The role of putative human enhancers in brain evolution and ocular diseases

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Abstract: The transcription factor SOX2 regulates several genes that, when mutated, are known to cause neurodevelopmental disorders (NDDs). SOX2 binds to distal enhancers, maintaining the integrity of the network of enhancer-promoter interactions, which in turn maintains appropriate levels of gene expression. Furthermore, we identified by ChIPseq and ChIA-PET in mouse neural stem cells (undifferentiated and differentiated into neurons/glia) about 10000 "epigenetic enhancers" and their long-range interactions with promoters. About 7500 of these are conserved in humans and are also referred to as human–mouse syntenic long-range interaction regions ("hmsLRIs"). Many of these overlap with NDD-associated DNA sequence variants; through our map of long-range interactions these enhancer-associated variants indicate novel candidate genes that contribute to NDD. Further we identified microdeletions/duplications in patients with microphthalmia/anophthalmia/coloboma (MAC), that remove/duplicate individual enhancers connected (in wild type) to eye-relevant genes, suggesting that loss/increase of these enhancer's activity may contribute to the disease. In a parallel study we identified 36 overlaps between hmsLRIs and some Human Accelerated Regions (HARs). HARs sequences are highly conserved across species but show substantial changes in humans. Moreover, HARs are enriched near genes involved in brain development, and several HARs have been shown to encode transcriptional enhancers with human-specific activity changes. We have focused on an hmsLRI that overlaps with a HAR and has extensive interaction with the BTG1 gene, which is important for mouse brain development. We will investigate the in vivo function of the enhancer connected to BTG1, as well as of four enhancers that have long-range interactions with genes associated with MAC condition using a transgenic enhancer assay in zebrafish. If we find enhancer activity in zebrafish, we could analyse the functional effects of the human-specific substitution (hSub) in hmsLRI BTG1 and of variants in hmsLRIs associated with MAC-related genes.