Which side are they on? Diagnosing primary ciliary dyskinesias in low- or middle-income countries: A review and case series

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Primary ciliary dyskinesia (PCD) is a rare genetic condition with a variable clinical presentation, making its diagnosis a challenge. We describe two unrelated sibling pairs with PCD: adult siblings in the first and perinatal/neonatal in the second. Both families highlight the more common and rarer clinical manifestations of PCD. We use these cases to highlight: (*i*) current understanding of the underlying genetic and pathophysiological mechanisms of PCD; (*ii*) the diversity of cardiac and respiratory features of PCD across a wide age range; (*iii*) aspects of the history and clinical examination that should raise suspicion of PCD; and (*iv*) the role of next-generation sequencing gene panel testing in confirmation of the diagnosis. We note genomic evidence predicting that PCD is relatively common in black African populations.
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Study synopsis

What the study adds. This review of two sibling pairs illustrates the variable histories, presentations, diagnostic processes and clinical courses of primary ciliary dyskinesia (PCD) in low- or middle-income countries (LMICs), highlighting the diagnostic challenges faced when encountering such patients in settings where there may not be access to specialised resources. Possible diagnostic tools that can be used are discussed, weighing up their pros and cons in an LMIC setting, and a potential diagnostic approach that can be adapted to the treating clinician's own context is provided.

Implications of the findings. Confirmation of the diagnosis of primary ciliary dyskinesia is no longer limited to well-resourced institutions, but can be done in less specialised environments using novel, highly accurate next-generation sequencing gene panel testing, reducing the need to transport patients as well as the overall cost to the healthcare system. Well-resourced institutions that see high volumes of patients with PCD can invest in new highly sensitive diagnostic tools such as high-speed video microscopy. There is a need for research investigating the validity of tools such as ciliary immunofluorescence in the South African population.

Primary ciliary dyskinesia (PCD) is a rare genetic condition that mainly affects the upper and lower respiratory tract and cardiovascular system. The best-known clinical feature is mirror-image dextrocardia and situs inversus of abdominal organs which, when combined, is referred to as situs inversus totalis (SIT) and raises a red flag for Kartagener syndrome, a condition associated with chronic respiratory tract problems caused by ciliary dysfunction. Based on studies of SIT, the incidence of PCD has for many years been considered to be approximately 1 in 15 000 - 20 000 live births, although it is known that PCD is markedly underdiagnosed.[1]

More than half of people with PCD do not have SIT, so individual cases often go unsuspected.[2,3] Of children with PCD, half have normal situs, and ~10% have heterotaxy, which is defined as a disorder comprising abnormal symmetry of the thoracoabdominal organs about the right-to-left axis.[4] Heterotaxy represents a failure to break symmetry during embryonic development, which results in either bilateral right-sidedness or left-sidedness of several thoracic and abdominal organs, termed termed right or left isomerism, respectively.

Right-sided isomerism is characterised by the atria both having right atrial morphology, and bilateral sinoatrial (SA) nodes with one being dominant. The lungs are trilobar bilaterally, and the right and left bronchi arise above the pulmonary arteries (eparterial morphology), which can be used to assist in the diagnosis of right-

sided isomerism.[5] Abdominal findings often include a rather centrally positioned liver that is palpable in the epigastrium.

Left-sided isomerism is characterised by both atria having left atrial morphology, with a hypoplastic or absent SA node and absence of the inferior vena cava with venous drainage typically via the azygous system.[6-8] The ventricles tend to have a more normal morphology, and cardiac defects are usually milder.[9] The lungs are typically bilobar with hyparterial bronchi (bronchi arising below the pulmonary arteries).[5] The liver may be symmetrical or asymmetrical.

We present four cases of PCD from two families that demonstrate a range of laterality disturbances, but also a range of respiratory findings that highlight how PCD can mimic common conditions such as neonatal respiratory distress syndrome and post-infectious bronchiectasis. We discuss associated clinical features, particularly those with greater specificity for PCD. We highlight new diagnostic modalities, and especially the role of next-generation sequencing (NGS) gene panel testing in facilitating diagnosis and management in an African setting.

Pathophysiology of PCD

The biological basis for PCD relates to abnormal function of the motile and nodal cilia. Motile cilia are minute hair-like structures on the surface of respiratory tract and certain other epithelial cells, that direct the flow of fluid or material in a particular direction. Fig. 1 shows the ultrastructure of a motile cilium, comprising a microtubular pair surrounded by nine microtubular doublets forming a cytoskeleton structure that is maintained by radial spokes and nexins. The microtubular doublets have inner and outer dynein arms, which are motor proteins that hydrolyse adenosine triphosphate to produce motion.[10]

Nodal cilia are found in the primitive node in early embryonic development. They are of similar structure to motile cilia except that they lack the central apparatus. As a result, they have a rotary motion that underlies embryological left/right axis determination in a manner that remains incompletely understood.[11]

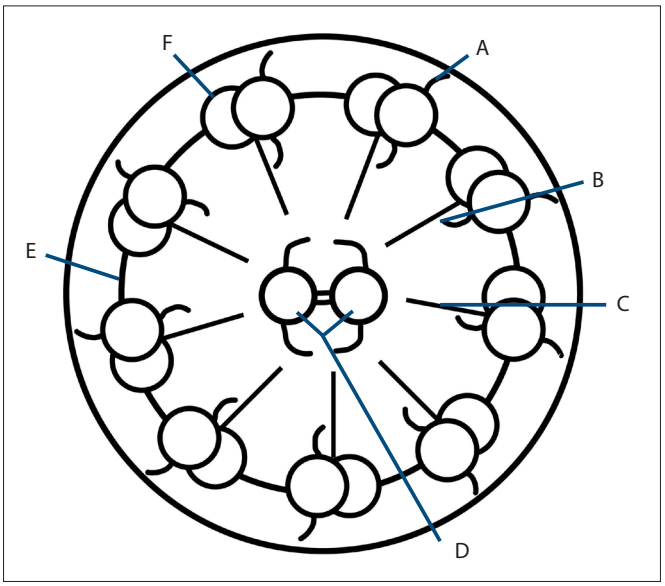

Fig. 1. Ultrastructure of a motile cilium. (A) outer dynein arm; (B) inner dynein arm; (C) radial spoke; (D) microtubular pair; (E) nexin; (F) microtubular doublet.

Case vignettes

Family A

A man in his late 20s (patient 1) was referred to a respiratory clinic with bronchiectasis presumed to be secondary to unconfirmed pulmonary tuberculosis (TB). Further enquiry revealed that he had had a cough and respiratory problems since childhood, as well as chronic upper respiratory tract problems, predominantly rhinosinusitis. Because of his chronic cough and unexplained weight loss, he had had many investigations over the years for pulmonary TB, which included sputum testing with GeneXpert, auramine staining and cultures for *Mycobacterium tuberculosis*. All were negative. He was admitted to a specialist TB hospital at the time of referral, and he was completing a course of empirical TB treatment but was not improving. His chest radiograph (Fig. 2A) showed chronic lung changes, mesocardia, and intestinal gas below both the right and left hemidiaphragm.

The initial differential diagnosis included post-TB bronchiectasis and cystic fibrosis. However, a computed tomography (CT) scan of the chest and abdomen (Fig. 2B) showed an abundance of pathological features including bilateral bronchiectasis, bilateral left atrial appendages, an interrupted hepatic inferior vena cava (IVC) with the IVC continuing into the azygous system, and polysplenia without a parent spleen. These findings strongly suggested heterotaxy with left isomerism in the context of the other features of PCD.

The final piece of the puzzle was volunteered by the patient, namely that his 34-year-old sister (patient 2) also suffered from upper respiratory problems and a chronic productive cough. Her chest radiograph demonstrated dextrocardia and SIT (Fig. 2C). In addition, she had clinical and radiological features of bilateral bronchiectasis, and primary infertility.

The findings in both patients were suggestive of PCD, although their clinical features differed. For confirmation of the diagnosis, an NGS gene panel test on patient 1 was requested via an international clinical genomics provider (Invitae Corp., USA). This included full sequencing of the exons and flanking DNA, and deletion/duplication analysis of 34 genes associated with PCD. A homozygous deletion of a single base pair in exon 11 of the *DNAAF3* (dynein axonemal assembly factor 3) gene was identified. The same homozygous variant was subsequently also found in patient 2 (Table 1).

The variant leads to a shift in the transcriptional reading frame, with a premature stop signal slightly downstream. This is expected to truncate the last 143 amino acids of the protein, and leads to a loss-of-function effect. Although this variant has not been described previously, it could be classified as pathogenic based on its characteristics, and was added to the ClinVar database of variants with known phenotype associations.[12] Homozygosity for this variant therefore confirmed a diagnosis of autosomal recessive PCD in both siblings.

Family B

A term male infant (patient 3) was born by normal delivery to a young, healthy and non-consanguineous couple. He had a birth weight of 3 060 g and good Apgar scores. Respiratory distress was noted later on day 1, and a chest radiograph showed clear lung fields but a boot-shaped heart. The baby was referred to a tertiary-level neonatal unit, where continuous positive airway pressure (CPAP), oxygen and first-line antimicrobial therapy were started. Echocardiography showed

a tetralogy of Fallot, which was considered a possible cause for the respiratory distress.

On day 5 there was respiratory deterioration due to right upper lobe atelectasis. Despite appropriate management, progressive bilateral atelectasis leading to ventilator dependence followed. This was complicated by infection with methicillin-resistant *Staphylococcus aureus* and persistent infection with *Pseudomonas aeruginosa*, and the baby eventually died at 48 days of age. Fig. 2A - D show the chest radiographic findings over several weeks.

The differential diagnosis for the respiratory findings included a congenital airway anomaly, cystic fibrosis and primary immunodeficiency. The trachea and bronchi were patent on a CT scan of the chest (Fig. 2E) and bronchoscopy, a 50-mutation panel for cystic fibrosis was negative, and the results of first-line immunological tests were normal. The presence of tetralogy of Fallot raised the possibility of DiGeorge syndrome, but no evidence of deletion at the 22q11.2 locus was found on genetic testing. After excluding these differential diagnoses as far as possible, the possibility of PCD as an explanation of the cardiac and respiratory features was considered at 1 month of age.

An NGS gene panel test of 31 genes associated with PCD was requested via the abovementioned clinical genomics provider. Two different heterozygous pathogenic variants in the *DNAAF1* gene were detected (Table 1), one being maternally derived and the other paternally derived. One was a single-nucleotide variant inducing a premature stop codon at amino acid position 271 (i.e. a 'nonsense' change), and the other a deletion of exon 7, resulting in a shift in the reading frame. The former variant has previously been reported as pathogenic, whereas the latter was novel but classified as pathogenic based on its characteristics. Taken together, these variants cause loss of function of both copies of the gene, confirming the diagnosis of PCD.

In the couple's second pregnancy, a fetal anatomy ultrasound scan at 21 weeks' gestation showed mirror-image dextrocardia and situs inversus. Following non-directive genetic counselling about a high probability of PCD recurrence, the couple decided to continue the pregnancy. A female infant (patient 4) was born at 39 weeks' gestation with a birth weight of 3 880 g and good Apgar scores. The infant had mild respiratory distress from day 1 and was treated with nasal CPAP and oxygen, and first-line antimicrobial therapy. There was ongoing respiratory distress, but no severe exacerbations. Nasal CPAP was required until day 21, and oxygen until day 24. A chest radiograph confirmed mirror-image dextrocardia and situs inversus, and the presence of bilateral lung infiltrates (Fig. 2F and G). An echocardiogram showed an atrial septal defect requiring expectant management only. Genetic testing for the 'family variants' confirmed the presence of both pathogenic variants in the *DNAAF1* gene (also known as the *LRRC50* gene). Treatment with physiotherapy and prophylactic antibiotics was continued after hospital discharge, and at 6 months of age the baby was growing and developing well, with no further respiratory complications.

Discussion

These four patients from two families highlight the diversity of respiratory and cardiac presentations of PCD. In each family, the initial patient did not have SIT that would raise early suspicion of Kartagener syndrome, but presented with other predominantly respiratory features that flag the possibility of PCD. Clinicians need to be aware of these other clinical features.

Respiratory presenting features

Neonatal respiratory distress has many common causes, including hyaline membrane disease, pneumonia, meconium aspiration syndrome and transient tachypnoea of the newborn. The respiratory distress of PCD can be difficult to suspect if typical cardiac findings are not present. Respiratory features that increase the chance of PCD include term gestation with normal delivery, a brief asymptomatic period before the onset of respiratory distress, persistent respiratory distress, and radiographic findings that include persistent pulmonary infiltrates or recurrent migratory atelectasis.[13] Patients 3 and 4 demonstrated several of these features.

If the patient survives the neonatal period, the respiratory distress tends to improve as the child, and the bronchi, grow. Respiratory symptoms persist, however: the chronic daily productive cough and chronic nasal obstruction found in family A are usually present from early childhood or even infancy, and sinusitis is often present when the sinuses complete their development in childhood. Bronchiectasis is common in childhood and nearly universal by early adulthood, most typically involving the middle and lower lobes.[14]

Bronchiectasis has diverse aetiologies, and an identifiable cause is not found in 35 - 50% of cases. However, it is still recommended to proceed with diagnostic work-up, since it may influence the management and prognosis.[15] Bronchiectasis is more common in low- and middle-income countries than in high-income ones, and more often attributed to pulmonary TB, HIV infection, and other types of bacterial and viral pneumonias (particularly adenovirus and varicella) that may have occurred in childhood or as adult.[16-18] Considering the high prevalence of TB in South Africa (SA), as well as the considerable variability of presentations of post-TB structural lung disease, it is often prudent to consider empirical TB treatment for untreated disease.[19,20] However, as was the case in patient 1, not considering other diagnoses can deflect attention for years from rarer possible diagnoses such as cystic fibrosis, primary immunodeficiency diseases and PCD. Where it has been studied, PCD accounts for 2.5 - 10% of bronchiectasis in adulthood.[21-23]

Laterality defects

With regard to laterality defects, the second patient in each family had typical SIT. In family A this helped to clarify the phenotype, and in family B it facilitated prenatal diagnosis. However, patients 1 and 3 had more clinically subtle disturbances of laterality. Patient 1 had heterotaxy with left isomerism, and patient 3 had apparently normal situs but with a congenital heart defect.

A study of 305 patients with PCD found that 47% had situs solitus (normal situs), 41% SIT, and 12.1% other situs anomalies – 9.5% with classic heterotaxy and 2.6% with subtle laterality defects.[24] Heterotaxy as found in patient 1 is therefore an uncommon presentation of a rare disease.

Congenital heart defects (CHDs) are common in PCD and can be found in patients with SIT and in those with apparently normal situs, but are particularly common in association with heterotaxy. Surprisingly, perhaps, our only patient with a congenital heart defect was the one with normal situs, and in this case the presence of a CHD

in addition to the persistent pulmonary findings was important in suspecting the diagnosis.

In patients with heterotaxy, CHDs are less common and usually less severe in those with left isomerism than right isomerism, with the result that patients reaching adulthood are more likely to have left isomerism, as with patient 1. Cardiovascular defects associated with left isomerism include interruption of the inferior vena cava with drainage via the azygous system (very common), atrioventricular septal defects, and partial anomalous pulmonary venous drainage (both moderately common). Right isomerism it is very often associated with more complex cardiac defects that may include one or more of complete atrioventricular septal defect with a single atrioventricular valve, total anomalous pulmonary venous drainage, and severe pulmonary stenosis or atresia.[6,9]

Heterotaxy of abdominal organs may also cause health problems. Polysplenia without a dominant spleen, as seen in patient 1, is a common feature of left atrial isomerism. This finding should prompt assessment for functional asplenia, which may occur and then requires the same prophylaxis and treatment as for the anatomical asplenia[25,26] found in right isomerism, where vaccinations against encapsulated organisms play a critical role. Intestinal malrotation may lead to obstruction or volvulus.[27] Biliary atresia may occur in early life, and should be suspected if an infant with PCD has jaundice.[28] None of these complications were detected in our patient.

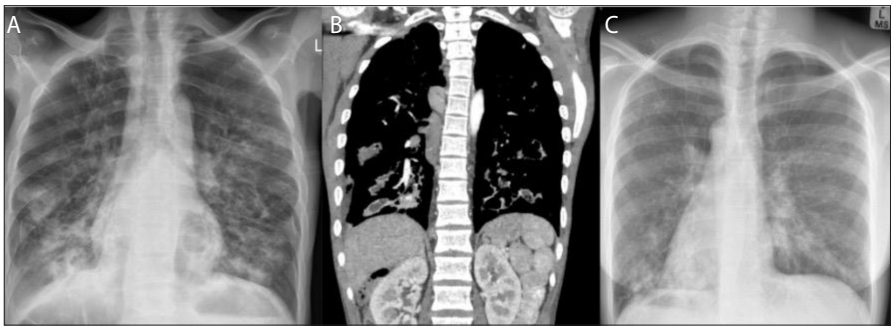

Fig. 2. Radiological findings in family A. Patient 1: (A) chest radiograph showing centrally positioned heart, bowel gas in both the right and left hypochondrium, and evidence of bronchiectasis in both lungs, most prominently in the lower lung fields; (B) coronal computed tomography scan showing polysplenia in the left hypochondrium. Patient 2: (C) chest radiograph showing evidence of mirror-image dextrocardia with situs inversus, as well as evidence of infiltrates and/or bronchiectasis in the lower lung fields.

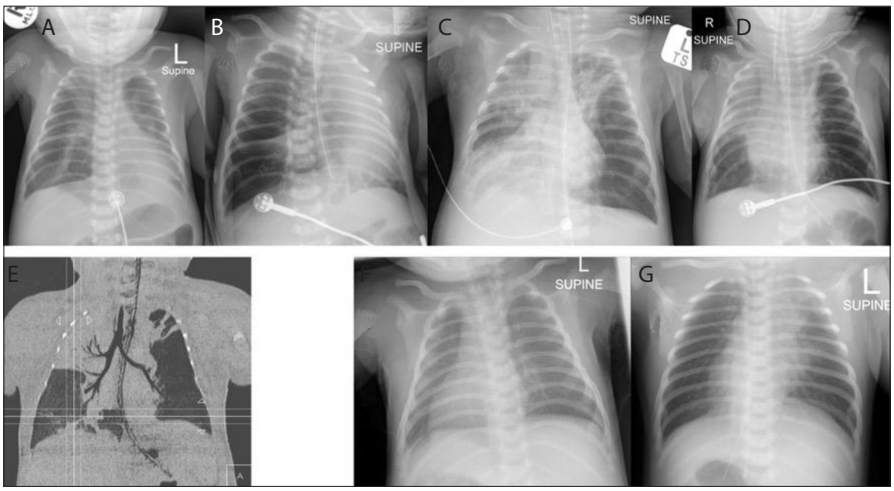

Fig. 2. Radiological findings in family B. Patient 3: (A - D) chest radiographs showing apparently normal position of the heart and abdominal organs. They also show (A) segmental right upper and lower lobe atelectasis; (B) complete left lung collapse and right middle lobe atelectasis; (C) right middle and lower lobe collapse and consolidation; and (D) right upper lobe collapse and consolidation and segmental left lower lobe collapse. A coronal computed tomography scan (E) shows collapse and consolidation of the right upper lobe and segmental left upper lobe, patent bronchi, and normal position of the nasogastric tube. Patient 4: chest radiographs on days 1 (F) and 4 (G) of life showing mirror-image dextrocardia and situs inversus and increasing infiltrates in the left lung.

Clinical predictive tools

To improve detection of PCD, other clinical clues must be specifically sought. Leigh *et al.*[1] found that four clinical features have good sensitivity and specificity for PCD in children and adolescents. These are: (*i*) unexplained neonatal respiratory distress, often from the day of birth; (*ii*) an early-onset year-round wet cough, often from infancy; (*iii*) early-onset perennial nasal congestion, often from infancy; and (*iv*) a laterality defect. The presence of all four features indicates a very high likelihood of PCD, and the presence of any two features should prompt diagnostic testing for PCD.[29] Absence of these features should allay suspicion.

Of our patients, all but patient 3 had at least two of the above features. Other features also contributed to making the correct diagnosis, particularly the presence of a family history compatible with autosomal recessive inheritance (in these families the presence of affected siblings of both sexes, but consanguinity should also be sought). This emphasises the importance of targeted assessment for specific clues in the personal and family history, clinical examination and chest radiographs.

Another published clinical tool is the PICADAR score, which looks for seven predictive parameters: full-term gestation, neonatal chest symptoms, neonatal intensive care admission, chronic rhinitis, ear symptoms, situs inversus and congenital cardiac defects. The presence of five features has a sensitivity and specificity of 90% and 75%, respectively.[30]

Screening and diagnostic tests

For many years, transmission electron microscopy (TEM) of a nasal mucosa biopsy specimen was considered the gold-standard diagnostic test for PCD, although the usefulness of TEM is reduced by its high capital costs and the expertise required for sample processing and interpretation. Fresher

evidence suggests that TEM has high specificity for PCD but has a 30% false-negative rate.[29,31]

Other diagnostic modalities have become available more recently (Table 2). These include measurement of the nasal nitric oxide (nNO) concentration and genetic testing. Ideally multiple diagnostic methods should be available because each has limitations, but in SA, genetic testing has assumed importance owing to its greater accessibility and usefulness at all ages.

Measurement of the nNO concentration has been shown to have value as a screening and diagnostic test.[29] In addition, nitric oxide analysers need not be unduly expensive, and are a possible testing option in lower-resource settings.[35] However, the value of nNO as a diagnostic tool is limited by the facts that it is not specific for PCD and that it requires a co-operative patient, and is therefore less useful in children aged <5 years.

Immunofluorescence is a standard microscopy technique, using fluorescently labelled monoclonal antibody probes targeting one or more specific proteins in the cilium. Loss of fluorescence with a particular probe indicates that the target protein is absent or defective. This technique is less costly than TEM and requires less specific expertise.[34] Furthermore, Shoemark *et al.*[34] found that a panel of fluorescent probes targeting six ciliary proteins had good specificity, although limited sensitivity, for the diagnosis of PCD in the UK.[34] Immunofluorescence therefore has some promise as a diagnostic test and is potentially relevant to low-resource settings. A difficulty is that the proteins associated with PCD are widely diverse, not all are known, and those that are more commonly associated with PCD may vary between populations. Without a good knowledge of the frequency with which specific proteins are associated with PCD in our population, it is difficult to select optimal antibody probes.

Given that proteins are encoded by genes, genetic testing encounters a similar difficulty to immunofluorescence. Over 200 genes are known to be associated with the motile cilia, and over 40 are known to be associated with PCD.[36] In addition, there is significant inter-individual variation in the DNA sequence of each gene. While some DNA changes are obviously pathogenic or benign, the appropriate classification of many variants is a work in progress, meaning that there remain many variants of uncertain significance (VUS). Compared with immunofluorescence tests, genomic tests such as NGS gene panels are more flexible and scalable. For example, genes that are newly associated with PCD can be added to NGS gene panels, and classification of VUS improves over time. Although the accuracy of genomic testing is less good in under-studied compared with well-studied populations, it is improving rapidly across the board. In addition, the costs of genomic testing are falling. For these reasons, genomic testing is a very promising diagnostic technique for the diagnosis of PCD.

A review published in 2018 found that NGS gene panels correctly detected PCD in 80% of cases, if >12 genes were included in the panel.[29] The detection rate increases with the number of relevant genes included, and if the method detects large deletions and duplications in addition to single-nucleotide variants.

While the above review related to the North American setting, there is evidence that genomic approaches are likely to be important in African populations. Hannah *et al.*[37] found that current genomic approaches identify many pathogenic variants in genes associated with PCD in populations from all continents, but with differences in the prevalence of particular variants and genes. Genomic testing is therefore expected to be useful in the diagnosis of PCD in Africa.

Using the fact that most PCD is associated with autosomal recessive inheritance, the same study[37] assessed carrier frequencies in populations from various continents and predicted that PCD should be relatively common in Africa, with a probable prevalence of at least 1 in 10 000.

Genomic testing is continuing to improve and is likely to increasingly become a mainstay of PCD diagnostics in all settings. However, current testing has deficiencies that are likely to be magnified in under-studied populations. It would therefore be ideal to complement genetic testing with tests such as nNO or immunofluorescence. Findings from these tests would also be useful in elucidating VUS, e.g. a lack of protein immunofluorescence for a gene with VUS findings would suggest pathogenicity of the VUS.

In our families, NGS gene panel testing, taken together with the clinical findings, provided effective confirmation of the diagnosis. They also demonstrated some of the complexities of interpreting genetic results: there was an incidental finding (the heterozygous pathogenic variant in *DNAH8* in patient 1 was considered an incidental carrier state), and several VUS in various genes associated with PCD. For this reason, and to discuss family implications, a genetics health professional should be involved in feedback of results.

Although the underlying genetic cause of PCD can be in any one of more than 40 genes encoding a variety of ciliary components, the genetic findings in our patients relate to the dynein arms that are important in generating motion in both primary and nodal cilia, and therefore explain both the respiratory and laterality features found. The *DNAAF1* gene encodes a component of the outer dynein arm,

Table 1. Actionable genetic variants detected on next-generation sequencing panel testing

	Gene	Variant	Classification	Zygosity
Family A				
Patient 1	*DNAAF3*	c.1396del (p.Ala466Profs*9)	Pathogenic	Homozygous
	DNAH8	c.2084del (p.His695Profs*3)	Pathogenic	Heterozygous
Patient 2	*DNAAF3*	c.1396del (p.Ala466Profs*9)	Pathogenic	Homozygous
Family B				
Patient 3	*DNAAF1*	c.811C>T (p.Arg271*)	Pathogenic	Heterozygous
	DNAAF1	c.1018_1019delAG (p.Gln341Argfs*10)	Pathogenic	Heterozygous
Patient 4	*DNAAF1*	c.811C>T (p.Arg271*)	Pathogenic	Heterozygous
	DNAAF1	c.1018_1019delAG (p.Gln341Argfs*10)	Pathogenic	Heterozygous

and the *DNAAF3* gene encodes a cytoplasmic factor that is essential for the assembly of dyneins into complexes before their transport into cilia.[38,39] Of note, neither of these two genes is among those predicted by Hannah *et al.*[37] to be most common in black Africans (or other populations on the African continent). This finding highlights the need to test a broad NGS gene panel.

Two final questions are whether a diagnosis, or specifically a genetic diagnosis, has clinical and/or personal usefulness. For PCD, like many other rare diseases, there are no randomised controlled trials to directly determine the benefit of specific diagnosis and treatment. However, it is evident that an earlier diagnosis in patient 1 would have spared him the 'harm' of multiple investigations, treatment, and hospital admissions for suspected TB. It is also possible that early diagnosis would have been beneficial: much of the recommended respiratory treatment for PCD is borrowed from treatment for cystic fibrosis, where early diagnosis to facilitate daily physiotherapy, regular follow-up and targeted antibiotic treatment has made a dramatic difference to life expectancy.[35]

With regard to the potential additional benefit of *genetic* diagnosis, current expert opinion endorses NGS gene panel testing to facilitate

Table 2. Screening and diagnostic tests used for PCD

Test	Technique	Validity and usefulness	Limitations
Screening tests			
nNO	Air measured from a single nostril and measured by either a stationary or portable chemiluminenscence NO analyser An nNO level <77 nL/min should prompt further investigation	Good sensitivity and specificity (>95%) in stationary analysers in experienced centres[32] Portable analyser may be a cheaper and easier alternative Requires patient co-operation (challenging in young (age <5 years) or unco-operative patients) Appropriate as a screening tool	Requires additional tests to confirm the diagnosis of PCD Not available at all health centres Lack of consensus for cut-off values Requires a high pretest probability to achieve accuracy Can have false positives in cystic fibrosis patients[32] Portable analysers may maintain a high sensitivity, but lack the specificity and robust evidence of stationary analysers
HSVM	Nasal ciliated epithelium obtained by brush sampling of inferior nasal turbinate Live cilia analysed under high magnification and ultra-high frame-rate video (usually 500 frames per second).	Highly sensitive for detecting PCD (up to 100% sensitive)	Requires confirmatory testing Expensive equipment Requires highly trained and experienced microscopists Cannot differentiate primary from secondary ciliary dyskinesias Improper sampling (damaging of cilia) can cause false positives
Confirmatory tests			
TEM	Direct assessment of the ciliary ultrastructure of nasal or bronchial tissue under TEM	Highly specific for detecting PCD (100% specific)[33]	Up 30% false negatives[33] Expensive equipment Experienced technical staff required
Ciliary immunofluorescence	Antibodies with fluorescent tags used to identify the lack of, or mislocation of, specific proteins	More cost-effective and requires less expertise than TEM Highly specific[34]	Limited sensitivity (12% of PCD were missed, and many results were inconclusive, in a study by Shoemark *et al.*[34]) In local populations the sensitivity and specificity is uncertain but probably lower, owing to lack of local data on the proteins that are more commonly affected
NGS	Saliva specimen utilised and tested with multigene panels using molecular assays	Reasonably sensitive (and improving with increased genomic testing) Highly specific (up to 100%) No technical skills required for sampling Specimens can be obtained at any health facility Reasonably cost-effective	Currently requires outsourcing to international laboratories Less sensitive but more specific than nNO and HSVM, and sensitivity improving rapidly

PCD = primary ciliary dyskinesia; nNO = nasal nitric oxide; NO = nitric oxide; HSVM = high-speed video microscopy; TEM = transmission electron microscopy; NGS = next-generation sequencing.

genetic counselling as well as for prenatal or preimplantation genetic diagnosis.[29] Infertility is the rule in affected males, and fertility is often reduced in females (as noted in patient 2), but it can be addressed with assisted reproductive techniques where these are available. Lastly, a genetic diagnosis has the potential to identify genotypes that are associated with poorer clinical outcome, and eventually to facilitate development of mutation-specific therapies.[29]

Conclusion

Primary ciliary dyskinesia is an uncommon but underdiagnosed condition that has a significant impact on individuals and families. In this review, we present vignettes of two families where access to genetic diagnostic testing for PCD facilitated improved clinical care. We show that a timeous diagnosis of PCD requires awareness of the condition, the variety of possible presentations, and attention to specific clues in the personal and family history, and on clinical examination and the chest radiograph. We discuss the increasing role of genomic testing in PCD diagnosis, and the fact that genomic studies predict PCD to be relatively common in Africa. Ongoing improvements in genomics have increased the availability of multigene NGS gene panel tests, and state-of-the-art gene panels have good clinical sensitivity and high specificity. As in one of the case vignettes, novel genetic variants found on diagnostic testing can be added to public databases that correlate genotypes and phenotypes, which will help improve the usefulness of genomic testing over time.

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Conflicts of interest. None.

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