In Vivo IS6110 Profile Changes in a Mycobacterium tuberculosis Strain as Determined by Tracking over 14 Years

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Transposition and homologous recombination of IS6110 appear in Mycobacterium tuberculosis along in vivo sequential infections. These events were checked in different clones of a successful strain, M. tuberculosis Zaragoza, with the focus on a variant in which integration of a copy of IS6110 in the origin of replication (oriC) region occurred.

Clonal Mycobacterium tuberculosis variants appear along the sequential infections of hosts involved in the same transmission cluster (1). IS6110 in M. tuberculosis has been one of the most widely used elements in molecular epidemiology and has a transposition rate estimated at ~18% over a period of 5 to 6 years (2). The IS6110 element itself modulates expression of neighboring genes and, depending on its location, could confer both transposition ability and virulence (3–5). Microevolution events due to the transposition of IS6110 are usually reflected in variations of restriction fragment length polymorphism (RFLP) patterns (6, 7). Minor variations in IS6110 fingerprints, spoligotypes, or mycobacterial interspersed repetitive-unit (MIRU)-variable-number tandem-repeat (VNTR) loci reveal microevolution in clonal infections (6, 8). These changes could have effects on the molecular structure of the mycobacterial genome and the biology of the bacillus (7).

Molecular epidemiological studies carried out for more than a decade have provided accuracy in the study of the transmission (9). The usefulness of the approach has been proven, even in countries with low incidences of tuberculosis (10). The M. tuberculosis Zaragoza strain spread in Zaragoza, Spain, and reached 18.7% of all isolates of M. tuberculosis in 2001 to 2004 (11). This strain was classified in principal genetic group 3 and in single-nucleotide polymorphism (SNP) cluster 6a (12), it demonstrated the rare spoligotype SIT773, and it carried 12 localized copies of IS6110 (13). In addition, in the context of a high-throughput survey of in vivo IS6110 transposition in multiple M. tuberculosis genomes, an additional IS6110 copy was detected in the oriC region in one isolate (14). The purpose of this study was to review the in vivo genomic changes of the M. tuberculosis Zaragoza strain, focusing on this variant.

Among the 2,348 isolates collected between 2000 and 2013, 246 were identified as M. tuberculosis Zaragoza by IS6110 RFLP typing (15) and spoligotyping (16) in the spoligotyping database, in contrast to the pattern of the H37Rv reference strain. The suspicion that homologous recombination between the two copies had occurred was corroborated by amplification of the expected sequence using the following external primers: DR43-F, 5′-ACCCGGTGCGATTCTGCG-3′; DR43-R, 5′-AAGGTGATCGAGGAGGATTGCG-3′. The amplicon obtained in variant 7 (1,660 bp) was sequenced, and BLAST searching was performed (http://genolist.pasteur.fr/TubercuList). The sequence confirmed the deletion of ~10 kb, including the genes from Rv2816c to the point of insertion of IS6110 in Rv2823c. The loss of this region is reflected in the lack of spacers 1 to 24 of the spoligotyping pattern (SIT585), classified as a Beijing-like pattern in the SITVITWEB database, in contrast to the pattern of the M. tuberculosis Zaragoza M. tuberculosis Zaragoza were confirmed by PCR (13). However, the two nonamplified copies were located a relatively small distance from each other (9,860 bp for the H37Rv reference strain). The suspicion that homologous recombination between the two copies had occurred was corroborated by amplification of the expected sequence using the following external primers: DR43-F, 5′-ACCCGGTGCGATTCTGCG-3′; DR43-R, 5′-AAGGTGATCGAGGAGGATTGCG-3′. The amplicon obtained in variant 7 (1,660 bp) was sequenced, and BLAST searching was performed (http://genolist.pasteur.fr/TubercuList). The sequence confirmed the deletion of ~10 kb, including the genes from Rv2816c to the point of insertion of IS6110 in Rv2823c. The loss of this region is reflected in the lack of spacers 1 to 24 of the spoligotyping pattern (SIT585), classified as a Beijing-like pattern in the SITVITWEB database, in contrast to the pattern of the M. tuberculosis Zaragoza
strain (SIT773). This process of homologous recombination has been described to explain the deletion of other extensive regions (17). Our finding agrees with other studies indicating that strains with large numbers of IS6110 copies have lost genomic regions due to recombination between copies (18). This mechanism was previously illustrated for other isolates of M. tuberculosis, demonstrating evolution of the spoligotyping pattern (19). Curiously, this microevolved clone did not spread to other patients.

In variant 3, a copy of IS6110 was detected, in the context of a high-throughput survey of in vivo IS6110 transposition, in the oriC region of the clone, which was not reflected in the RFLP pattern. Accordingly, it has been described that RFLP analysis can underestimate the real copy number for the IS6110 pattern. Accordingly, it has been described that RFLP analysis can underestimate the real copy number for the IS6110 pattern. This mechanism has been described to explain the deletion of other extensive regions (18). This mechanism was previously illustrated for other isolates of M. tuberculosis, demonstrating evolution of the spoligotyping pattern (19). Curiously, this microevolved clone did not spread to other patients.

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In conclusion, in monitoring of the M. tuberculosis Zaragoza strain for 14 years, different clones have been detected. Although the turnover of the markers used is not in the range of the pace of transmission, the insertion of an IS6110 copy in oriC allowed us to distinguish one large subgroup. Variant 3 was able to be transmitted in a circumscribed area of the city. Interestingly, this well-documented outbreak will allow us to perform further research comparing variants belonging to the same isogenic group and therefore possessing the same genetic background. In addition, whole-genome sequencing would be useful for determining the microevolution of the M. tuberculosis Zaragoza strain during the well-documented outbreak and would be useful to validate the directionality and sequence of transmission (28).

The study and the protocols for collecting the bacterial strains were approved by the ethics committee of the Aragon government.

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REFERENCES


