Reanalysis of whole exome sequencing data to identify copy number variants in patients with primary ciliary dyskinesia

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Introduction

Primary ciliary dyskinesia (PCD) is a rare autosomal recessive condition affecting ~1/20,000, characterised by the dysfunction of motile cilia. This leads to impaired mucous clearance from airways and if untreated can result in infections and bronchiectasis, requiring lung transplant in some cases. If treated, pulmonary deterioration can be arrested and patients are able to live full and active lives[1].

However, a barrier to commencing treatment is diagnostic age which is currently achieved at a median of 5 years. Genetic testing provides an opportunity to detect PCD much earlier but the molecular diagnostic rate is only 65%[2]. It is predicted that a proportion of the molecularly unresolved cases may be due to copy number variants (CNVs) which are not routinely looked for.

Conventional genetic diagnostic techniques include whole exome sequencing (WES) which maps the protein coding regions of DNA. It works by matching up multiple fragments of DNA copied from a person's genome to create a consensus of the correct sequence. The number of fragments aligned at a particular point is referred to as read depth, which is primarily taken as a quality control indicator. Bioinformatic analysis of read depth data can reveal CNVs by comparing values for an individual patient with those of a wider cohort. For example, a heterozygous deletion would be revealed if the read depth for a DNA segment was half of the expected average[3].

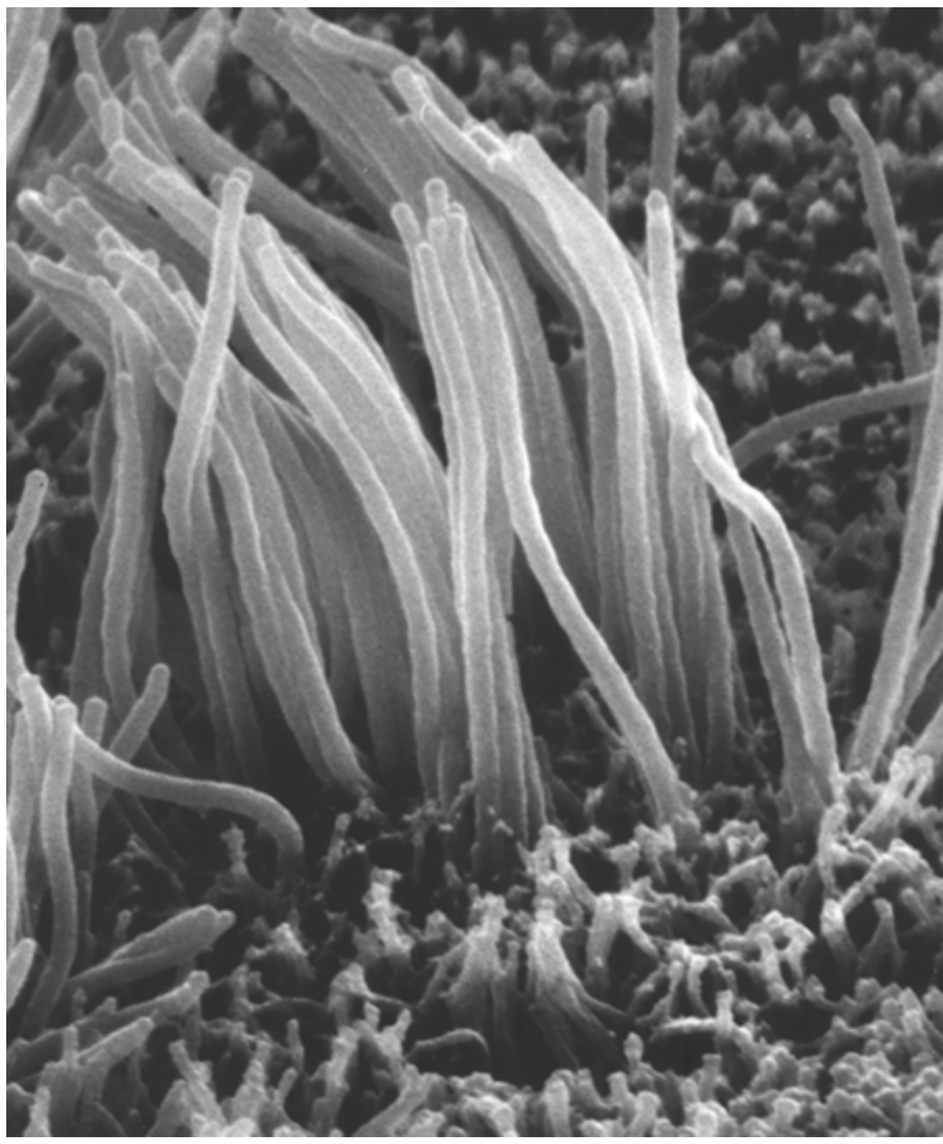

Fig 1: Scanning electron micrograph of cilia. Courtesy of Southampton Biomedical Imaging Unit.

Aim: To investigate PCD cases unresolved after whole exome sequencing (WES) through CNV analysis of read depth data.

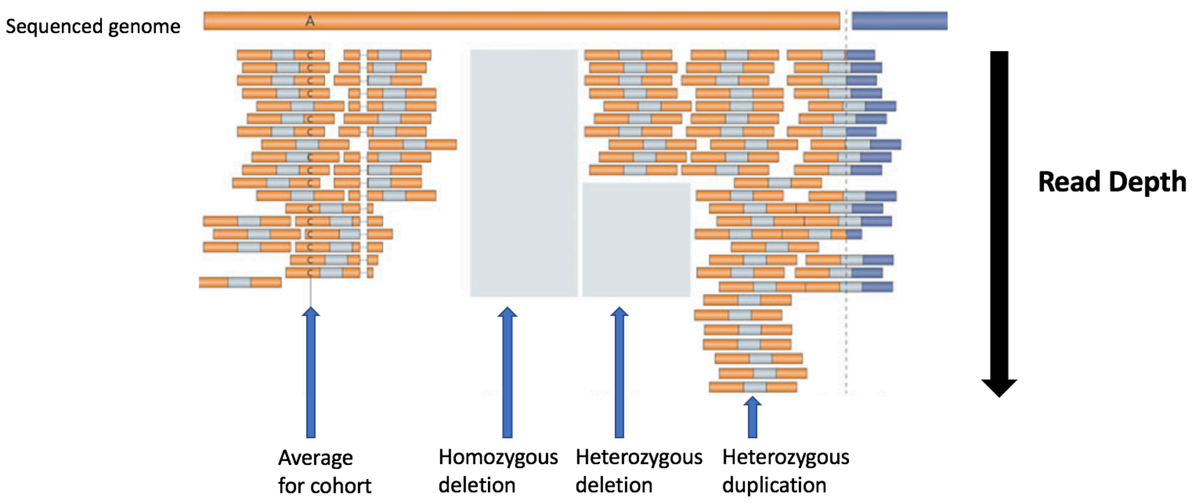

Fig 2: Read depth of DNA segments demonstrating how quantification of differences from a cohort average can reveal CNVs. Figure taken from Meyerson *et al.*, 2010[4].

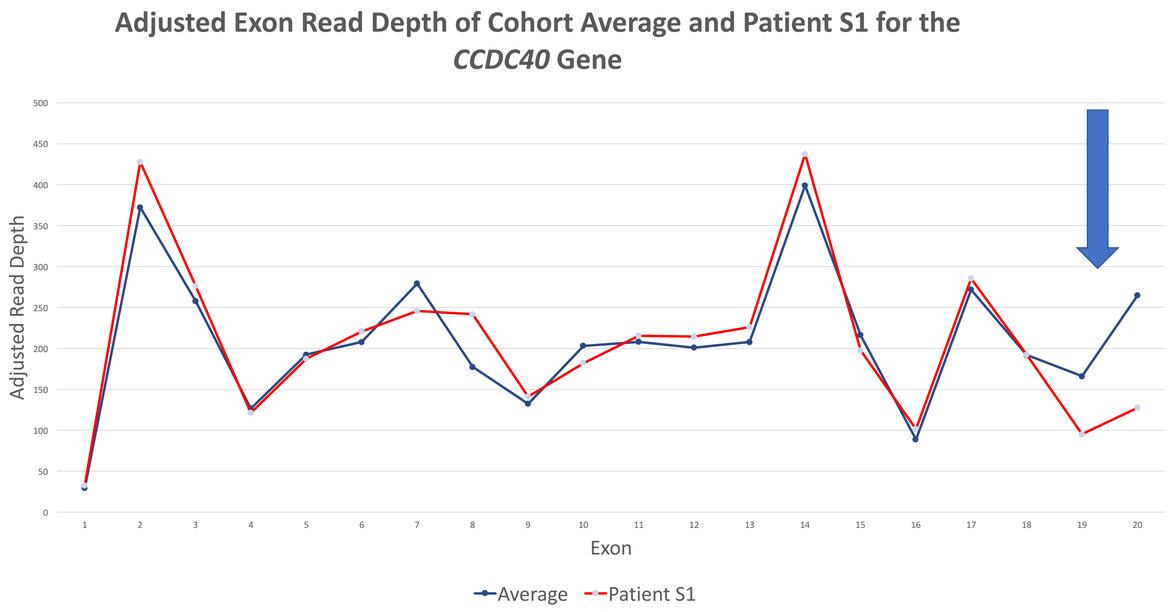

Fig 3: Graph shows a reduced read depth for exons 19-20 in patient S1, indicating a heterozygous deletion in the *CCDC40* gene.

Methods

Targeted CNV analysis of WES read depth data was conducted in 4 patients (S1-4) where a single heterozygous variant had been identified in established PCD-genes. Exon read depths were compared against average values for a cohort of 24 patient exomes (Fig 2).

Results

Patient S1 was found to have a single heterozygous variant (c.248delC) and a deletion of exons 19-20 (Fig 3) in the *CCDC40* gene. Both variants are classed as pathogenic according to the American College of Molecular Genetics guidelines[5,6].

It is predicted that each variant is inherited from different parents of S1, to fit the autosomal recessive pattern reported in PCD, resulting in disease through compound heterozygous null expression of *CCDC40*.

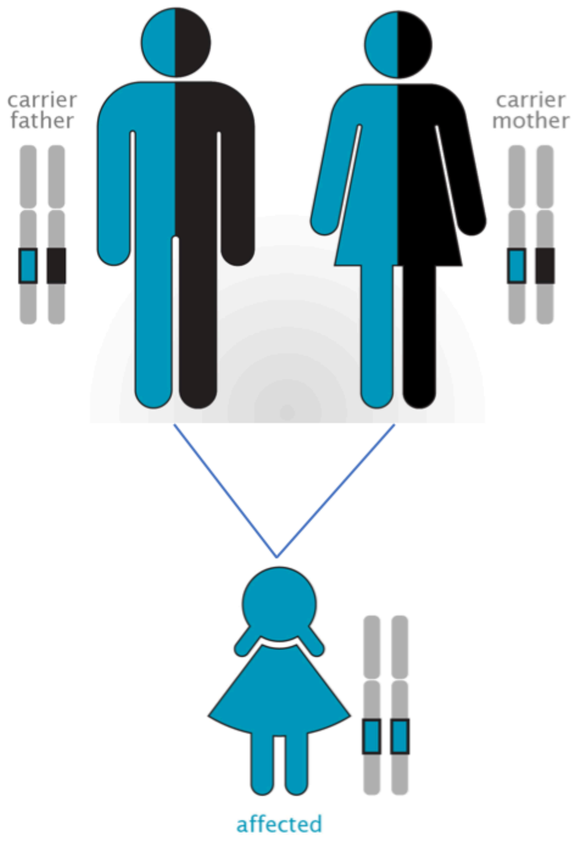

Fig 4: Autosomal recessive inheritance

Discussion

The clinical phenotype for patient S1 (disarranged cilia shown through transmission electron microscopy) is consistent with reports of PCD caused by defects in the CCDC40 gene[7]. The suspected molecular diagnosis of PCD in this case should be confirmed through a well established CNV analysis method however, it represents 25% of PCD cases molecularly unresolved after WES. This suggests that there could be a significant uplift in genetic diagnostic rates for PCD through conducting CNV analysis in established PCD-genes. The bioinformatic approach used can increase the diagnostic rate for WES by analysing the data from a different perspective.

References

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