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SHORT REPORT
Association of *Mycobacterium tuberculosis* Beijing genotype with tuberculosis relapse in Singapore

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SUMMARY

The relationship of *Mycobacterium tuberculosis* Beijing genotype with tuberculosis relapse was examined. Beijing strains were detected from 32 out of 45 (71%) relapsed cases and 148 out of 290 (51%) non-relapsed cases. Multivariate logistic regression analysis revealed that Beijing genotype was significantly associated with tuberculosis relapse (OR 2·64, 95% CI 1·30–5·34, $P=0·005$).

Mycobacterium tuberculosis Beijing genotype was first described in 1995 because of its predominance in the Beijing area of China, the high similarity of IS*6110* restriction fragment length polymorphism (RFLP) patterns and the identical polymorphism pattern of the direct repeat region of *M. tuberculosis* genome between isolates [1]. Studies since have shown that the Beijing genotype was prevalent throughout the world, and predominant in many areas in Asia and North America [2, 3].

In addition to their wide distribution, Beijing strains have been reported to be associated with drug resistance in Vietnam, New York, Cuba, and Estonia, but not in other areas, such as Hong Kong, Thailand, and Indonesia. Furthermore, the association of the Beijing genotype with younger patients demonstrated in Vietnam [4] was not found in other areas [3]. Therefore, the relationship of the Beijing genotype with drug resistance and patient's age remains a matter of debate. The Beijing genotype was recently associated with treatment failure and tuberculosis relapse in Vietnam [5]. Here, we present our finding that the Beijing genotype is significantly associated with tuberculosis relapse in Singapore.

Drug-susceptible *M. tuberculosis* isolates from 364 culture-proven patients were consecutively collected from the Central Tuberculosis Laboratory, Department of Pathology, Singapore General Hospital between August and December 1994. This laboratory is the national reference centre for mycobacteria in Singapore. This collection represented the population of *Mycobacterium tuberculosis* from this urban setting. Isolates were genotyped by IS*6110* RFLP according to the internationally standardized protocol [6], spoligotyping [7], and mycobacterial interspersed repetitive units–variable number tandem repeat (MIRU–VNTR) typing [8]. Beijing genotype strains were identified by spoligotyping on the basis of a new definition which defines them as strains hybridizing to at least three of the nine spacers 35–43 and with absence of hybridization to spacers 1–34 [9]. The IS*6110* RFLP patterns were analysed at the National Institute of Public Health and the Environment (RIVM), The Netherlands using the Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Clustered isolates by identical IS*6110* fingerprints (isolates with identical IS*6110* RFLP patterns) were further discriminated using

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MIRU–VNTR typing regardless of IS*6110* copy number.

Demographic data of age, sex, and ethnicity as well as the information of current episode (recurrence or first episode) were obtained retrospectively from the National Tuberculosis Registry database. Patients who had previously had tuberculosis and who were culture negative after treatment at that time, were categorized as recurrent cases when infection occurred and isolates were obtained in this study period. Patients who had their first episode of disease during the period of study were defined as non-recurrent cases. Recurrent cases who were infected with clustered *M. tuberculosis* isolates by IS*6110* and MIRU–VNTR typing were defined as exogenously re-infected patients, whereas patients in this group who were infected with a unique strain by IS*6110* and MIRU–VNTR typing were defined as relapsed cases. The non-recurrent and re-infected cases were categorized as non-relapsed cases.

Univariate χ^2 and Fisher's exact tests and multivariate logistic regression were used to assess the association of tuberculosis relapse with Beijing genotype, patient's age, sex, and ethnicity. Student's *t* test was performed to compare the mean ages between the relapsed and non-relapsed groups. A *P* value of $<0{\cdot}05$ was considered statistically significant.

Of the 364 isolates, 196 (53·8%) belonged to the Beijing genotype, with the remainder belonging to other non-Beijing genotypes. Information on patient's age, sex, ethnicity, and current episode were available for 335 patients. The 29 patients with missing data had a similar distribution of the Beijing genotype and randomly distributed sampling dates during the collection period. Therefore, the exclusion of the 29 patients from the total sample is unlikely to introduce bias into the study.

The 335 patients (243 males, 72·5%) ranged in age from 6 to 91 years, with a mean age of 50 years (S.D. = 19 years). Forty-five patients (37 males, 82·2%) were categorized as relapsed tuberculosis cases; among the remaining 290 patients, 283 had first-episode disease. Seven recurrent cases (five were infected with Beijing strains, two with non-Beijing strains) were regarded as having exogenous re-infection as they were members of seven IS*6110* and MIRU–VNTR fingerprint-defined clusters. Beijing strains were found in 32 (71·0%) of the 45 relapsed cases and in 148 (51·0%) of the 290 first-episode and re-infected (non-relapsed) cases. As shown in the Table, univariate analysis revealed that there was association of the Beijing genotype with tuberculosis relapse ($P=0{\cdot}019$). The mean age was 61 years (S.D. = 16 years) in the relapsed group, but it was 48 years (S.D. = 19 years) in the non-relapsed group, the difference is statistically very significant (*t* test, $P<0{\cdot}01$). All but one relapsed case were older than 30 years, and $\sim$50% relapsed cases were $\geqslant$65 years. Tuberculosis relapse was found to be strongly associated with older patients by univariate analysis ($P<0{\cdot}001$). However, no association of tuberculosis relapse with sex ($P=0{\cdot}166$) and ethnicity ($P=0{\cdot}375$) was shown.

Multivariate logistic regression analysis, which included genotypes (dichotomous as Beijing and non-Beijing) and age (continuous variable), confirmed the significant association of Beijing genotype with tuberculosis relapse (OR 2·64, 95% CI 1·30–5·34, $P=0{\cdot}005$), consistent with the recent observation in Vietnam [5]. Patient's age was also independently associated with tuberculosis relapse, the odds that an older patient had relapsed tuberculosis increased 4% over that of a younger patient with each year of age (OR 1·04, 95% CI 1·02–1·06, $P<0{\cdot}001$).

The relationship between *M. tuberculosis* Beijing genotype and patient's age was also examined. The mean ages were 49 years (S.D. = 20 years) and 51 years (S.D. = 18 years) for the patients infected with Beijing and non-Beijing strains respectively. In comparison to a previous study [4], there was no association between the Beijing genotype and patient's age in Singapore (logistic regression analysis, $P=0{\cdot}244$).

Recurrent tuberculosis may develop as the result of reactivation of the endogenous primary infection (relapse) or as a result of a recent exogenous infection (re-infection) [10]. The conclusive method to differentiate these two events from each other is to fingerprint *M. tuberculosis* isolates of the primary and recurrent episodes. If the paired isolates of primary and recurrent episodes of one patient are identical (or very similar) in their DNA fingerprints, the recurrent event is regarded as a reactivation; otherwise, if the paired isolates exhibit different DNA fingerprints, the recurrent event is considered to be a re-infection. As strain typing was not done in the previous episodes of tuberculosis for the recurrent cases in this study, we were unable to define these cases as relapse or re-infection by this method. Nevertheless, if re-infection is an event of recent transmission from person to person, isolates involved in this event are epidemiologically related, showing identical or very similar DNA fingerprints; but

Table. *Analysis of relapsed and non-relapsed tuberculosis cases based on the* M. tuberculosis *genotypes and demographic factors*

	No. of patients	Patient (%)		Univariate analysis*		Multivariate analysis†	
		Relapsed	Non-relapsed	OR (95% CI)	*P*	OR (95% CI)	*P*
Genotype					0·019		0·005
Beijing	180	32 (17·8)	148 (82·2)	2·36 (1·20–4·64)		2·64 (1·30–5·34)	
Non-Beijing	155	13 (8·4)	142 (91·6)	1		1	
Age group (yr)					0·000		
<25	35	1 (2·9)	34 (97·1)	1			
25–44	110	6 (5·5)	104 (94·5)	1·96 (0·30–12·74)			
45–64	103	17 (16·5)	86 (83·5)	6·72 (1·09–40·78)			
⩾65	87	21 (24·1)	66 (75·9)	10·82 (1·76–65·31)			
Age (yr)‡	335	45 (13·4)	290 (86·6)			1·04 (1·02–1·06)	0·000
Sex					0·166		
Male	243	37 (15·2)	206 (84·8)	1·89 (0·86–4·14)			
Female	92	8 (8·7)	84 (91·3)	1			
Ethnicity					0·375		
Chinese	248	32 (12·9)	216 (87·1)	1			
Malay	54	8 (14·8)	46 (85·2)	1·18 (0·52–2·67)			
Indian	21	5 (23·8)	16 (76·2)	2·11 (0·75–5·96)			
Unknown	12	0 (0)	12 (100)	Not done			

* χ^2 and Fisher's exact test; OR, odds ratio; CI, confidence interval.
† Logistic regression analysis. OR for genotype and age is adjusted by each other.
‡ Used as continuous variable in logistic regression analysis. OR (1·04) indicates that the odds of relapse increases 4% for every 1 year older.

isolates from reactivated cases were distinct from each other. Based on this notion, we have defined each of the recurrent cases by relapse or re-infection and demonstrated the strong association of *M. tuberculosis* Beijing genotype with tuberculosis relapse in Singapore.

The limitation of our definitions for relapse and non-relapse is the risk of misclassification of true relapsed cases into re-infection or true re-infected cases into relapse. However, this possibility is unlikely to counter the strong association between the Beijing genotype and tuberculosis relapse for several reasons. The relative contribution of exogenous re-infection to recurrent tuberculosis depends on the incidence of tuberculosis in a community [10, 11]. Although the incidence in Singapore was moderate (49/100 000 people) in 1994, re-infection appeared to be not significant. We observed 21 drug-resistant tuberculosis patients who had multiple isolates sampled during the period of August 1994 to December 1996. The time intervals between the first and last isolates for a given patient ranged from 45 to 815 days (mean = 258 days). Twenty-six subsequent isolates had identical or highly similar (only one band difference) IS*6110* RFLP patterns and identical MIRU–VNTR patterns with the respective initial isolate (data not shown). This indicates that exogenous re-infection was very unlikely in these patients. The mean age of the relapsed group was significantly older than that of the non-relapsed group, suggesting that the recurrences in the relapsed group were more probably due to endogenous reactivation. By our definition of re-infection, more recurrent cases infected with Beijing strains (five cases) were classified as re-infected cases compared to those infected with non-Beijing strains (two cases).

A previous study in Vietnam [4] has found that the Beijing genotype was associated with younger patients and a trend association between Beijing genotype and younger patients was detected which could explain the emerging of Beijing strains in that country. Nevertheless, Beijing strains were not found to be associated with patient's age in this study. Our observation is consistent with previous ones obtained from Hong Kong, Thailand, Indonesia, and Estonia [3]. More well-designed molecular epidemiological studies are needed to address this inconsistency.

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DECLARATION OF INTEREST

None.

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