Beware next generation sequencing gene panels as the first line genetic test in Charcot-Marie-Tooth disease

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Dear Editor,

The testing strategy for genetic conditions has evolved in recent years. Initially, sequential single gene tests were the mainstay. This was followed by gene panels performed through targeted gene panel sequencing. Now in many countries 'virtual panels' are applied to whole exome (WES) or whole genome sequencing (WGS) as first line tests, where multiple genes can be tested in parallel. Improved reliability and cost efficiency of WES or WGS, combined with advancing bioinformatic technology, mean that next generation sequencing (NGS), which includes WES and WGS, is preferable. One exception is for diseases where there is a common genetic diagnosis, and a single gene test is still more cost-efficient e.g. Charcot-Marie-Tooth disease (CMT) type 1A. Another is where the pathogenic genetic defects are not easily detectable with NGS, including some complex copy number variants (CNVs; large deletions, duplications, rearrangements or translocations e.g. deletion of exon 7 and 8 of *SMN1* in spinal muscular atrophy) or repeat expansions (e.g. amyotrophic lateral sclerosis caused by repeat expansion in *C9orf72)*. Many CNVs are now reliably detected by bioinformatic pipelines, but historical pipelines were less robust and CNVs were missed. For these reasons, multiplex ligation-dependent probe amplification (MLPA) is still commonly used as the first line single gene test in these settings.

CMT1A is by far the most common form of CMT, accounting for up to 62% of genetic diagnoses.[1] It is caused by a 1.5Mb duplication in the short arm of chromosome 17, incorporating *PMP22*. For this reason, in patients with demyelinating CMT (CMT1), MLPA of chromosome 17 remains the first line genetic test in most laboratories. In the past, other methods have been employed including microsatellite analysis, though this method comes with around a 2% false negative rate.[2]

In the last three years our specialist service has diagnosed CMT1A in three patients referred for a diagnostic opinion because the common genetic causes of CMT had been excluded, in whom we have unexpectedly detected *PMP22* duplication through NGS. The first was a patient in their 60s with typical CMT1, in whom no sequencing variants were detected in our CMT panel, though abnormal *PMP22* dosage was flagged through our diagnostic laboratory's CNV analysis of exome sequencing. The assumption was made that the patient had previously undergone *PMP22* dosage analysis, since a CMT 'genetic screen' had been performed prior to referral; in the UK this usually includes MLPA for CMT1A and a NGS CMT gene panel. The second, another patient in their 60s, was referred with a diagnosis of severe axonal CMT (CMT2) and had been enrolled in the 100,000 Genomes Project (100K GP) with no primary findings detected. Our neurophysiology surprisingly demonstrated a demyelinating neuropathy, and analysis of the 100K GP data in the research environment showed 1.5x the read depth of *PMP22* compared with other parts of the genome (Figure 1). Lastly, a patient in their 40s was referred with a 'normal *PMP22* dosage'. On assessment the patient's phenotype was typical for CMT1A. Reviewing the original *PMP22* dosage test, done in an external laboratory, we noted this was negative but had been done by microsatellite analysis. They had been enrolled in the 100K GP, again with no primary findings reported, and retrospective review of this data suggested *PMP22* duplication, as seen in the second case. MLPA subsequently confirmed *PMP22* duplication in all three cases.

In conclusion, our cases act as a reminder to neurologists and geneticists, in an era of gene therapies where a molecular diagnosis is more important than ever, that NGS is not always the right test. Firstly, careful review of prior testing (*'Has PMP22 dosage been done?'*) can avoid unnecessary further expensive tests, and the possibility that the diagnosis will still be missed (Case 1). Secondly, patient phenotype is critical to guide testing *('Is the clinical*

diagnosis correct'?); MLPA is still the first line test for CMT1A (Case 2). Lastly, the method of prior testing must be scrutinised (*'Exactly what test was done?'*), considering that both microsatellite analysis and older NGS pipelines can miss the *PMP22* duplication (Case 3).

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Ethics statements

Ethics approval: Participant data was collected in line with the ethically approved study 'Charcot-Marie-Tooth Disease and related disorders: A Natural History Study', reviewed by the London Queen Square Research Ethics Committee (REC No.: 09/H0716/61).

Patient consent for publication: not applicable

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Competing interests

None declared.

References

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Figure 1 Integrated Genomics Viewer (IGV) showing our patient's bam file with 1.5x the read depth of *PMP22* (right pane) compared with other parts of the genome (chromosome 1, left pane). This indicates a duplication of chromosome 17 in the region of *PMP22*, confirmed by multiplex ligation-dependent probe amplification.