Whole-genome sequencing of *Mycobacterium tuberculosis* as an epidemiological marker

DNA fingerprinting of *Mycobacterium tuberculosis* has revolutionised the study of the transmission of tuberculosis. Since the early 1990s, IS6110 restriction fragment length polymorphism (RFLP) typing and variable number of tandem repeat (VNTR) typing have progressed and are being used to answer longstanding epidemiological questions. DNA fingerprinting of *M. tuberculosis* isolates enables the visualisation of transmission and allows a much more sensitive investigation of this important aspect of tuberculosis control. Although having these possibilities is an advantage to tuberculosis control in daily practice, the findings of possible epidemiological links as shown with DNA fingerprinting are only partly confirmed through the standard epidemiological investigation by patient interviews. As a result, DNA fingerprinting is used to indicate possible epidemiological links, but the verification of these links is dependent on whether we can understand how transmission occurred; if contact between the patients is not thought to be possible, transmission is ruled out. This verification, however, does not include incidental contacts in the public domain and might lead to an underestimation of active transmission. Nevertheless, with RFLP or VNTR typing, transmissions can often be excluded and possible links are at least indicated much more sensitively than through interviews alone. Some of the uncertainties, however, about the usefulness of RFLP or VNTR typing are also caused by the slow turnover of these markers; RFLP typing has a half-life of 3–4 years and the change in patterns for VNTR typing has not yet been established accurately. However, most *M. tuberculosis* strains retain their RFLP or VNTR profiles during transmission and the sources—eg, primary, secondary, or tertiary—cannot be distinguished within DNA fingerprint clusters.

In 2010 and 2011, the first results of *M tuberculosis* DNA fingerprinting with whole-genome sequencing (WGS) were reported and it became apparent that this new approach has a higher resolution than conventional typing. Clusters of cases according to RFLP or VNTR typing were subdivided into actual transmission chains and this new information was more in agreement with the contact tracing information. Previous studies of the usefulness of WGS have been retrospective, but in Timothy Walker and colleagues’ study in Oxfordshire, UK, WGS was applied in the first line to ascertain clustering of tuberculosis cases and to quantify active transmission. This WGS was done with a pairwise determination of the number of single nucleotide polymorphisms (SNPs) separating the isolates; a difference of a maximum of 12 SNPs was applied to rule in a possible transmission. Although this approach is elegant and valid there are still further considerations in the validation process of WGS. As for RFLP and VNTR typing, the usefulness of WGS depends on the population structure of *M tuberculosis* in the setting in which it is used. In Walker and colleagues, only in a subset of the pairs of cases with fewer than 12 SNPs difference was an epidemiological link confirmed with interviews and transmission judged likely; these findings are in agreement with those of an earlier study in Amsterdam. Does this indicate that strains with little or no difference are circulating in these areas without any epidemiological link between the respective cases? Or are we again, and this time with a more accurate and higher resolution marker, not able to understand how transmission of tuberculosis occurred? Is it simply impossible to confirm transmission through interviews with patients? This apparent lack of resolution in typing is also related to the remarkable genomic stability of *M. tuberculosis*. In a study in the UK, a turnover of 0·5 SNPs per genome per year was noted, whereas in the Netherlands a highly similar rate of change of 0·36 SNPs was found.

Another major concern that has not been sufficiently addressed and investigated generally is that *M tuberculosis* isolates are not at all homogeneous populations. They comprise several populations with different SNP profiles. With the current WGS techniques only the SNP profile in the predominating population is visualised; however, this predominance might shift when the strain is passed on to another person.

In view of the concerns described above, WGS is also not an ideal epidemiological marker, but still undoubtedly a major step forward in DNA fingerprinting compared with the conventional RFLP and VNTR typing. In the molecular epidemiology of tuberculosis it would be ideal if at each transmission a minor, but recognisable, difference occurred in the DNA profile of *M tuberculosis*. Such a difference would enable the deduction of all transmission
Pulmonary hypertension is a pathophysiological disorder defined as an increase in pulmonary arterial pressure, as assessed by right heart catheterisation (mean pressure >25 mm Hg at rest). Pulmonary hypertension can arise in various clinical disorders, which have been classified by WHO into five clinical groups, on the basis of mechanisms, with different pathogenic, prognostic, and therapeutic features. The first group is defined as pulmonary arterial hypertension, whereas the third group encompasses pulmonary hypertension due to lung diseases or hypoxaemia, including hypertension due to chronic obstructive pulmonary disease (COPD), interstitial lung disease, and sleep-disordered breathing. In patients with COPD, the presence of COPD-associated pulmonary hypertension has been linked with reduced exercise capacity, impaired quality of life, and increased risk of mortality. Therefore, interventions that alleviate COPD-associated pulmonary hypertension are needed to improve symptoms, prevent right heart failure, and prolong survival.

Prostanoids, endothelin receptor antagonists, and phosphodiesterase type-5 (PDE5) inhibitors are the main treatment options for patients with pulmonary arterial hypertension. PDE5 inhibitors increase cyclic guanosine monophosphate (GMP), the final mediator in the nitric oxide pathway, in smooth muscle cells of the pulmonary artery, causing pulmonary arterial vasodilation. PDE5 inhibitors have proven effectiveness in pulmonary arterial hypertension, enhancing exercise capacity and quality of life. However, in patients with COPD-associated pulmonary hypertension, treatment with the short-acting PDE5 inhibitor sildenafil did not improve exercise capacity or quality of life.

Why was tadalafil ineffective in patients with COPD-associated pulmonary hypertension in this trial?