Gene therapy and CRISPR/Cas9 epigenomic editing for treating neurodevelopmental disorders

Rett syndrome (RTT) is an incurable neurodevelopmental disorder caused by mutations in the gene encoding for methyl-CpG binding-protein 2 (MeCP2). We aim at establishing safe and effective gene therapy protocols to restore gene function in the brain to treat this and other neurodevelopmental diseases. By employing the AAV-PHP.eB, we delivered throughout the brain an instability-prone Mecp2 transgene cassette which limits supraphysiological Mecp2 protein levels. Intravenous injections of the PHP.eB-iMecp2 virus in symptomatic male and female Mecp2 mutant mice significantly ameliorated the disease progression with improved locomotor activity, coordination, lifespan and normalization of altered gene expression. Our objective is to advance vector design, AAV capsid engineering and viral delivery to establish safe and effective gene transfer strategies into the brain to provide new treatments for genetic neurodevelopmental disorders. Moreover, new approaches based on CRISPR/Cas9 genome and epigenome editing are actively exploited as new strategies of precision medicine with minor off-target effects. On this line, we have explored CRISPRa, a system which modulates the expression of endogenous genes by directly targeting their promoters. CRISPRa-mediated gene activation can rescue Scn1a haploinsufficiency in a mouse DS model and restore physiological levels of its gene product, the Na\textsubscript{V}1.1 voltage-gated sodium channel. We identified a specific sgRNA that increases Scn1a gene expression levels in cell lines and primary neurons with high specificity. Na\textsubscript{V}1.1 protein levels were augmented, as was the ability of wild-type immature GABAergic interneurons to fire action potentials. Our results pave the way for exploiting CRISPRa-based gene activation as an effective and targeted approach to Dravet syndrome and other disorders resulting from altered gene dosage.

References:

Keywords: gene therapy, AAV, CRISPR/Cas9, genome editing, neurodevelopmental disorders.

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