fourth International Spoligotyping Database,3 which classified 39,295 strains of M tuberculosis from 141 countries into 62 clades or lineages, indicated that the Beijing and the Beijing-like strains represent about 50% of strains in east Asia, and at least 13% of the isolates worldwide. However, to date, spoligotyping has mostly been used in high-income countries, and data from many high prevalence settings, especially in southeast Asia and the former Soviet Union states, are missing. As a result, the true contribution of Beijing strains to the worldwide epidemic might be higher. In the worldwide survey done within the framework of a European project, the prevalence of tuberculosis attributable to the Beijing genotype varied substantially between areas: high in Asia (except in the Indian subcontinent), and increased further east; intermediate in the USA and Cuba; low in parts of Africa, Latin America, western Europe, eastern Europe (other than the former Soviet Union), and in the Middle East.1

There are several indications that the Beijing genotype is an emerging strain. First, a time-trends analysis among non-immigrants from studies over more than 3 years revealed a slight increase in the occurrence of Beijing genotype in all western European countries (although only reaching significance in the Netherlands).9 Emergence of Beijing strains in this large study was defined as a significant correlation between the Beijing genotype and young age in patients with tuberculosis. Young age is suggestive of active spread, as confirmed by DNA fingerprinting in the Netherlands, which showed that unique DNA fingerprints are associated with advanced age (endogenous reactivation of remote infections) and DNA fingerprint clusters (recent transmission) with young age.9 Whether this difference in the spread of tuberculosis in different age categories can be extrapolated to countries with high prevalence remains to be proven.

Another approach to investigate possible dynamics in the population structure of M tuberculosis is the typing of bacteria in archived specimens. In South Africa, spoligotyping was applied to M tuberculosis in paraffin-embedded clinical material from consecutive time periods. Beijing strains were absent in histological samples from the period 1930–65, rare in samples from 1966–95, and increasingly common in samples from the period 1996–2005. The proportion of Beijing strains causing tuberculosis in children increased from 13% in 2000, to 33% in 2003.27 Finally, a recent study established the time of divergence, population diversity, and spread of M tuberculosis complex by MIRU typing a collection of 355 strains representing all well-defined primary branches of M tuberculosis complex. Compared with other clades, the Beijing genotype displayed the largest population increase that has taken place in the past 180 years.28 The Beijing genotype is therefore one of the most predominant M tuberculosis genotypes, and there are indications that it is increasing in certain areas.

Factors contributing to worldwide emergence
The emergence of Beijing genotype might be because of natural selection, possibly skewed by the two major measures against tuberculosis in the past century: BCG vaccination and antituberculosis treatment.29 BCG vaccination might be less protective against Beijing genotype strains than against other strains. Similarly, antituberculosis treatment might be less effective in eradicating Beijing strains than other strains. However, the spread of Beijing strains might already have started long before the introduction of vaccination and antibiotic treatment,29 which would suggest that these strains have an intrinsic advantage over other M tuberculosis genotypes in terms of virulence (ie, transmission, progression from latent to active tuberculosis, acquisition of drug resistance, or disease chronicity).30 Specific characteristics of this genotype might render it more virulent or better able to resist or evade the human immune system.

BCG vaccination might be a selective force favouring the spread of the Beijing genotype. In BALB/c mice and in a rabbit model of tuberculosis, BCG vaccination was less protective against subsequent infection by Beijing strains than to infection with M tuberculosis strains of other lineages.31,32 However, another study, which used C57BL/6 mice, was unable to confirm strain-specific
resistance to BCG vaccination. Newly developed candidate vaccines might be more protective: a vaccine developed from recombinant BCG mutants that secrete listeriolysin induced strong protection against infection by Beijing strains, whereas parental BCG failed to do so. If BCG vaccination is indeed less protective against infection with Beijing strains, one would expect to find a higher proportion of Beijing strains among BCG-vaccinated individuals compared with non-vaccinated individuals. Although most studies did not find any association, a recent study that subdivided Beijing strains into typical and atypical lineages isolated typical Beijing strains more frequently from BCG-vaccinated people than from non-BCG-vaccinated people, suggesting that BCG vaccination is less protective against typical Beijing strains. The underlying mechanism for this observation might be related to certain biochemical and immunogenic properties of Beijing strains, which will be discussed below.

In the present era of tuberculosis treatment, drug resistance might drive the spread of a particular *M tuberculosis* genotype. Epidemiological studies have examined the association of drug resistance with Beijing strain (table 1). A comprehensive review on this issue revealed four patterns: endemic prevalence of Beijing strains, not associated with drug resistance; epidemic, associated with drug resistance; epidemic, but drug sensitive; and very low level or absent. The difference between these patterns might be related to the variation in treatment regimens, compliance to treatment protocols, and varying quality of drugs. Alternatively, it might be because of spread of different and not yet distinguished sublineages of the Beijing strains.

In many countries with a high prevalence of Beijing strains (ie, Azerbaijan, Ukraine, Uzbekistan, Estonia, Latvia, Lithuania, Mongolia, and China), more than 5% of new tuberculosis cases have been shown to involve multidrug-resistant tuberculosis. In 2003–07, 2494 isolates of multidrug-resistant tuberculosis from 24 European countries were subjected to IS6110 RFLP typing to investigate possible international transmission of multidrug-resistant tuberculosis across Europe. About 39% of the examined cases were attributable to clusters and 84% of these likely transmissions were caused by Beijing strains. This is remarkable because only 6–7% of susceptible strains in Europe belong to the Beijing genotype. Strikingly, one large cluster of 174 cases infected with multidrug-resistant tuberculosis was associated with the spread of one Beijing strain. Obviously, these findings are affected by migration of people to Europe from areas with more multidrug-resistant tuberculosis or a higher prevalence of Beijing strains.

Logically, many researchers have examined possible associations between the Beijing genotype and the distribution of mutations in genes underlying resistance to antituberculosis drugs (table 1). Genotypic studies looking at *katG*315, the most important mutation encoding for resistance to isoniazid, did indeed report more mutations among Beijing strains. The association is less clear for mutations in the *rpoB* gene, which account for more than 90% of drug resistance to rifampicin, because studies comparing Beijing and other genotype strains have examined the distribution (exact genetic location), rather than the overall frequency of *rpoB* mutations. Some researchers found that the Beijing strains did not show substantial differences in frequency of the most commonly encountered mutations in the *rpoB* gene compared with non-Beijing genotype strains. A substantially higher proportion of the *rpoB* S531L mutation was found in Beijing genotype strains from Germany and Russia, whereas in Korea, researchers found the opposite. In a study from Russia, Beijing strains showed more mutations in the *embB*306 gene. No studies have been published on associations of the Beijing genotype with other drug-resistance genes such as *pncA*, *gyrA*, and *rpsL/rrs*, which are responsible for resistance against pyrazinamide, fluoroquinolone, and streptomycin, respectively. Clearly, more studies are needed to clarify this important issue.

What might cause Beijing strains to be more drug resistant? First, Beijing genotype strains might generally show a higher mutation frequency. This hypothesis was driven by the finding that some Beijing strains have alterations in so-called putative mutator genes, resulting in altered DNA repair and an increased mutation rate. More recently, the *M tuberculosis mutT2* gene was found to play a part in the general slowdown of metabolism when mycobacteria are deprived of essential nutrients. Later studies on 3R (DNA repair, recombination, and replication) genes revealed that strains of other *M tuberculosis* genotypes, such as Haarlem, have mutations in these genes and it is therefore not yet clear whether alterations in these genes play a significant part in the adaptation of Beijing strains. The second hypothesis is that specific characteristics of the cell-wall structure of Beijing strains lead to suboptimum intracellular concentrations of antituberculosis drugs and acquisition of drug resistance. Both these hypotheses need further proof: in vitro, Beijing strains did not seem to acquire drug resistance more easily when exposed to antituberculosis drugs. However, these

---

**Table 1: Approaches to examine the relation of M tuberculosis Beijing genotype with drug resistance**

<table>
<thead>
<tr>
<th>Finding</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemiological studies of phenotypic drug resistance</td>
<td>Associations with drug resistance in certain parts of the world, but not all</td>
</tr>
<tr>
<td>Mutations in drug-resistance genes</td>
<td>Higher rates of mutations reported for ppol codon S531L, katG, embB; different distributions of ppol mutations</td>
</tr>
<tr>
<td>In-vitro exposure to anti-tuberculosis drugs</td>
<td>No difference in acquisition of drug resistance</td>
</tr>
<tr>
<td>Examination of underlying molecular mechanisms</td>
<td>More mutations in mutator genes reported by some studies, but not all</td>
</tr>
</tbody>
</table>

---
studies were based on a limited number of strains of unknown Beijing lineage and selection with a limited number of drugs at particular concentrations. The third hypothesis is that increased virulence of Beijing strains leads to more persistent infection and treatment failures with prolonged exposure to antituberculosis drugs, increasing the risk of acquisition of drug resistance.

In summary, many, but not all, epidemiological studies have shown associations between the Beijing genotype and drug-resistance mutations. So far, however, experimental studies and in-depth molecular studies have failed to reveal the underlying mechanisms for these associations (table 1).

### Biochemistry and immunogenicity

*M. tuberculosis* Beijing genotype strains might be more virulent as a result of intrinsic biochemical properties and their interaction with human host defence system. Studies in vitro have focused on protein expression, lipid structures, and immunogenicity of different *M. tuberculosis* genotypes (table 2). In a proteomic study, Beijing strains showed increased expression of α-crystallin protein homologue (16 kDa protein; an *M. tuberculosis* virulence factor) and decreased expression of heat shock protein Hsp65, phosphate transport protein PstS1, and 47 kDa protein compared with other clinical isolates and control strain H37Rv. Another proteomic study using human monocytic cell-line U-937, showed that two proteins, Mb1363 (probable glycogen phosphorylase GlgP) and MT2656 (haloalkane dehalogenase LinB), had a higher expression after phagocytosis of *M. tuberculosis* K strain (a Beijing strain) compared with H37Rv, H37Ra, and *Mycobacterium bovis* BCG. Highly expressed GlgP protein in K strains might induce macrophage activation of migration inhibitory factor, which might be advantageous for the bacteria. Although the role of these proteins is not yet clear, they might have an important function in the pathogenesis of tuberculosis.

Beijing strains also show differences in the cell-wall-associated lipid structures. In vitro, Beijing strains produce a biologically active lipid—polyketide synthase-derived phenolic glycolipid—which was found to inhibit the release of proinflammatory mediators and was associated with lethal infection in animals. Recently, Beijing strains were found to accumulate large quantities of triacylglycerides in an aerobic culture in vitro, coinciding with upregulation of Rv3130c, a member of the DosR dormancy regulon, which is involved in growth in microaerophilic or anaerobic environments during

<table>
<thead>
<tr>
<th>Experimental approach</th>
<th>Control strain</th>
<th>Findings associated with Beijing genotype strains*</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pfeiffer et al51</td>
<td>Proteomics</td>
<td>F23 strain, H37Rv</td>
<td>Increased virulence; evasion of host immune response</td>
</tr>
<tr>
<td>Reed et al51</td>
<td>Lipid expression</td>
<td>CDC1551, NHN5</td>
<td>Lower release of inflammatory mediators</td>
</tr>
<tr>
<td>Reed et al51</td>
<td>Lipid expression, gene expression</td>
<td>H37Rv</td>
<td>Increased in triacylglycerides; increased expression of dosR regulon gene</td>
</tr>
<tr>
<td>Ryoo et al51</td>
<td>Proteomics</td>
<td>H37Rv, H37Ra, BCG</td>
<td>Not yet determined</td>
</tr>
<tr>
<td>Immuneogenicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chacon-Salinas et al54</td>
<td>Infection of murine macrophages</td>
<td>Canetti, H37Rv</td>
<td>Pro-inflammatory cytokine response</td>
</tr>
<tr>
<td>Rocha-Ramirez et al59</td>
<td>Lipid fraction vs human monocyte-derived macrophages</td>
<td>H37Rv, Canetti</td>
<td>Increased in TNF and interleukin 10; decrease in TLR2, TLR4, and MHC class II expression</td>
</tr>
<tr>
<td>Sohn et al56</td>
<td>Infection of human monocytic THP-1 cells</td>
<td>H37Rv</td>
<td>Decrease in TNF, interleukin 6, and interleukin 12b; increase in necrotic cell death</td>
</tr>
<tr>
<td>Virulence in animal models</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lopez et al54</td>
<td>Intrathecal injection of BALB/c mouse</td>
<td>H37Rv</td>
<td>Increased in extensive pneumonia, early but ephemeral TNF, iNOS expression, earlier mortality, and higher bacillary load</td>
</tr>
<tr>
<td>Dormans et al55</td>
<td>Intrathecal injection of BALB/c mouse</td>
<td>H37Rv, Canetti</td>
<td>Severe pathological response, higher mortality and bacillary load</td>
</tr>
<tr>
<td>Tsenova et al55</td>
<td>Intracisternal injection in rabbits</td>
<td>CDC1551</td>
<td>Higher bacillary load; increased dissemination of bacilli to other organs, persistent concentrations of TNF; increase in leukocytosis, and more severe clinical manifestations</td>
</tr>
<tr>
<td>Manca et al54</td>
<td>Aerosol in B6D2/F1 mice</td>
<td>CDC1551</td>
<td>Increase in type I interferons; decrease in TNF, interleukin 12, and T-cell activation; lower survival</td>
</tr>
<tr>
<td>Rios-Barrera et al58</td>
<td>Intratracheal injection of BALB/c mice</td>
<td>H37Rv</td>
<td>Increased in peak progressive increase of apoptotic T-helper 1 lymphocytes</td>
</tr>
</tbody>
</table>

*Beijing lineage family included HN8778, Beijing strains 210, W10, W4, and K. iNOS—inducible isoform of nitric oxide synthase; PGL—polyketide synthase-derived phenolic glycolipid, TLR=Toll-like receptor; TNF=tumour necrosis factor.

Table 2: Biochemical characteristics, immunogenicity, and virulence of *M. tuberculosis* Beijing genotype strains
infection, and might therefore be related to the success of Beijing strains. However, this glycolipid was only synthesised by a subset of Beijing strains, so this is unlikely to be the only virulence factor.

Another hypothesis is that Beijing genotype strains induce a different immune response, or undermine an effective host response. This has been the focus of research in vitro and in animal studies (table 2). In the human acute monocytic leukaemia cell line THP-1 infection with Beijing genotype strains induced low production of several pro-inflammatory cytokines, such as tumour necrosis factor (TNF), interleukin 6, and interleukin 12b, which favours outgrowth of M. tuberculosis. However, macrophages infected with Beijing isolates expressed a cytokine pattern associated with control of tuberculosis (ie, high concentrations of proinflammatory cytokines mRNA for inducible isoform of nitric oxide synthase [iNOS], interleukin 1b, TNF, interleukin 12, and low concentrations of the anti-inflammatory cytokine interleukin 10). Macrophages stimulated with lipid fractions of Beijing strains had a higher production of TNF and interleukin 10, but downregulation of Toll-like receptor (TLR) 2, TLR4, and MHC class II expression, which might lower immune recognition and antigen-presentation of M. tuberculosis. By contrast, lipids from a Mycobacterium canetti strain induced almost the opposite: lower concentrations of TNF and interleukin 10, and upregulation of TLR2 and TLR4, without modifying MHC class II expression. In THP-1 cells, compared with the control strain H37Rv, the highly virulent Beijing (K) strain induced significantly higher levels of necrotic cell death than apoptosis. These results suggest that Beijing strains inhibit cellular apoptosis, a host defence mechanism that probably limits outgrowth of mycobacteria in macrophages, while inducing necrosis, which most likely favours outgrowth of mycobacteria.

Animal models
Animal models suggest that Beijing genotype strains are more virulent, and more effectively resist or evade the host immune response. BALB/c mice intratracheally infected with Beijing strains showed more histopathological changes, bacterial outgrowth, and higher and earlier mortality compared with animals infected with H37Rv and other genotype strains. This coincides with early TNF response and iNOS expression.

Similarly, when BALB/c mice were challenged with 19 different M. tuberculosis strains of 11 major genotype families, Beijing strains induced more severe histopathological changes and higher mortality. In a rabbit model, CNS infection with HN878 or W4 resulted in higher bacillary loads in the cerebrospinal fluid and brain, increased dissemination of bacilli to other organs, persistent concentrations of TNF, higher leukocytosis, and more severe clinical manifestations compared with the M. tuberculosis clinical isolate CDC1551 (a highly immunogenic strain). In addition, in B6D2/F1 mice, these same Beijing strains (HN878 and W4) induced higher mortality compared with the highly transmissible strain CDC1551, coinciding with higher concentrations of type I interferons, lower concentrations of TNF and interleukin 12, and reduced T-cell activation. Intratracheal injection of BALB/c mice by a Beijing strain induced a two times higher percentage of apoptotic activated macrophages than in mice infected by H37Rv, and earlier progressive pneumonia that contained numerous macrophages, with vacuolated cytoplasm. Vacuolated macrophages might induce apoptosis of T-helper 1 cells that favours disease progression, and is related to the virulence of the mycobacterial strains.

In animal studies, the immunological response after infection by Beijing strains seems to be less adequate than after infection by other strains. This could affect the required synergistic effect of the immunological response of a patient to drug treatment, and lead to a longer time to sputum conversion, which in turn could increase the risk of drug-resistance development.

Taken together, there is clear evidence that Beijing strains show altered expression of proteins, glycolipids, and triglycerides, which may contribute to increased virulence, or to evasion or suppression of protective host defence mechanisms. However, there is no conclusive evidence to explain the overall success of Beijing strains in circumventing BCG-induced immunity or enabling the development of resistance.

Clinical phenotype
If Beijing strains are more virulent, one would expect a higher proportion of infected patients to develop active tuberculosis. This hypothesis is supported by a small study in The Gambia. In a cohort of tuberculosis patients and household contacts, transmission rates between patients exposed to M. tuberculosis and Mycobacterium africanum were similar, but rates of progression to disease were lower in contacts exposed to M. africanum. Within M. tuberculosis, patients infected with the Beijing strain were most likely to progress to disease. Differences between Beijing genotype and other M. tuberculosis complex genotypes were less clear, and the overall number of investigated cases was relatively small.

Taking the relative success of Beijing strains into consideration, one might also expect that patients infected with Beijing genotype strains present with a different or a more severe clinical phenotype. Several studies have investigated this hypothesis. In a small study in Indonesia, patients infected with Beijing genotype strains were more likely to develop fever unrelated to disease severity during the first stage of treatment. However, this was not confirmed in studies from Singapore and Russia, which actually reported less fever and night sweats in patients infected with Beijing strains.

Studies comparing chest radiographic abnormalities of patients infected with Beijing and other genotype strains
have also met with conflicting results. In Russia, associations were found between the extent and severity of radiological abnormalities, whereas in the Netherlands, no such associations were found. However, the correlation between Beijing bacteria and drug resistance might be much stronger in former Soviet Union states than in the Netherlands, resulting in more persistent infections and, hence, more lung damage. A high bacterial load in sputum increases the risk of transmission, but to date, no study has reported associations between bacterial load in the lung or sputum of patients infected with different genotype strains.

If Beijing strains are more virulent, this might also be shown by a higher proportion of patients with disseminated disease. In Arkansas, USA, patients with extrapulmonary tuberculosis were found to be three times more likely to be infected with Beijing strains after correcting for potential confounders. However, there was no evidence of such an association in children from Cape Town, South Africa. In Vietnam, patients infected with HIV with meningeal tuberculosis caused by Beijing strains had a shorter duration of illness before presentation, and a lower cerebrospinal fluid leucocyte count than patients with meningitis caused by other strains. In conclusion, in some, but not all, studies, patients infected with M tuberculosis Beijing strain showed more severe disease than patients infected by non-Beijing strain. This might be because of differences between studies in design, patient selection, and methods used, and to geographical differences in Beijing sublineages or the genotypic distribution of non-Beijing strains.

Increased virulence of Beijing strains might also result in a lower response to tuberculosis treatment. Indeed, several cross-sectional studies have reported higher rates of recurrent tuberculosis in patients infected with Beijing strains. In the only prospective cohort study published to date, patients infected with Beijing strains had more positive sputum cultures after 6 months of treatment than patients infected with non-Beijing strains, also after adjustment for differences in drug resistance.

### Co-evolution

Geographic differences in the population structure of M tuberculosis might be because of a so-called founder effect, with a higher chance of finding a particular genotype family closer to where it originated. The population structure might also be related to environmental factors, migration of the human populations, or differences in tuberculosis control. Alternatively, particular M tuberculosis lineages may have adapted to specific properties of the immune system in particular human populations (genetic co-evolution). Similar to geographical phylogenetic differences (phylogeography) of M tuberculosis, host immune genes also show geographical differences. For instance, functional polymorphisms in TLR4, one of the pattern recognition receptors capable of recognizing M tuberculosis, show a unique global distribution.

Several lines of research support the notion that the geographical variation of M tuberculosis and the human host genotype are related. Particular M tuberculosis lineages have shown preferential spread in particular populations. One might argue that people of the same background or immigration group tend to live together in a foreign country, thereby spreading their native strains to people of the same ethnicity. However, preferential spread of a particular genotype in a specific ethnic group might also be the result of co-evolution of host and pathogen. So far, three studies have examined possible associations between genetic characteristics of tuberculosis patients and the genotype of M tuberculosis isolates. In a study in Vietnam, a particular mutation in TLR2 was more common in patients infected with M tuberculosis Beijing genotype strains than among patients infected with other strains. In a study from Ghana, polymorphisms in the promoter of AOX5, which encodes leukotrienes involved in regulation of the immune response to M tuberculosis, were most frequent in patients infected with M africanum. Finally, in a recent study from Indonesia, the Beijing genotype was strongly associated with polymorphisms in SLC11A1.
(formerly NRAMP1), one of the most important genes associated with susceptibility to tuberculosis. These findings provide further support for the hypothesis that evolutionary adaptation of particular *M. tuberculosis* lineages to certain human populations contributes to the marked geographical variation in *M. tuberculosis* genotypes, and that host gene polymorphisms, particularly those related to innate immunity against *M. tuberculosis*, confer increased susceptibility of certain populations to the Beijing genotype. Clearly, this concept of genetic co-evolution needs further investigation.

**Conclusion**

The Beijing strains have emerged worldwide as a genetically conserved genotype of *M. tuberculosis*, often in association with drug resistance. This worldwide change in the population structure of *M. tuberculosis* is probably driven by man-made factors (antibiotic treatment and vaccination), and perhaps also linked with intrinsic mycobacterial characteristics (table 3).

The Beijing genotype family has been studied most extensively, but other predominant genotypes like the Haarlem and African genotypes may undergo similar changes. An evolutionary shift of *M. tuberculosis* towards a new population of bacteria that are more difficult to treat and that have a greater ability to circumvent vaccination will hamper our efforts to control tuberculosis. Therefore, there is an urgent need to better understand the mechanisms underlying the emergence of *M. tuberculosis* Beijing genotype strains, and to examine the implications for tuberculosis control.

In our view, future research should focus on three issues. First, more structural genotyping should be done in high-burden areas, and the molecular epidemiology of tuberculosis should be monitored in time to have more insight into possible changes in the population structure of *M. tuberculosis*. This analysis should be related to vaccination and spread of drug resistance. Second, to understand the success of Beijing and certain other genotype strains, clinical phenotyping of patients with tuberculosis should be combined with in-depth studies of those patients’ mycobacterial isolates and their interaction with the human host or their behaviour in animal models. Third, more fundamental studies are needed into the evolutionary changes that take place in emerging genotype families, including the 3R genes and other genomic regulators, by applying whole genome sequencing and transcriptomics.

**Contributors**

IP and RvC wrote the first draft of the paper and the tables. All authors provided critical input and approved the final version of the Review.

**Conflicts of interest**

We declare that we have no conflicts of interest.

**References**

Review


