Functional characterization of putative non-coding regulatory elements in Autism Spectrum Disorder risk

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Abstract: Autism spectrum disorder (ASD) is highly inherited and arises from a complex interplay between three major categories of genetic risk: common polygenic variation, rare inherited and *de novo* mutations. The efforts to identify rare variants of large effect have mainly focused on the common variants in coding regions of the genome. However, rare genetic variants, especially in non-coding regions, remain elusive. Most identified DNA variants associated with ASD indeed map to non-coding regions of the genome, which often carry epigenetic features of enhancers. Often located far away on the linear chromosome map, enhancers are associated with the regulated promoter of the gene by long-range interactions (LRI). We previously performed RNApolIII-ChIA-PET and ChIP-seq profiling of mouse brain-derived neural stem cells (mNSCs), and identified over 10,000 enhancers linked to LRI, many of which are syntenic to human enhancers with neurodevelopmental relevance. Several such LRIs connect “epigenetic enhancers” to high-confidence ASD genes. The most promising enhancers hosting prioritized variants are presently being selected, to identify *de novo* variants that are specific to patients, that map onto enhancers, and that may contribute to the disease, looking for variants that are predicted to interfere with transcription factor binding sites. An interesting case is an enhancer connected to the FOXG1 gene (whose mutation causes an autism-related disorder), which is the object of microdeletions in various ASD patients, that leave the FOXG1 gene intact. We decided to analyse and evaluate potential mechanisms for the contribution of regulatory elements likely corresponding to neural enhancers to the regulation of the gene(s) connected to them by LRI, by silencing variant-containing regulatory regions using a CRISPR interference (CRISPRi) system *in vitro* and analysing the effect on the expression of the gene connected by LRI. To ask whether the identified enhancers do regulate the connected gene, we introduced vectors expressing guide RNAs (gRNAs) targeting a specific enhancer region, and the gene transcription start site (TSS) as a positive control in mNSC stably expressing dCas9/KRAB protein from nuclei. qRT-PCR analysis showed strong (>90%) downregulation of the connected FOXG1 gene following anti-FOXG1-TSS gRNA expression in our preliminary experiments. We are presently comparatively testing how repression of the enhancer connected with FOXG1 affects FOXG1 mRNA levels.