Unusual inheritance of primary ciliary dyskinesia (Kartagener’s syndrome)

Deepak Narayan, Santosh N Krishnan, Madhvi Upender, Thanjavur S Ravikumar, Maurice J Mahoney, Thomas F Dolan Jr, Ahmad S Teebi, Gabriel G Haddad

Abstract
Primary ciliary dyskinesia syndrome is characterised by chronic sinusitis, bronchiectasis, and, in 50% of cases, dextrocardia. It is generally believed to be inherited as an autosomal recessive disorder. In this report, we describe a family consisting of a mother and her five male children, the offspring of three different fathers, all of whom have this syndrome. This argues for either an X linked or autosomal dominant pattern of inheritance. Cytogenetic and FISH (fluorescent in situ hybridisation) analyses were done on the mother and one son and were found to be normal.

In 1933, Kartagener described a syndrome characterised by the triad of findings of sinus inversus, bronchiectasis, and chronic sinusitis. It was not until 1976 that the cellular basis of the disease was described. Although a multitude of phenotypes has been attributed to this syndrome, it has been traditionally characterised genetically as an autosomal recessive disease. To our knowledge, there have been no instances described of other possible genetic modes of inheritance. In this report, we describe a family which has a pedigree that very strongly suggests that an alternative inheritance such as autosomal dominant or X linked is also possible.

Since a number of non-human models of primary ciliary dyskinesia (PCD) have been described and the chromosome locations of mouse models of primary ciliary dyskinesia have syntenic counterparts in the human genome, we decided to see if we could detect any alterations in these areas as candidates for the lesion in the case reported here. These include WIC-Hyd rats, hpv mice, iv mice, and various flagellar mutants in Chlamydomonas. These mutants show the full spectrum of human primary ciliary dyskinesia. The two mouse mutants have been linked to specific chromosomes which have syntenic counterparts in humans. Chromosome 6, on which hyp has been mapped in the mouse, is syntenic with human chromosome 12. Similarly, chromosome 12, onto which iv has been mapped in the mouse, is syntenic with human chromosome 14. We therefore used traditional G banding and R banding as well as fluorescent in situ hybridisation (FISH) to determine if any alterations in these chromosomes or the X chromosome could be discerned.

Case report
The proband (fig 1, subject III.4) is a 33 year old female of Cherokee descent who was referred to the Yale-New Haven Hospital in 1978 for evaluation of chronic sinusitis and frontal headaches. Her physical examination was remarkable as were her routine laboratory tests. Her medical problems began in early life with nasal congestion, mucoid rhinorrhoea, frontal headaches, chronic maxillary sinusitis, productive cough, and recurrent bronchitis that necessitated frequent use of antibiotics. Her school performance was below average, and at puberty she was noted to have moderate truncal obesity. At the age of 26, she was diagnosed by CT scan to have a polyp in the right maxillary sinus. Two years later, she developed multiple brownish naevi on the back and the left breast. Excision biopsy of these naevi showed condylomatous atypia with no malignant cells. A diagnosis of PCD was confirmed based on the absence of ciliary structures in an electron microscopic examination of a nasal mucosal biopsy on two different occasions (fig 2B). A family history indicated that her mother had initially had four children, three females (III.2, III.3, and III.4) and one male (III.1), sired by a male (II.2) said to be of Native American descent. One of these children, a female (III.2), died of pneumonia at the age of 2 months. The remaining three children, including the proband, were adopted by their stepfather (II.23) when their mother remarried. Subsequently, III.1 died in a car accident. Five offspring resulted from this union, two males (III.6 and III.7) and three females (III.5, III.8, and III.9). A maternal grandmother (I.1) is alive in a convalescent state.
home. All the above mentioned persons are said to be asymptomatic except for the proband.

The proband's first husband (III.10), a white male (asymptomatic for PCD), produced two male children (IV.1 and IV.2). Both of them had the diagnosis of PCD confirmed by electron microscopy. Their defect is a lack of both inner and outer dynein arms. In addition, one of them (IV.1) shows dextrocardia and, as determined by an upper GI series, a partial malrotation of the foregut. He also underwent an adenoectomy at the age of 3 and bilateral mastoidectomies at the age of 7. The proband had one miscarriage after the failure of an intrauterine contraceptive device (not shown in pedigree).

The proband subsequently married a Puerto Rican Hispanic male (III.11), also said to be asymptomatic for PCD. One male child (IV.3) resulted from this union. He was shown to have PCD based on electron microscopy studies which showed the defect to be a lack of inner dynein arms alone. He had a bilateral myringotomy and an adenoectomy at the age of 14 months.

After a third marriage to a clinically normal white male (III.12), the proband had two male offspring (IV.4 and IV.5). Both of these children (IV.4) have PCD confirmed by electron microscopic analysis. These children also are missing the inner dynein arms alone. The youngest child (IV.5) has suffered from chronic upper respiratory tract infections since birth, resulting in one admission to hospital for pneumonia. The relevant clinical findings for the entire family are summarised in table 1. All the children were born from non-consanguinous unions.

Methods

ELECTRON MICROSCOPY

Material for ultrastructural examination was obtained from III.4, IV.1, IV.2, IV.3, IV.4, and IV.5 under general anaesthesia from the inferior turbinate. All persons were biopsied when they had no acute respiratory disease. While III.4 is a smoker and IV.1 has just started smoking, the other children do not smoke. After a 24 hour glutaraldehyde fixation, the tissue was rinsed with cacodylate buffer and refixed for one hour in 1% osmium tetroxide. The blocks were stained in uranyl acetate for three hours and then dehydrated in a graded series of alcohols. The biopsy material was embedded in Epox 812. Silver to grey thin sections were cut on an ultra microtome and stained with uranyl acetate and lead citrate. Subsequently, they were analysed and photographed on a Hitachi 11B electron microscope.

CYTOGENETIC AND FLUORESCENT IN SITU HYBRIDISATION ANALYSIS

Human metaphase chromosome spreads were prepared from phytohaemagglutinin (PHA) stimulated cultured lymphoblasts by standard methods of Colcemid arrest, hypotonic treatment, and methanol/acetic acid fixation. Blood was drawn after obtaining informed consent from the patients following a protocol approved by the Yale University School of Medicine Human Investigation Committee (protocol 6697). Giemsa and bromodeoxyuridine staining was used to analyse G banding and R banding. DNA probes were labelled with biotin or digoxigenin by nick translation, sized on a 2% agarose gel, passed through a Sephadex G-50 spin column, and hybridised in situ under suppression hybridisation conditions as reported elsewhere.10 Biotinylated DNA was used in single probe hybridisations and detected via avidin conjugated fluorescein isothiocyanate (FITC). To visualise chromosome bands, chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI) hydrochloride.10 For confirmation of chromosome assignment and for detailed regional mapping, the biotinylated probes were co-hybridised with chromosome specific DNA probes labelled with digoxigenin. The following probes were used: (1) the DNA libraries, pBSX, pBS2, pBS12, and pBS14, derived from sorted and amplified human chromosomes X, 2, 12, and 14 respectively and generously provided by Joe Gray (Lawrence Livermore Laboratory); (2) SalI, a probe for

Table 1 Summary of clinical features in the family

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Inner dynein</th>
<th>Outer dynein</th>
<th>9 + 2 complex</th>
<th>Dextrocardia</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>III.4</td>
<td>33y</td>
<td>No ciliary</td>
<td>Structures</td>
<td>seen</td>
<td></td>
<td>Learning disability, malrotation of foregut</td>
</tr>
<tr>
<td>IV.1</td>
<td>15y</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>Learning disability</td>
</tr>
<tr>
<td>IV.2</td>
<td>13y</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV.3</td>
<td>11y</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV.4</td>
<td>2y</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV.5</td>
<td>8 mth</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
proximal 14q; (3) a telomere probe; and (4) a ribosomal DNA centromere probe. For each probe, a minimum of 35 metaphase spreads were evaluated and at least 80% of the metaphases showed specific signals on all four chromosomes.

Results
In order to identify any chromosomal lesions associated with the inheritance of PCD in this family, we used cytogenetics and FISH analysis. Metaphase spreads were prepared from subjects III.1 and IV.1. No abnormalities were detected by G banding or R banding. The mean band resolution for the G banding studies was 550. Chromosome painting using FISH can detect small translocations, but will not identify small deletions, duplications, or inversions. Chromosome specific probes for the X chromosome and chromosomes 2, 12, and 14 showed no abnormalities (probes pBSX, pBS2, PBS12, and pBS14). These chromosomes were studied since they are syntenic with murine chromosomes bearing mutations that affect ciliary function (see Introduction). Ribosomal DNA probes which hybridised to the centromeric regions of chromosomes 13, 14, 15, 21, and 22 and a probe for all telomeres ([\(T_AG_T_{30}\) - 42mer] gave normal hybridisation patterns. Finally single copy Sal7 (RNA component of human RNAase P gene-14q prox) and 12C12 (12pter) also showed no abnormalities. We are therefore unable to identify any chromosomal defect as the underlying cause of PCD in this family.

Discussion
Since the elucidation of the defect underlying PCD in 1976, the scope of the disease has widened to include other apparently inherited disorders of the ciliary axoneme, such as the absence of radial spokes,11 transposition of cilia,12 and ciliary dysmotility not associated with ultrastructural defects.13 This has led to a new nosological designation of "primary ciliary dyskinesia" for conditions that have a genetic and structural basis.14 Genetic analysis of ciliary ultrastructural phenotype is also founded by the fact that transient ciliary abnormalities may be produced by environmental toxins or infectious agents. Afzelius15 has carefully reviewed the possible ciliary ultrastructural alterations from acquired causes. It is important to emphasise that none of these reported ciliary features was seen in the electron microscopic pictures of ciliary structure in this family. In addition, the members of this family have additional symptoms associated with this disorder such as otitis media. Therefore, we can rule out the possibility that the ciliary defects seen in this family are secondary to some other process such as chronic infection. Instead, they are the primary manifestation of a loss of genetic information needed to construct the ciliary apparatus for proper function.

Dyneins are microtubule stimulated ATPases that induce movement towards the minus end of microtubules. They are characterised by an enormous (\(-500\) kDa) polypeptide and a single type of associated polypeptides 14-120 kDa in size.16 Since there are seemingly many genes that control the expression of multiple ciliary proteins, it is then likely that there may be different modes of inheritance involved in these processes and that these may all lead to the final common pathway of dynein dysmorphology or dysfunction or both.

We analysed chromosomes X, 2, 12, and 14 with chromosome specific libraries without detecting any significant gross additions of deletions. Chromosome 12 and 14 single copy sequence probes, telomeric probe, and ribosomal centromeric probes also failed to show any abnormalities. The lack of abnormalities in G banded and R banded chromosomes is in keeping with previously reported cytogenetic studies17 in patients with PCD.

PCD is considered to be an autosomal recessive disorder.4 Most familial cases have been confined to sibs who have had normal parents and a negative family history. There are a small number of reports, however, of families having one or more features of PCD with inheritance patterns which are not compatible with autosomal recessive inheritance (table 2). In these families, the inheritance pattern appears X linked, autosomal dominant, or autosomal dominant with incomplete penetrance. In the family reported here with an affected mother and children from three different marriages, autosomal recessive inheritance can, in all likelihood, be excluded. Instead, either autosomal dominant inheritance, with the mother having a fresh mutation, or mitochondrial inheritance is likely. X linked inheritance, either dominant or recessive, perhaps with predominant inactivation of the normal allele, is also possible.

The family as well as those previously published support the concept of genetic heterogeneity in PCD. These findings have important implications for genetic counselling of affected families. Until specific molecular characterisation becomes available, differentiation among possible inherited forms will remain extremely difficult.

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Table 2 Published families with persons having one or more components of Kartagener's syndrome suggestive of inheritance patterns other than autosomal recessive

<table>
<thead>
<tr>
<th>Abnormalities</th>
<th>Affected family members</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Situs inversus</td>
<td>Uncle and nephew, 2 families</td>
<td>18</td>
</tr>
<tr>
<td>Situs inversus + bronchiectasis</td>
<td>Son of a sister</td>
<td>23</td>
</tr>
<tr>
<td>Situs inversus + bronchiectasis + sinus troubles</td>
<td>Mother and daughter</td>
<td>19</td>
</tr>
<tr>
<td>Situs inversus + bronchiectasis</td>
<td>Father and son</td>
<td>20</td>
</tr>
<tr>
<td>Situs inversus + bronchiectasis + sinus troubles</td>
<td>Father and child</td>
<td>21</td>
</tr>
<tr>
<td>Situs inversus + sinusitis</td>
<td>A man and maternal grandfather</td>
<td>22</td>
</tr>
<tr>
<td>Situs inversus + respiratory troubles</td>
<td>Sisters and maternal great uncle</td>
<td>23</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>A woman (situs inversus and bronchiectis), mother and daughter (bronchiectis and sinusitis)</td>
<td>23</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>Father and children 3 families</td>
<td>27</td>
</tr>
</tbody>
</table>
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