

## Gene surgery **Scientists have demonstrated the feasibility of exon skipping for patients with dysferlin-deficient diseases**

Nicolas Lévy's team at the *Université de la Méditerranée* (Inserm UMR\_S 910 "Medical Genetics and Functional Genomics" research unit, Marseilles Faculty of Medicine), working closely with teams led by Luis Garcia and Vincent Mouly/Gillian Butler-Browne at the *Institut de Myologie* (UPMC/Paris 6/Inserm UMR\_S 974, CNRS UMR 7215, Pitié-Salpêtrière Hospital), has just demonstrated the relevance and feasibility of exon skipping in certain cases of dysferlin-deficient muscular dystrophy. Exon skipping – already being studied in humans for the treatment of Duchenne muscular dystrophy - may therefore open up new avenues for the treatment of other neuromuscular diseases. The research was published online on 1 December 2009 in *Human Mutation* and was notably funded by Telethon donations.

Dysferlin deficiencies represent a heterogeneous group of recessive muscular dystrophies in which the common factor is a defect in the dysferlin gene. This protein is notably involved in muscle fibre membrane repair. The most common of these diseases are type 2B limb-girdle muscular dystrophy (LGMD2B) and Miyoshi distal myopathy. The first of these primarily affects the muscles of the shoulders (the shoulder girdle) and the pelvis (pelvic girdle), while the second mainly involves the extremities of the limbs (lower legs, feet, forearms, hands).

So far, more than 350 different gene defects have been identified, with these mutations causing symptoms of varying severity depending on the patient. But in 2006, while studying a family affected by a dysferlin-deficient disease, Michael Sinnreich's team at the Montreal Neurological Institute observed that the mother of two severely affected girls was herself affected very late on and to a mild degree. On closer examination, the researchers discovered that this mother had the same mutation as her daughters on one of the two chromosomes but that on the other chromosome the exon 32 was naturally absent.

On the basis of these results, Nicolas Lévy's team at Inserm (French National Institute for Health and Medical Research) and the *Université de la Méditerranée* (Marseilles), working closely with teams from the *Institut de Myologie* (Myology Institute - Paris), came up with the idea of reproducing this natural exon skipping in the laboratory on the cells of a patient with exon 32 mutations. The aim was therefore to reproduce on these cells carrying an exon 32 mutation, a similar situation to that observed for the patient described by Michael Sinnreich's team, which was associated with very moderate impairment. To achieve this, and with the support of teams led by Luis Garcia and Vincent Mouly/Gillian Butler-Browne, they employed a variety of techniques currently being studied for dystrophin, the protein that is deficient in Duchenne and Becker muscular dystrophy, as well as other neuromuscular diseases. First of all, three synthetic antisense oligonucleotides\* were tested, leading to identification of two genome zones to be targeted to promote exon 32 skipping. The scientists then targeted these two zones using a U7 gene producing an antisense RNA, carried to the cells via a lentivirus. At the end of these experiments the scientists were able to observe a truncated RNA in which the exon 32 had been deleted in the treated cells, both with the antisense oligonucleotides alone and those vectored by a lentivirus. It was therefore possible to delete the mutation previously present in the exon 32 in the patient's cells. In other words, they had demonstrated the feasibility and relevance of exon 32 skipping.

The results of this research pave the way for a possible therapeutic trial on exon skipping for patients with defects located in exon 32. Scientists already have at their disposal a UMD-DYSF database, developed in collaboration with Christophe Bérroud's team (IURC Montpellier), containing all the mutations reported in the literature to date, more than half of which come from patients followed up in Marseilles and Paris. Using this database and the Dutch Leiden Muscular Dystrophy pages© base, doctors in Marseilles and Paris have identified around twenty patients with dysferlin deficiencies liable to be concerned for this single exon 32. Finally, although certain parts of the dysferlin gene are essential for its correct function, the scientists believe that it may be possible to "target" exons other than exon 32 using an exon skipping therapeutic approach.

\*small fragments of DNA produced synthetically in the laboratory which stick to pre-messenger RNA during its conversion into messenger RNA.

### What is exon skipping?

A gene is made up of introns and exons, with the latter designating the encoding part of the gene, which contains the information necessary for synthesis of the protein. Exon skipping consists in intervening during transcription of the gene into protein to eliminate the exons carrying genetic defects. The resulting protein is therefore truncated but functional.

### **To find out more**

#### ***"Efficient Bypass of Mutations in Dysferlin Deficient Patient Cells by Antisense-Induced Exon Skipping"***

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