Molecular methods for tuberculosis trials: time for whole-genome sequencing?

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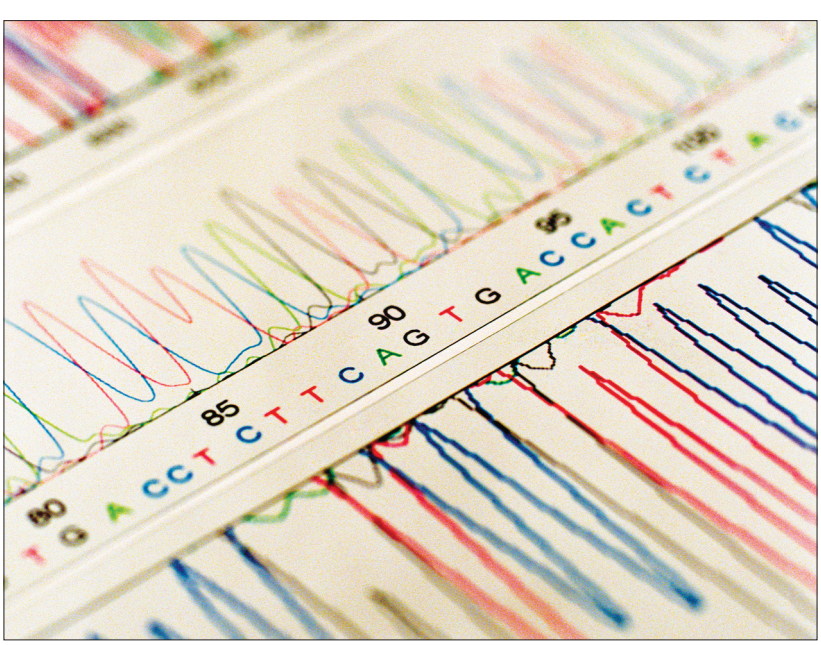
The first genetic sequencing of *Mycobacterium tuberculosis* was a momentous achievement that required years of painstaking effort and substantial funding.[1] It is remarkable that 15 years later, advances in laboratory techniques and informatics have enabled whole-genome sequencing to be done for hundreds of *M tuberculosis* isolates, making this information available for epidemiological studies.[2,3] The increasing availability of whole-genome sequencing has raised questions about the interpretation of this new method,[3] as well as its public health and clinical applications and utility.

In the *Lancet Respiratory Medicine*, Josephine Bryant and colleagues[4] report a study comparing molecular typing by mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) versus whole-genome sequencing for isolates of *M tuberculosis* from 47 consecutive participants in a phase 3 trial of tuberculosis treatment. These participants had recurrent positive cultures after at least 17 weeks of treatment; the two molecular methods were used to determine whether these recurrences were a result of relapse or re-infection. None of these patients had treatment failure, none acquired drug resistance, and 11 were HIV-positive. Five recurrences were single isolated positive cultures followed by repeated negative cultures without treatment; four of these were attributed to lab contamination. On the basis of whole-genome sequencing of the remaining 42 pairs, 33 were judged to be relapses, three were considered re-infection, four had evidence of mixed infection initially, of which one of the two initial strains relapsed, two had single strains initially but mixed infection with two strains was identified at the time of recurrence implying that both relapse and re-infection had occurred.

Several findings of this study are worthy of comment. Of the 42 participants with clinically significant disease recurrence, at most five (12%) could be considered to be a result of re-infection (including two with mixed infection at the time of recurrence), whereas relapse accounted for 37 recurrences (including the four patients with evidence of mixed infection initially). This proportion of re-infections is substantially lower than reports from other studies from South Africa, in which more than half of those with recurrent tuberculosis had evidence of re-infection.[5,6] However, in those studies, most patients were HIV positive with advanced immune suppression, whereas in the Bryant study only 22% had HIV and patients with advanced immune suppression were excluded from the parent trial.[3] For patients with HIV—as long as it is well controlled—the primary determinant of long-term outcomes seems to be the adequacy of their tuberculosis treatment.

Although whole-genome sequencing is the new test being compared with MIRU-VNTR, it is still generally assumed to be the new gold standard, in view of the wealth of detailed data provided by analysis of single nucleotide polymorphisms (SNPs). MIRU-VNTR and whole-genome sequencing were concordant in the three patients judged to have re-infection, and of the 33 paired isolates that had few or no SNP differences by whole-genome sequencing, 27 also had identical MIRU-VNTR at all 24 loci. As with many clinical and epidemiological comparisons, the discordant cases are the most informative. Of the six discordant results deemed relapses on the basis of whole-genome sequencing, five (83%) were discordant by a single MIRU-VNTR locus, meaning



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that if one defined relapse as MIRU-VNTR differences of not more than one locus, then the two tests would have been concordant for all but one case (from the published data, concordance of the two methods is difficult to judge for the six patients with evidence of mixed infections). Walker and colleagues[3] studied concordance of MIRU-VNTR and whole-genome sequencing in carefully characterised patients, and reported that of 14 paired isolates with 1–2 locus differences by MIRU-VNTR, ten (71%) had 12 or fewer SNP differences. They decided that such a threshold discriminated between same and different isolates. In the same study,[3] of 75 isolates from epidemiologically unlinked cases, 62 differed from at least one other isolate by fewer than five SNPs; raising questions about the specificity of whole-genome sequencing.

Bryant and colleagues suggest that whole-genome sequencing should be used to classify recurrences in all randomised tuberculosis treatment trials, because this is the most important and frequent bacteriological outcome. Re-infection after treatment is completed cannot be affected by the regimens evaluated in the trial, so will introduce random misclassification and reduce the power of the trial by biasing the study towards the null. This problem is overcome by molecular methods that can accurately distinguish relapse from re-infection. In this article, whole-genome sequencing was better than MIRU, but if a locus difference with MIRU-VNTR of zero or one was judged to indicate relapse, then the difference would have been trivial. The earlier methodological study of Walker raised important questions about specificity of whole-genome sequencing. An even more important question is that of cost, complexity, and accessibility of whole-genome sequencing. At present, the unit costs are still substantial and the complexity of this test limits it to a few highly specialised centres. By contrast, MIRU is simpler to do and less costly, although no studies of head-to-head cost comparisons have been published.

Is whole-genome sequencing ready to have a central role in tuberculosis clinical trials? For the moment, MIRU might still be more practical and cost-effective—enabling investigators to allocate more funds to the conduct of the trial itself. But if the cost and accessibility of whole-genome sequencing continue to improve, this could change. In view of the rapid evolution of this technology in the past decade, this seems possible.

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I declare that I have no conflicts of interest.

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