Altered airway ciliary orientation in patients with X-linked retinitis pigmentosa

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ABSTRACT

Previous reports suggested links between respiratory ciliary dysfunction and primary ciliopathies such as X-linked retinitis pigmentosa (XLRP). To investigate if patients with XLRP have abnormal airway ciliary structure or function, we assessed respiratory ciliary beat pattern and ultrastructure, including ciliary orientation, in 12 patients with XLRP without respiratory disease and 10 control subjects. Patients with XLRP had normal ciliary ultrastructure but significantly (p=0.004) increased mean ciliary deviation (33.8°±9.4°) compared with normal subjects (14.8°±5.4°). Altered orientation was associated with impaired ciliary beat pattern in six patients with XLRP. These findings indicate that XLRP mutations, affecting non-motile cilia of the photoreceptors in the retina, can have effects on motile cilia in the respiratory tract. The observation of disrupted ciliary orientation in patients with XLRP is suggestive of a defect in planar cell polarity.

INTRODUCTION

Motile cilia are an essential component of normal airway mucociliary clearance.[1] Inherited defects of motile ciliary structure and function result in primary ciliary dyskinesia (PCD), characterised by symptoms of impaired airway clearance, including chronic cough and progressive suppurative lung disease.[1] While motile cilia have a 9+2 microtubule arrangement, with inner and outer dynein arms on outer doublets, non-motile cilia, with a 9+0 arrangement and lacking dynein arms, are found in diverse organ systems including the kidney and eye.[2] Inherited conditions resulting from developmental abnormalities of non-motile cilia are termed primary ciliopathies and include conditions such as X-linked retinitis pigmentosa (XLRP).[2]

Traditionally, it was thought that people with PCD did not display symptoms of primary ciliopathies. Several case reports have described patients with symptoms consistent with both motile and primary ciliary disease, including reports of retinitis pigmentosa and proven PCD in individual patients.[3–7] We examined the possible relationship between XLRP and impaired airway cilia structure/function by analysing airway ciliary ultrastructure and function in 12 patients with XLRP without respiratory symptomatology.

METHODS

Twelve patients with genetically proven XLRP were recruited. Patients completed the PICADAR (PrImary CiliARy DyskinesiA Rule) screening questionnaire for PCD.[8] Control data were obtained from 10 patients referred to the PCD diagnostic clinic, whose testing was normal. Studies were done at least 6 weeks after any reported respiratory tract infection. Ciliated epithelium was obtained by nasal brushing as described previously.[9] Recordings of ciliary beating were obtained through an inline video camera and video recorder at 500 frames/s and used to measure ciliary beat frequency.[10] Waveform quality was assessed qualitatively on two separate occasions by two experienced PCD clinicians, who were blinded to sample identity, using a technique previously described by Chilvers.[10] To be classed abnormal, beat pattern had to be considered abnormal on at least three of the four occasions observers described beat patterns (two observers, two occasions).

Cilia ultrastructure was examined for defects including missing dynein arms, central complex defect and missing radial spokes. Secondary defects including compound cilia, swollen outer membranes, disorganisation of the microtubular doublets, extra or missing microtubules were also recorded.[9] Ciliary orientation was measured as described previously[11] using the angle measuring function of ImageJ (National Institutes of Health). A minimum of 20 measurements was made for each patient. The study was reviewed and approved by the local ethics committee (www.rch.org.au/ethics).

Statistical analyses were conducted using GraphPad Prism (v7.04). As Shapiro–Wilk normality test showed that ciliary deviation data were normally distributed, an unpaired t-test with Welch's correction was used to examine if ciliary deviation was significantly different ($p<0.05$) between controls and patients with XLRP.

RESULTS

None of the 12 patients (mean age±SD=25.5±16.9 years) had a PICADAR score higher than 3 (vs controls mean score 6). Results were compared with 10 controls 14.1±10.6 years who were known not to have PCD on specific testing. There was no significant difference in mean ciliary beat frequency at room temperature between patients with XLRP (5.92±1.6 Hz) and controls (7.95±1.9 Hz). Although ciliary ultrastructure was normal in all patients in both groups, with normal 9+2 microtubule pattern and inner and outer dynein arms (figure 1), patients with XLRP had significantly greater (p=0.004) mean ciliary deviation (33.75°±9.4°; n=8) than controls (14.76°±5.4°; n=10) (figure 2).

Six of 10 available video samples showed dys-synchronous ciliary beating (online supplementary

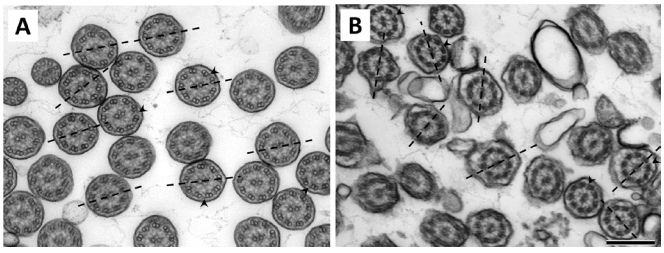

Figure 1 Electron micrographs showing a field of cilia in transverse section from a normal subject (A) and a patient with X-linked retinitis pigmentosa (XLRP) (B). In both normal and XLRP patients, the cilia show the normal 9+2 conformation of microtubules and dynein arms (arrowheads) are evident. In the normal subjects the orientation of cilia, as indicated by the dashed lines, is closely aligned, whereas in the patient with XLRP ciliary orientation was more divergent and variable. Ciliary angles for each cilium are measured with respect to a reference line (vertical axis in these images); differences from the mean angle were averaged to calculate standard ciliary deviation. Scale bar, 200 µm.

videos 1-5) when viewed side on, although a clear arc-like effective stroke was seen. In four of the six cases, cilia on plan view appeared uncoordinated and dys-synchronous (table 1). There was no correlation between the level of ciliary deviation and ciliary beat patterns.

DISCUSSION

We describe impairment of ciliary structure and function, specifically ciliary orientation and beat synchronicity, in a group of patients with XLRP with a low probability of associated PCD. While several previous reports have suggested a relationship between non-motile ciliopathies, including XLRP and PCD, this is the first study to examine a possible relationship in a large number of patients with XLRP with a low probability of having PCD. Our results suggest that even in the absence of a true motile ciliary defect, such as in PCD, motile cilia from patients with XLRP display subtle structural and functional abnormalities

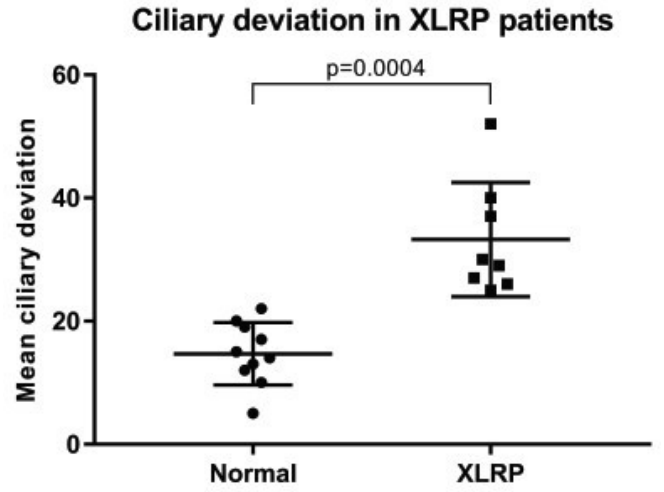

Figure 2 Ciliary deviation in patients with X-linked retinitis pigmentosa (XLRP) (n=8) is significantly greater (two-tailed, unpaired t-test with Welch correction) than in normal subjects (n=10). Ciliary orientation was measured as described previously,[11] using the angle-measuring function of ImageJ (National Institutes of Health). Data from minimum of 20 fields for each patient were pooled to calculate a mean ciliary deviation. At least 25 cilia were counted for each subject.

Table 1 YouTube links to cilia videos of four patients with X-linked retinitis pigmentosa and one control patient

Disorientated 1	https://youtu.be/0QCE7-GHoew
Disorientated 2	https://youtu.be/6mvMGvhuLfA
Disorientated 3	https://youtu.be/hYvYGRXDm-M
Disorientated 4	https://youtu.be/aYdLmdH_DLQ
Normal	https://youtu.be/XblwgZOOMVk

without overt clinical consequences. Arden and Fox[3] reported an increased incidence of airway cilia with abnormal axonemal microtubular structure and compound cilia in 11 patients with XLRP although assessment of ciliary orientation or ciliary beat pattern was not reported. Bukowy-Bierytto *et al*[12] described an association between *RPGR* (Retinitis Pigmentosa GTPase regulator) mutations and a PCD phenotype in two unrelated patients. Their analysis of ciliary structure and function by electron micrograph (EM) and high-speed videomicroscopy did not reveal changes in ciliary structure in either patient although one showed abnormal ciliary beat coordination and disturbed ciliary orientation. While both patients had PCD, in this present study, despite all 12 patients not having PCD and beat frequency being not significantly different from controls, ciliary beat patterns were dys-synchronous in six patients.

It is unlikely that our patients with XLRP have undiagnosed PCD as not only were no ultrastructural abnormalities detected on EM analysis, predictive scores using PICADAR revealed a very low probability of PCD. The PICADAR score is designed to assess the probability of PCD in patients with a productive cough.[8] As no patient described a regular productive cough, the calculated PICADAR score may represent an overestimation of risk.

Defects of ciliary orientation, such as found in our patients with XLRP, are suggestive of aberrant planar cell polarity signalling, a process regulated by the non-canonical Wnt PCP (planar cell polarity) pathway.[13] Recent studies have shown that PCP signalling is critical during ciliogenesis for positioning and orienting cilia basal bodies in the apical cytoplasm of epithelial cells and thus regulating the orientation and beat direction of cilia.[13] The localisation of RPGR to the transitional zone of photoreceptor cilia and to basal bodies in ciliated cells[14] suggests mutations of *RPGR* and other XLRP genes may disrupt planar polarity, resulting in aberrant ciliary orientation and dys-synchronicity of ciliary beating. Consistent with this, knockdown of *RPGR* in epithelial cells disrupts the actin cytoskeleton via RhoA activity, a central mediator of the PCP pathway.[15]

In summary, we describe alterations of ciliary orientation in patients with XLRP, who have no clinical symptoms suggestive of PCD. In 60% of these cases, ciliary orientation abnormality was associated with impaired ciliary beat pattern. While these abnormalities do not appear to result in clinical impairment of airway clearance, they re-enforce the commonalities between motile airway cilia and non-motile ciliopathies and suggest that alterations in the PCP pathway may be involved.

Correction notice This article has been corrected since it was published Online First. The author name Robb de Iongh was corrected.

Contributors GM, PG, PM, RdI, JR, PR were all involved in protocol development. JR and GM performed patient selection, ethics submission was constructed by PR. Nasal brushing and processing was performed by GM with assistance of PG and PM. Cilial orientation was assessed by GM and RdI. Video analysis was assessed by PG and PR. Data analysis were performed by GM, PR and RdI. Manuscript preparation was performed by GM, PR and RdI. All

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