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ARTICLE

# Primary ciliary dyskinesia: a genome-wide linkage analysis reveals extensive locus heterogeneity

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**Primary ciliary dyskinesia (PCD), or immotile cilia syndrome (ICS), is an autosomal recessive disorder affecting ciliary movement with an incidence of 1 in 20000–30000. Dysmotility to complete immotility of cilia results in a multisystem disease of variable severity with recurrent respiratory tract infections leading to bronchiectasis and male subfertility. Ultrastructural defects are present in ciliated mucosa and spermatozoa. Situs inversus (SI) is found in about half of the patients (Kartagener syndrome). We have collected samples from 61 European and North American families with PCD. A genome-wide linkage search was performed in 31 multiplex families (169 individuals including 70 affecteds) using 188 evenly spaced (19 cM average interval) polymorphic markers. Both parametric (recessive model) and non-parametric (identity by descent allele sharing) linkage analyses were used. No major locus for the majority of the families was identified, although the sample was powerful enough to detect linkage if 40% of the families were linked to one locus. These results strongly suggest extensive locus heterogeneity. Potential genomic regions harbouring PCD loci were localised on chromosomes 3p, 4q, 5p, 7p, 8q, 10p, 11q, 13q, 15q, 16p, 17q and 19q. Linkage analysis using PCD families with a dynein arm deficiency provided 'suggestive' evidence for linkage to chromosomal regions 8q, 16pter, while analyses using only PCD families with situs inversus resulted in 'suggestive' scores for chromosomes 8q, and 19q.** *European Journal of Human Genetics* (2000) **8**, 109–118.

**Keywords:** immotile cilia syndrome; primary ciliary dyskinesia; Kartagener syndrome; situs inversus; heterogeneity; linkage analysis

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## Introduction

Primary ciliary dyskinesia (PCD), also known as immotile cilia syndrome (ICS), (OMIM 242650), is an hereditary disorder affecting ciliary movement, with an incidence of 1 in 20000–30000.[1–3] Dysmotility to complete immotility of cilia predisposes to recurrent pulmonary and upper respiratory tract infections resulting in bronchiectasis. Male infertility is frequently reported, whereas subfertility in females has also been suggested.[4,3] Dextrocardia usually with situs inversus totalis, is observed in about 50% of patients;[1] PCD with situs inversus (SI) is also referred to as Kartagener syndrome (KS), (OMIM 244400). About two-thirds of patients suffer from chronic headaches.[5]

In PCD patients, cilia and the sperm flagella, demonstrate defective motion;[3,6,7] the movement defects are usually associated with similar abnormal morphology. Numerous short cilia cover the epithelia of the airways, whereas each spermatozoon has a single long flagellum with a similar central axoneme structure. Electron microscopy revealed nine peripheral microtubular doublets plus a central pair in cilia and flagella. Each microtubule is composed of globular protein molecules, the tubulins. One of the microtubules of each peripheral doublet shows two short linear extensions laterally, the dynein arms, oriented towards the next doublet. The outer and inner arms act in conjunction with microtubules of the adjacent doublets to create and coordinate a sliding force required for the beating of cilia and flagella.

About half the patients with PCD have an abnormality of inner and/or outer dynein arms (IDA, ODA, complete absence or reduction in their number, or abnormalities in their orientation).[8] The remaining half have various other types of anomalies in ciliary structure or no apparent changes. Although ultrastructural analysis suggests that the primary defect(s) in PCD may lie in constitutive proteins of the cilia,[9,8,10] no mutant gene associated with the disease has been identified to date.

In the majority of families, PCD is transmitted as an autosomal recessive trait. However, a few rare cases of apparently dominant or X-linked inheritance have been reported.[11,12]

In order to determine the chromosomal localisation of genes involved in PCD, we performed a genome-wide linkage analysis using a panel of multiplex families (at least two affecteds per family). We failed to identify a common locus for PCD, but several chromosomal regions yielded lod scores which suggest the presence of loci linked to the phenotype. These results confirm that PCD is genetically highly heterogeneous; a much larger sample is needed to establish the chromosomal localisation of some of the responsible genes. A candidate gene approach for the cloning of PCD genes may be more fruitful than positional cloning.

## Families and methods

### Collection of families with PCD

Families with PCD were enrolled in the study after informed consent. The clinical protocols were approved by the University of Geneva, University College London and local institutions. Diagnosis was established by electron microscopy in at least one affected individual in most families. Affected individuals demonstrated symptoms that included recurrent infections of upper respiratory tract, nasal polyps, sinusitis, recurrent bronchopneumonia, SI, reduced mucociliary clearance, subfertility, abnormal ciliary movements and ciliary abnormalities. DNA was extracted from all members of these families and has been stored anonymously.

### DNA genotyping

Genomic DNA was purified from blood lymphocytes according to standard extraction methods. DNA polymorphisms were analysed by PCR amplification of short tandem repeat sequences.[13] The markers selected were from the Généthon and CHLC collections, version 6.0, of Research Genetics.[14–17] Genotypes were independently determined by two different investigators. Family information and linkage analysis files were created using the pedigree computer program Cyrillic (Cherwell, Oxford, UK).

### Linkage analysis

We estimated the power of the family sample for linkage analysis, under either homogeneity or at different levels of heterogeneity using the SLINK program.[18,19] Two-point parametric linkage analysis was performed using the ILINK, MLINK programs of LINKAGE v5.2,[20] or FASTLINK v3.0.[21] We used the ANALYSIS package to estimate heterogeneity,[22] and identity by descent (IBD) sib-pair allele sharing.[23] Multipoint parametric and non-parametric analyses were performed using the Genehunter program.[24] Linkage analysis computations were performed either with the informatics facility of Geneva University Medical Center or the UK HGMP (http://www.hgmp.mrc.ac.uk/). Maximum lod and location scores were calculated for each marker by assuming autosomal recessive inheritance with 90% penetrance. The gene frequency of PCD was estimated at 1 in 141 (corresponding to a disease frequency of 1 in 20000). The allele frequencies were considered equal. For multipoint linkage analysis the intermarker distances were based on the marker map (Weber, version 6.0) (http://www.marshmed.org/genetics/sets/scrset6.txt). Analyses were conducted independently on all multiplex families, multiplex families with SI, and with inner and/or outer dynein arm deficiency (DAD).

## Results

### Recruitment of families with PCD

We identified and collected 61 families with individuals diagnosed with PCD (OMIM 242650) from Europe and North America (Table 1). There were 99 affected and 178 non-affected individuals in the sample. Over half (60%) of the families had DAD. There were 29 families in which at least one member had SI.

**Table 1** Families with PCD. Number of families and individuals collected for the molecular analysis of PCD. The multiplex families (at least two affecteds per family) were also subdivided into sub-groups with SI and DAD. Several families (14) showed both SI and DAD

| | *Families* | *Individuals* | *Affected individuals* | *Non-affected individuals* |
|---|---|---|---|---|
| Families with PCD | 61 | 277 | 99 | 178 |
| All multiplex | 31 | 169 | 70 | 99 |
| Multiplex with SI | 18 | 106 | 39 | 67 |
| Multiplex with DAD | 20 | 114 | 47 | 67 |

We selected 31 multiplex families with at least two affected individuals for a genome-wide linkage analysis (70 affected and 99 non-affected) (Figure 1). Among these were 46 sib-pairs and 51 affected pairs. In 18 of these 31 families, SI was present in one or more affected individuals (in 12 of these SI was not present in all affecteds). In one family, one monozygotic twin had SI, whereas the other did not (another such family has recently been described[25]). This latter family was not among the 31 families used for linkage analysis. Consanguinity was observed in family UCL01 only.

### Sample power

In order to estimate the power of our family sample to detect a PCD locus, pedigrees were used for a two-point linkage simulation assuming either genetic homogeneity or various levels of heterogeneity based on 200 replicates of genotype combinations. Under homogeneity using a 6-allele marker, the expected maximum lod score (ELOD) value would reach 24.9 at $\theta = 0$, with an average ELOD score of 20.89 at $\theta = 0$. Under the model of 50% unlinked families the maximum ELOD score was 16.37 at $\theta = 0$, and the mean ELOD was 4.83 at $\theta = 0$. Under an extensive heterogeneity model (90% of families unlinked to a locus), the maximum ELOD score was 3.51 at $\theta = 0$, and the mean ELOD was 0.32 at $\theta = 0$. The sample was adequate to detect a mean ELOD of 3.02 at $\theta = 0$ for a model in which 60% of the families were not linked to a locus. Since our genome-wide scan was done with a map density of markers at 19 cM average interval, we also performed power calculations using $\theta = 0.10$ (worst case scenario). Under homogeneity the ELOD score was 18.22 with an average ELOD of 15.43. Under the model of 50% unlinked families the maximum ELOD was 12.84, and the mean ELOD was 3.74. The sample was adequate to detect a mean ELOD of 3.74 at $\theta = 10$ for a model in which 50% of the families were not linked to a locus.

### Genome wide linkage analysis

The 31 multiplex families were analysed for linkage between the PCD phenotype and the 188 microsatellite markers evenly spaced throughout the 22 autosomes with an average interval distance of 19 cM.

Genotypes were determined and analyzed initially using parametric linkage with a recessive mode of inheritance. Since this analysis failed to detect a single chromosomal region with a significant lod score we used a non-parametric linkage analysis of affected pairs.

The highest scores for each chromosome are shown in Tables 2 and 3. The chromosomal localisation of the most positive scores are displayed in Figure 2.

### PCD (all 31 families) phenotype

***Two-point linkage analysis*** The probability of linkage was first estimated in a two-point analysis with recessive inheritance (for parametric) and sib-pair IBD allele-sharing analysis (non-parametric). We considered suggestive linkage when the lod scores were (Z/ZH) $\geq 2.0$, and 'potentially interesting' linkage when Z/ZH was 0.9–2.0.

Table 2 shows the maximum lod score for each chromosome, assuming homogeneity (Z), or heterogeneity (ZH). This table also contains the results of the non-parametric linkage. *p*-values for the sib-pair analysis of IBD allele sharing is also indicated in the Sib pair column.

The best two-point lod scores exceeding 2.0 were obtained for chromosomes 4q and 5p at markers D4S2368 (ZH = 2.20 at $\theta = 0.12$) and D5S1473 (ZH = 2.20 at $\theta = 0.08$) respectively. These scores do not provide significant evidence for linkage and are considered as suggestive. The sib-pair analysis for D4S2368 resulted in $p = 0.000435$ which is also in the 'suggestive' range.[26] For D5S1473, sib-pair analysis yielded $p = 0.0071$.

In the group of 'potential, interesting' results (Z/ZH between 0.9 and 2.0) we noted markers in seven additional chromosomal regions at 3p (D3S2432, ZH = 1.37 at $\theta = 0$), 7p (D7S2201, ZH = 0.90 at $\theta = 0$), 8q (D8S1179, ZH = 1.49 at $\theta = 0.04$), 15q (D15S642, ZH = 0.93 at $\theta = 0.24$), 16p (D16S2619, ZH = 0.90 at $\theta = 0$), 17q (D17S928, ZH = 1.03 at $\theta = 0$), 19q (D19S601, ZH = 1.64 at $\theta = 0.02$) (Figure 2B). The sib-pair analysis for each of these markers showed the following *p*-values: D3S2432 ($p = 0.271$); D7S2201 ($p = 0.287$); D8S1179 ($p = 0.021$); D15S642 ($p = 0.025$); D16S2619 ($p = 0.180$); D17S928 ($p = 0.080$) and D19S601 ($p = 0.021$). In addition, the sib-pair two-point linkage analysis yielded 'potential, interesting' scores for D3S1766 ($p = 0.017$), and D16S748 ($p = 0.024$).

***Multipoint linkage analysis*** We also performed a chromosome-wide multipoint analysis using a fixed map and the Genehunter program (Table 2). No chromosomal regions showed Z-all values that were 'significant' or 'suggestive' for linkage. The analysis showed highest allele sharing on chromosomes 8q (Z-all$_{max} = 1.90$, $p = 0.025$, at position 120 cM from 8pter) and 16pter (Z-all$_{max} = 1.85$, $p = 0.028$, at position 0).

Additional potential regions of interest may include chromosomes 3q (Z-all$_{max} = 1.55$, $p = 0.054$, at position 120), 5p (Z-all$_{max} = 1.32$, $p = 0.086$, at position 22), 10p (Z-all$_{max} = 1.33$, $p = 0.084$, at position 23), 15qter

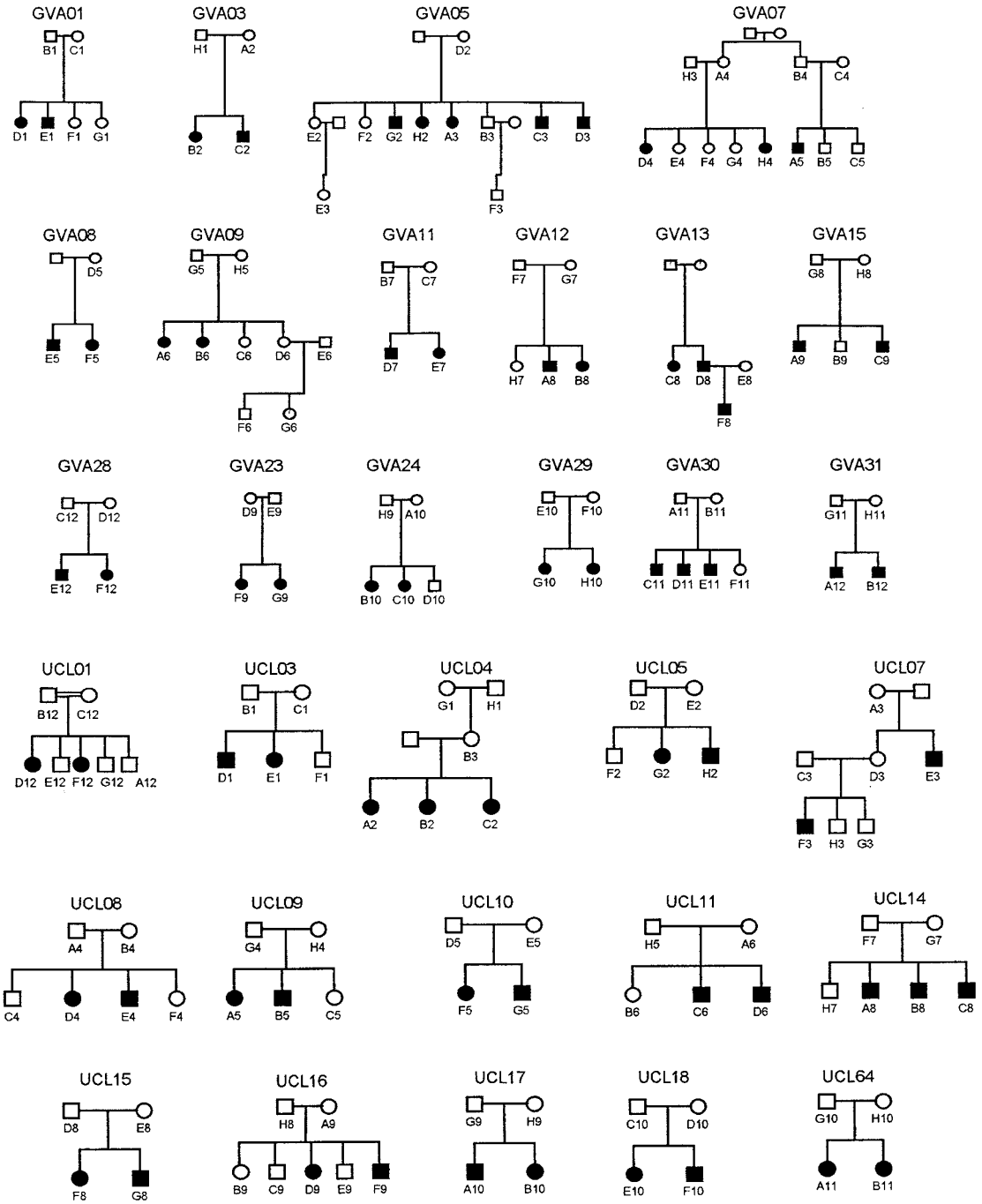


**Figure 1** A schematic representation of the 31 nuclear families with PCD included in the genome-wide linkage analysis.

(Z-all$_{max}$ = 1.60, $p$ = 0.049, at position 109), and 17qter (Z-all$_{max}$ = 1.39, $p$ = 0.078, at position 135) (Figure 2B).

### PCD-SI subphenotype

We also performed two-point and multipoint linkage analysis in sub-groups of PCD families. One major sub-group of 18 families is that with SI in at least one affected per family. The highest parametric two-point 'suggestive' lod score (Table 3A) were obtained for chromosomes 8q (D8S1179, ZH = 2.50 at $\theta$ = 0.08; the ZH for all 31 PCD families was 1.49) and 19q (D19S601, ZH = 2.46 at $\theta$ = 0.14; the ZH for all 31 PCD families was 1.64). Non-parametric two-point sib-pair analysis for marker D8S1179 on 8q was $p$ = 0.0012 ($p$ for all

**Table 2** Results of genome-wide linkage analysis on the 31 multiplex families. The highest scores obtained for each chromosome are given for both parametric (left panel) and non-parametric (right panel) analyses. For the two-point analyses, the marker loci with the highest scores and their corresponding distance from pter of the genetic map are shown. For the multipoint analysis, the position of the highest score is determined by the distance from pter of the genetic map

| | *Parametric* | | | | | | *Non-parametric* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Two point* | | | | | | *Two point sib pairs* | | | *Multipoint all pairs* | | |
| *Chromosome* | *Z* | *θ* | *ZH* | *θH* | *Markers* | *Distance from pter* | P | *Markers* | *Distance from pter* | *Z-all* | P | *Distance from pter* |
| | *No heterogeneity* | | *Heterogeneity* | | | | | | | | | |
| 1 | 0.11 | 0.32 | 0.11 | 0.32 | D1S1660 | 216 | 0.177 | D1S2134 | 72 | 0.82 | 0.196 | 61 |
| 2 | 0.34 | 0.28 | 0.53 | 0 | D2S1326 | 155 | 0.081 | D2S1391 | 198 | 0.96 | 0.159 | 188 |
| 3 | 0.62 | 0.3 | 1.37 | 0 | D3S2432 | 55 | 0.017 | D3S1766 | 77 | 1.55 | 0.054 | 120 |
| 4 | 2.2 | 0.12 | 2.2 | 0.12 | D4S2368 | 168 | 0.000435 | D4S2368 | 168 | 0.73 | 0.223 | 167 |
| 5 | 2.17 | 0.1 | 2.2 | 0.08 | D5S1473 | 40 | 0.0071 | D5S1473 | 40 | 1.32 | 0.086 | 22 |
| 6 | 0.06 | 0.34 | 0.77 | 0 | D6S474 | 114 | 0.262 | D6S1009 | 134 | 0.92 | 0.17 | 165 |
| 7 | 0.35 | 0.28 | 0.9 | 0 | D7S2201 | 0 (pter) | 0.104 | D7S559 | 193 | 0.93 | 0.169 | 194 |
| 8 | 1.07 | 0.24 | 1.49 | 0.04 | D8S1179 | 136 | 0.021 | D8S1179 | 136 | 1.90 | 0.025 | 120 |
| 9 | 0.1 | 0.36 | 0.1 | 0.36 | D9S925 | 23 | 0.09 | D9S934 | 124 | 0.53 | 0.29 | 58 |
| 10 | 0.63 | 0.26 | 0.63 | 0.26 | D10S1412 | 22 | 0.148 | D10S1412 | 22 | 1.33 | 0.0845 | 23 |
| 11 | 0 | 0.5 | 0.61 | 0 | D11S2359 | 145 | 0.068 | D11S1984 | 0 | 1.14 | 0.121 | 0 (pter) |
| 12 | 0.24 | 0.32 | 0.3 | 0.18 | D12S1064 | 92 | 0.156 | D12S392 | 168 | 0.70 | 0.234 | 0 (pter) |
| 13 | 0.51 | 0.28 | 0.73 | 0.08 | D13S285 | 119 | 0.095 | D13S285 | 119 | 1.24 | 0.1 | 100 |
| 14 | 0 | 0.5 | 0.007 | 0 | D14S1280 | 10 | 0.44 | D14S592 | 54 | 0.13 | 0.446 | 44 |
| 15 | 0.93 | 0.24 | 0.93 | 0.24 | D15S642 | 110 (qter) | 0.025 | D15S642 | 110 | 1.60 | 0.049 | 109 (qter) |
| 16 | 0.24 | 0.3 | 0.90 | 0 | D16S2619 | 20 | 0.024 | D16S748 | 13 | 1.85 | 0.028 | 0 (pter) |
| 17 | 0.72 | 0.26 | 1.03 | 0 | D17S928 | 135 (qter) | 0.08 | D17S928 | 145 | 1.39 | 0.078 | 135 (qter) |
| 18 | 0.08 | 0.36 | 0.1 | 0.22 | D18S535 | 73 | 0.31 | D18S535 | 73 | 0.08 | 0.462 | 0 (pter) |
| 19 | 1.28 | 0.2 | 1.64 | 0.02 | D19S601 | 78 | 0.0206 | D19S601 | 78 | 1.54 | 0.056 | 12 |
| 20 | 0.004 | 0.4 | 0.015 | 0.04 | D20S1085 | 74 | 0.31 | D20S1085 | 74 | –0.33 | 0.63 | 0 (pter) |
| 21 | 0.09 | 0.34 | 0.09 | 0.34 | D21S1432 | 0 (pter) | 0.19 | D21S1432 | 0 | 1.14 | 0.124 | 0 (pter) |
| 22 | 0.02 | 0.4 | 0.18 | 0 | D22S683 | 34 | 0.35 | D22S420 | 0 | 0.36 | 0.354 | 0 (pter) |

31 families was 0.021) and D19S601 on 19q was $p = 0.007$ ($p$ for all 31 families was 0.02).

The highest multipoint Z-all$_{max}$ value has been obtained for chromosome 8q (Z-all$_{max}$ = 2.03, $p = 0.017$, at position 137 cM from 8pter; Z-all$_{max}$ for all 31 families was 1.90 for position 120 cM from 8pter). In some cases 'potential, interesting' Z-all$_{max}$ values for novel chromosomal regions were observed. These are on chromosomes 3 (Z-all$_{max}$ = 1.68 at position 120), 5q (Z-all$_{max}$ = 1.45 at position 1.71), 11q (Z-all$_{max}$ = 1.52 at position 128), 13q (Z-all$_{max}$ = 1.47 at position 57), 16pter (Z-all$_{max}$ = 1.49 at position 0), 17p (Z-all$_{max}$ = 1.58 at position 135), 19q (Z-all$_{max}$ = 1.71 at position 78) (Figure 2B).

### PCD-DAD subphenotype

Another sub-group of PCD, composed of 20 families in our sample, is that with outer and/or inner dynein arm deficiency (DAD), and was also analysed (Table 3B).

The highest parametric lod scores were obtained on chromosomes 8q (D8S1179, ZH = 1.91 at $\theta = 0.04$; the ZH for all 31 families was 1.49) and 19q (D19S601, ZH = 1.77 at $\theta = 0.1$; the ZH for all 31 families was 1.64).

Non-parametric two-point sib-pair analysis yielded 'suggestive' $p$-value for D8S1179 on 8q, which was $p = 0.002$ (the corresponding $p$ for all 31 families was 0.021) and D16S748 on 16p was $p = 0.008$ (the $p$ for all 31 families was 0.024).

The highest 'suggestive' Z-all$_{max}$ multipoint values have been obtained for chromosomes 16pter (Z-all$_{max}$ = 2.96, $p = 0.001$, at position 0 cM from 16pter); the Z-all$_{max}$ = for all 31 families was 1.85 for position 0 cM from 16pter), and 8q (Z-all$_{max}$ = 2.85, $p = 0.002$, at position 120 cM from 8pter; the Z-all$_{max}$ = for all 31 families was 1.90 for position 120 cM). In some cases 'potential, interesting' Z-all$_{max}$ values for other chromosomal regions have been observed. These are on chromosomes 15pter (Z-all$_{max}$ = 1.86 at position 109), and 17qter (Z-all$_{max}$ 1.55 at position 135) (Figure 2B). We did not further sub-categorise our sample into families with only inner or only outer dynein deficiency since the numbers of such families was too small for linkage analyses.

## Discussion

Primary ciliary dyskinesia (PCD), is a phenotype with recognised clinical variability. One example of the variable expression is that only half the patients within a given family have SI. A set of monozygotic twins has been reported in which one twin had SI and the other did not.[25] We also observed one such case in family UCL013.

PCD is likely to be a genetically heterogeneous disorder since there is a variety of ciliary abnormalities and a plethora of proteins are involved in the construction and motility of the cilia. Nearly 250 different polypeptides have been identified within the ciliary axonemes of lower organisms.[9]

Our genome-wide linkage analysis of 31 multiplex families provided strong evidence against a single locus responsible for the majority of families with PCD. Furthermore, we failed

**Table 3** Results of genome-wide linkage analysis on the PCD sub-phenotypes (SI, DAD). Both parametric and non-parametric analyses were performed on **a**, families with SI and on **b**, families with DAD. The highest scores obtained for each chromosome are given for both two-point and multipoint analyses

| | *Parametric* | | | | | | *Non-parametric* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Two point* | | | | | | *Two point* | | *Multipoint* | | |
| *Chromosome* | *Z* | $\theta$ | *ZH* | $\theta H$ | *Markers* | *Distance from pter* | P | *Markers* | *Z-all* | P | *Distance from pter* |
| **a**: Situs inversus | | | | | | | | | | | |
| 1 | 0.11 | 0.34 | 0.11 | 0.34 | D1S2141 | 235 | 0.204 | D1S2141 | 0.54 | 0.284 | 225 |
| 2 | 0.01 | 0.38 | 0.1 | 0 | D2S423 | 10 | 0.863 | D2S410 | 1.04 | 0.133 | 0 (pter) |
| 3 | 0.50 | 0.3 | 1.25 | 0 | D3S2432 | 55 | 0.0191 | D3S2459 | 1.68 | 0.039 | 120 |
| 4 | 1.57 | 0.12 | 1.57 | 0.12 | D4S2368 | 168 | 0.0028 | D4S2368 | 0.90 | 0.169 | 155 |
| 5 | 1.13 | 0.2 | 1.13 | 0.2 | D5S1471 | 186 | 0.0494 | D5S1471 | 1.45 | 0.065 | 171 |
| 6 | 0.42 | 0.22 | 0.72 | 0.02 | D6S1056 | 99 | 0.159 | D6S1056 | 0.41 | 0.322 | 0 (pter) |
| 7 | 0.44 | 0.26 | 0.44 | 0.26 | D7S559 | 193 | 0.023 | D7S559 | 0.73 | 0.217 | 194 (qter) |
| 8 | 2.43 | 0.14 | 2.51 | 0.08 | D8S1179 | 136 | 0.0012 | D8S1179 | 2.03 | 0.017 | 137 |
| 9 | 0.29 | 0.26 | 0.29 | 0.26 | D9S915 | 144 | 0.132 | D9S915 | 0.33 | 0.359 | 58 |
| 10 | 0.04 | 0.38 | 0.53 | 0 | D10S1225 | 82 | 0.266 | D10S1225 | 1.06 | 0.130 | 100 |
| 11 | 0.31 | 0.28 | 0.31 | 0.28 | D11S912 | 125 | 0.125 | D11S912 | 1.52 | 0.054 | 128 |
| 12 | 0.27 | 0.3 | 0.32 | 0.18 | D12S392 | 168 (qter) | 0.193 | D12S392 | 0.62 | 0.255 | 164 (qter) |
| 13 | 0.71 | 0.24 | 0.86 | 0.08 | D13S285 | 119 (qter) | 0.042 | D13S285 | 1.47 | 0.063 | 57 |
| 14 | 0 | 0.5 | 0 | 0.5 | D14S53 | 75 | 0.948 | D14S53 | –0.19 | 0.579 | 44 |
| 15 | 0.74 | 0.24 | 0.74 | 0.24 | D15S642 | 110 (qter) | 0.073 | D15S642 | 1.45 | 0.064 | 109 (qter) |
| 16 | 1.38 | 0.14 | 1.38 | 0.14 | D16S403 | 37 | 0.0098 | D16S403 | 1.49 | 0.059 | 0 (pter) |
| 17 | 1.01 | 0.24 | 1.01 | 0.24 | D17S928 | 145 (qter) | 0.037 | D17S928 | 1.58 | 0.054 | 135 |
| 18 | 0.34 | 0.26 | 0.38 | 0.18 | D18S535 | 73 | 0.095 | D18S535 | –0.47 | 0.689 | 4 |
| 19 | 2.46 | 0.14 | 2.46 | 0.14 | D19S601 | 78 | 0.007 | D19S601 | 1.71 | 0.037 | 78 |
| 20 | 0.004 | 0.4 | 0.01 | 0.02 | D20S1085 | 74 | 0.270 | D20S1085 | –0.28 | 0.605 | 58 |
| 21 | 0.002 | 0.44 | 0.002 | 0.44 | D21S1432 | 0 (pter) | 0.407 | D21S1432 | 0.33 | 0.364 | 0 (pter) |
| 22 | 0.08 | 0.34 | 0.43 | 0 | D22S683 | 34 | 0.330 | D22S683 | 0.02 | 0.489 | 34 (qter) |
| **b**: Dynein arm deficiency | | | | | | | | | | | |
| 1 | 0.12 | 0.32 | 0.12 | 0.32 | D1S3669 | 32 | 0.135 | D1S3669 | 1.37 | 0.075 | 22 |
| 2 | 0 | 0.5 | 0.06 | 0 | D2S423 | 10 | 0.904 | D2S423 | 0.63 | 0.25 | 160 |
| 3 | 0.46 | 0.3 | 0.96 | 0 | D3S2432 | 55 | 0.212 | D3S2459 | 1.19 | 0.112 | 120 |
| 4 | 1.19 | 0.16 | 1.19 | 0.16 | D4S2368 | 168 | 0.221 | D4S403 | 1.18 | 0.113 | 145 |
| 5 | 0.47 | 0.22 | 0.70 | 0 | D5S807 | 19 | 0.027 | D5S807 | 1.32 | 0.09 | 0 (pter) |
| 6 | 0.54 | 0.18 | 0.56 | 0 | D6S305 | 163 | 0.118 | D6S305 | 1.32 | 0.087 | 165 |
| 7 | 0.58 | 0.22 | 0.75 | 0.08 | D7S1802 | 25 | 0.035 | D7S1802 | 1.44 | 0.071 | 25 |
| 8 | 1.78 | 0.18 | 1.91 | 0.04 | D8S1179 | 136 | 0.002 | D8S1179 | 2.85 | 0.0017 | 120 |
| 9 | 0.59 | 0.2 | 0.59 | 0.2 | D9S915 | 144 | 0.034 | D9S925 | 1.31 | 0.094 | 18 |
| 10 | 0.16 | 0.3 | 0.16 | 0.3 | D10S1423 | 43 | 0.199 | D10S1423 | 0.77 | 0.212 | 128 |
| 11 | 0.61 | 0.26 | 0.62 | 0.22 | D11S1984 | 0 (pter) | 0.037 | D11S1984 | 1.02 | 0.147 | 0 (pter) |
| 12 | 0.05 | 0.36 | 0.13 | 0 | D12S372 | 0 (pter) | 0.207 | D12S372 | 0.67 | 0.243 | 0 (pter) |
| 13 | 0.79 | 0.24 | 0.88 | 0.12 | D13S285 | 119 (qter) | 0.061 | D13S285 | 1.42 | 0.073 | 100 |
| 14 | 0.02 | 0.38 | 0.02 | 0.38 | D14S592 | 54 | 0.383 | D14S592 | 0.08 | 0.464 | 117 |
| 15 | 0.74 | 0.24 | 0.74 | 0.24 | D15S642 | 110 (qter) | 0.020 | D15S642 | 1.86 | 0.029 | 109 (qter) |
| 16 | 1.07 | 0.22 | 1.08 | 0.18 | D16S748 | 13 | 0.008 | D16S748 | 2.96 | 0.001 | 0 (pter) |
| 17 | 0.57 | 0.28 | 0.57 | 0.28 | D17S928 | 135 (qter) | 0.04 | D17S928 | 1.55 | 0.057 | 135 (qter) |
| 18 | 0 | 0.5 | 0 | 0.5 | D18S843 | 33 | 0.426 | D18S843 | –0.23 | 0.592 | 0 (pter) |
| 19 | 1.61 | 0.18 | 1.77 | 0.1 | D19S601 | 78 | 0.015 | D19S601 | 1.23 | 0.104 | 78 |
| 20 | 0.10 | 0.26 | 0.24 | 0 | D20S1085 | 74 | 0.162 | D20S1085 | –0.33 | 0.63 | 59 |
| 21 | 0.02 | 0.38 | 0.02 | 0.38 | D21S1432 | 0 (pter) | 0.372 | D21S1432 | 0.71 | 0.237 | 0 (pter) |
| 22 | 0.11 | 0.32 | 0.34 | 0 | D22S683 | 34 | 0.375 | D22S683 | 0.54 | 0.283 | 34 |

to detect the existence of only two major loci, each of which could be responsible for at least 40% of the families with PCD. As it is apparent from the power analysis, our sample is sufficient for the detection of two major loci. Our results strongly suggest that PCD is a very heterogeneous autosomal recessive disorder with three or (most likely) more genes responsible for this phenotype.

The failure to detect a significant lod score in this study could also be due to erroneous assignment of the phenotype. It is, however, unlikely that many affected individuals would be wrongly diagnosed since we used strict criteria for diagnosis. The normal phenotype could also have been wrongly assigned due to reduced penetrance, but the misclassification of a few individuals would not have had a significant effect on the resulting scores.

It is likely that PCD is a heterogeneous condition because the cilium structure is an assembly of numerous distinct proteins, primarily dyneins, MAPs and tubulins.[7] This has

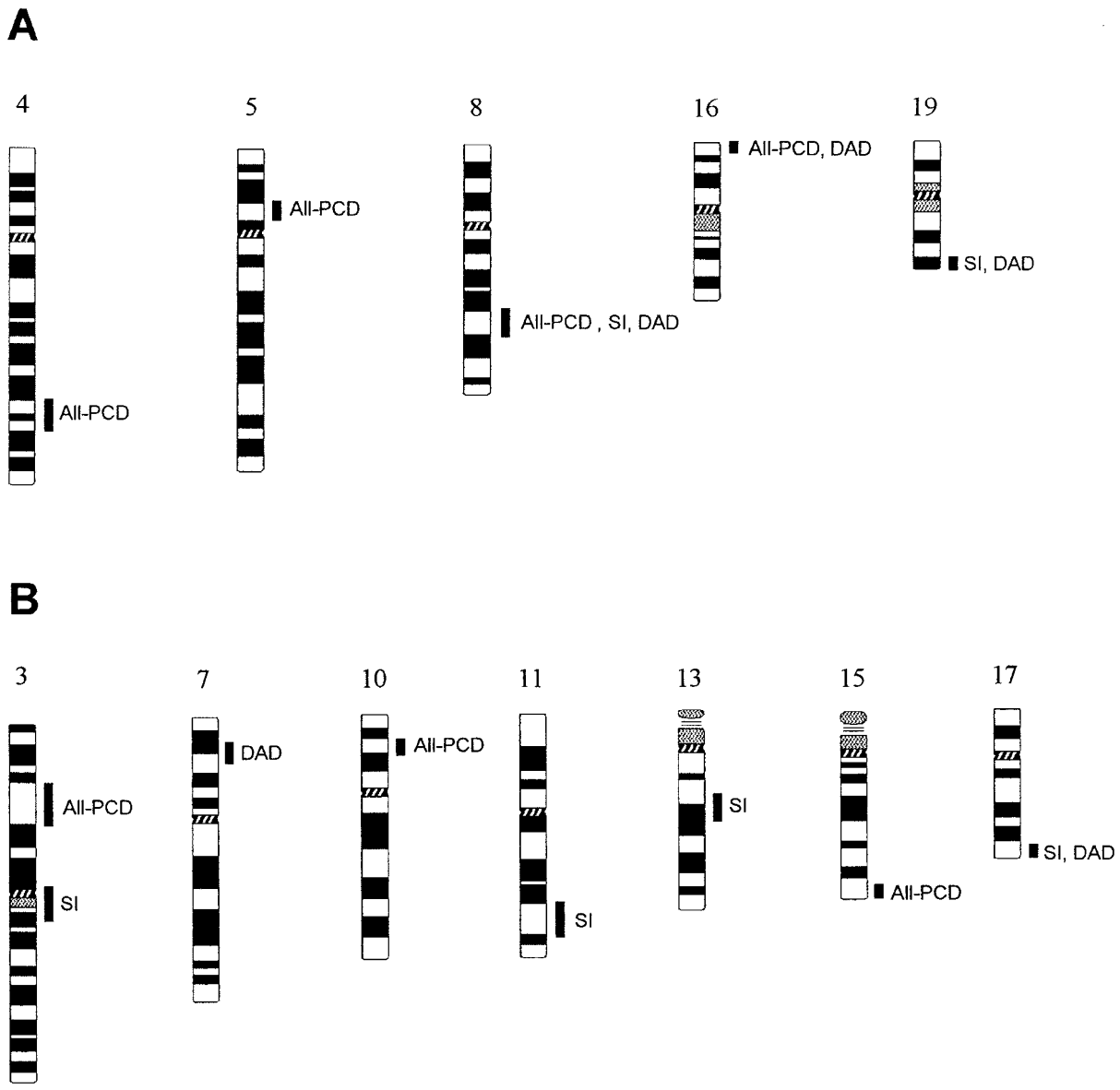


**Figure 2** Schematic representations of the regions with the highest scores obtained from the genome-wide linkage analysis on the PCD families. Scores are classified as suggestive (**A**) or 'potential, interesting' (**B**). Results are shown for all 31 multiplex PCD families (All-PCD), in subgroups of PCD families with situs inversus (SI or PCD with dynein arm deficiency DAD).

been documented in *Chlamyodomonas*, in which immotile strains are associated with many distinct, heritable mutations;[27,28] in that study 12 loci were responsible for defects in the assembly of the outer dynein arms. In our study we used not only parametric, but also non-parametric linkage analysis. This is apparently peculiar given the recessive mode of inheritance in PCD. However, we used the non-parametric linkage because of the uncertainty of the mode of inheritance in some families, the uncertainty of the penetrance of the phenotype, the possibility of digenic inheritance or more complex heritability, and the probable genetic heterogeneity.

Another potential explanation for the failure to detect a major locus in PCD is the assumption of the mode of inheritance. It is possible that, in some families, PCD is not a classic recessive phenotype but rather a digenic disorder in which the co-occurrence of two non-allelic mutations in two different genes is required for the phenotype. In this case the non-parametric analysis would theoretically detect the contribution of some of these loci. The only documented

disorder of digenic inheritance is that of retinitis pigmentosa due to mutations in the unlinked genes *ROM1* and peripherin/*RDS*.[29] There are rare cases with dominantly inherited PCD[30,11] and in our sample families GVA13 and UCL07 showed modes of inheritance compatible with non-recessive models. The digenic mode of inheritance is also compatible with some cases of apparent dominant inheritance.

We observed differences in the linkage results between the PCD–SI or PCD–DAD groups of families versus all PCD families. These differences may be due to the possibility that the PCD–SI or PCD–DAD families belong to a less heterogeneous group, or that the genes contributing to the PCD–SI or PCD–DAD phenotypes are different from those in families with PCD only. Witt *et al*[31] performed linkage analyses using microsatellite markers spanning chromosome 7 in 30 PCD Polish families. They concentrated on chromosome 7 because of a case with uniparental disomy of chromosome 7 and a phenotype of cystic fibrosis, complete SI, and immotile (although ultrastructurally normal) bronchial ciliary apparatus.[32] Pairwise and multipoint lod scores for 17 microsatellite markers on chromosome 7 provided no support for linkage. However, pairwise and multipoint lod scores for families with ciliary dysfunction only (without SI) detected a possible linkage to chromosome 7. The highest multipoint lod score was 1.41 at 7p15 (markers D7S493 or D7S629) where a gene for the heavy chain of the outer dynein arm has been localised.[33] In our study, no evidence for linkage has been obtained for chromosome 7p using all families, or families with SI. However, it is intriguing that all the Z-all$_{max}$ for the DAD families on chromosome 7 was 1.44 for a region of 25 cM from 7pter.

The different dynein genes are excellent candidates for the phenotypes of PCD. Abnormalities in the inner or outer dynein arms are common in PCD. In addition, a missense mutation Glu-to-Lys in a highly conserved amino acid between the second and third P-loop of the axonemal dynein heavy-chain gene, left/right-dynein (Lrd) was found in both legless (lgl) and inversus viscerum (iv/iv) mice.[34] In humans 18 different dynein gene sequences have been partially characterised and six have been mapped to different chromosomes (3p21–p14, 5p or 8q, 6p21.3, 7p, 17p, 17q25) (http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/). These include 13 axonemal heavy chain polypeptides, and 6 axonemal or cytoplasmic heavy, intermediate and light polypeptides.[35–40] No full length cDNA sequence of any of the human axonemal heavy chain dynein genes has yet been published and no deleterious mutations have been reported. The concordance of dynein mapping positions with the PCD linkages are found on chromosomes 3p (ZH = 1.37), 5p (ZH = 2.197, 2-point sib pairs $p = 0.0071$, Z-all$_{max}$ = 1.32), 8q (ZH = 1.49, multipoint Z-all$_{max}$ = 1.90) and 17q (ZH = 1.0, Z-all$_{max}$ = 1.40).

Additional candidate genes for PCD include those encoding the ciliary proteins such as tubulins, connexins, kinesins, nexins and microtubule-associated proteins (MAPs).[41–45] Furthermore, human homologues of mouse genes, mutations in which cause left–right axis malformations in mice, are excellent candidates for PCD. These include the human nodal on chromosome 10q21–q23, HFH-4 on 17q25, inv (either on 6q21.1–q23 or 9q33), Acrv2b (activin receptor type IIB) on 3p22–p21.3, Pitx2 (pitituary homeobox) on 4q25–q26.[46–50] From our results on the SI subgroup, chromosome 10q yielded Z-all$_{max}$ = 1.06, 17q a Z-all$_{max}$ = 1.58, 3p a ZH = 1.25, and 4q ZH = 1.57. These results do not exclude the possibility that some of these genes are responsible for PCD in certain families.

Another excellent candidate for PCD-SI is ZIC3, since its mouse homologue is mutated in a murine SI model.[51] Analysis of the SI subgroup with a marker from the pseudoautosomal region of chromosome X (DXYS1107) showed a poor lod score to Xq25–26 (ZH = 0.7 at $\theta = 0.0$).

The identification of PCD loci may require a much larger sample size and a genome-wide scan of a much denser set of polymorphic markers. Also, homozygosity mapping in large consanguineous families, or in population isolates[52] may reveal the chromosomal position of some PCD genes. Another option is to perform mutation analysis in all candidate genes encoding proteins that either contribute to the structure or motility of the cilia, or are responsible for ciliary or laterality phenotypes in the mouse or other organisms (*Chlamydomonas*, *Xenopus*).[28,34,53] These studies will undoubtedly elucidate the genetics and pathophysiology of PCD and may establish interesting novel aspects of monogenic or oligogenic inheritance.

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