Letters to the Editor

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Exclusion of Chromosome 7 for Kartagener Syndrome but Suggestion of Linkage in Families with Other Forms of Primary Ciliary Dyskinesia

To the Editor:

We read with great interest the recent letter by Pan et al. (1998), in which they report a case of uniparental disomy, of chromosome 7, associated with cystic fibrosis (CF), complete situs inversus, and immotile (although ultrastructurally normal) bronchial ciliary apparatus. Those authors appropriately suggest that linkage studies be conducted in families with Kartagener syndrome (KS), to evaluate chromosome 7 as a candidate location for the gene underlying this disorder.

KS (MIM 244400) is recognized on the basis of a classic triad of symptoms: situs inversus (complete mirror-image reversal of left-right asymmetry of the chest and abdominal organs [MIM 270100]), bronchiectasis, and chronic sinusitis (Afzelius 1976; Schidlow 1994; Afzelius and Mossberg 1995). In families with a KS proband, approximately half of the proband's affected siblings display the full triad of symptoms, whereas the other affected sibs exhibit only bronchiectasis and chronic sinusitis but have normal left-right organ asymmetry. KS is clinically considered a subgroup of primary ciliary dyskinesia (PCD), formerly known as "immotile cilia syndrome" (ICS [MIM 242650]). However, it is unclear whether KS has the same genetic etiology as PCD without situs inversus. These disorders are characterized by dysmotility or immotility of the cilia in airway epithelial cells, spermatozoa, and other ciliated cells of the body. Clinical consequences of PCD cover a wide spectrum of symptoms mainly involving both lower and upper airways and the male reproductive system. Ciliary immotility is caused by various ultrastructural defects of cilia, with major or subtle anomalies detectable, by electron microscopy, in all or nearly all patients (Teknos et al. 1997). The structural defects are predominantly a total or partial absence of dynein arms (70%–80% of cases), defects of radial spokes, nexin links, and general axonemal disorganization with microtubular transposition (Afzelius and Mossberg 1995).

Estimates of the incidence of PCD are in the range 1/16,000–1/60,000 live births, with KS accounting for approximately half (1/32,000–1/120,000 live births) of these (Afzelius and Mossberg 1995). Inheritance in most cases is autosomal recessive, although some examples of dominant or X-linked modes of inheritance have been reported (Narayan et al. 1994*b*). Nearly 200 different polypeptides have been identified within the ciliary axoneme of lower organisms; at least the same number of proteins can be expected in axonemes of humans (Luck et al. 1982). Mutations within many of these 200 genes coding for ciliary proteins might cause the same or similar pathologic consequences of ciliary dysfunction. However, as noted in OMIM (MIM 242650), if this were true, then we might expect that the incidence of PCD would be much higher than that which actually occurs. It is possible that mutations in many of these genes might be lethal—and thus not be found among viable offspring. Alternatively, there may be functional redundancy of some proteins, such that loss of one gene's product may be compensated by other proteins and thus occur without ciliary dysfunction.

Support for PCD genes potentially located on chromosome 7 is provided by several observations. First, 7q33-q34 is syntenic to a fragment of murine chromosome containing the *hop* mutation (previously named "*hpy*")—mice homozygous for this mutation have a dynein defect in cilia and flagella that is similar to that seen in some cases of PCD (i.e., dynein arms are missing from A-tubules of the outer doublets) (Handel 1985). Second, the gene for the β heavy chain of the outer dynein arm maps to 7p15 region (Kastury et al. 1997), and additional genes containing sequences highly homologous to the dynein-gene family map to 7q21-q22 (GenBank accession number AC002452) and to 7p21 (GenBank accession number AC004002). Third, there is the case of chromosome 7 uniparental disomy and other chromosomal anomalies with KS-like symptoms, summarized in the letter by Pan et al. (1998). PCD candidates on other chromosomes include the following: (1) the HLA region of chromosome 6p, containing the β-tubulin gene (TUBB) (Volz et al. 1994), although limited data reported elsewhere (Gasparini et al. 1994) did not support the motylin gene (MLN), also residing in this region, as being a candidate for involvement in PCD

etiology; (2) chromosome 14q32, containing the gene for echinoderm microtubule–associated protein (EMAP), a candidate for Usher syndrome type 1A (the USH1A gene) (these patients exhibit, in the axonemes of their respiratory cilia ultrastructural defects similar to those in PCD) (Bonneau et al. 1993; Eudy et al. 1997); (3) the dynein heavy-chain gene located on 14qter (Narayan et al. 1994*a*); and (4) numerous other dynein, nexin, and other microtubule-related genes rapidly accumulating in the genomics databases. Many genes have been implicated recently in the control of the left-right asymmetry of body development such as that observed in KS (Overbeek 1997; Srivastava 1997; Wood 1997; Levin and Mercola 1998). However, with the exception of the dynein defect associated with the iv mouse mutant (Supp et al. 1997), homologous to the human heavy-chain dynein gene located on chromosome 14 qter, none of these are associated with ciliary dysfunction.

We performed linkage analyses using microsatellite markers spanning chromosome 7 in 30 PCD families recruited in Poland. Each family had at least one member diagnosed with PCD, and no other anomalies or dysmorphologies were present. For linkage analyses, families were further classified either as KS families, if at least one affected member was diagnosed as having KS (i.e., as exhibiting situs inversus), or as ciliary dysfunction only (CDO) families, if none of the affected members had situs inversus. Our sample comprised 23 KS families with 25 KS-affected individuals and 7 CDO-affected individuals and 7 CDO families with 9 CDO-affected individuals. Data from all of these families were consistent with autosomal recessive transmission (i.e., there were no nonsibling affected relatives). Among the KS families, there were four pairs of affected siblings and two trios of affected siblings; among the CDO families, there were two pairs of affected siblings. Two additional KS families were ascertained as having the disease in multiple generations, consistent with a dominant mode of inheritance, but, to date, we have been unable to recruit a sufficient number of members to make these families informative for linkage analysis. We chose to analyze the KS and CDO families separately because of the possibility that different molecular (hereditary) pathologies might underlie these forms of PCD; for example, the *hop* mouse mutation exhibits CDO (without situs inversus).

Seventeen microsatellite markers spanning chromosome 7, with average interval of 10.8 cM, were analyzed by fluorescence-based, semiautomated DNA-sizing technology (Ziegle et al. 1992) using Applied Biosystems 373 Automated DNA Sequencers and GENESCAN and GENOTYPER software (Applied Biosystems/Perkin-Elmer). Pairwise LOD-score analyses were performed by means of the FASTLINK program (Schäffer 1996). LOD scores allowing for locus heterogeneity (Ott 1991) were calculated by means of a program developed for this purpose (S. R. Diehl, unpublished data). Our unpublished program performs the same simple admixture calculation as is performed by publicly available programs such as HOMOG (and provides identical results in numerous benchmark comparisons), and it is used for data-formatting convenience only. A copy of our program is available on request from the corresponding author. Multipoint LOD scores for all of chromosome 7 were calculated by means of the GENEHUNTER program (Kruglyak et al. 1996) and the sex-average map distances

Table 1

LOD Scores for 23 KS Families

Marker	Map Position (cM)	Summed LOD Score under Homogeneity, for θ = .00001	.01000	.05000	.10000	Maximum LOD Score for Heterogeneity	Alpha at Maximum LOD Score
D7S531	5.28	−12.768	−7.357	−3.647	−2.121	−.01141	.05
D7S517	7.44	−12.722	−7.056	−3.357	−1.885	−.01106	.05
D7S513	17.74	−13.868	−8.068	−3.937	−2.262	−.01309	.05
D7S507	28.74	−8.319	−4.576	−2.133	−1.161	−.00457	.05
D7S493	34.69	−8.053	−4.332	−1.963	−1.056	−.00425	.05
D7S629	37.51	−9.616	−5.046	−2.114	−1.032	.07877	.20
D7S484	53.50	-9.594	−5.027	−1.993	−.850	.06800	1.00
D7S519	69.03	−5.742	−3.035	−1.093	−.331	.15600	1.00
D7S502	78.65	−9.215	−5.308	−2.410	−1.262	−.00372	.05
D7S669	90.42	−4.445	−1.861	−.238	.250	.38000	1.00
D7S657	104.86	−11.184	−6.100	−2.916	−1.630	−.00768	.05
D7S527	108.59	−6.591	−3.671	−1.763	−.989	−.00445	.05
D7S486	124.08	−1.096	−.191	.323	.428	.51927	.55
D7S530	134.55	−7.525	−3.492	−1.162	−.374	.28454	.30
D7S640	137.83	−6.547	−2.833	−.645	.031	.31760	.60
D7S684	147.22	−3.048	−1.115	−.041	.249	.28700	1.00
D7S550	178.41	−6.711	−3.420	−1.198	−.437	.04600	1.00

reported by the Marshfield Medical Research Foundation. For all LOD-score analyses, we assumed a recessive mode of inheritance, 50% penetrance for homozygous-mutant genotypes, 0.000013% penetrance for wild-type and heterozygous genotypes (i.e., PCD phenocopies), and PCD disease-allele frequency of .00514. These assumptions yield a population prevalence consistent with that reported for PCD.

Pairwise and multipoint LOD scores for the KS families are shown in table 1 and figure 1, respectively. The last column in the table ("Alpha at Maximum LOD Score") refers to the estimated proportion of families in which there is linkage to the marker under locus heterogeneity (i.e., the maximum LOD score obtained by varying both the recombination fraction and the proportion of families linked). Pairwise LOD scores under locus homogeneity for 17 microsatellite markers of chromosome 7 are all negative and range between -1.096 and -13.868, at a recombination fraction (θ) of .00001, providing no support for linkage. Even if we allow for locus heterogeneity within the KS families (i.e., some KS families have linkage to a gene on chromosome 7, whereas others do not), the maximum pairwise LOD score obtained for any of the 17 markers is only 0.52. Multipoint LOD scores under the assumption of homogeneity exclude (at LOD < -2.0) a KS-susceptibility locus from most of chromosome 7 (fig. 1). The only part of the chromosome not formally excluded is the region between the last two markers on the chromosome, where a gap of >31 cM exists, and even this region is not positive but only lacks power for exclusion. If we allow for locus heterogeneity, the highest multipoint LOD score for the entire chromosome is still only 0.27, which could easily be due to chance. By contrast, pairwise and multipoint LOD scores for the CDO families, shown in table 2 and figure 1, respectively, provide at least a weak suggestion of possible linkage to chromosome 7. We note that, interestingly, the highest multipoint LOD scores for the CDO families, 1.41, occurs at precisely the position on chromosome 7p15 where the gene for the β heavy chain of the outer dynein arm is located (Kastury et al. 1997). The same maximum LOD score is obtained when we allow for locus heterogeneity within the CDO families, since all families provide evidence of linkage to this region (i.e., no evidence of recombination with markers D7S493 or D7S629; see table 2). Analyses of combined KS and CDO families provide no significant evidence of linkage, with a maximum multipoint LOD score, calculated by the GENEHUNTER program, of only 0.56 for the entire chromosome, occurring at the same location where the highest LOD score (1.41) for the CDO families occurs.

Since our linkage results exclude chromosome 7 as a candidate location for a KS gene, the cytogenetic evidence suggested that this region should be reevaluated. Ciliary structural anomalies are always or almost always found in cases of KS (Teknos et al. 1997); however, despite the absence of normal ciliary motion, no visible

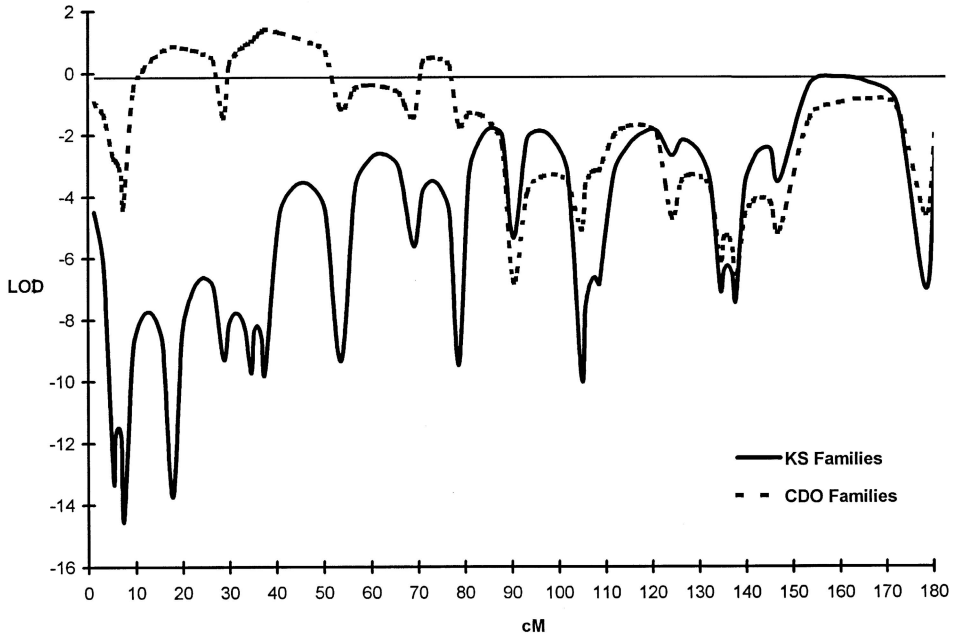

Figure 1 Multipoint LOD scores for KS families and CDO families, for chromosome 7, under the assumptions of recessive inheritance and locus homogeneity.

Table 2

LOD Scores for Six CDO Families

Marker	Map Position (cM)	Summed LOD Score under Homogeneity, for θ = .00001	.01000	.05000	.10000	Maximum LOD Score for Heterogeneity	Alpha at Maximum LOD Score
D7S531	5.28	−1.942	−1.008	−.379	−.139	.03100	1.00
D7S517	7.44	−4.170	−2.188	−.934	−.450	−.00035	.05
D7S513	17.74	.833	.812	.723	.607	.83300	1.00
D7S507	28.74	−1.760	−.780	−.192	.005	.09500	1.00
D7S493	34.69	.893	.876	.801	.695	.89200	1.00
D7S629	37.51	.762	.728	.598	.455	.76200	1.00
D7S484	53.50	−1.398	−.496	.021	.152	.15800	1.00
D7S519	69.03	−2.117	−1.119	−.470	−.213	.01030	.85
D7S502	78.65	−1.750	−.745	−.092	.138	.21900	1.00
D7S669	90.42	−6.693	−3.680	−1.790	−1.029	−.00567	.05
D7S657	104.86	−4.870	−2.738	−1.429	−.875	−.00680	.05
D7S527	108.59	−2.540	−1.526	−.819	−.496	−.00308	.05
D7S486	124.08	−4.700	−2.453	−1.149	−.627	−.00311	.05
D7S530	134.55	−4.470	−2.479	−1.192	−.666	−.00335	.05
D7S640	137.83	−7.445	−4.200	−2.217	−1.368	−.01008	.05
D7S684	147.22	−4.761	−2.748	−1.394	−.812	−.00497	.05
D7S550	178.41	−4.643	−2.404	−1.124	−.619	−.00333	.05

ciliary ultrastructural defects were found in the patient with situs inversus and paternal isodisomy of chromosome 7 who was reported by Pan et al. (1998). It is possible that the situs inversus of this patient was caused by a gene solely involved in heterotaxy but without any ciliary ultrastructural anomaly (Overbeek 1997; Srivastava 1997; Wood 1997; Levin and Mercola 1998). Two other studies have reported cytogenetic anomalies of chromosome 7 that are associated with laterality defects (Genuardi et al. 1993; Koiffman et al. 1993). Furthermore, the lack of normal ciliary motion without ciliary structural anomalies, as was observed in Pan et al.'s patient, may represent a secondary effect of cytolysis and cell destruction of bronchial epithelial cells in CF (Cheung and Jahn 1976), although this finding remains controversial (Rutland et al. 1983). Alternative explanations include involvement of genes involving ciliary function (in addition to a gene causing situs inversus). Such a ciliary-function gene might also be located on chromosome 7, or, coincidentally, the patient studied by Pan and colleagues might have a mutation in a gene influencing ciliary function and located elsewhere in the genome. Our suggestive LOD score of 1.41 at the chromosomal location of a dynein gene in the CDO families that we have studied is consistent with the involvement of a ciliary-function gene on chromosome 7, but the ultimate resolution of these issues will require additional data. Although this LOD score is quite small, it may be especially noteworthy in view of the fact that the limited number of CDO families available can produce a maximum LOD score of only 1.49, even under complete linkage and full marker informativeness, as we have determined by means of the SLINK program (Weeks et al. 1990). The KS families, by contrast, can yield LOD scores as high as 3.95, under complete linkage and complete informativeness.

We conclude from our data presented here that the gene(s) responsible for KS is (are) not likely to be located on chromosome 7. Our suggestion of possible linkage for the CDO families should be taken with caution, because of the small size of the sample analyzed; however, especially because the β heavy chain of the outer dynein arm maps to the same location as our positive LOD score of 1.41, we suggest that this region should be considered a high-priority location for follow-up linkage studies in additional CDO families.

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Electronic-Database Information

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html (for 7q21-q22 [accession number AC002452] and 7p21 [accession number AC004002])

Marshfield Medical Research Foundation (Center for Medical Genetics), http://www.marshmed.org/genetics/ (for sex-average map distances)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for ICS [MIM 242650], KS [MIM 244400], and situs inversus [MIM 270100])

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