Identification and Functional Study of Non-Coding DNA Variants in Microphthalmia, Anophthalmia, and Coloboma Patients

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Abstract:

Microphthalmia, Anophthalmia, and Coloboma (MAC) are severe ocular malformations resulting from mutations in both protein-coding genes, such as the transcription factor SOX2, and non-coding regulatory elements. Approximately 50% of affected individuals lack a definitive molecular diagnosis based on exome sequencing aimed at detecting protein-coding genes mutations, suggesting that mutations in non-coding regions may play a critical role in disease pathogenesis.

We previously performed RNApolII-ChIA-PET analysis on neural stem cells derived from the mouse brain, mapping long-range chromatin interactions between enhancers and gene promoters. These interactions were subsequently mapped to the human genome, identifying 7.698 syntenic regions, termed human-mouse syntenic long-range interactions (hmsLRI). DNA sequence variants associated with MAC (identified through whole-genome sequencing of affected individuals) were then overlapped with this enhancer dataset.
This comparison identified significant copy number variants (CNVs) associated with genes involved in eye development and inherited ocular diseases, though not affecting protein-coding regions; our study focuses on four CNVs. We started from this selection to plan further functional studies, including transgenesis experiments in zebrafish and the generation of brain and eye organoids from human pluripotent stem cells. For in vivo analysis, CNVs were cloned into a Gateway vector, then transferred to a ZED vector optimized for transgenesis in *Danio rerio*. This vector enables precise expression of transgenes and real-time monitoring of gene activity using fluorescent reporters. Additionally, CRISPR-Cas9-based genome editing will be employed to manipulate these enhancers, enabling the assessment of their effects on the expression of the connected gene and their contribution to ocular development. These tools will provide valuable insights into how the identified CNVs influence eye development and contribute to inherited ocular diseases.