ELECTRONIC LETTER

Linkage analysis localises a Kartagener syndrome gene to a 3.5 cM region on chromosome 15q24–25

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Background: Primary ciliary dyskinesia (PCD) is a genetic disorder caused by ciliary immotility/dysmotility due to ultrastructural defects of the cilia. Kartagener syndrome (KS), a subtype of PCD, is characterised by situs inversus accompanying the typical PCD symptoms of bronchiectasis and chronic sinusitis. In most cases, PCD is transmitted as an autosomal recessive trait, but its genetic basis is unclear due to extensive genetic heterogeneity.

Methods: In a genome-wide search for PCD loci performed in 52 KS families and in 18 PCD families with no situs inversus present (CDO, ciliary dysfunction-only), the maximal pairwise LOD score of 3.36 with D15S205 in the KS families indicated linkage of a KS locus to the long arm of chromosome 15. In the follow-up study, 65 additional microsatellite markers encompassing D15S205 were analysed.

Results: A maximal pairwise LOD score of 4.34 was observed with D15S154, further supporting linkage of the KS, but not the CDO, families to 15q24–25. Analysis of heterogeneity and haplotypes suggested linkage to this region in 60% of KS families.

Conclusions: Reinforced by the results of multipoint linkage, our analyses indicate that a major KS locus is localised within a 3.5 cM region on 15q, between D15S973 and D15S1037.

Primary ciliary dyskinesia (PCD), formerly known as immotile cilia syndrome (MIM 244400; 242650), is a systemic disease caused by inherited dysfunction of the ciliary apparatus.^{1,2} Analogous to mitochondrial, lysosomal, and peroxisomal diseases, PCD therefore belongs to a group of disorders involving cellular organelles.³ The clinical consequences of PCD cover a wide spectrum and mainly affect the lower and upper airways and the male reproductive system. In general, prognosis remains good, but morbidity can be considerable if the disease is not correctly managed.⁴ All forms of PCD are characterised by dysmotility or immotility of cilia in airway epithelial cells, spermatozoa, and other ciliated cells of the body.^{3,5,6}

Estimates of PCD incidence range from 1/16 000 to 1/60 000 live births.⁵ Half of all PCD cases are classified as Kartagener syndrome (KS, 1/32 000 and 1/120 000 live births), where bronchiectasis and chronic sinusitis are also accompanied by situs inversus (SI), a reversal of the usual left-right asymmetry of the abdominal and thoracic organ locations.

All forms of PCD are characterised by dysmotility or immotility of cilia in airway epithelial cells, spermatozoa, and other ciliated cells of the body.^{3,5,6} The cases of ciliary dysmotility or immotility reported to date are associated with ultrastructural defects of the cilia, such as a total or partial

absence of dynein arms (70–80% of all electron microscopy detectable defects), defects of radial spokes or nexin links, and general axonemal disorganisation with microtubular transposition.⁵ Discoordination of ciliary function can also be caused by random orientation of the cilia.⁷ Inheritance of PCD in most families is autosomal recessive, although pedigrees showing autosomal dominant or X-linked modes of inheritance have also been reported.^{8–10} Although within families PCD appears to be transmitted via a single locus, a broad spectrum of ciliary ultrastructural defects clearly suggests that PCD is genetically heterogeneous.

The molecular basis of PCD is far from being clear. Up to 250 different polypeptides have been identified within the ciliary axoneme of the model unicellular alga *Chlamydomonas reinhardtii*; at least the same number of proteins can be expected in the axonemes of humans.¹¹ It is rather unlikely that mutations within as many as 250 different genes coding for various ciliary proteins cause the same or similar pathologic consequences of ciliary dysfunction. If this were true, one might expect the incidence of PCD to be much higher than it actually is.³ It is possible that mutations in some ciliary protein gene(s) may be lethal even if heterozygous, while mutations in other genes may not affect ciliary function at all. Hence, one would expect involvement of a limited number of genes in the pathogenesis of this disorder. This potential reduction of locus heterogeneity provides cautious optimism for the success of gene mapping efforts.

So far, only two known human genes, *DNAI1* and *DNAH5*, have a documented causative effect in PCD. *DNAI1*, located in 9p13–21, codes for the intermediate chain of the axonemal dynein and is an ortholog of the IC78 dynein gene in *Chlamydomonas*.^{12–14} *DNAH5*, located in 5p15–p14, codes for the heavy chain of the axonemal dynein and is related to the *Chlamydomonas* gene coding for the dynein γ -heavy chain.¹⁵ Mutations in both these genes cause an outer dynein arms defect phenotype. The relatively broad spectrum of low frequency mutations in both these genes enhances the genetic heterogeneity of PCD. Together, mutations in *DNAI1* and *DNAH5* have been shown to account for ~24% of PCD.¹⁶

Obviously, mutations in genes other than *DNAI1* and *DNAH5* must be responsible for most PCD.

In the past, a number of genome-wide scans in PCD families have been performed, resulting in several chromosomal regions being considered as candidates for PCD gene(s) locations. A genome-wide, low density linkage search performed in 31 PCD families revealed potential PCD loci on 3p, 4q, 5p, 7p, 8q, 10p, 11q, 13q, 15q, 16p, 17q, and 19q, but failed to identify a major disease locus, confirming extensive locus heterogeneity.¹⁷ A genome scan in five PCD families of Arabic origin (four with reported consanguinity) indicated a possible PCD locus on 19q13.3.¹⁸ Another genome-wide scan and subsequent fine mapping in Faroe Island and Israeli

Abbreviations: CDO, ciliary dysfunction-only; KS, Kartagener syndrome; PCD, primary ciliary dyskinesia

Druze families pointed to two PCD linked loci, on 16p12.1–12.2 and 15q13.1–15.1, respectively.¹⁹

In our previous study on Polish PCD families, linkage analysis performed separately in families with KS and families with CDO (ciliary dysfunction-only, that is PCD not accompanied by situs inversus), indicated that these two subtypes of the disorder are caused by genes located in different genomic regions.²⁰ We excluded linkage of a KS locus to chromosome 7,²⁰ contrary to the suggestion of other authors.²¹ At the same time, however, our results provided suggestive evidence of linkage to 7p15 in the non-KS subset of Polish PCD families. Subsequently, *DNAH11*, the gene for the axonemal dynein heavy chain, has been mapped to the 7p15 region,²² but its involvement in the pathogenesis of PCD remains unclear.

Here we report the chromosomal localisation of a putative KS locus based on linkage analysis in Polish PCD families. The results of both pairwise and multipoint analyses suggest that ~60% of the KS families are linked to a 3.5 cM region on 15q24–25. In contrast, the CDO families are excluded from linkage to chromosome 15, thus demonstrating locus heterogeneity for PCD in general.

METHODS

We recruited a total of 70 PCD families, 66 from Poland and four from Slovakia. The families studied in this report did not come from a genetic isolate. Each family had at least one member diagnosed as having PCD with no other major anomalies or dysmorphologies present. In 60% of the families, the clinical diagnosis was confirmed by transmission electron microscopy (EM) analysis of bronchial cilia ultrastructure; of 46 PCD patients analysed by EM, 32 displayed outer and inner dynein arms defects, 11 outer dynein arms defects, and three inner dynein arms defects.

Families were classified as KS if at least one PCD affected member exhibited situs inversus (52 families), or as CDO if none of the affected members exhibited situs inversus (18 families). The mode of inheritance in all families studied was consistent with autosomal recessive transmission (that is, both sexes affected at comparable frequencies, no affected parents or distant relatives). In the KS families, 60 affected subjects (including eight independent pairs of affected siblings and one affected trio) and 139 unaffected family members were genotyped. One KS family with a consanguinity loop, initially included in the study group, was excluded from further calculations after haplotype analysis. In the CDO families, 21 affected subjects (including three independent pairs of affected siblings) and 39 unaffected members were analysed.

A whole genome scan with 462 microsatellite markers (11–32 per chromosome) was performed. Markers were distributed with a mean intermarker distance of ~8 cM. There were nine intervals between adjacent markers greater than 20 cM; the three largest intermarker intervals were 35, 26, and 26 cM. In the extended analysis of chromosome 15q, 62 marker loci encompassing D15S205 were added, with a mean heterozygosity of 0.7 and intermarker distances of 0–2 cM. Markers were assigned genetic map positions using the Decode map.²³ For markers positioned at exactly the same location or not included in the Decode genetic map,²³ the latest Ensembl assembly (v27.35a.1) was used to determine marker order and to estimate genetic map distances for multipoint linkage analyses.

All PCR amplified microsatellite markers were genotyped using fluorescence based, semi-automated DNA sizing technology,²⁴ based on Applied Biosystems 373 Automated DNA Sequencers and GENESCAN and GENOTYPER software (Applied Biosystems, Perkin Elmer, Foster City, CA).

Pairwise LOD score analyses were performed using FASTLINK.²⁵ Multipoint LOD scores allowing for locus heterogeneity were calculated using HOMOG on data generated by LINKMAP.²⁶ Multipoint LOD scores for markers on chromosome 15 were calculated using GENEHUNTER.²⁷ We assumed a recessive mode of inheritance with full penetrance, 0.0001 phenocopies, and a PCD disease allele frequency of 0.005.²⁰ These assumptions were designed to yield a population prevalence for the disease consistent with that reported for PCD. A model with no phenocopies was used to determine heterogeneity in the analysed KS families. Haplotypes were generated using GENEHUNTER software and verified manually.

RESULTS

To assess the statistical power of our collection of PCD families, we calculated the maximum pairwise LOD score for a theoretical marker locus with nine alleles and a heterozygosity of 0.73, linked at different θ 's to the disease locus. The results indicated that linkage would be detected resulting in a LOD score >3.3 (at $\theta=0$ and assuming locus homogeneity) with both the entire collection of 70 PCD families and with the subset of 52 KS families, but not with the 18 CDO families (the expected maximal LOD score in the CDO subset was below the value of 2, reflecting the low power of detecting linkage in those families).

A genome-wide scan was performed with 462 microsatellite markers. In the 52 KS families, one single marker locus on the long arm of chromosome 15 (D15S205) revealed a LOD score >2 ; the maximal LOD score value for D15S205 was 3.36, at $\theta=0.05$. As expected, none of the markers showed pairwise LOD scores >2 in the set of 18 CDO families; combining both subsets (that is, the KS and CDO families) resulted in pairwise LOD scores below the level of statistical significance. This preliminary analysis was followed by typing the 52 KS families with 62 additional microsatellite markers spanning ~20 cM surrounding D15S205, roughly corresponding to 21 Mb of the total physical distance. The highest pairwise LOD score value of 4.34 (at $\theta=0$) was obtained for D15S154 located on 15q24–25 (table 1).

To examine whether all the analysed KS families were likely to be linked to this chromosome 15q region, we performed tests of heterogeneity (HOMOG software). The test results supported heterogeneity (H2: linkage, heterogeneity ν H1: linkage, homogeneity; likelihood ratio 487.33) indicating that chromosome 15q is likely to be involved in 60% of the KS families. Six pedigrees out of 52 were indicated as being unlinked to the region. Analysis of haplotypes (not shown) revealed that in four of those pedigrees affected and unaffected siblings shared both parental haplotypes, while in two other pedigrees affected siblings had different parental haplotypes. Another 15 pedigrees were not informative and were eliminated from further calculations. Analysis of the remaining 31 pedigrees confirmed linkage under homogeneity (H1: linkage, homogeneity ν H0: no linkage; likelihood ratio 8.511×106).

Results of the pairwise LOD scores obtained for the 31 KS families “homogenised” for their linkage to 15q are shown in table 1. The highest pairwise LOD score value (5.75) was obtained for D15S1005. The result of the multipoint linkage analysis performed on the homogenised set of 31 KS families (fig 1) confirmed the pairwise LOD score results and excluded close linkage of more distantly located marker loci with KS. The multipoint LOD scores reduced abruptly at the genetic map positions 80.8 and 87.2 cM (at markers D15S991 and D15S115, respectively). The 95% confidence interval ($\text{LOD}_{\max}-1$) localised the putative KS gene to a 3.5 cM region between D15S973 and D15S1037.

Table 1 Pairwise linkage findings for the high density follow up on chromosome 15 in the KS families

Marker*	cM	LOD-all†	θ-all†	LOD-31fam‡	θ-31fam‡
D15S967	75.67	0.702202	0.15	1.546949	0.1
D15S131	76.52	0.720699	0.1	2.470191	0.05
D15S204	77.60	1.466857	0.05	2.880023	0.05
D15S197	77.69	0.851782	0	2.018382	0
D15S124	78.37	0.278873	0.15	1.658671	0.05
D15S192	78.38	1.036385	0.1	2.333415	0.05
D15S818	79.03	1.016548	0.1	2.980691	0.05
D15S984	80.37	2.860638	0.05	3.80305A	0.05
D15S989	83.75	0.445265	0.15	2.075293	0
D15S1005	84.19	2.339443	0.05	5.758866	0
D15S969	84.31	1.373751	0.05	3.693551	0
D15S1037	85.81	2.860638	0.05	3.80305	0.05
D15S1047	85.83	1.180228	0.05	2.358563	0.05
D15S211	85.84	2.248682	0.05	3.033275	0.05
D15S206	87.02	2.35253	0	2.024841	0.05
D15S115	87.24	3.641433	0	2.124683	0.1
D15S205	89.09	3.364761	0.05	4.176748	0.05
D15S154	89.10	4.344967	0	2.636754	0.05
D15S653	89.22	1.681151	0.05	1.591634	0.1
D15S539	89.22	1.651598	0.05	2.47482	0.05
D15S152	89.30	3.506446	0	2.372452	0.1
D15S972	89.31	2.727519	0.05	3.466998	0
D15S999	89.30	1.290613	0.05	2.626094	0.05
D15S979	93.21	2.15897	0.05	2.175463	0.1
D15S1045	94.83	2.358117	0	2.914872	0
D15S116	95.49	2.829704	0.05	2.067284	0.1
D15S127	97.26	0.775935	0.1	1.525221	0.1

*Only markers with LOD>1.5 are shown; †calculated in 52 KS families; ‡calculated in 31 KS families (without the six unlinked and 15 uninformative families).

Interestingly, no extended common haplotype encompassing marker D15S1005 could be detected among the affected members of different KS families (fig 2). Two longest common haplotypes, one encompassing three markers to the left and two right of D15S1005, and another encompassing five markers to the right of D15S1005, were shared by only two families each. Even if only three markers including D15S1005 were considered, a common haplotype was shared by at most four families. This contradicts the notion of the ancestral haplotype carrying the major common mutation.

Both pairwise and multipoint LOD scores calculated for the set of 18 CDO families were negative for the entire KS region, excluding close linkage of this region to PCD without situs inversus. Even when genetic heterogeneity within the CDO subgroup of PCD was assumed, allowing only some of the families to be linked to this region, the maximum pairwise LOD scores for all markers in this region were still non-significant (not shown).

DISCUSSION

In our previous study on Polish PCD families, we excluded linkage of a KS locus to chromosome 7,²⁰ contrary to the suggestion of other authors.²¹ At the same time, however, our results provided suggestive evidence of linkage to 7p15 in the subset of PCD families without situs inversus. Our disparate results for linkage of PCD to chromosome 7 in the KS and CDO families¹¹ indicated that the genetic basis of these two forms of PCD might be different, consistent with some postulated animal models such as the *hop* mouse mutation, which exhibits CDO without situs inversus.²⁸ In a search for putative PCD genes on other candidate chromosome(s), we examined our collection of PCD families after splitting them into subsets of KS and CDO families. Both the genome scan and the follow-up linkage analyses confirmed linkage to chromosome 15q in the KS but not in the CDO families. Importantly, when both subsets of PCD families, that is, the KS and CDO families, were combined, the pairwise LOD

Figure 1 The multipoint LOD score plot for 31 KS families. Disease gene frequency 0.005; penetrance 1.0; phenocopies 0.0.

Figure 2 Segregation of haplotypes in six selected families, thus narrowing the region of interest.

scores for the markers that were significant in the KS families, dropped below the level of statistical significance. This indicated that the failure to find linkage in CDO families, rather than merely reflecting the low number of families,

revealed the real lack of linkage of the analysed chromosome 15 region to CDO. These gene mapping findings are consistent with the hypothesis that there is no single gene of major effect that controls the risk for both forms of PCD.

Had such a gene existed, the combined set of the KS and CDO families would have yielded stronger support for the linkage.

Linkage analysis in genetically heterogeneous recessive disorders presents a difficult task. Large pedigrees with many affected persons are rare and heterogeneity obscures the linkage signal by introducing noise from unlinked families. We have successfully applied a strategy that was used in mapping Bardet-Biedl syndrome, a heterogeneous recessive disorder with eight loci reported so far.^{29–30} Once the threshold of LOD score value showing significant linkage to chromosome 15q was reached and confirmed in the follow up analysis with more markers, we focused our efforts on homogenising the analysed set of KS families. Statistical analysis of heterogeneity, performed under a more stringent disease model with full penetrance and no phenocopies, indicated six families with negative LOD scores in the D15S1005 region. Excluding those families resulted in the increased LOD scores for D15S1005 and its closest neighbours and enabled us to narrow the region containing the putative KS gene. As can be seen in table 1, by removing the unlinked families we eliminated noise from our data: LOD scores maximise at $\theta = 0$ for the immediate neighbours of D15S1005 and at $\theta = 0.05$ for the more distant markers within 10 cM.

Another genome-wide scan and subsequent fine mapping in Israeli Druze PCD families pointed to a PCD locus at 15q13.1–15.1.¹⁹ Although in Druze families several patients exhibited situs inversus and partial loss of inner dynein arms, the 15q region implicated in KS is located proximally from the region identified in this study.

The physical size of the KS candidate region defined by the physical map position of the markers D15S973 and D15S1037 is ~2.8 Mb. This candidate region contains 35 genes and coding sequences, but no known functional candidate genes with a clear involvement in the ciliary structure and/or relevant to KS pathogenesis were identified among them. However, a number of interesting candidates reside in this region. The gene coding for the cellular retinoic acid binding protein 1 (*CRABP1*), located at 15q24, is one of the potential candidates. Retinoic acid is an important factor during development of the organism.³¹ It is involved in promoting development of ciliated cells in lungs,³² and also plays a role in spermatogenesis; moreover, changes in its concentration in the murine embryo may lead to situs aberrations.³³ Other potential candidates include the *MESDC1* and *MESDC2* (mesodermal development candidates 1 and 2) genes located at 15q25: *MESDC1* might be involved in anterior-posterior axis development in mouse.³⁴ The potential role of these genes in the pathogenesis of KS remains to be elucidated.

In conclusion, our linkage analysis confirms the concept of locus heterogeneity in the pathogenesis of PCD, at least when its different forms – with and without situs inversion – are considered. Most likely localisation of the purported KS locus is within the 3.5 cM region on 15q24–q25.

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REFERENCES

- 1 **Witt M.** *DNAI1* and *DNAH5* and primary ciliary dyskinesia and Kartagener syndrome. In: Epstein C, Erickson RP, Wynshaw-Boris A, eds. *Inborn errors of development: the molecular basis of clinical disorders of morphogenesis*. New York: Oxford University Press, 2004:934–45.
- 2 **Geremek M, Witt M.** Primary ciliary dyskinesia: genes, candidate genes and chromosomal regions. *J Appl Genet* 2004;**3**:347–61.
- 3 **McKusick V**, ed. #244400 Kartagener syndrome. See: Online Mendelian Inheritance in Man, 2005, <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=244400>.
- 4 **Bush A, Cole P, Hariri M, Mackay I, Phillips G, O'Callaghan C, Wilson R, Warner JO.** Primary ciliary dyskinesia: diagnosis and standards of care. *Eur Respir J* 1998;**12**:482–8.
- 5 **Afzelius BA, Mossberg B.** Immobile-cilia syndrome (primary ciliary dyskinesia) including Kartagener syndrome. In: Scriver C, Beaudet AL, Sly W, Valle D, eds. *The metabolic and molecular bases of inherited diseases*, 7th ed. New York: McGraw-Hill, 1995:3943–54.
- 6 **Schidlow DV.** Primary ciliary dyskinesia (the immotile cilia syndrome). *Ann Allergy* 1994;**73**:457–70.
- 7 **Rutland J, de Jongh RU.** Random ciliary orientation: a cause of respiratory tract disease. *N Engl J Med* 1990;**323**:1681–4.
- 8 **Narayan D, Krishnan SN, Upender M, Ravikumar TS, Mahoney MJ, Dolan TF Jr, Teebi AS, Haddad GG.** Unusual inheritance of primary ciliary dyskinesia (Kartagener syndrome). *J Med Genet* 1994;**31**:493–6.
- 9 **Krawczynski MR, Witt M.** PCD and RP: X-linked inheritance of both disorders? *Pediatr Pulmonol* 2004;**38**:88–9.
- 10 **Krawczynski MR, Dmenska H, Witt M.** Apparent X-linked primary ciliary dyskinesia associated with retinitis pigmentosa and a hearing loss. *J Appl Genet* 2004;**45**:107–10.
- 11 **Luck DJL, Huang B, Piperno G.** Genetic and biochemical analysis of the eukaryotic flagellum. *Symp Soc Exp Biol*, 1982;**35**:399–407.
- 12 **Pennarun G, Escudier E, Chapelin C, Bridoux AM, Cacheux V, Roger G, Clement A, Goossens M, Amselem S, Duriez B.** Loss-of-function mutations in a human gene related to *Chlamydomonas reinhardtii* dynein IC78 result in primary ciliary dyskinesia. *Am J Hum Genet* 1999;**65**:1508–19.
- 13 **Guichard C, Harricane M-C, Lafitte J-J, Godard P, Zaegel M, Tack V, Lalau G, Bouvagnet P.** Axonemal dynein intermediate-chain gene (*DNAI1*) mutations result in situs inversus and primary ciliary dyskinesia (Kartagener syndrome). *Am J Hum Genet* 2001;**68**:1030–5.
- 14 **Zariwala M, Noone PG, Sannuti A, Minnix S, Zhou Z, Leigh MW, Hazucha M, Carson JL, Knowles MR.** Germ-line mutations in an intermediate chain dynein cause primary ciliary dyskinesia. *Am J Respir Cell Mol Biol* 2001;**25**:577–83.
- 15 **Olbrich H, Häffner K, Kispert A, Völkel A, Volz A, Sasmaz G, Reinhardt R, Hennig S, Lehrach H, Konietzko N, Zariwala M, Noone PG, Knowles M, Mitchison HM, Meeks M, Chung EMK, Hildebrandt F, Sudbrak R, Omran H.** Mutations in *DNAH5* cause primary ciliary dyskinesia and randomization of left-right asymmetry. *Nat Genet* 2002;**30**:143–4.
- 16 **El Zein I, Omran H, Bouvagnet P.** Lateralization defects and ciliary dyskinesia: lessons from algae. *Trends Genet* 2003;**19**:162–7.
- 17 **Blouin JL, Meeks M, Radhakrishna U, Sainsbury A, Gehring C, Sail GD, Bartoloni L, Dombi V, O'Rawe A, Walne A, Chung E, Afzelius BA, Armengot M, Jorissen M, Schidlow DV, van Maldergem L, Walt H, Gardiner RM, Probst D, Guerne PA, Delozier-Blanchet CD, Antonarakis SE.** Primary ciliary dyskinesia: a genome-wide linkage analysis reveals extensive locus heterogeneity. *Eur J Hum Genet* 2000;**8**:109–18.
- 18 **Meeks M, Walne A, Spiden S, Simpson H, Musaffi-Georgy H, Hamam HD, Fehaid EL, Cheehab M, Al-Dabbagh M, Polak-Charcon S, Blau H, O'Rawe A, Mitchison HM, Gardiner RM, Chung E.** A locus for primary ciliary dyskinesia maps to chromosome 19q. *J Med Genet* 2000;**37**:241–4.
- 19 **Jeganathan D, Chodhari R, Meeks M, Faeroe O, Smyth D, Nielsen K, Amirav I, Luder AS, Bisgaard H, Gardiner RM, Chung EM, Mitchison HM.** Loci for primary ciliary dyskinesia map to chromosome 16p12.1–12.2 and 15q13.1–15.1 in Faroe Islands and Israeli Druze genetic isolates. *J Med Genet* 2004;**41**:233–40.
- 20 **Witt M, Wang Y-F, Wang S, Sun C, Pawlik J, Rutkiewicz E, Zembrak J, Diehl SR.** Exclusion of chromosome 7 for Kartagener syndrome but suggestion of linkage in families with other forms of primary ciliary dyskinesia. *Am J Hum Genet* 1999;**64**:313–18.

- 21 **Pan Y**, McCaskill CD, Thompson KH, Hicks J, Casey B, Shaffer LG, Craigen WJ. Paternal isodisomy of chromosome 7 associated with complete situs inversus and immotile cilia. *Am J Hum Genet* 1998;**62**:1551–5.
- 22 **Bartoloni L**, Blouin J-L, Pan Y, Gehrig C, Maiti AK, Scamuffa N, Rossier C, Jorissen M, Armengot M, Meeks M, Mitchison HM, Chung EMK, Delozier-Blanchet CD, Craigen WJ, Antonarakis SE. Mutations in *DNAH11* (axonemal heavy chain dynein type 1) gene cause one form of situs inversus totalis and most likely primary ciliary dyskinesia. *Proc Natl Acad Sci U S A* 2002;**99**:10282–6.
- 23 **Kong A**, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G, Shlien A, Palsson ST, Frigge ML, Thorgeirsson TE, Gulcher JR, Stefansson K. A high-resolution recombination map of the human genome. *Nat Genet* 2002;**3**:241–7.
- 24 **Ziegler JS**, Su Y, Corcoran KP, Nie L, Mayrand PE, Hoff LB, McBride LJ, Kronick MN, Diehl SR. Application of automated DNA sizing technology for genotyping microsatellite loci. *Genomics* 1992;**14**:1026–31.
- 25 **Schäffer AA**. Faster linkage analysis computations for pedigrees with loops or unused alleles. *Hum Hered* 1996;**46**:226–35.
- 26 **Ott J**. *Analysis of human genetic linkage*, 3rd rev ed. Baltimore: Johns Hopkins University Press, 1999.
- 27 **Kruglyak L**, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and non-parametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 1996;**58**:1347–63.
- 28 **Handel MA**. Allelism of *hop* and *hpy*. *Mouse News Lett* 1985;**72**:124.
- 29 **Leppert M**, Baird L, Anderson KL, Otterud B, Lupski JR, Lewis RA. Bardet-Biedl syndrome is linked to DNA markers on chromosome 11q and is genetically heterogeneous. *Nat Genet* 1994;**7**:108–12.
- 30 **Katsanis N**, Lewis RA, Stockton DW, Mai PM, Baird L, Beales PL, Leppert M, Lupski JR. Delineation of the critical interval of Bardet-Biedl syndrome 1 (BBS1) to a small region of 11q13, through linkage and haplotype analysis of 91 pedigrees. *Am J Hum Genet* 1999;**65**:1672–9.
- 31 **Smith SM**, Dickman ED, Power SC, Lancman J. Retinoids and their receptors in vertebrate embryogenesis. *J Nutr* 1998;**128**(2 Suppl):467S–70S.
- 32 **Million K**, Tournier F, Houcine O, Ancian P, Reichert U, Marano F. Effects of retinoic acid receptor-selective agonists on human nasal epithelial cell differentiation. *Am J Respir Cell Mol Biol* 2001;**25**:744–50.
- 33 **Van Keuren ML**, Layton WM, Iacob RA, Kurnit DM. Situs inversus in the developing mouse: proteins affected by the *iv* mutation (genocopy) and the teratogen retinoic acid (phenocopy). *Mol Reprod Dev* 1991;**29**:136–44.
- 34 **Hsieh J-C**, Lee L, Zhang L, Wefer S, Brown K, DeRossi C, Wines ME, Rosenquist T, Holdener BC. Mesd encodes the LRP5/6 chaperone essential for specification of mouse embryonic polarity. *Cell* 2003;**112**:355–67.