Primary Ciliary Dyskinesia (PCD)

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Summary. This article summarizes the current state of the scientific and clinical knowledge that relates to primary ciliary dyskinesia (PCD). Although PCD is a rare disease with a prevalence of 1 in 20,000 it has a well recognized morbidity. It is believed that an accurate diagnosis and the application of appropriate management can significantly reduce this morbidity. The cilia themselves are highly complicated organelles that perform important functions, particularly in the respiratory and reproductive tracts, and they have been the focus of many years of research.

INTRODUCTION

Primary ciliary dyskinesia (PCD) is an inclusive term for diseases that occur as a direct result of congenital defects in cilia. It includes Kartagener syndrome and the immotile cilia syndrome, as well as ciliary dysmotility and primary orientation defects. The condition was first described in 1904 by Siewert1 and then in 1935 by Kartagener2 as the triad of situs inversus, sinusitis, and bronchiectasis. The main features of PCD are now more accurately characterized as recurrent sinopulmonary infections, subfertility, in association with situs inversus. The diagnosis of PCD is an important one to make as it has implications for the management of upper and lower respiratory tract disease. This includes the prevention of bronchiectasis and the avoidance of ear, nose, and throat procedures that may be inappropriate in these patients.5

There are also implications regarding the future fertility of individuals with the disease.6 Although the excess mortality of PCD is small, there is a significant morbidity which can be modified by appropriate interventions.7

This paper is divided into three sections. The first section is devoted to basic science and begins with an introduction to the epidemiology and genetics of PCD. This is followed by a review of the structure of cilia and the ciliary molecular motor dynein, the function of cilia in mucociliary clearance, current knowledge of defects of laterality, and the animal models of PCD. The second section is concerned with the clinical diagnosis and management of patients with PCD. The final section discusses new developments.

EPIDEMIOLOGY AND GENETICS OF PCD

Epidemiology

PCD is a rare genetic condition with a prevalence that has been estimated from radiographic studies as 1:20,000. These studies determined the prevalence of situs inversus in the general population as 1:10,000 and that of Kartagener syndrome (situs inversus associated with

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bronchiectasis) as 1:40,000.3-4 The prevalence of PCD can thus be calculated as 1:20,000. These calculations rely on a number of assumptions: that radiographic screening is a sensitive method of diagnosing bronchiectasis, that all patients with PCD will develop bronchiectasis, and that situs inversus occurs in exactly 50% of individuals with PCD. The first two of these assumptions are clearly inaccurate, and recent data suggest that the last assumption also has its limitations.4 These factors together suggest that the figure of 1:20,000 is a significant underestimate of the true prevalence of the condition.

Using the quoted figure for prevalence, the number of individuals with PCD in the United Kingdom should be approaching 3,000. The actual number of individuals with PCD known to the family support group in the United Kingdom is between 130 and 150. This trend is reflected throughout the world and may be influenced by the observation that only a handful of centers have the facilities to accurately diagnose this condition. It is interesting to note that the cases of PCD identified in Britain tend to cluster around specialist centers.

Genetics of PCD

The inheritance of PCD in the majority of cases is autosomal recessive,8 although other inheritance patterns have been described.9 In common with other autosomal recessive diseases, there is a higher incidence of PCD among the children of consanguineous couples and within inbred populations. The affected genes have yet to be identified, but several chromosomal regions have been suggested as potentially interesting. In 1992 a paper was published in which the authors postulated linkage of the PCD phenotype to the HLA locus on chromosome 6,10 and a number of groups have also suggested that a gene for PCD lies on chromosome 7.11,12 It is highly likely that PCD is a genetically heterogeneous condition, and that mutations in different genes are responsible for the PCD phenotype in different families. There are at least 200 proteins within a single cilium, each encoded by a separate gene, and many other genes are involved in the assembly and regulation of cilia. The number of possible candidate genes is therefore large. Identification and characterization of the genes causing PCD will lead to an improved understanding of cilia structure and function and the association of ciliary defects with situs inversus. It may also lead to improved diagnostic methods and the identification of subtle PCD phenotypes. Eventually, it may be possible to show a genotype/phenotype correlation, enabling prognosis to be more accurately predicted in individual patients.

Cilia

Cilia are eukaryotic organelles that are found throughout the animal kingdom, within protozoa, and in some plants. They are highly complex structures and share morphological similarities with spermatozoal flagellae. In humans they are found lining the upper and lower respiratory tract, including the sinuses and middle ear, the ependyma of the brain, the ductuli efferentes of males, and the female oviduct. The presence of dyskinetic cilia in the female Fallopian tubes may lead to ectopic pregnancies. In males infertility may result from azoospermia as a result of dyskinetic cilia in the vas deferens, or immotile spermatoza because of similar ultrastructural defects in the flagella of the sperm tail. Modified cilia are also found in the rods and cones of the eye as well as in olfactory cells.

Throughout the animal kingdom, motile cilia function to propel a single cell through liquid or to move fluid across the surface of a layer of cells. The core axoneme consists of the well described nine peripheral microtubular doublets surrounding a central pair of microtubules. This 9 + 2 structure has been evolutionarily conserved within motile cilia,13 but the associated ultrastructural detail differs between organisms. Each of the microtubular doublets is constructed from heterodimers of alpha and beta tubulin, assembled into the 13 protofilaments of the A tubule and the 11 protofilaments of the B tubule. The microtubules of the central pair are composed of 13 protofilaments of tubulin and are oriented with respect to the central pair of adjacent cilia. This orientation is important in the production of a coordinated ciliary waveform. Other important structural proteins are known as microtubule-associated proteins (MAPs). The dynein arms were the first of these to be discovered and have also been the most extensively studied.14-19 They are classified as inner or outer dynein arms and each has a different function in the generation of the ciliary beat; the outer dynein arms have an effect on beat frequency, while the inner arms influence the beat waveform. Their detailed structure and function are described below.

Additional MAPs are the nexin links which connect the peripheral microtubule doublets to each other, and the radial spokes which connect each doublet to the central pair of microtubules (Fig. 1). A dynein regulatory complex that interacts with the radial spokes and inner dynein arms to regulate the ciliary beat has also been recently described.20,21 Along the length of the axoneme, a basic

Abbreviations
ATP Adenosine 5’-triphosphate
ENT Ear, nose, and throat
ICSI Intracytoplasmic sperm injection
MAP Microtubule-associated proteins
NO Nitric oxide
PCD Primary ciliary dyskinesia
repeating unit of 96 nm can be identified. This contains four identical outer dynein arms separated by 24 nm, three or four different inner dynein arms, one spoke group with a periodicity of 29 nm, six pairs of central sheath projections with a periodicity of 14 nm, and one nexin link with a periodicity of 86 nm. Transverse section along this basic repeat unit provides the characteristic transmission electron micrograph (Fig. 2).

Abnormalities of ciliary ultrastructure can be divided into the primary defects seen in PCD or those secondary to the effects of infection, smoking, or pollutants. These secondary ciliary abnormalities consist predominantly of microtubular defects and can occur in up to 10% of cilia in normal individuals. The first primary defect of cilia to be described in humans was absent dynein arms (Fig. 3). A number of well characterized ultrastructural abnormalities have now been recognized as causing...
PCD, and these are listed in Table 1. In some patients with the clinical phenotype of PCD, the ultrastructure of the cilia appears normal, but the cilia are not normally orientated with respect to one another. This disorientation defect can occur secondary to infection or as a primary defect.

The average thickness of tissue fixed for examination by electron microscopy is 100 nm, and the basic repeat unit of the axoneme is 96 nm. As a result, the electron micrograph picture used to classify the ultrastructural defect is an amalgamation of superimposed inner dynein arms, outer dynein arms, radial spokes, and nexin links from adjacent repeat units. This information, together with a detailed knowledge of the ultrastructural complexity of the axoneme, explains the hypothesis that an apparently identical ultrastructural phenotype may be the result of a number of different genetic mutations. At present, the clinical phenotype does not appear to be related to the type of ultrastructural defect, but this may change with improved classification of ultrastructural subtypes.

### Dynein

Dynein is the most extensively studied protein within the cilia and has a crucial role in both ciliary structure and function. It is a high molecular weight protein that belongs to the group of mechanochemical ATPases which include myosin and kinesin. The dyneins are classified as cytoplasmic or axonemal, and both of these classes of dynein function as minus end directed motors. Cytoplasmic dyneins function to move proteins along microtubules within the cytoplasm to perform functions such as organization of the spindle poles during meiosis and trafficking of vesicles in interphase cells. Axonemal dynein has an important role in causing the sliding of the microtubular doublets relative to one another within cilia and flagella. The integrated structure of the other microtubule-associated proteins within cilia cause this sliding movement to be converted into the ciliary bend (Fig. 4).

The dynein molecules of the outer dynein arms are constructed of two heavy chains (α and β), two or more intermediate chains, and 4–8 light chains. The heavy chains contain the important motor domain that is responsible for the hydrodynamic coupling of nucleotide hydrolysis with the mechanical movement of dynein along the microtubules. The nucleotide sequence of some of the genes encoding these dynein heavy chains was recently determined, and their human map positions were published. The intermediate and light chains are also being characterized and mapped to human chromosome locations.

The inner dynein arms have a similar basic structure to the outer dynein arms, but there are three structurally distinct inner arms that contain different dynein isoforms known as I1, I2, and I3. This reflects their more complicated function in the generation of the ciliary waveform. The I1 inner dynein arm interacts with the dynein regulatory complex and the radial spokes.

### Mucociliary Clearance

There are an estimated 10^9 cilia/cm^2 lining the respiratory tract, and these are orientated with respect to the central microtubules. Each cilium has a diameter of 250 nm and a length of 6 μm. The function of these cilia is to beat in a coordinated and controlled manner as part of the mucociliary defense system. The second important component of the mucociliary clearance system is mucus, a concentrated mixture of glycoproteins, proteoglycans, and lipids. Mucus is a non-Newtonian viscoelastic fluid that coalesces into sheets that lie above the periciliary fluid layer. The cilia lining the respiratory tract beat with a waveform which consists of an effective stroke that penetrates the layer of mucus and a recovery stroke that dives beneath it into the periciliary fluid. The coordinated beating of these cilia sweeps the mucus layer towards the oropharynx, where it is swallowed (Fig. 5). Small particles and bacteria are thus cleared from the peripheral airways. Between beats the cilia rest in the position obtained at the end of their effective strokes, pointing downstream and preventing the mucus from sliding back into the peripheral airways. The cilia are stimulated to beat by both intracellular factors under neural control and external stimulation including drugs such as isoproterenol. Adjacent cilia are recruited as a direct result of hydrodynamic forces and intracellular communication to form the characteristic metachronal wave. The mucociliary defense system obviously has an important role in preventing any colonization by bacteria. Both primary and sec-
Secondary ciliary defects or abnormalities of mucus can interfere with this normal host defense mechanism. The consequences of delayed mucociliary clearance are recurrent sinopulmonary infections and subsequent bronchiectasis.

Laterality

A useful nomenclature of laterality defects is as follows: situs solitus is the term applied to the normal visceral arrangement of thoracic and abdominal contents; situs inversus to the complete reversal of this arrangement; and situs ambiguous or heterotaxy to any other arrangement. Abnormalities of laterality can occur independent of ciliary abnormalities or in association with them. The earliest sign of asymmetry within a developing vertebrate is the dextral bending of the heart loop, but asymmetrical gene expression and subtle variations in polarity exist prior to this. Some of the developmental pathways responsible for the determination of left-right asymmetry in vertebrates are currently being elucidated.

In the majority of cases, the abnormality of laterality in PCD is complete mirror image situs inversus with a structurally normal heart, but more complicated congenital cardiac defects have been described in up to 12% of patients.30 The identification of individuals with ciliary...

Fig. 4. Sliding filament hypothesis. The relative movement of the microtubules that occurs as a consequence of the ATPase action by dynein is converted into a ciliary bend in the intact cilia.

Fig. 5. Mucociliary clearance occurs as a consequence of the effective stroke of the cilia propelling the mucus forward, as shown in red.
defects who have no associated abnormalities of laterality led to the hypothesis of random situs determination. This hypothesis states that the determination of situs in the developing embryo occurs in a random way when ciliary defects are present. This would explain the 50% incidence of situs inversus observed in individuals with PCD, and the observation that even siblings with PCD within the same family may not have the same visceral arrangement. Afzelius\textsuperscript{31} postulated a number of theories to explain these observations. One of these theories suggested that the beating of primitive cilia might be important in the left-right determination of the early embryo. Mouse studies have provided evidence for this mechanism involving primary cilia.\textsuperscript{32,33} Until recently, the more popular theory was that very early in embryologic development, the cytoskeleton had somehow lost the ability to differentiate right from left. The importance of molecules such as dynein and microtubules that have a well defined polarity and roles in both the cytoskeleton and axoneme lend weight to this argument. As individuals with PCD are being subclassified on the basis of their ultrastructural phenotype, it is becoming evident that some defects in ultrastructure are not associated with abnormalities. This may further clarify the relationship between cilia and laterality.

Several important genes involved in the embryological pathways which determine laterality have now been discovered from studies in \textit{xenopus}, \textit{chicken}, and \textit{mouse}, and a mutation in a gene encoding a zinc finger transcription protein (ZIC3) was recently found in humans with X-linked isomerism sequence.\textsuperscript{34} Several of the genes identified belong to the transforming growth factor-\(\beta\) family of genes that are important extracellular signalling molecules. Each of these genes has a characteristic spatial and temporal expression pattern within the developing embryo. The “switching on” and “switching off” of these genes in particular predefined groups of cells are obviously essential to the determination of normal left-right asymmetry. There are subtle differences in the exact pathways identified in the different organisms studied, but some of the genes such as nodal appear to have a role in all vertebrates. The exact pathway determining left-right asymmetry may not be absolutely conserved within vertebrates, and further studies are indicated to resolve this highly complex issue.

The way in which primary ciliary defects are related to left-right asymmetry remains a hypothesis and has not yet been confirmed. However, there are two further important points which are of interest to this relationship; it would seem sensible to propose that genetic mutations leading to complete mirror image situs inversus are likely to occur at an early stage in development, as the only true defect is one of lateral orientation. Mutations that are expressed at a late date and once complicated cell differentiation has begun would seem more likely to result in complex abnormalities. However, this is an obvious oversimplification. It is also highly relevant to discover that the gene mutated in the mouse model \textit{iv/iv} in which 50% of homozygous offspring have laterality defects is a novel axonemal dynein heavy chain which has been named left right dynein (Lrd).\textsuperscript{35} A role for dynein in both cilia and the determination of laterality has therefore been confirmed at least in mice.

**Animal Models**

A phenotype similar to PCD occurs naturally in both dogs and pigs.\textsuperscript{36–39} Several breeds of dog, including springer spaniels and English setters, suffer from primary ciliary defects, and the phenotype is identical to that seen in humans, except that hydrocephalus is a prominent feature. Pigs are known to have a high incidence of respiratory disease and bronchiectasis, and a significant proportion of these cases may be caused by primary ciliary defects. Hydrocephalus is also a feature of the disease in pigs in contrast to that seen in humans.

The rodent models include the WIC-Hyd rat,\textsuperscript{40} in which the inheritance of ciliary defects appears to be X-linked recessive; the mouse models include \textit{iv/iv, inv/inv, and hyp/hop}.\textsuperscript{41–43} Homozygous \textit{iv/iv} mice have well described laterality defects in 50% of offspring, but ciliary abnormalities have not been described. A mutation in the \textit{lrd} gene was identified in these mice in 1997,\textsuperscript{35} and is of interest to those studying the molecular genetics of the human disease; the region to which it is likely to map in humans is on chromosome 7q. In the \textit{inv} mouse model there are no known ciliary abnormalities, but 100% of the homozygous offspring have laterality defects. The gene responsible for this phenotype has also been cloned, but further functional studies are needed before its role in development is fully understood. The \textit{hyp-sterile} and \textit{hop-sterile} mice are allelic and both of these models demonstrate ciliary abnormalities and male infertility; in contrast to the other mouse models of PCD, they do not show laterality defects. This gene has not yet been cloned, but maps to mouse chromosome 6, which is syntenic with human chromosome 12.

Studies of \textit{Chlamydomonas} have been important in improving our understanding of the structure and function of cilia. \textit{Chlamydomonas} is a eukaryotic unicellular green algae that resembles flagellated protozoa but contains chloroplasts for photosynthesis. It possesses two flagellae with a similar axonemal ultrastructure to cilia that enable it to be propelled through water. Studies of the ultrastructure of \textit{Chlamydomonas} flagellae in wild-type \textit{Chlamydomonas} and a wide variety of mutants have yielded important information about the role of specific proteins in axonemal structure and their contribution to the generation of the ciliary beat. In many cases, the genes responsible for the mutants have been cloned, and
some of these may be candidate genes for PCD in humans.

**DIAGNOSIS AND MANAGEMENT OF PCD**

**Clinical Phenotype**

The clinical aspects and management issues pertaining to PCD have been reviewed recently. A knowledge of the basic physiology of cilia and mucociliary clearance allows us to predict the respiratory symptoms of recurrent sinopulmonary infections in PCD. These are present from birth or shortly afterwards and include persistent rhinitis, chronic and severe secretory otitis media, sinusitis, and pneumonia. These symptoms may be accompanied by complete mirror image situs inversus or more complicated congenital defects. The presence of dyskinetic cilia in the female fallopian tubes may lead to ectopic pregnancy, and in males this may result in infertility due to dyskinetic cilia in the vas deferens or immotile spermatozoa. It is important to emphasize that not all male patients will be infertile, and that definitive diagnosis of infertility should await spermatozoal analysis.

There is large variation in the severity of the clinical phenotype of PCD and the predominant symptoms at various ages. The diagnosis of PCD in the neonatal period is strongly suggested in term neonates with tachypnea or pneumonia with no obvious predisposing risk factors, neonates with significant rhinitis, and those with defects of laterality or complex congenital heart disease. It should also be considered in neonates with a diagnosis of esophageal atresia, biliary atresia, hydrocephalus, or a positive family history of PCD. In the infant and older child, PCD may present as atypical asthma, chronic cough with sputum production, and severe gastroesophageal reflux, as well as the already predicted symptoms of rhinosinusitis, pneumonia, bronchiectasis, and chronic secretory otitis media. The symptoms in adults are similar to those of older children, but the secretory otitis media appears to be of less significance. The diagnosis of PCD in adults may also be made only as a result of the clinical presentation of subfertility or infertility.

The investigation of individuals in whom PCD is suspected can be divided into three main categories: the exclusion of differential diagnoses including cystic fibrosis and immunodeficiencies; the investigation of ciliary structure and function; and the quantification of any end organ damage. Initial investigations would therefore include a chest X-ray, sweat test, immunoglobulins and subclasses, cystic fibrosis genotype, and age-appropriate lung function tests.

The specific investigations for PCD include assessment of mucociliary clearance, measurement of ciliary beat frequency, and identification of the ultrastructural phenotype (Fig. 6). Tests of mucociliary clearance are difficult to perform accurately in children under 12 years and are often omitted in this age group. In older children and adults the test can be performed using a small particle of saccharin or technetium-labelled albumin placed on the inferior turbinate. If saccharin is used, the time taken for the patient to taste the saccharin is measured and should be less than 1 hr. The ability of the patient to taste saccharin must also be verified. When radiolabelled albumin is used, mucociliary clearance can be measured directly using information obtained by a gamma counter. In both tests the patient must sit quietly and refrain from sniffing, sneezing, and any other activity that might influence mucociliary clearance.

To visualize cilia under light microscopy, epithelial cell brushings can be obtained by direct vision from the inferior or middle turbinate using a modified bronchoscopy cytology brush. The ciliary beat frequency can then be calculated and an experienced observer can also describe the ciliary waveform. Alternatively, biopsies can be taken from the middle turbinate or from the bronchi if bronchoscopy is being performed for some other indication. Electron microscopy should be performed if there is a strong suspicion of PCD. A suggested algorithm for these investigations is outlined in Figure 6. This is meant as a guide, and an experienced respiratory physician will combine the clinical presentation with the results of investigations in making a diagnosis of PCD. The ciliary beat frequency can be affected by bacterial infections, and secondary ultrastructural defects can also cause confusion in making the diagnosis.

An integrated approach to management is essential as in the management of any child with chronic disease. It involves general practitioners, respiratory physicians, physiotherapists, clinical nurse specialists, and ENT and audiology experts, as well as urologists and gynecologists. At present, the mainstay of treatment for lung complications is regular daily physiotherapy and prompt prolonged treatment of intercurrent respiratory infections. This is combined with bronchodilator treatment where it has been demonstrated to be effective. Hearing should be carefully monitored, and ENT procedures such as insertion of myringotomy tubes should be avoided because of the common complication of prolonged and severe chronic otorrhea in these patients. In the management of a significant hearing deficit, hearing aids may be prescribed, or occasionally unilateral myringotomy-tube insertion may be justified. The condition spontaneously improves and the prognosis for long-term hearing does not appear to be affected by the specific management in the short term. The development of new in vitro fertilization techniques such as intracytoplasmic sperm injec-
tion has improved the outcome for patients with reduced fertility.

**NEW DEVELOPMENTS**

Nitric oxide (NO) has recently surfaced as a subject of interest in patients with PCD. In contrast to patients with asthma, the levels of NO in exhaled air from adults and children with PCD is significantly reduced when compared to levels in normal individuals. The exact reason for this observation remains to be established, but it may be that the diffusion of NO from the sinuses is not as effective in the presence of ciliary immotility. Alternatively, the production of NO may in some way be related to normal ciliary function, since it is known to be an important signalling molecule. NO may in the future have a role as a noninvasive screening test for PCD, but its use in this way needs to be further validated. The effect of NO manipulation on ciliary beat frequency and mucociliary clearance is also under investigation.

The problem of secondary ciliary ultrastructural defects causing confusion in the diagnosis of PCD has already been alluded to. In vitro tissue culture systems of epithelial cells taken from the middle turbinate have recently been established, and these allow the regeneration of cilia in an infection-free environment. The resulting cilia can then be examined under light and electron microscopy for primary defects. At present this technique remains a research tool, but has implications for improving the availability of diagnostics.

There are a number of groups actively investigating the molecular genetics of PCD, and some of the genes responsible should be identified in the near future.

**CONCLUSIONS**

There are significant implications in making the diagnosis of PCD, and many individuals with the disease are not being diagnosed. It is, therefore, important for medical practitioners to actively consider the diagnosis in a number of clinical situations. An improved availability of noninvasive diagnostic screening techniques would al-
low identification of specific individuals for referral to specialist centers where specific ciliary investigations can be performed. In common with many diseases, the management of PCD requires an integrated, multidisciplinary approach involving a number of clinical specialties. The mainstay of care will be provided by the general practitioner and local hospital, but there should be easy access to specialist input in order to optimize care and minimize the associated morbidity in each individual case.

It is hoped that some of the potential new developments outlined in this article may lead to improved methods of screening for the disease, accurately diagnosing the subclasses of disease, and ultimately improving treatment. The identification of the genes causing PCD has an important role to play in these potential developments, but only with the close communication and interaction of those involved in molecular genetics, the molecular biology of cilia, and the care of individuals with PCD.

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