Division de Génétique
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# Study of the genetic etiology of Primary Ciliary Dyskinesia : A summary of progress in the identification of disease-causing genes

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Dear Collaborator,

We first contacted some of you several years ago with a call for patients for a research project on the genetic basis of the Primary Ciliary Dyskinesia (PCD), also known as Immotile Cilia Syndrome (ICS) or Kartagener syndrome.

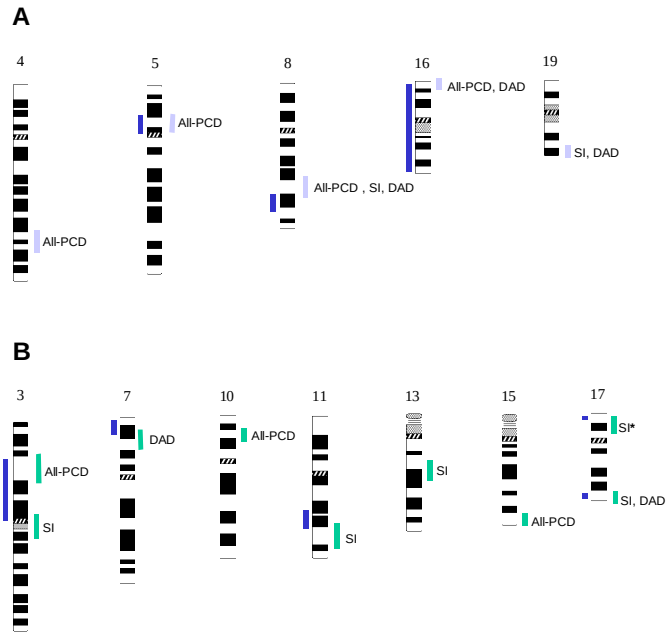
With the DNA samples which you and others provided, our major achievement to date has been the chromosomal localization of genes possibly involved in PCD; this step was accomplished through familial segregation analysis (also know as linkage analysis). We also began the investigation of candidate gene sequences in patients, in order to identify DNA mutations responsible for the disease phenotype. This part of the study is ongoing, as new gene sequences become available through our own work and through data provided by the Human Genome Project.

## Chromosomal localization of PCD Genes

Briefly, we compared the allelic DNA content of affected and non-affected individuals from 31 multiplex families (at least 2 affecteds per family), using 188 probes evenly-spaced every 19 centiMorgans of genetic distance throughout the 22 autosomal chromosomes. The probes used detected DNA microsatellite polymorphisms having a variable number of tandem repeats of simple nucleotide motifs [$(CA)_n$, $(CGG)_n$, $(GATA)_n$, etc], which can distinguish the parental origin of segments of DNA on any chromosome. This work has been published (Blouin and collaborators, Primary ciliary dyskinesia: a genome-wide linkage analysis reveals extensive locus heterogeneity. *Eur. J. Hum. Genet.2000,* 8:109-118).

The results of this analysis provide strong evidence against the proposition that a single locus is responsible for PCD in the majority of families; they support the existence of a highly heterogeneous autosomal recessive phenotype with at least 3 (most likely estimate) genes responsible for this phenotype in different families. This heterogeneity is compatible with the hypothesis that PCD can result from an anomaly in any of the multiple structural or functional components of normal cilium and the spermatozoa tail. None of the investigated loci showed highly significant values for linkage, but several chromosomal regions were identified with possible association to the disease since they co-localize with genes coding for dyneins, a major family of ciliary structural proteins. These dynein genes had been mapped to human chromosomes either directly, or indirectly by comparison with mouse localizations (Mouse-Human chromosomal synteny; figure 1). Among these potential loci, chromosomal arms 16p and 8q were highly suggestive, with localization scores approaching significance, at 2.96 and 2.85 respectively (a localization score is significant when it meets or exceeds a value of 3.00, which is the $\log_{10}$ of linkage likelihood at 1000:1). Other potential loci are suggested on chromosomes 4, 5, 19, 3, 7, 10 ,11, 13, 15, and 17 (Figure 1).

Figure 1: Results of the PCD gene localization



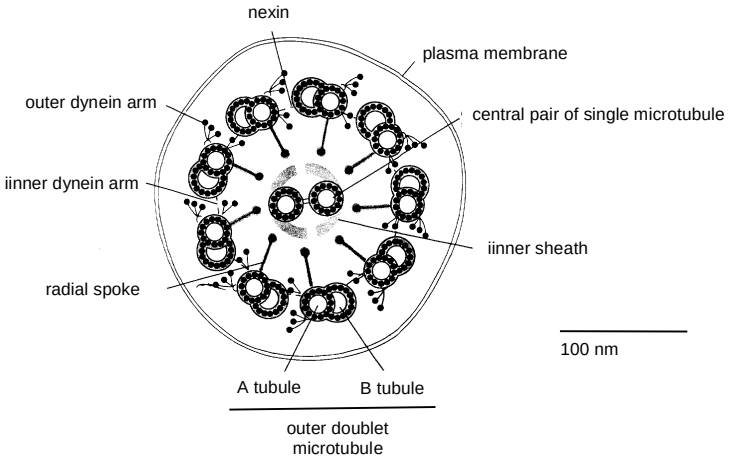

Panel A: the most convincing chromosomal loci (regions) identified
Panel B: additional candidate chromosomal loci also to be considered

Bars on the right side of each chromosome denote the family segregation findings; bars on the left side of chromosomes indicate the approximate localization of genes coding for Dyneins.

## Analysis of candidate gene sequences in patients

A second phase in research for the genes involved in the Immotile Cilia Syndrome began 2-3 years ago. We now pinpoint and isolate candidate genes, in order to screen their sequences for mutations in patients. Over the past three years we have focused on 2 of the numerous genes coding for dynein proteins (DNAH9 and DNAH11), the major component of the dynein arms that link peripheral doublets of microtubules in the cilia (see figure 2).

**Figure 2: Schematic representation of the cross section of a normal cilium (at its base)**

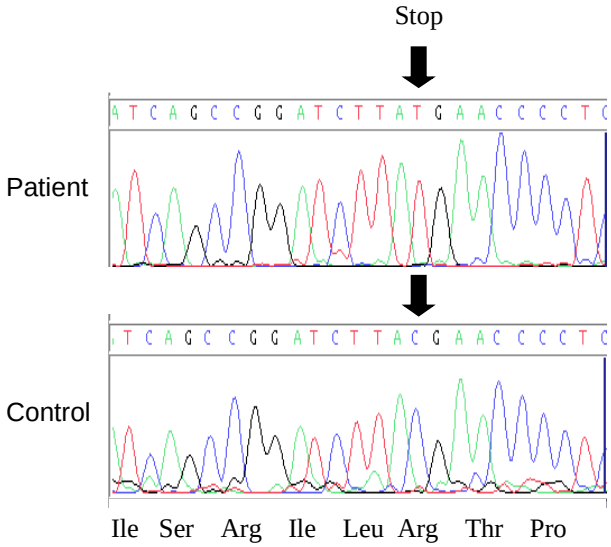



from Alberts B et al. Molecular Biology of The Cell, 2nd edition, Garland Publishing Inc., New York & London, 1989.

The DNAH9 gene maps on chromosome 17p (close to its upper extremity, or telomere) in a region that co-localized with a family segregation analysis. We characterized the full length sequence of this gene (this is one of the largest genes known in humans, with 69 exons dispersed over 400'000 nucleotides of DNA) and searched for mutations in 2 families in which the sharing of DNA alleles suggested that the disease-causing gene might be located there (see publication by Bartoloni and collaborators. Axonemal Beta Heavy Chain Dynein DNAH9: cDNA Sequence, Genomic Structure, and Investigation of Its Role in Primary Ciliary Dyskinesia. *Genomics 2001. 72:21.-33*). No really convincing DNA sequence variation (mutation) was found in these two families.

We also isolated and characterized the gene coding for dynein DNAH11, which is also a very large gene since it is composed of 78 exons. A mutation was found in one patient who inherited two identical chromosomes 7 from her father (uniparental isodisomy) and who also has cystic fibrosis due to homozygosity for the most common deletion in the CFTR gene (ΔF508). The mutation in the dynein DNA of this patient changes a nucleotide C into a T (as shown in figure 3 below) and causes truncation of the DNAH11 protein (published by Bartoloni and collaborators: The DNAH11 (axonemal heavy chain dynein type 11) gene is mutated in one form of Primary Ciliary Dyskinesia. Am.J.Hum.Genet. 67(supplement 2), oral presentation [98] 27- Annual meeting of the Americam Society of Human Genetics. 2000).

Figure 3: Mutation of the DNAH11 gene in patient with a UPD7




Finally, we have studied the Hfh4 gene, previously found to be mutated in a mouse strain with situs inversus and complete absence of cilia. A mutation search in the first 8 families did not reveal any modification of sequence that would explain the disease phenotype (published by Maiti and collaborators: No deleterious mutations in the FOXJ1 (alias HFH-4) gene in patients with primary ciliary dyskinesia (PCD). Cytogenet.Cell Genet.2000, 90:119-122).

Other candidate genes are currently under study in our laboratory and as part of an international collaboration with other research groups.

Our results are in agreement with studies from others which indicate that PCD is probably due to mutations in several, even many, genes in different patients. The dynein gene hypothesis is now even stronger given the discovery by our group of a mutation in DNAH11, and in another gene, the axonemal intermediate chain dynein gene DNAI1 on chromosome 9 by the group of S. Amselem in Paris (Published by Pennarun and collaborators: Loss-of-function mutations in a human gene related to Chlamydomonas reinhardtii dynein IC78 result in primary ciliary dyskinesia. Am.J.Hum.Genet. 65:1508-1519, 1999) and confirmed recently by another group (Guichard and

collaborators. Axonemal dynein intermediate-chain gene (dnai1) mutations result in situs inversus and primary ciliary dyskinesia (kartagener syndrome). Am.J.Hum.Genet. 2001. 68:1030-1035).

However, the genetics of PCD may not turn out to be as simple as a classical recessive single gene disorder, since defects in two distinct but interacting components of the cilium could also lead to the phenotype. This strongly suggests that we may have to test a large number of genes in many patients in order to identify most, if not all, genetic causes of PCD. With this perspective, we will need to collect a second blood sample from all affected individuals (patients only) who initially joined the study, in order to include them in the upcoming large-scale investigations. In addition, we now invite patients who are the only individuals affected in their families (sporadic cases) to participate in our research. The essential component in the advancement of this study will be the collaboration of a large number of patients, without whose help the search for PCD genes will be much more complicated and therefore slower.

In summary, thanks to the participation of numerous families and their physicians, the regions of the human genome carrying genes responsible for PCD have now been pinpointed. The second phase, the search for mutations in specific genes, is underway and has allowed definition of causative mutations in a handful of patients. In order to identify other responsible genes and their specific mutations--an essential step towards improving diagnosis and treatment—we need the collaboration of a larger number of individuals affected with PCD.

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