The genetics of fluttering cilia

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The movement of the vibrant cilia which line our respiratory airways enables inhaled impurities to be eliminated. In people suffering from primary ciliary dyskinesia (PCD), the structures of these cilia have anomalies which bring about chronic respiratory problems. Within the context of a European project, researchers at the University of Liège are studying this disease in bobtails (Old English sheepdogs), a breed of dog subject to PCD, in order to bring to light the genes which are responsible.

Often termed 'man's best friend' because of its loyalty, the services it can provide humanity or quite simply its companionship, the dog has for a long time occupied an important place at the side of human beings. In effect, besides the qualities we had already recognised in it, the dog is proving to be a particularly interesting model for research into diseases which occur in both it and humanity. That is the case for primary ciliary dyskinesia (PCD). 'This hereditary disease, which manifests itself through chronic respiratory problems is observed in both humans and various animals, notably dogs, and more specifically in certain specific breeds,' adds Professor Michel Georges, Director of the Unit of Animal Genomics at the ULg’s GIGA-Research Unit.

In Belgium around a hundred cases of primary ciliary dyskinesia are detected each year. Over the course of time, whilst the disease develops, the chronic respiratory problems of the patients become worse and worse. They are due to a poor functioning of the body's vibrant cilia, and in particular the cilia of the cells which line the respiratory system. The latter are covered with mucus and their movement forms a kind of conveyor belt called the 'mucociliary escalator.' 'This escalator takes back up the fluids which line our respiratory airways and which keep in check inhaled dirt. When it doesn't function, this dirt accumulates and that causes secondary infections,' explain the researchers. At the present time no treatment allows the primary lesions to be cured. The only thing doctors can do for the patient is to alleviate the symptoms, which are very incapacitating on a daily basis.
The vibrant cilia, present in our respiratory airways, are made up of an axis containing elements of the cytoskeleton, the axoneme, and a ciliary root which is separated from the axoneme by two structures: the basal body and the basal plate. The axoneme is made up of microtubules which follow a very precise spatial organisation. The vibrant cilia enable mucous secretions to be transported.
The vibrant cilia, a complex cog in the machinery

'One in two patients has a situs inversus, in other words a reversal in a mirror position of organs in relationship to the normal position. That is connected to the role played by the cilia in establishing organ symmetry over embryo development. When we see a situs inversus in someone, it's a sign and we think of PCD straightaway,' points out the scientist. Subsequently - or for patients which do not have a situs inversus but instead present frequent respiratory infections - a biopsy allow a diagnosis to be made/confirmed. The analysis of these ciliary cells by electronic microscope and the placing of these cells in a culture reveals if their functioning and/or structure has anomalies. For, if the movement of these cilia is not optimal in the case of a PCD, that flows from modifications which appear in their structure.

But what is a vibrant cilia made of? Briefly, a cilia is made up of nine peripheral pairs and one central pair of microtubules. Each doublet of peripheral microtubules is equipped with external and inner dynein arms, a complex protein also called a 'movement protein' (see the diagram). 'For the cilia to flutter there has to be slippage between these microtubules, which is orchestrated by the dynein,' explain the researchers. The dynein arms can in effect hang on to the preceding microtubule and thus 'bend' the cilia.

'The vibrant cilia are complex structures which contain numerous components. The defects we can observe within the structure of the cilia arise from the mutation of several genes,' adds Michel Georges.
When the bobtail lends its genome

The study of PCD in humans is complex as it does not concern a disease with a single origin, and several genes are responsible for its development. ‘It is what we call a heterogeneous disease,’ states Michel Georges. ‘The families of patients in which PCD appears have mutations in different genes. That makes studying this disease more difficult as each family is unique,’ he continues.

Individuals within the same breed being very closely related, the breed can be considered as a single large family. ‘Studying PCD in a breed of dog comes down to studying a genetically homogenous disease. The genetics of diseases within it is comfortably more simplified in comparison with humans,’ explains the professor.

In the context of the European LUPA project (‘Unravelling common human diseases using dog genetics’), Anne-Christine Merveille, Anne-Sophie Lequarré, Michel Georges and their colleagues set out about taking an interest in the genes responsible for primary ciliary dyskinesia in the bobtail. Several puppies of this breed, suffering from chronic bronchitis, were examined at the clinic of the ULg’s Faculty of Veterinary Medicine. The analysis and comparison of the DNA of bobtails affected by PCD and healthy dogs enabled the identification of a region of canine chromosome 34 linked to this disease. Ten of the 151 genes situated in this region in effect code for the proteins involved in cilia functioning. In taking the analyses further the researchers were able to more precisely put their finger on a mutation of gene CCDC39. 'In checking if the equivalent gene in
humans also had mutations in the families of patients affected by PCD, we realised that these mutations could explain 5 to 10% of the cases of patients suffering from this complaint,’ reveal the researchers. The results of this study have been published in the *Nature Genetics* journal (1).

**From prenatal diagnosis to gene therapy**

Once the mutations of CCDC39 had been detected what remained was an understanding of how they affect the movement of vibrant cilia. 'We could observe that the loss of function of this gene reduces the mobility of the cilia but we didn't know if that was due to a direct or indirect effect of the mutation.' Thanks to the functional analyses of gene CCDC39 the researchers succeeded in demonstrating that the components of the inner dynein arms and the dynein regulatory complex (DRC), an important intermediary of ciliary mobility, are linked to the expression of this gene. 'The CCDC39 gene plays a key role in the assembly of the cilia's internal structures, and its mutation affects the correct functioning of the inner dynein arms and the dynein regulatory complex,' point out the researchers. It is in this way that people who have such mutations have a sort of rigidity in the fluttering of their vibrant cilia.

'The discovery of the genes responsible for PCD has an undeniable clinical impact. The families with this disease often discover it in a brutal manner, with the birth of a child who is affected. Couples often want to know if other children will be affected or not. They don't want to see a second child suffering in such a way. Thanks to the identification of predisposition genes, they can make use of prenatal diagnosis,’ explains the researchers. In addition, in the longer term the bringing to light of such genes could permit gene therapy to be envisaged. *The dog is a privileged model for developing such a therapy*’ add the researchers.

Humans are thus now counting on the dog to better understand and treat the diseases which affect them. Besides the impact this approach could have on human health, it also serves veterinary medicine and thus once again tightens a little more the links between humanity and its 'best friend.'