Primary ciliary dyskinesia: Meeting the challenges of diagnosis in South Africa

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Primary ciliary dyskinesia (PCD) is an inherited ciliopathy that results in impaired mucous clearance and affects primarily the respiratory tract, causing upper airway disease, bronchial inflammation and bronchiectasis. The prevalence of PCD in low- and middle-income settings, including South Africa (SA), is unknown, largely owing to challenges with diagnosis, and identifying children or adults with PCD is challenging in a setting with a high prevalence of other infectious diseases, including lower respiratory tract infections and tuberculosis. No single test is diagnostic of PCD, and while some tests are costly, others are labour intensive and require highly specialised laboratory expertise. In the SA setting, awareness and opportunities for the diagnosis of PCD need to be created. In this commentary, we provide a pragmatic approach to identifying which children and adults require further investigations for PCD using a range of diagnostic tests or tools that are available. Furthermore, we recommend that designated centres of expertise for PCD diagnosis are created in SA. This would be an important step towards improving accessibility of diagnostic tests and developing local expertise to improving PCD diagnosis, especially in early childhood, to prevent long-term irreversible respiratory sequelae.

Keywords: *ciliary dyskinesia, PCD*

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Ciliary dyskinesias are abnormalities in cilial function, and when present in the respiratory system can result in impaired mucous clearance from the respiratory tract, initiating a vicious cycle of bronchial inflammation, bacterial colonisation and bronchial wall damage that may result in bronchiectasis.[1] Causes of ciliary dyskinesia can be primary or secondary, and result in transient or persistent ciliary impairment. The focus of this article will be primary ciliary dyskinesia (PCD), which is an inherited ciliopathy.[2]

Columnar epithelial cells lining the respiratory tract typically bear 200 - 300 apical cilia. Cilia are motile, slender (0.3 µm in diameter), hair-like (6 - 7 µm in length) cell membrane projections enclosing a matrix with an axoneme (cytoskeletal microtubular core) (Fig. 1). The axoneme is composed of nine pairs of peripheral microtubules connected to two centrally positioned ensheathed central microtubular apparatus by radial spokes. Each pair of peripheral microtubules has molecular proteins arranged as hook-like outer and inner dynein arms attached to the α-microtubule. These cilia beat in the periciliary fluid layer and sweep the overlying mucus layer produced by epithelial goblet cells with their tips. This unidirectional movement of debris and mucus in a metachronal wave form (one after the other) ensures that mucus moves in a cephalad direction towards the laryngeal opening, where it is expelled out of the respiratory system (Fig. 2). Movement of mucus is therefore impeded by ciliary defects.[3]

Axonemal structure and function are controlled by numerous mechano-regulatory components and proteins.[4] Disruption or abnormal expression of any of these ciliary proteins involved in motile ciliogenesis, structure and/or function will lead to abnormal or absent ciliary beating and PCD disease. PCD is considered an uncommon disease caused by mutations in a PCD-causing gene, which typically are inherited in an autosomal recessive manner (biallelic autosomal), although heterozygous and X-linked gene mutations have been described. The estimated prevalence ranges from 1:2 300 in highly consanguineous cohorts to 1:15 000 in some European and East Asian cohorts.[5,6] More than 50 PCD-associated pathogenic genes have been identified to date, and 70 - 75% of clinically suspected PCD cases can be confirmed by genetic sequencing.[7] Biallelic mutations in *DNAH5*, *DNAH11* and *CCDC40* are most commonly reported in European ancestry, *CCDC39* mutations in a North African (Tunisian) cohort, *CCDC39, DNAH11* and *DNAAF11* mutations in a Palestinian cohort and the *DRC1* copy number variation the most important genetic identifier in the Japanese population.[8-10] There is a paucity of data on the penetrance and most prevalent PCD gene mutations in sub-Saharan Africa. A recent publication on PCD in two South African

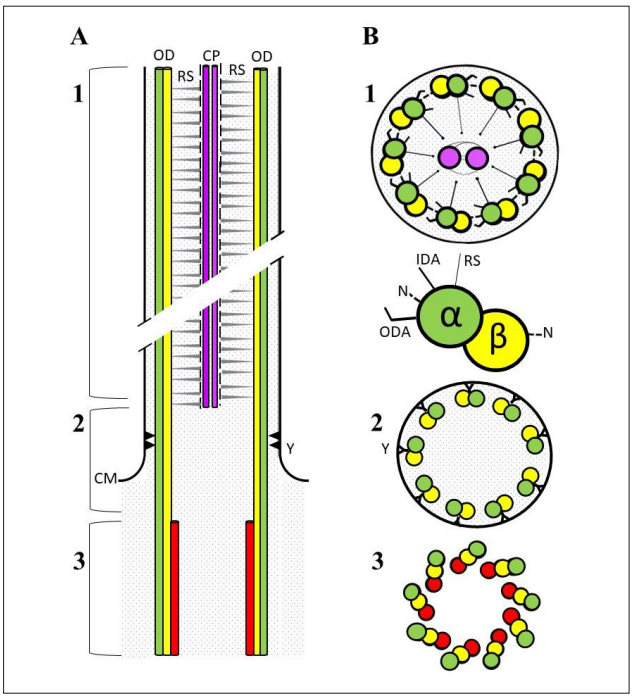

Fig. 1. Schematic diagrams of the structure of a cilium in longitudinal (A) and transverse (B) sections, illustrating: (1) the axoneme, which is composed of nine pairs of peripheral microtubules (outer doublets, OD) and two centrally positioned, ensheathed singlet microtubules (central pair, CP). Each pair of peripheral microtubules has 96 nm-repeats of molecular motor proteins arranged as hook-like outer and inner dynein arms (ODA and IDA), attached to the a-microtubule (Fig. B1 enlargement). These dynein arms generate ATP-dependent sliding forces between the microtubules of the doublets. The sliding motions are controlled by flexible nexin linkages (N) between adjacent doublets to enable bending of the axoneme. Outer doublets are also connected to the ensheathed central microtubular apparatus by radial spokes (RS) which work in concert with the other mechanoregulatory components and proteins to control axonemal movements; (2) the transition region between the axoneme and the basal body, typified by Y-linkages (Y) of the peripheral doublets to the cell membrane (CM), and (3) the absence of the central pair of microtubules; the basal body of triplet microtubules which is embedded in the cell cytoplasm and docked at the cell membrane.

(SA) families identified a homozygous variant in *DNAAF3* and two pathogenic heterozygous variants in *DNAAF1*,[11] neither of which were predicted among the top five African/Afro-American PCD-associated genes.[8]

Signs and symptoms of PCD are nonspecific and present variably depending on the genotype and age of the patient. Suggestive clinical presentation includes neonatal respiratory distress occurring 12 - 24 hours after birth (as opposed to present at birth seen in transient tachypnoea of the newborn or congenital pneumonia), pre-school chronic otitis media and/or recurrent lower respiratory tract infection, and a chronic wet cough or nasal polyps in older school-going children. Bronchiectasis, infertility (male or female) and/or chronic sinusitis may be presenting features in childhood, adolescence or adulthood.[12] Disorders of lateralisation such as *situs inversus totalis*, dextrocardia and heterotaxy syndromes occur in ~50% of cases, and are therefore an important flag for underlying PCD diagnosis.

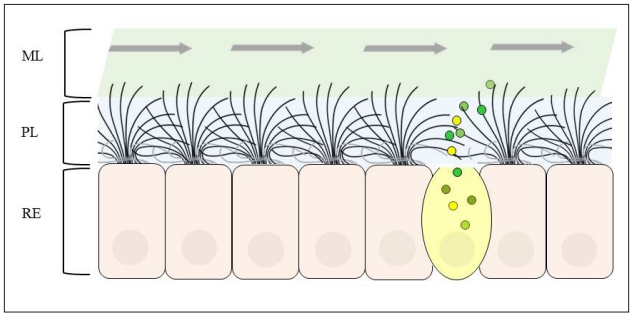

Fig. 2. Schematic representation of respiratory epithelium (RE), with the typical ratio of five multiciliated, columnar epithelial cells to one mucus-producing goblet cell. A metachronal wave of ciliary beating occurs in the periciliary layer (PL), a fluid layer that facilitates ciliary motion (power strokes in black, recovery strokes in grey). At their most extended power strokes, the tips of the cilia move through a more viscous, overlying mucus layer (ML) in which allergens, pathogens and debris are embedded, thus propelling the mucus out of the respiratory tract (arrows).

Making the diagnosis of PCD

Diagnosis of PCD, even in high-income settings, is challenging. There is no single diagnostic test, and confirming the diagnosis is often achieved through algorithms. Two main diagnostic algorithms have been described, and a third (PCD-UNiBe, University Children's Hospital, Inselspital Bern, Switzerland) was more recently described.[13-15] All three have good agreement, but are established for high-income settings where capacity and expertise to diagnose PCD is more widely available.[13] Certain signs and/or symptoms are entry points of the algorithm – the American Thoracic Society suggests that two of the following four signs and symptoms should prompt further investigation: (*i*) unexplained neonatal respiratory distress in a term infant; (*ii*) year-round daily cough beginning before 6 months of age; (*iii*) year-round daily nasal congestion beginning before 6 months of age; and (*iv*) organ laterality defects.[14] The European Respiratory Society algorithm suggests that several of the following signs and symptoms should prompt further investigation:[15] persistent wet cough, situs anomalies, congenital cardiac defects, persistent rhinitis, chronic middle-ear disease with or without hearing loss, neonatal upper/lower respiratory symptoms in a term neonate and neonatal intensive care admission.

Children and adults with suspected PCD should be referred to pulmonologists for specialised diagnostic investigations, as listed in Table 1, where available.[2,11,15] All three algorithms start with nasal nitric oxide (nNO) measurements,[13] followed by, and at the very minimum, genetic testing and transmission electron microscopy imaging of the ciliary structure. Although these algorithms cannot be currently applied to most centres in SA, a joint call to funders is urgently needed to improve accessibility to the diagnostic tests across various regions. Until such time, a pragmatic approach is warranted.

Pragmatic approach to diagnosing PCD in South Africa

In the SA setting, awareness and opportunities for the diagnosis of PCD need to be created. Importantly, guidance for identifying which children and adults require further investigations are needed. SA has a high infectious disease burden dominated by HIV and TB that results in respiratory sequelae; some of these presentations often overlap with the clinical features of PCD.

Table 1. Tests used in the diagnosis of PCD in SA setting

Test	Description	Accessibility and feasibility of testing in SA
Nasal nitric oxide (nNO) measurements	Using fixed chemiluminescent meters or portable electrochemical devices (not standardised and less sensitive)	nNO, the first recommended test in each algorithm, is largely unavailable in the public sector and challenging to perform in young children. Although tidal breathing nNO measurement is advised by the ERS,[16] in SA, the necessary equipment is not easily available at an affordable cost, except in research settings. Recent guidelines indicate that measurement of nNO in infants <1 year is of little diagnostic value.[17] Notably, nNO levels may be higher in confirmed cases of PCD with normal ciliary ultrastructure, and cut-off levels for nNO may need to be increased from 77 nL/min to 107.8 nL/min.[18] Additionally, there are at least 15 PCD-associated genes that do not affect nNO values.[19] Despite these limitations, nNO is recommended as a minimal requirement for the diagnosis of PCD in each of the three algorithms and should be procured by lung function laboratories.
High-speed video microscopy analysis (HSVMA)	To assess ciliary function and beat frequency	Light microscopy is a simple bedside investigation that examines the beating pattern of cilia from nasal brushings.[20] Nasal brushings can be collected in awake patients. The technique requires a special transport medium and a simple inverted light microscope. Recognition of normal or abnormal ciliary beat patterns is, however, subjective, and requires experience and expertise. HSVMA to measure precise beat frequency (Hertz/second) is currently unavailable in SA.
Transmission electron microscopy (TEM)	To assess ciliary ultrastructure	TEM is the most commonly available diagnostic testing modality in SA, and despite the international consensus guidelines for TEM reporting which have greatly improved the diagnostic standardisation,[21] TEM is labour and resource intensive, and requires expertise in the analyses of ciliary ultrastructure. Adequacy of a diagnostic sample is also crucial – using a cytology brush, nasal brushings of the ciliated epithelium from the inner turbinate(s) can be performed in the doctor's rooms. Nasal mucosal biopsies performed by ear, nose and throat surgeons in theatre are invasive, and these specimens tend to have less ciliated epithelial tissue than brushings. About 30% of the PCD spectrum is reported to have normal ciliary ultrastructure, therefore highlighting the importance of other diagnostic modalities to establish a PCD diagnosis.
Genetics	To identify known biallelic mutations	Genetic testing is expensive and largely restricted to the private sector or through collaborations with out-of-country laboratories. Nationally, a six-gene panel will be available in 2024 from the Department of Human Genetics, Braamfontein, National Health Laboratory Service, while a 17-gene panel is available through private laboratories, and up to 47 gene panels available through international laboratories.[22] Variants of unknown significance or mono-allelic heterozygous variants are commonly reported and add complexity to interpretation, especially in untested populations such as in SA. Importantly, identification of two or more variants of unknown significance in the same gene still requires confirmation by functional and structural tests (HSVMA, immunofluorescence microscopy and TEM).
Immunofluorescence microscopy (IF) – fluorescently labelled and visualised by microscopy	To detect key ciliary proteins	In the same way that genetics testing is limited to a selection of known pathogenic variants, immunofluorescent labelling of ciliary proteins can only reveal the antigen being labelled – so the effect of non-biallelic variants, for example, is unknown. IF testing for PCD in SA is currently precluded by availability of trained staff.
Air-liquid interface culture of nasal epithelial cells	Facilitates differentiation of PCD from secondary ciliary defects	Laboratories with expertise do not currently exist in SA.

PCD = primary ciliary dyskinesia; SA = South Africa.
Note: mucociliary clearance tests using inhaled radiolabelled aerosol from the lung, or the mucus transport rate of a marker (saccharin or charcoal technique) are no longer recommended.[23]

Deciding who to investigate with the limited diagnostic capacity is important to avoid overloading the health system. We therefore propose criteria and pathways for referral and investigation of PCD in children and adults in SA (Fig. 3). The special investigations are detailed in Table 1.

If no testing or referral facilities are available, we recommend that children or adults with a high PICADAR (PrImary CiliARy DyskinesiA Rule) score (>10) be managed as PCD until testing becomes available.[24] The PICADAR score was developed and validated in Europe and serves as a useful screening tool in paediatric patients with a chronic wet cough. Seven signs and symptoms most associated with a diagnosis of PCD are scored.[25] These include situs inversus, gestational age (full term), neonatal chest symptoms, neonatal unit admission, congenital cardiac defect, rhinitis and ear/hearing symptoms. Children with a score ≤5 have only a 10% probability of PCD (sensitivity and specificity of PICADAR in PCD diagnostics are 0.90 and 0.75, respectively), and therefore the negative predictive value is most useful.[25] The PICADAR score provides a numerical number for the presence of the following clinical characteristics: (*i*) Was the patient born full term? (2 points); (*ii*) Did the patient experience chest symptoms in the neonatal

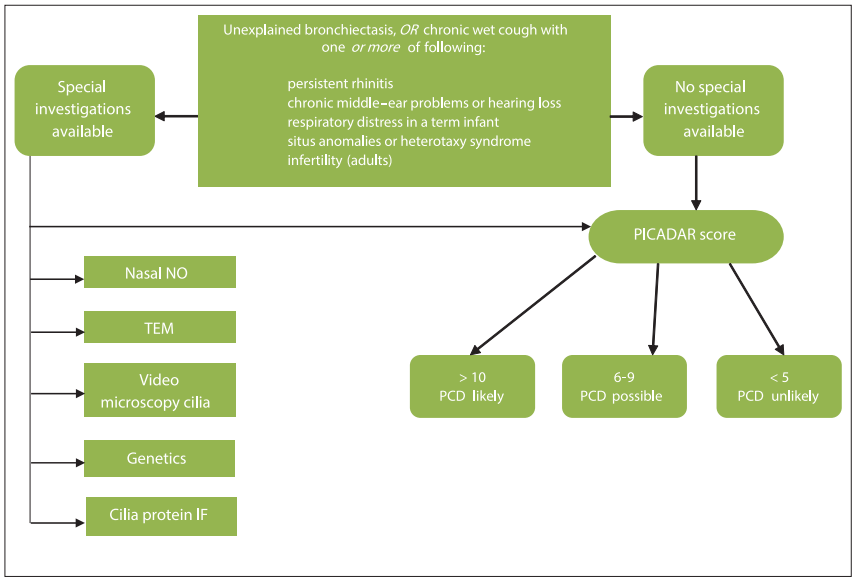

Fig. 3. Overview of recommended primary ciliary dyskinesia (PCD) diagnosis steps for South African setting. (PICADAR = (PrImary CiliARy DyskinesiA Rule); NO = nitric oxide; TEM = transmission electron microscopy; IF = immunofluorescence microscopy.)

period (e.g. tachypnoea, cough, pneumonia)? (2 points); (*iii*) Was the patient admitted to a neonatal unit? (2 points); (*iv*) Does the patient have a situs abnormality (situs inversus or heterotaxy)? (4 points); (*v*) Does the patient have a congenital heart defect? (2 points); (*vi*) Does the patient have persistent perennial rhinitis? (1 point); (*vii*) Does the patient experience chronic ear or hearing symptoms (e.g. glue ear, serous otitis media, hearing loss, ear perforation)? (1 point).

Leveraging on existing platforms

Delivering highly specialised care for rare orphan diseases in SA is complicated by the fragmented public and private healthcare sectors, and the high costs of some of these investigations (or equipment). Centres of expertise exist, but standards of care are highly variable and poorly co-ordinated. PCD, cystic fibrosis (CF) and bronchiectasis share similar clinical manifestations and require overlapping health needs and expertise. A successful model that partners with the CF community exists for CF care in SA, and could serve as a useful framework for building similar centres of excellence for PCD diagnosis and care in SA. A national CF registry covering the public and private health sector was established in 2018 and serves as a powerful research and advocacy tool for CF in SA (https://sacfa.org.za/). Similarly, prioritising a national bronchiectasis registry to serve as a research and advocacy tool for PCD in SA will help strengthen access to optimal diagnostics and care for those living with PCD. Creating designated centres of expertise for paediatric and adult PCD diagnosis and care would be an important step towards developing local expertise and improving PCD diagnosis, especially in early childhood, to ensure appropriate management and prevent long-term morbidity, in SA and this region of Africa.

Conclusion

PCD is likely underdiagnosed and under-recognised in SA due to limited diagnostic capacity. Greater awareness and collaboration between clinicians, geneticists and laboratory scientists is required to improve PCD diagnosis and care in SA. Establishing specialised PCD centres will improve access to the various modalities of diagnostic testing that are aligned with international standards.[9,26]

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