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DEVELOPMENT OF THERAPIES FOR HEREDITARY DISEASES (DUCHENNE MUSCULAR DYSTROPHY, FRIEDREICH ATAXIA AND FAMILIAL ALZHEIMER DISEASE) BY MODIFYING GENES WITH THE CRISPR/CAS9 TECHNOLOGY

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The new CRISPR/Cas9 technology permits to target with a single guide RNA (sgRNA) a precise genomic sequence of 18 to 20 nucleotides followed by a Protospacer Adjacent Sequence (PAM). This permits with an active Cas9 nuclease to induce double strand breaks (DSBs) at a precise site in the genome. Friedreich ataxia is due to a reduced expression of frataxin due to an elongated GAA repeat in intron 1 of that gene. We have used an active Cas9 to remove this long GAA repeat by inducing DSBs before and after the repeat. This increased the expression of the frataxin protein. We have also used an active Cas9 to modify a mutated dystrophin gene responsible for Duchenne Muscular Dystrophy (DMD) by cutting in an exon that precedes and in an exon follows the patient deletion. This permitted to form a hybrid exon that not only restored the correct reading frame but which also coded for a dystrophin protein with an adequate spectrin-like repeat. The CRISPR technology also permits to target a promoter sequence with 1 or several sgRNAs and an inactive Cas9 protein fused with VP64 and to induce the expression of a gene. We have thus increased the expression of the alpha-1 chain of laminin by targeting its promoter. This protein formed a complex with beta-1 and gamma-1 laminin chains and made links with the alpha-7 beta-1 integrin to reduce the severity of DMD. Finally, a new variation of the CRISPR/Cas9 technology, called base editing used a Cas9 nickase fuse with a cytidine or an adenosine deaminase to chemically modify a cytidine into a thymine or an adenosine into an inosine (equivalent to guanine). We have use this technology to insert in the APP gene 3 point mutations (A673T, A673V and H684R) each individually able to reduce the formation or the aggregation of amyloid peptides. This will eventually prevent the accumulation of amyloid plaques responsible for the progression of Alzheimer disease.

Conclusion & Significance: CRISPR/Cas9 technology will permit to develop therapies for these diseases as well as for many other hereditary diseases by either inducing DSBs to modify a gene, inducing the expression of a gene or by modifying a single nucleotide.

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