Transcriptome Atlas of the Craniofacial Sutures: A FaceBase2 Spoke Project

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In craniofacial sutures, growth occurs along bone margins (osteogenic fronts) separated by non-osseous suture mesenchyme. The distinction between osteogenic and non-osteogenic subregions is maintained by the differential expression of genes such as *Fgfr2* and *Twist1*. Sutures vary in the lineage from which their subregions are derived, location and exposure to compressive or tensile forces, physical structure, and susceptibility to the pathological obliteration that occurs in craniosynostosis (CS). We hypothesize that these differences are reflected in characteristic gene expression profiles. Knowledge of the gene expression profiles, specific or common to the various sutures and their subregions, is crucial to obtain a better understanding of the formation and maintenance of craniofacial bones and sutures, and may aid in the development of better clinical management of CS. We are using laser capture microdissection to isolate the osteogenic fronts and suture mesenchyme from 11 major craniofacial sutures in wild-type (WT) and $Fgfr2^{+/S252W}$ Apert CS model mice, and from two of these sutures in the $Twist1^{+/-}$ Saethre-Chotzen CS model mice, at multiple stages of embryonic craniofacial development. From these subregions we are creating RNA-Seq libraries that allow analysis of gene expression profiles within and across sutures in WT and pathological conditions. The final transcriptome atlas will consist of 635 RNA-Seq datasets. To date, we have analyzed 70 RNA-Seq datasets derived from interfrontal and coronal sutures of WT and $Twist1^{+/-}$ mice. Cluster analysis of the WT interfrontal suture shows robust differential expression of 1,272 genes between the osteogenic fronts and suture mesenchymes at both E16.5 and E18.5. Enriched gene ontology (GO) categories among genes that are upregulated in the osteogenic front versus mesenchyme are highly consistent with bone formation, and the expression of major bone matrix genes, such as *Ibsp,* are highly enriched in the osteogenic front, validating our technique and analysis. Further comparisons of suture subregions, embryonic ages, suture types, and genotypes show clear differences between i) osteogenic fronts versus suture mesenchymes within interfrontal or coronal sutures at E16.5 or E18.5 in either WT or $Twist1^{+/-}$ mice, ii) coronal versus interfrontal suture osteogenic fronts or suture mesenchymes at E16.5 and E18.5, and iii) WT versus $Twist1^{+/-}$ coronal sutures. There were less significant differences in gene expression between WT and $Twist1^{+/-}$ interfrontal sutures. The creation of a murine suture expression atlas will be a powerful resource for hypothesis-driven research into the etiology of CS and the developmental and evolutionary biology of the vertebrate skull. Supported by grant NIH/NIDCR U01

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