MECP2 gene therapy for Rett syndrome: adding regulation.

Ralph D. Hector[1,2], Andrew McCracken[1,2], Mark E.S. Bailey[2], Stuart R. Cobb[1]

[1]Institute of Neuroscience and Psychology, and [2]School of Life Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow

Gene therapy approaches to the treatment of Rett syndrome (RTT) have been inspired by the finding that the phenotype in *Mecp2* knockout mice is reversible after restoration of expression of the endogenous gene. Gene augmentation therapy approaches in the mouse model using viral vectors based on AAV9 have shown promising results and further studies investigating the efficacy of this approach are ongoing. However, recent work has suggested that achieving good levels of phenotypic recovery with minimal toxicity effects may be challenging and may require cellular levels of MeCP2 to be maintained within relatively narrow bounds. We have approached this problem by identifying a proposed set of sequence-level and chromatin-level regulatory elements in *MECP2* that appear to show most promise in governing *MECP2* mRNA, and potentially protein, expression levels. We have identified, by *in silico* analysis, all detectable functional transcriptional regulatory elements, as well as proposed functional elements in the 3'-UTR, and we have updated a number of previously defined sets of such elements. We have selected the most promising of these proposed regulatory regions and combined them in panels of constructs designed to test, in reporter expression assays, whether improved expression characteristics can be obtained in cell lines and in primary neurons and glia. This combined *in silico* and *in vitro* approach will set the scene for AAV vector optimisation experiments in the mouse model *in vivo*. Such constructs should allow finer control of exogenously-derived *MECP2* gene expression and may therefore be more appropriate for translational RTT gene therapy applications.