Genetic and structural characterization of outer dynein arm variants causing primary ciliary dyskinesia

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Introduction: Primary ciliary dyskinesia (PCD) is a heterogeneous, recessive disease, characterized by dysfunction of motile cilia that arises from structural defects. Symptoms include chronic pulmonary disease, rhinosinusitis, otitis media, laterality defects, congenital heart disease and subfertility. The most commonly affected cilia structure is the outer dynein arm (ODA), a complex structure composed of a docking complex and multiple heavy, light and intermediate dynein chains. An understanding of the relationship between the genetic and structural phenotype of ODA variants will allow patient stratification and improve diagnosis through verification of new candidate genes effecting the ODA structure.

Methods: 195 PCD patients were genotyped using next generation sequencing. Candidate variants were confirmed by Sanger sequencing and familial segregation analysis. For selected ODA mutation patients, electron tomography, an extension to transmission electron microscopy, was used to produce high-resolution 3D models of axonemal microtubular doublets and ODA volume ratios. The data were analysed to determine the impact of eight different gene mutations causing different structural defects of the ODAs.

Results: 39% of patients had bi-allelic mutations identified affecting the ODA. These include variants in known PCD genes: DNAH5 (n=39), DNAH11 (n=18), DNAI1 (n=8), DNAI2 (n=5), ARMC4 (n=3), CCDC114 (n=2), DNAL1 (n=1) and mutations in the novel candidate DNAH9. Variants in DNAH9 have been suggested as a cause of PCD previously but disregarded due to lack of phenotypic evidence.

3D models of the ODA complex identified genotype specific changes in the ODA complex in PCD. ODA structure in PCD was different in the proximal region, in proximity to the microvili, when compared to the distal region, towards the tip of the axoneme. A significant deficiency in the ODA volume was detected at the distal part of the axoneme in the patient with DNAH9 defects, whereas the proximal portion was unaffected, reflecting the protein position of DNAH9 and suggesting a structural affect of the variant.

Conclusion: 3D electron tomography can be used to detect subtle changes in the ultrastructure of the ODA in PCD patients with differences detected in the impact of mutations in proximal versus distal regions of the cilia.