Genetic testing and variant classification in Primary Ciliary Dyskinesia reveals considerable genetic heterogeneity despite a high proportion of homozygous cases.

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WRGL
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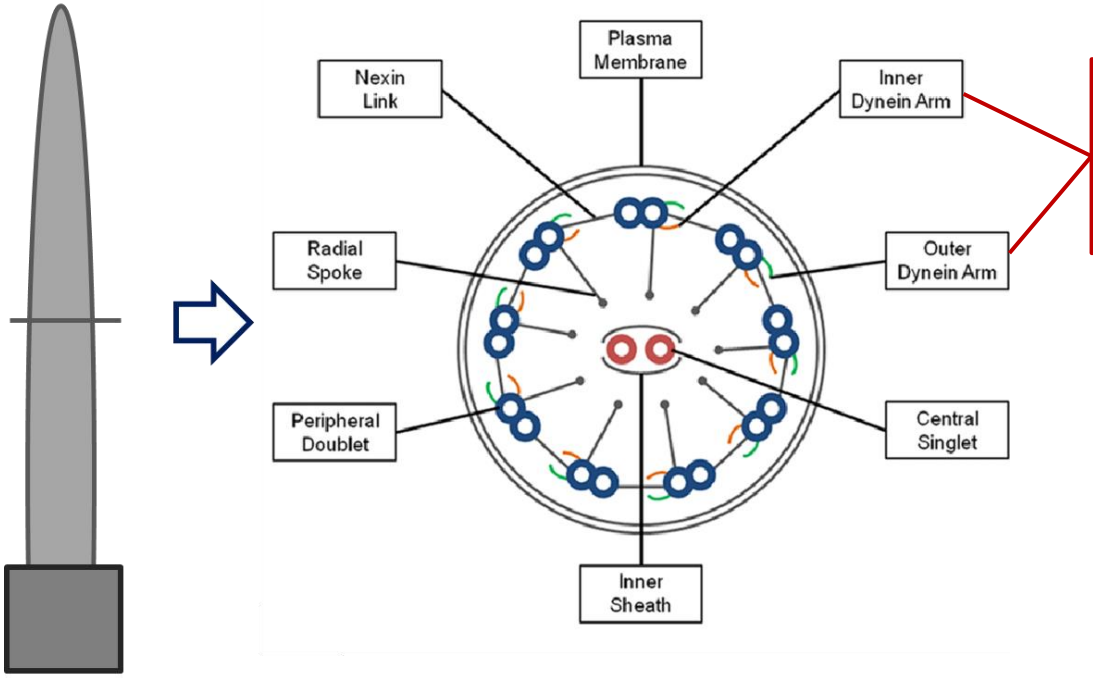
Structure and function of motile cilia

Cilia are hair-like organelles that play an essential role in clearance of fluids, mucus, bacteria and cellular debris.

Motile cilia exhibit a 9+2 configuration — a pair of single microtubules surrounded by nine peripheral doublets.



Connected to each doublet is an inner and an outer dynein arm, that provide energy for movement.

The inner and outer dynein arms are large multi-subunit complexes that are pre–assembled and then transported from the cytoplasm.

Impaired ciliary function results in absent or abnormal motility which is described as Primary ciliary dyskinesia (PCD).

Clinical features of Primary Ciliary Dyskinesia

Motile cilia are present on the surface of epithelial cells in the respiratory tract, inner ear, brain ventricles and fallopian tubes. Therefore there is a fairly wide clinical presentation.

PCD is a lifelong disease but generally presents in childhood.

Patients suffer with chronic or recurrent infections of the airways and ears in childhood and this can lead to widening of the airways (bronchiectasis) and hearing problems respectively.

PCD also affects reproductive organs and can cause infertility.

In approximately 50% of patients, the establishment of left-right axis (laterality) is disrupted causing abnormal organ positioning (situs inversus).

Genetics of Primary Ciliary Dyskinesia

PCD is an inherited genetic disorder and a large number of genes encode the structural components of motile cilia.

In addition mutations have also been identified in genes which encode proteins involved in the assembly and transport of the dynein arms.

PCD is pre-dominantly an autosomal recessive condition, although mutations in a few X-linked genes have also been described.

In humans, mutations in at least 40 different genes are now associated with PCD.

The incidence of PCD is estimated to be around 1 in 10,000 births. The presence of founder mutations and the rate of consanguinity strongly affect the incidence in specific populations e.g. British Asians and Irish travellers.

Diagnosis of Primary Ciliary Dyskinesia

The clinical features of PCD are now well recognised, but the diagnosis is still challenging, especially when patients present with non-specific symptoms.

Most mutations associated with PCD result in an identifiable ultrastructural defect of cilia and/or changes in the pattern of motility.

Diagnosing PCD requires a combined approach utilising characteristic phenotypes and complementary methods for detection of defects of ciliary function and ultrastructure: high-speed video-microscopy, transmission electron microscopy and immunofluorescence; in addition to measurement of nasal nitric oxide.

Genetic testing is an important component of PCD diagnosis and the large number of genes that can cause PCD is suited to NGS panel testing.

Primary Ciliary Dyskinesia testing in Wessex

58 patients tested using 19 gene virtual panel (TruSight One)

21 with a positive molecular diagnosis (36%)

58 patients tested using 29 gene virtual panel (TruSight One Extended)

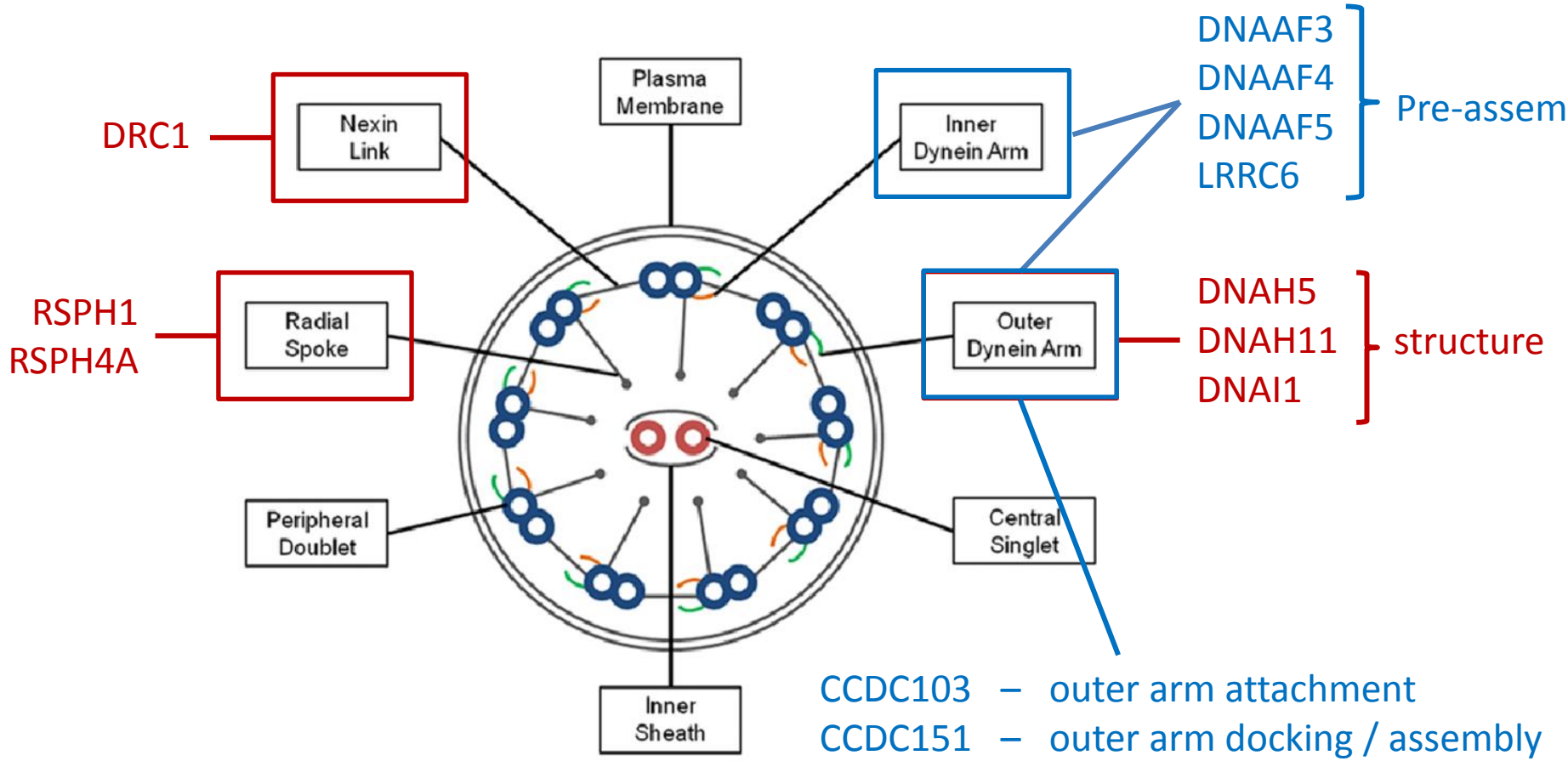
16 with a positive molecular diagnosis (28%)

12 novel patients and 8 cases negative for 19 gene panel tested by 100KGP (31 or 32 gene virtual panel)

6 with a positive molecular diagnosis

Total of 43 patients with a positive molecular diagnosis.
Mutations identified in 14 different genes.

Functional consequence of mutations identified

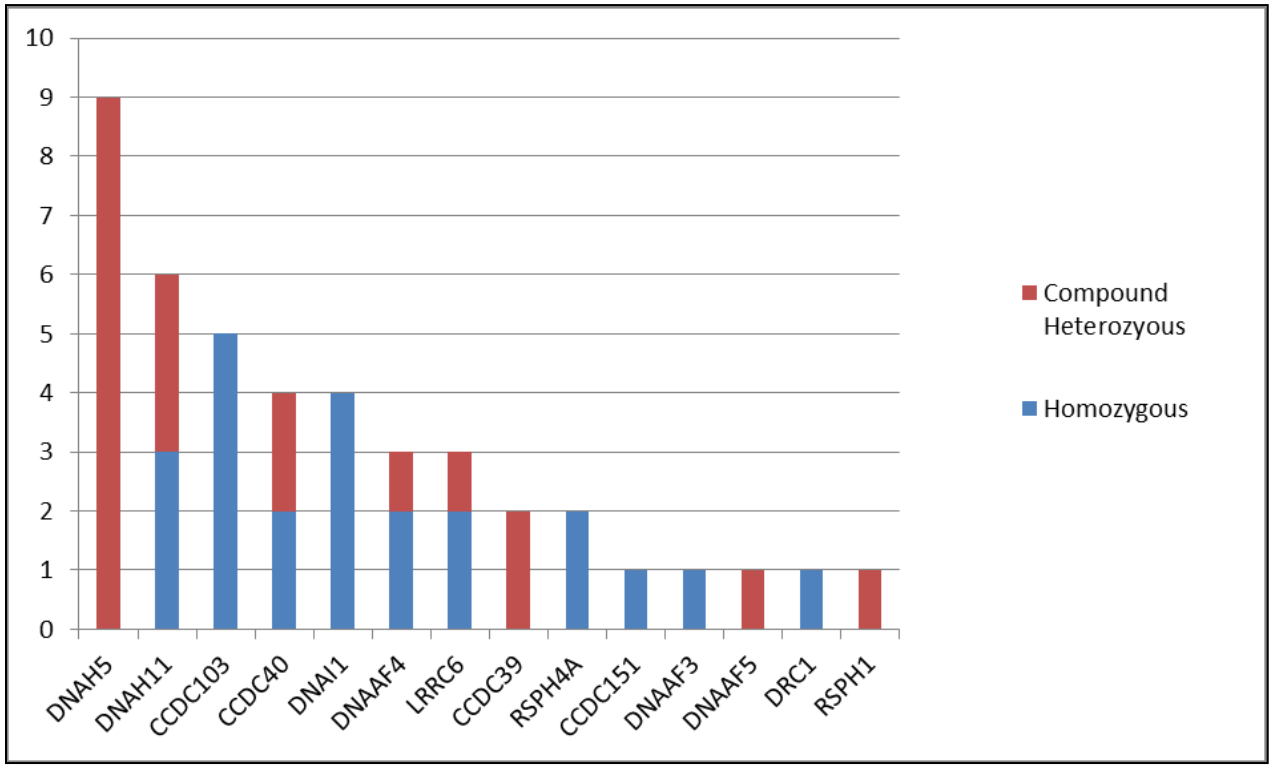


CCDC39
CCDC40
Cilia repeat ruler (maintain regular spacing of structural components)

Breakdown of 43 positive cases

All 43 patients had bi-allelic mutations in one of 14 recessive genes.

- 3 genes accounted for >50% of positive cases
- 23 homozygous
- 20 compound heterozygotes



Distribution of specific mutations

50 different mutations identified in the 86 alleles

- 33 observed as only a single allele
- 17 > 1 allele (including both homozygotes and compound heterozygotes)

Gene	Variant	Total Allele Count	Homozygous Cases (Alleles)
CCDC103	c.461A>C p.(His154Pro)	10	5 (10)
CCDC40	c.248delC:p.Ala83ValfsTer84	6	2 (4)
DNAI1	c.48+2dupT	4	2 (4)
LRRC6	c.630delG: p.Trp210CysfsTer12	4	2 (4)
DNAH5	c.10815delT:p.Pro3606HisfsTer23	3	0 (0)
DNAH11	c.4333C>T:p.Arg1445Ter	3	1 (2)
DNAAF4	CNV: c.784-1037_894-2012del	3	1 (2)

Classes of pathogenic mutation

Mutation Type	Number of alleles	Number of unique variants
Nonsense	26	21
Frameshift	26	12
Splicing	12	7
Missense	16	6
CNV	**4**	**3**
Initiation Codon	2	1
Total	86	50

Majority of missense substitutions classified as VUS

Predominantly SNVs but small number of CNVs identified

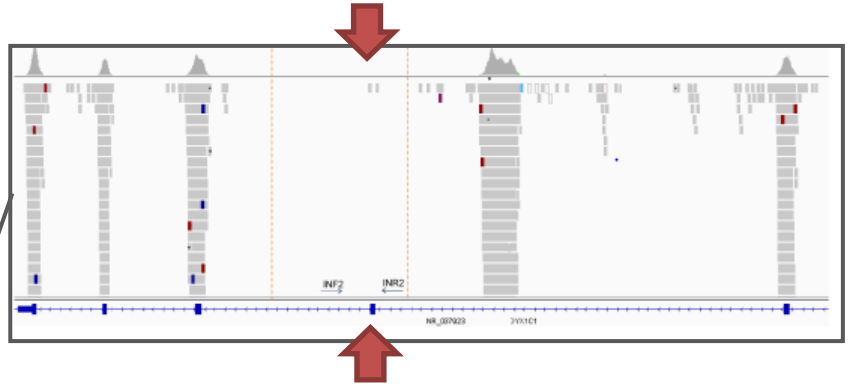
100KGP CNV case

DNAH5 intra-genic deletion identified in one 100KGP case
- confirmed by PCR

CNV cases

DNAAF4 (DYX1C1) single exon deletion (c.784-1037_894-2012del) - Homozygous

- large consanguineous family with multiple affected sibs
- no pathogenic variants identified by sequencing
- review of coverage data identified absence of reads for a single exon
- literature search identified same/similar mutation
- homozygosity confirmed by PCR



nature genetics

DYX1C1 is required for axonemal dynein assembly and ciliary motility

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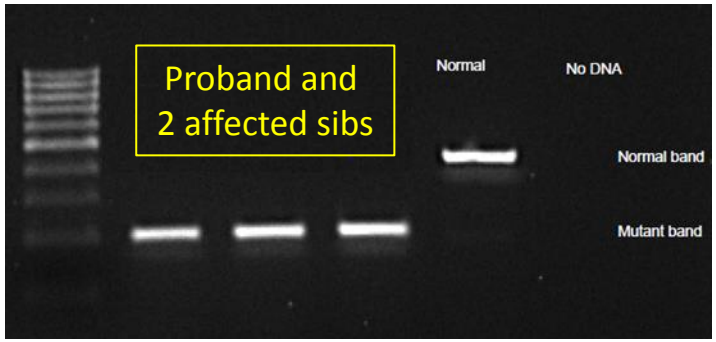


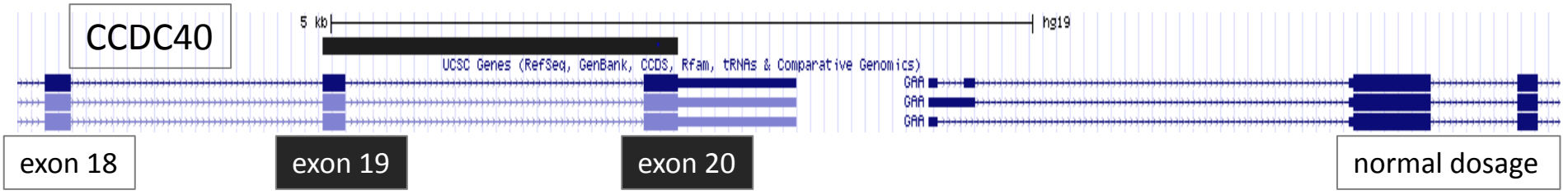
CNV cases

DNAAF4 single exon deletion (c.784-1037_894-2012del) - Heterozygous

- diagnostic test identified single pathogenic sequence mutation in DNAAF4
- screened by PCR for presence of common single exon deletion
- compound heterozygote for the deletion and SNV

CCDC40 deletion of exons 19 and 20

- frameshift mutation identified in two affected brothers
- review of CCDC40 identified apparent deletion of last two exons in both brothers
- not yet confirmed



Breakpoint interval

Breakpoint interval

Limitations of testing

Diagnostic yield of 32 %
Large numbers without a molecular diagnosis

Gene content
11 / 14 genes in which we found mutations were tested on all patients
> 40 known PCD genes but maximum of 32 tested

CNV analysis
CNVs identified in 4/43 patients
No systematic analysis of CNVs in Wessex patients

12 cases with single pathogenic mutation identified
3 possible splice mutations – RNA studies
1 in-frame deletion and 4 missense – DM in HGMDPro and/or one additional line of evidence could be sufficient to re-classify as likely pathogenic

ACMG Classification: commonly applied criteria

Including both bi-allelic and “carrier” results, **76 variants** were classified as (likely) pathogenic using ACMG / ACGS best practice guidelines.

PVS1	(loss of function variants)	64	
PM2	(absent from population databases)	60	
PVS1 + PM2	(sufficient for likely pathogenic)	55	
PS4	(observed in multiple patients)	33	(moderate)
		11	(supporting)
PM3	(in trans with a known pathogenic variant) Many homozygous cases.	34	

ACMG Classification: rarely applied criteria

PS2 / PM6	(de novo)	All mutation tested were inherited
PS3	(functional studies)	Only 3 cases and all RNA studies
PP1	(segregation)	Follow up testing of parents only
PM1	(hotpsot or domain)	Recessive genes tolerant of missense changes and common in population control databases.
PM5	(same codon affected)	
PP2	(gene intolerant of missense)	

ACMG Classification: comments

HGMDPro

Invaluable resource to prioritise non-loss of function variants:

- all PCD variants absent from HGMD ended up as VUS
- majority of PCD variants present classified as (likely) pathogenic

PP4 (phenotype of family history highly specific for gene)

PCD displays a clinical spectrum and multiple genes.

However:

- phenotype can be highly specific (TEM, video, NO)
- strong phenotype-genotype correlations
- Immunofluorescence can show absence of a specific gene

Acknowledgements

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