Distant Acting Enhancers in Craniofacial Development


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1 Background
- The shape of the human face and skull is highly heritable, but the genetic factors that contribute to normal variation in craniofacial morphology remain poorly defined.
- Arrays of non-coding elements modulate the expression of ‘core craniofacial genes’. Variation within these elements could contribute to natural phenotypic variation of face morphology and represent risk factors for craniofacial birth defects.
- To identify craniofacial / palatal in vivo enhancers we have performed ChIP-seq on these tissues with the enhancer-associated protein p300. We validated the potential of identified bound regions as craniofacial enhancers through mouse transgenesis for a subset of them.
- To investigate the functional contribution of these enhancers to normal craniofacial development, we have selected mouse transgenesis for a subset of them.

2 Facebase Update - Data available on the Hub
- P300 ChIP-seq (8 matched RNA-seq)
  - Collection of E11.5 facial tissues
  - Collection of secondary palates
  - 5864 putative enhancers
  - 5508 putative enhancers
- Optical Projection Tomography
  - OPT scans are available for every identified craniofacial enhancer

3 Craniofacial Enhancer knockouts
- Selection criteria for enhancers to knockout
  - Highly reproducible craniofacial enhancers
  - Non-redundant in vivo activity with neighbouring enhancers
  - Map within a craniofacial gene locus
- Gene expression phenotypes (qPCR)
  - Subregions of the faces were dissected for both WT and KO e11.5 mouse embryos (littermates)

4 Micro-CT scans
- Morphometric analysis of WT and KO adult skulls (8 weeks old)
  - 20 adult KO mice / genotype
  - 60 matched WT (littermates) with same genetic background (C57/Sv112)
  - Additional matched WT (non-littermates) with same genetic background
  - Analysis of 50 hallmarks

5 Conclusions
- Using p300 ChIP-seq in e11.5 faces and e13.5 secondary palates we identified thousands of distant-acting enhancers that likely orchestrate gene expression during craniofacial/palatal development.
- In vivo dissection of the regulatory landscape of craniofacial genes reveals the complexity of enhancer arrays involved in their regulation.
- In vivo functional characterization of a subset of craniofacial enhancers demonstrates that enhancers can contribute to normal phenotypic variation and support the notion that they likely contribute to pathological aberrations of craniofacial morphology.