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Situs inversus and ciliary abnormalities What is the connection?

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ABSTRACT The finding of men with living but immotile sperm tails has initiated a search for the cause of the disorder. The sperm tails were found to lack dynein arms or to have some other ultrastructurally visible defect and the cilia were found to have the same defects. The disorder was hence named the immotile-cilia syndrome. Two more groups with the same clinical symptoms were later found, characterized by ciliary dysmotility or ciliary aplasia. In each group there are several subgroups. Many of the affected persons have situs inversus totalis; in some subgroups the incidence of situs inversus is probably 50%; there is, thus, a random determination of visceral asymmetry. Five hypotheses have been forwarded that attempt to explain the connection between ciliary defects and loss of laterality control. Support for, or evidence against, these five hypotheses have been sought in some animal models of the syndrome. Whereas immotile-cilia syndrome in dogs and pigs is very similar to the human one, an animal model in the rat differs from the human syndrome in that mainly the males are affected. Two animal models in the mouse differ in that one has ciliary defects but no increased incidence of situs inversus and the other has a random determination of visceral laterality and no ciliary defects. The connection between ciliary defects and random determination of laterality remains enigmatic.

KEY WORDS: *immotile cilia syndrome, Kartagener syndrome, ciliary axoneme, animal models*

An unexpected discovery was made in the mid 70's, when the spermatozoa from some men were found to be immotile and to have no dynein arms (Afzelius *et al.*, 1975; Pedersen and Rebbe, 1975). As dynein arms are the force-producing entities in cilia and flagella, which by their work make the cilia and flagella bend (Afzelius, 1959), an absence of dynein arms in these spermatozoa could be expected. This was not the surprising finding. The surprise came from some additional information.

Two of the men were brothers; one of them had situs inversus totalis. A third patient turning up at about the same time had identical dynein arm-less and immotile spermatozoa; he too had situs inversus totalis. A fourth patient examined by Pedersen and Rebbe (1975) had dextrocardia, probably also complete situs inversus, although he could not be reached for further examination, as he had changed his address (Pedersen, personal communication). Thus, of the four first persons seen to be devoid of dynein arms in their spermatozoa, three had (and have) situs inversus or at least dextrocardia, and the fourth has a brother with situs inversus. Situs inversus has a prevalence in Scandinavia of about 1 in 8000 (Torgersen, 1947, 1949). Clearly, there must be a connection between the lack of dynein arms and situs inversus. What connection?

The two Swedish men with situs inversus were further diagnosed to have Kartagener syndrome, which is defined as situs inversus, bronchiectasis and chronic sinusitis (Kartagener, 1933); the brother had the same features except that his visceral asymmetry was the normal one. The connection between the three components of Kartagener's triad have long remained puzzling and several explanations of the connection have been suggested: a malrotation of the umbilical cord (Varano and Merklin, 1960), a malrotated heart that causes a weakness of the bronchial tree (Kartagener, 1933; Miller and Divertie, 1972), an altered secretory activity of the respiratory tract (Holmes, 1979), an allergic constitution that affects the entire respiratory tract (Fornatto *et al.*, 1956), an immunological factor that causes vascular fragility (Douchez and Appel, 1975), or an environmental factor that acts during the embryological development (Gorham and Marselis, 1959). In short, all components of the airways had been suspected to be the causative factor in Kartagener syndrome – except the cilia.

With the discovery that dynein arms were missing in spermatozoa from these four men with Kartagener syndrome, it was logical to examine the structure and function of their cilia. The axoneme of the sperm tail and that of a cilium are very similar. As a first step the mucociliary clearance of the lungs was investigated. This test is performed by letting patients inhale radioactively labeled aerosols and measuring the rate of clearance of the labels from the lungs. Mucociliary clearance was found to be severely retarded, or even absent (Camner *et al.*, 1975). When cilia were observed in the electron microscope, they were found

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to have the same type of defect in their axoneme as has the sperm flagellum (Figs. 1 to 4) (Afzelius, 1976; Camner *et al.*, 1979).

A name of the disease was then proposed, the immotile-cilia syndrome (Eliasson *et al.*, 1976). It includes both persons with situs inversus and those with situs solitus (= the normal asymmetry of the viscera). Kartagener syndrome is regarded as a subgroup of the immotile-cilia syndrome. Whether the cilia in any particular case are immotile, are dysmotile, or are lacking, is impossible to determine without an examination of the cilia by electron microscopy or vital microscopy. The clinical consequences are the same in all three cases. About twenty alternative names for the syndrome and its various subgroups have been proposed since its first characterization, for instance primary ciliary dyskinesia and ciliary aplasia. The original term will be used here.

Kartagener syndrome is now interpreted as a disease caused by defective (or absent) cilia. Studies can be focused on organs that have ciliated epithelia, rather than on immunological or environmental factors, which were previously believed to be of etiological importance. The ciliated organs in man are relatively few: upper airways including nasal sinuses and cavities, lower airways with trachea, bronchi and bronchioles, middle ear, brain ventricles, oviducts and ductuli efferentes between testis and epididymis. In most cases the functions of these organs were affected, although not as seriously as could be expected a priori (review in Afzelius, 1985). Thus, some women with the disease have been able to become mothers, which shows that oviduct cilia are not mandatory for female fertility. No case of ectopic, tubal pregnancies has been noted.

Animal models

In order to understand the etiology of the immotile-cilia syndrome it is instructive to compare the human disease with some animal models.

Immotile-cilia syndrome in man

Clinical problems come from sites that have ciliated epithelia, although not from the ductuli efferentes, as far as known, and only rarely from the brain. Development of hydrocephalus has, however, been reported (Olsen, 1943; Greenstone *et al.*, 1984; Jabourian *et al.*, 1986; De Santi *et al.*, 1990; Zammarchi *et al.*, 1993) and enlargement of the brain ventricles has been reported (Afzelius, 1979). The ependymal lining the brain ventricles, and the Sylvian aqueduct in particular, is ciliated and blockage of the aqueduct is one cause of hydrocephalus.

The syndrome is a highly heterogeneous one with three main groups: ciliary immotility, ciliary dysmotility and ciliary aplasia, each with several subgroups characterized by their diverse ultrastructures. About 50% of the cases are further characterized by their situs inversus; this seems to hold true for at least some of the subgroups (review in Afzelius, 1985). Many pedigrees have been examined and indicate an autosomal recessive inheritance (Afzelius, 1981; Sturgess *et al.*, 1986). In a single pedigree the mode of inheritance seems to be either X-linked or of the autosomal, dominant type: a mother and her five male sons – the offspring of three different fathers – all have immotile-cilia syndrome (Narayan *et al.*, 1994b).

Immotile-cilia syndrome in dogs

Immotile-cilia syndrome or its sub-group Kartagener syndrome has been described from 15 breeds of dog (border collie, English setter, Doberman pinscher, springer spaniel, English sheepdog, golden retriever, English pointer, Dalmatian dog, rottweiler, miniature poodle, chihuahua, chow-chow, Chinese shar pei, bichon frise, and Gordon setter) (reviews in Maddux *et al.*, 1991 and Crager, 1992). Edwards *et al.* (1989) studied three generations of springer spaniel dogs, of which about half of those with airway problems had situs inversus and of which many had dilated brain ventricles. Heredity could be determined to be autosomal recessive. The disease was characterized by a deficiency of the outer dynein arms. The English sheepdog studied by Randolph and Castleman (1984) lacked inner dynein arms and had enlarged brain ventricles. A bichon frise male was seen to have immotile spermatozoa and to have a decreased, possibly absent, mucociliary clearance (Vaden *et al.*, 1991). Of the four puppies of a litter of English pointer, all had immotile-cilia syndrome, one had situs inversus totalis and another one isolated dextrocardia (Morrison *et al.*, 1987). Immotile-cilia syndrome in dogs is very similar to its human equivalent, except that the incidence of hydrocephalus seems higher.

Immotile-cilia syndrome in pigs

Roperto *et al.* (1991) have studied six siblings of pigs with the immotile-cilia syndrome, two of which also had dextrocardia (and probably situs inversus totalis). Most cilia sampled from the oviduct were reported to lack both dynein arms.

The WIC-Hyd mutant in rats

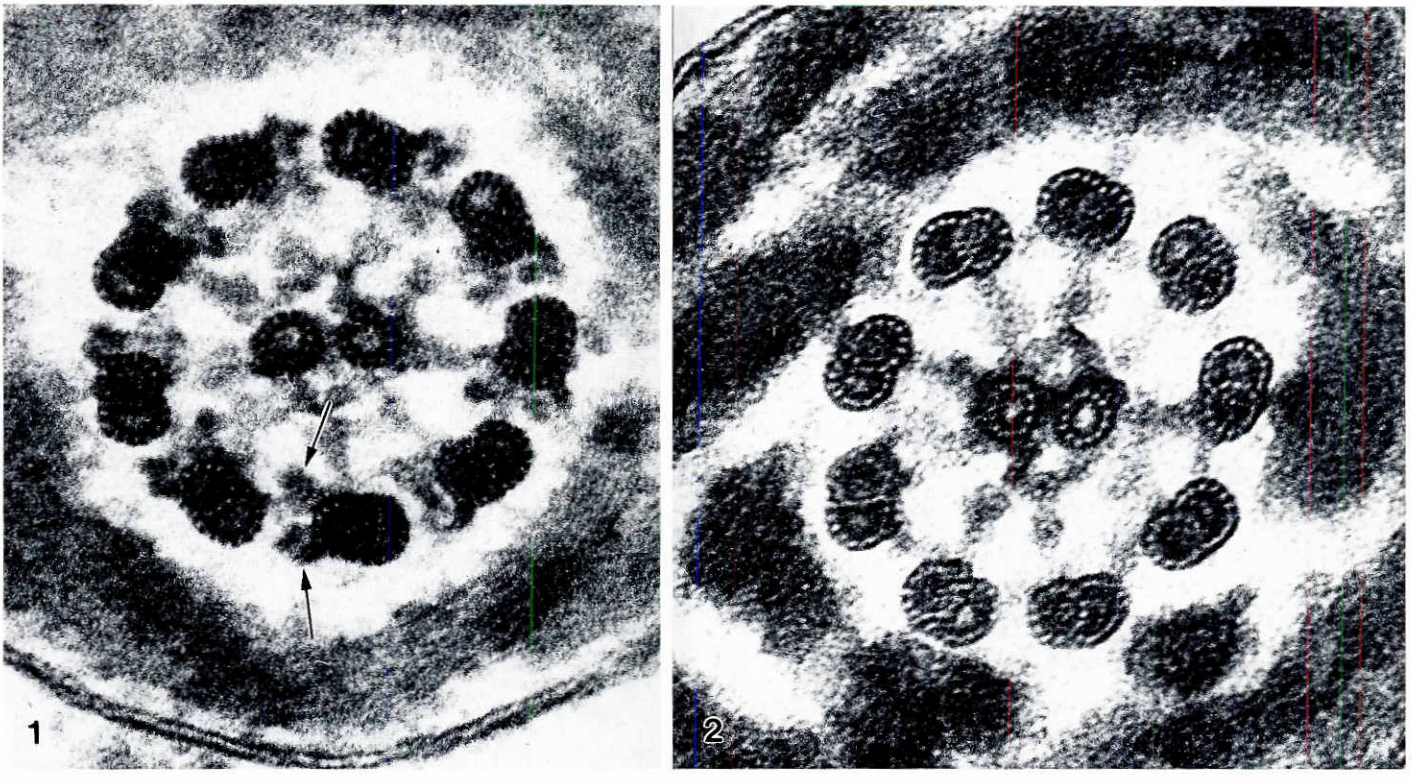
A rat mutant with spontaneous hydrocephalus has been isolated (Torikata *et al.*, 1991). In the males, hydrocephalus is severe and about half of the hydrocephalic male littermates have situs inversus. Cilia on the ependyma in the brain were found to be immotile (Nakamura and Sato, 1993), as were the respiratory cilia in males. In spite of this, male (and female) rats did not suffer from chronic sinusitis or bronchiectasis. Respiratory cilia from the females are motile. (The name comes from the Csk: Wistar-Imamichi rats and from hydrocephalus).

The iv/iv mutant of the mouse

Fifty percent of the homozygous *iv/iv* mice have situs inversus regardless of whether their parents had situs inversus or situs solitus (=normal visceral laterality) (Layton, 1976). Thus it seems that the normal allele at the *iv*-locus specifies for situs solitus, whereas in the absence of control, situs is determined in a totally random fashion. The *iv/iv* mice are derived from a strain that originally was sickly; now they are healthy and their cilia normal. The mice have no respiratory or reproductive problems. Ciliary ultrastucture has been examined and found to be normal, as is the sperm tail (Handel and Kennedy, 1984). (The name is from inversed viscera).

The hpy-mutant in the mouse

This is a mouse mutant characterized by defective cilia in trachea, oviduct and ependyma and by a defective sperm tail (Bryan, 1983). The males are sterile but there is no indication of respiratory distress in males or females. The mice have poly-



Figs. 1 and 2. A cross-sectioned sperm tail that has dynein arms (arrows) and hence a normal ultrastructure (1) and a sperm tail that lacks the dynein arms and is immotile (2). *Magnification x300,000.*

dactyly and develop hydrocephalus. No increased incidence of situs inversus has been reported from this mouse mutant. (The name comes form hydrocephaly and polydactyly).

From this short survey of animal models it is seen that ciliary defects may be inherited without involvement of situs position and the reverse.

Hypotheses on the curious connection

The puzzling feature of the immotile-cilia syndrome remains to be explained. Why do persons with congenitally immotile cilia have situs inversus of a much higher incidence than in the general population: 50% versus about 0.01% in the general population. Some hypotheses have been formulated:

1) On the various epithelia of an embryo there are cilia that have determined positions and a fixed beat direction, much the same as they have on the epidermis of amphibian embryos (Löfberg, 1974). Ciliary beating in normal embryos is assumed to be instrumental in pushing the heart to the left side, whereas chance alone will determine whether viscera will take up the normal or the reversed position during embryogenesis, when there is no regular ciliary motility (Afzelius, 1976).

2) A loss of control of the proper asymmetry of the viscera may be due to a mutation in a gene located close to, but separate from, the gene coding for a protein that is necessary for synthesis or assembly of ciliary and flagellar structures. The two defects are presumed to be genetically closely linked (anonymous, 1976).

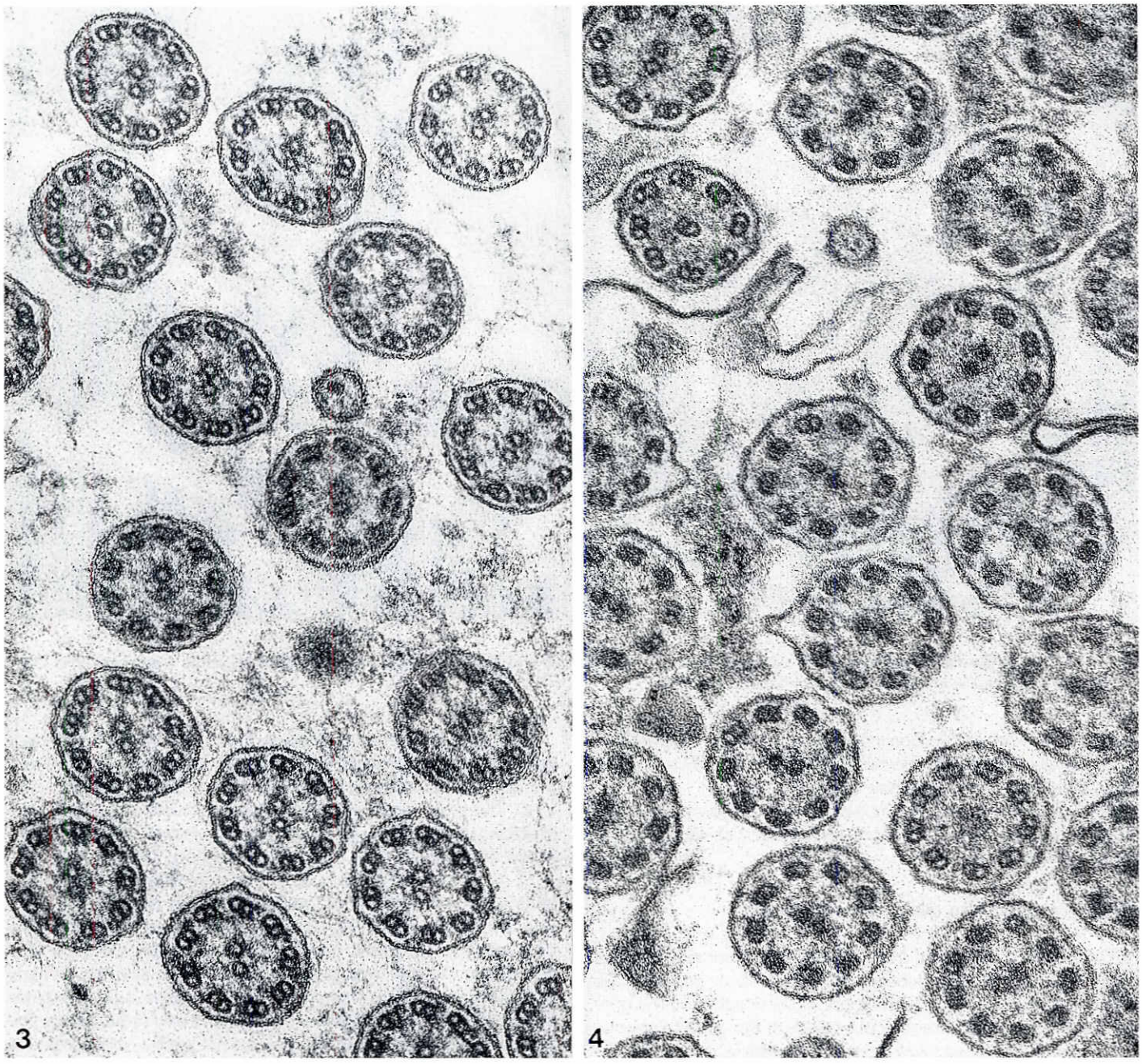
3) The basic defect resides in the cytoskeleton, which has lost its assumed ability to distinguish between right and left and thereby has lost its developmental control (Layton, 1978).

4) The embryonic cells are unable to achieve structural coordination. As a consequence hereof, orientation of the cilia becomes random rather than regular (Zanon, 1986).

5) A 'ciliation-or-division switch' is defective. Centrioles can either stay in the interior of the cell and there become the poles of a mitotic spindle, or they can move to the cell surface where they act as basal bodies for cilia. The non-ciliated cells are able to divide and grow, whereas the ciliated cells are not. In early gastrulation one side has a growth advantage over the other side. The side of growth advantage is normally regulated by the ciliation-or-division switch. In the immotile-cilia syndrome the switch is defective and growth advantage starts at random on one of the sides (Pansera, 1994).

Discussion of the hypotheses

In favor of the *first hypothesis* is its simplicity and the fact that many subgroups of the immotile-cilia syndrome are associated with situs inversus. Against the hypothesis is the fact that the trait situs inversus occurs isolated in the mouse mutants *iv/iv* and the trait ciliary defects isolated in the *hpy/hpy* mice. A test of the hypothesis was attempted in collaboration with Professor William M. Layton (Hanover, N.H., USA). We tried to find out whether there are cilia at the proper embryonic stage and proper site of the first visible asymmetry, namely close to the forming



Figs. 3 and 4. Cross-sectioned human nasal cilia that have a normal ultrastructure (3) and nasal cilia that lack dynein arms (4). *Magnification x90,000.*

heart of the 8 day-old embryo. Although epithelial cells were found to have cilia, so-called primary cilia or mono-cilia, these lacked dynein arms and are hence unlikely to be able to nudge the heart to the left side.

The *second hypothesis* is also rather simple. It will be refuted or accepted with the collection of more data on the genetics of situs inversus and of defective cilia. It is now evident that many genes exist for either of these two traits. The locations of several genes for the determination of visceral laterality are known. The *iv*-gene is thus located on mouse chromosome 12, at a locus that is equivalent to a site on human chromosome 14 q between the gene for α-antitrypsin and that for the immunoglobulin-heavy chain constant region complex (Igh-c) (Brueckner *et al.*, 1989). A gene for a cytoplasmic dynein heavy chain has recently been localized – also to human chromosome 14 q (Narayan *et al.*, 1994a). Coincidence or not?

Other genes, also involved in determination of visceral laterality, are claimed to be located on human chromosomes 10, 13

and 18 (Carmi *et al.,* 1992), chromosomes 7, 8 (Koiffmann *et al.,* 1993), and on the X chromosome (Casey, 1993). One pedigree has been described in which the trait situs inversus seems to be X-linked: 5 out of 9 boys in a generation plus 4 boys in the next generation have situs inversus, whereas the 8 girls have not (Mathias *et al.,* 1987). Some of the boys also have hydrocephalus. The cilia were seen to have a normal ultrastructure and a normal motility.

These various genes code for random determination of situs inversus. By contrast, Yokoyama *et al.* (1993) have characterized a gene in the mouse *inv,* which in contrast to the *iv*-gene seems to be a true situs inversus mutation, in that all of the homozygous transgenic mice have situs inversus. The gene for this trait is located on mouse chromosome 4 and the name of the disorder, *inv,* comes from inversed viscera.

There are more than 200 protein species in the ciliary axoneme (Luck *et al.,* 1982), so the number of ciliary genes must also be high. Their locations remain to be determined; presumably they are scattered over a wide range in the chromosome map. There is no reason to believe that the two sets of genes, those for cilia and those for laterality control, are located close together.

Support for the *third hypothesis* can be obtained from studies on the behavior of neutrophil leukocytes. According to several investigators, leukocyte migration is somewhat defective in the immotile-cilia syndrome (reviews in Palmblad *et al.,* 1984 and Afzelius, 1985). As leukocytes are devoid of cilia, the migratory abnormalities must depend on defects in parts of the cytoskeleton other than a cilium, perhaps the centriole. The defective component may either be a sensory or a motor component. A weakness of this hypothesis is that it is difficult to test.

The *fourth hypothesis* resembles the third one in that the primary defect is supposed to reside in the cytoskeleton. Ciliary insufficiency is believed to be secondary of cytoskeletal disorganization. This hypothesis provides no answer to the problem why cilia have abnormal ultrastructures.

A characteristic of the *fifth hypothesis* is that the error is thought to occur already at the gastrulation stage. Brown and Wolpert (1990) assume that there is a mechanism for converting asymmetry at the molecular level to handedness at the cellular and multicellular level and that this mechanism operates in three steps. The handedness would in principle be detectable as a gradient in the single cells, and the handedness might hence be detectable already in the zygote.

A possible route to follow in the search of the mechanism would be to isolate the gene product of the *iv*-locus and of the WIC-Hyd-locus and to explore at what stages and in what parts of the cell or of the embryonal body the gene products are found. The first step in this direction has been taken by Van Keuren *et al.* (1991), who have characterized the protein that is of importance in the determination of laterality in the *iv*-mouse.

The enigma remains. There is still no good explanation of the connection between defective cilia and a lost genetic control of visceral laterality. During the nearly 20 years that have gone since the connection was found, the problem has become more knotty. We are no longer dealing with one disease caused by one defect in one gene, but of several classes of diseases that involve different parts of the human or animal body and many different genes.

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Note added in proof:

Since the submission of this manuscript some theories about the origin of situs inversus have been proposed. Horwich and Brueckner (1993) suggest that cytoplasmic dynein associated with the spindle apparatus of the early embryo (one-cell or few-cell stage) plays a role in left-right determination. Yost (1995) assumes that left-right orientation might be due to a left-right asymmetry in the microtubules-dependent rotation that establishes the dorsoventral axis during the first cell cycle. Klar (1994) proposes that DNA replication produces different chromatids that are non-randomly segregated to the daughter cells to specify the left-right axis.

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