The 4th FaceBase Annual Meeting

Project updates on Functional Genomics, Imaging Analyses, and Rescue of Cleft Palate
PI: Yang Chai
University of Southern California

Team Members:
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Pedro Sanchez
Thach-Vu Ho
Richard Pelikan
Carolina Parada
Bridget Samuels

U01 DE020065 NIDCR, NIH
What did we propose to do and what have been accomplished

1. Global and specific gene expression analyses and 3D imaging study
   - 143 sets of microarray data uploaded to the hub
   - 87 hard and 104 soft tissue microCT images uploaded to the hub and how to use microCT images to study hard and soft tissue structures
   - Development of a data presentation interface with FaceBase

2. High-throughput analysis of Tgf-β downstream target genes in our Tgfbr mutant models and to test whether manipulation of altered Tgf-β downstream signaling molecules offers the opportunity to rescue cleft palate in vivo.
   - Discovery of an alternative Tgf-β signaling pathway and rescue of cleft palate in vivo (Iwata et al., JCI, 2012; Iwata et al., Development, 2013; Iwata et al., JBC 2011, 2012)
Gene expression in the secondary palate

Select the tissue of interest (click at the tissue of interest to get a list of the genes expressed in that tissue)

<table>
<thead>
<tr>
<th>Tissues:</th>
<th>Epithelium</th>
<th>Oral epithelium</th>
<th>Nasal epithelium</th>
<th>Midline epithelium</th>
<th>Basal epithelium</th>
<th>Peridermal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenchyme</td>
<td>Nasal region</td>
<td>Oral region</td>
<td>Anterior region</td>
<td>Posterior region</td>
<td>Palatal bone primordium</td>
<td></td>
</tr>
<tr>
<td>Muscles of the soft palate</td>
<td>Levator</td>
<td>Tensor</td>
<td>Palatoglossus</td>
<td>Palatopharyngeous</td>
<td>Uvula</td>
<td></td>
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<tr>
<td>Species:</td>
<td>Mouse</td>
<td>Rat</td>
<td>Human</td>
<td>Other</td>
<td></td>
<td></td>
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</table>

Color code:
- Nasal mesenchyme
- Oral mesenchyme
- Anterior mesenchyme
- Midline mesenchyme
- Posterior mesenchyme
- All mesenchyme
- Nasal epithelium
- Midline epithelium
- Oral epithelium
- All epithelium

<table>
<thead>
<tr>
<th>E12.5</th>
<th>E13.5</th>
<th>E14.5</th>
<th>E15.5</th>
<th>E16.5</th>
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<tr>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
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</tbody>
</table>

3D images µCT scans
Home page

Welcome to Craniofacial Central, a resource created and maintained by the Center for Craniofacial Molecular Biology at the Ostrow School of Dentistry of the University of Southern California.

To browse the resources that are currently available, please use the menu on the left. To search the site (e.g., for a specific gene), please use the search box below.

Supported by the FaceBase Consortium (U01DE020065 NIDCR, NIH).

Please send your comments and suggestions to Bridget Samuels (bdsamuel@usc.edu).

http://face.usc.edu/

1. To share our data immediately with the research community
2. To serve as an interface with the hub
Gene expressions

The color coding illustrated below is used on gene expression illustrations throughout this site.

![Gene expressions diagram](image-url)
Category Archives: Transcription factors

Dlx5 gene expression

Dlx5 (Distal-less homeobox 5)  Description: Dlx5 is expressed in the nasal mesenchyme along the AP axis of the E13.5 palatal shelf. Source: Han et al., 2009 Indirect modulation of Shh signaling by Dlx5 affects the oral-nasal patterning of palate and rescues ...

Posted in Anterior region, Mesenchyme, Mouse, Nasal region, Posterior region, Transcription factors |

Gli1 gene expression

Description: Gli1 is expressed in the oral mesenchyme and oral epithelium along the AP axis at E13.5. At E14.5, expression is restricted to the oral mesenchyme of the anterior region of the palate. Sources: Han et al. (2009) Indirect modulation of ...

Posted in Anterior region, Epithelium, Mesenchyme, Mouse, Oral epithelium, Oral region, Posterior region, Transcription factors |

Irf6 gene expression

Irf6 (Interferon regulatory factor 6) Description: Irf6 is expressed in the palatal epithelium along the AP axis throughout development, including the oral and nasal epithelia and in the MEE from E14.5 to E15.5. Source: Immunofluorescence. Unpublished data from Chai's lab.

Posted in Epithelium, Midline epithelium, Mouse, Nasal epithelium, Oral epithelium, Transcription factors |

Msx1 gene expression

Msx1 (Mash homeobox 1) Description: Msx1 is expressed in the mesenchyme of the anterior region of the developing palate, on both nasal and oral sides. Sources: Zhang et al. (2002) Rescue of cleft palate in Msx1-deficient mice by transgenic Bmp4 reveals ...

Continue reading →
Msx1 gene expression

Msx1 (Msh homeobox 1)

Description: Msx1 is expressed in the mesenchyme of the anterior region of the developing palate, on both nasal and oral sides.

Sources:

Unpublished data from Chai’s lab.
Classification of cleft palate in mutant mouse models and examples

Class I: Palatal shelf hypoplasia
- *Fgf12−/−*

Class II: Palatal shelf fusion with the tongue or the mandible
- *Fgf2−/−;
- *Irf6−/−*

Class III: Palatal shelves fail to meet at midline
- *Ctgf−/−
- *Mx3t1−/−
- *Wnt1-Cre;Alk5−/−
- *Wnt1-Cre;Shh−/−
- *Wnt1-Cre;Tgfbr2−/−*

Class IV: Submucosal cleft palate and/or persistence of medial edge epithelial cells
- *K14-Cre;Alk5−/−
- *K14-Cre;Tgfbr2−/−
- *Osr2-IresCre;Bmp1−/−
- *Tbx1−/−
- *Tbx22−/−*
Craniofacial phenotype analysis: Wnt1-Cre;Tgfbr2fl/fl

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wnt1-Cre;Tgfbr2fl/fl</th>
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<tbody>
<tr>
<td>Symbol (MGI)</td>
<td>Tgfbr2tm1.Him</td>
</tr>
<tr>
<td>Known defects</td>
<td>Cleft palate, bone defects, small tongue</td>
</tr>
<tr>
<td>Known human diseases</td>
<td>Loeyes-Dietz Syndrome</td>
</tr>
<tr>
<td>Histology</td>
<td>E18.5</td>
</tr>
<tr>
<td>SEM</td>
<td>E18.5</td>
</tr>
<tr>
<td>μCT</td>
<td>E18.5: soft - hard</td>
</tr>
<tr>
<td>3D μCT</td>
<td>E18.5: soft - hard</td>
</tr>
<tr>
<td>μMRI</td>
<td>E18.5: soft</td>
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<tr>
<td>Gene expression analysis</td>
<td>E14.5</td>
</tr>
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<td>Protein expression analysis</td>
<td></td>
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</tbody>
</table>

References

Ito Y et al., Development 2003
Sasaki T et al., Development 2006
Oka K et al., Dev Biol 2007
Oka K et al., Dev Biol 2008
Iwata J et al., J Biol Chem 2010
Iwata J et al., J Clin Invest 2012
Iwata J et al., J Biol Chem 2012

Comments are closed.
Dynamic microCT data for analysis of skull development and malformations

Control_E18.5

Wnt1-Cre;Tgfbr2^{fl/fl}_E18.5
1. Anterior point of maxilla  
2. Lateral point of premaxillary-maxillary suture  
3. Tip of zygomatic process of maxilla  
4. Anterior-medial point to zygomatic process  
5. Posterior point of maxilla  
6. Posterior-lateral point of the palatal process of maxilla  
7. Posterior-medial point of the palatal process of maxilla  
8. Anterior-medial point of palatal process of maxilla  
9. Anterior-lateral point of the palatal process of maxilla  
10. Medial point of premaxillary-maxillary suture

1. Anterior point of palatine bone  
2. Anterior point of the ridge of palatine bone  
3. Lateral point of the pyramidal process of palatine bone  
4. Posterior point of the pyramidal process of palatine bone  
5. Posterior-medial point of the horizontal plate of palatine bone
Control  Wnt1-Cre;Tgfbr2\textsuperscript{fl/fl}  Wnt1-Cre;Tgfbr2\textsuperscript{fl/fl};Alk5\textsuperscript{fl/+}

Control  Wnt1-Cre;Tgfbr2\textsuperscript{fl/fl}  Wnt1-Cre;Tgfbr2\textsuperscript{fl/fl};Alk5\textsuperscript{fl/+}
**Craniofacial phenotype analysis:**

**Wnt1-Cre;Tgfbr2fl/fl**

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<td>(\mu)MRI</td>
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<td>Ito Y et al., Development 2003</td>
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Comments are closed.
Microarray: E14.5 Tgfbr2 Palate

Whole-genome transcriptome profiling by microarray of palatal tissue of E14.5 murine embryos with and without a conditional inactivation of Tgfbr2 in the cranial neural crest.

See a heatmap of the top 10 most differentially expressed genes between mutant and control samples for the following signaling pathways:

- BMP/TGF-Beta signaling pathway
- FGF signaling pathway
- Hedgehog signaling pathway
- Wnt (canonical) signaling pathway

See an interactive pie chart of the enriched Gene Ontology (GO) biological processes for:

- Genes upregulated in Wnt1-Cre;Tgfbr2^{0/0} mutant mice
- Genes downregulated in Wnt1-Cre;Tgfbr2^{0/3} mutant mice

Download the original CEL files [here](#).

Comments are closed.
Click on a sector to see a list of genes in that category.

Based on the HighCharts JavaScript library.

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Click here to go back to Craniofacial Central.
Functional Categories Enriched With Genes Downregulated in Wnt1-Cre;Tgfbr2 fl/fl mutant

Relative Proportions of Category Representation

- 73.17% - cellular process
- 59.35% - metabolic process
- 51.22% - regulation of biological process
- 42.28% - cellular component organization
- 32.52% - developmental process
- 31.71% - response to stimulus
- 28.46% - cell cycle
- 18.70% - cell communication
- 11.38% - transport
- 9.76% - immune system process
- 8.94% - reproduction
- 8.94% - system process
- 8.13% - apoptotic process
- 8.13% - homeostatic process
- 7.32% - cell adhesion
- 0.00% - generation of precursor metabolites and energy

Click on a sector to see a list of genes in that category.

Based on the HighCharts JavaScript library.

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Click here to go back to Craniofacial Central.
<table>
<thead>
<tr>
<th>GO Term (GO ID)</th>
<th>Genes Annotated to the GO Term</th>
<th>GO Term Usage in Gene List</th>
<th>Genome Frequency of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>cellular process (GO:0009987)</td>
<td>4632434111Rik, Ablim3, Actn2, Adey8, Angpt1, Anp32a, Birc5, Bub1, Bub1b, Calea, Car2, Ccdc99, Ccna2, Ccnb1, Cdk5, Cdc25c, Cdc45, Cdc6, Cdfca3, Cdfca5, Cdh4, Cdhn3, Ceph, Crym, Cyp26b1, D2fertd750e, Depdc1b, Dsccl1, Dusp9, Eme1, Ephe4, Esco2, Efy5, Exol1, Fhbp4, Fbn1, Fbn3, Fbxo5, Fes1, Fgg, Gas2, Ginko, Gmnn, Gs2, H2afv, Hfe2, Hist1h3a, Hmgpa2, Hmgb2, Irx2, Kif11, Kif20b, Kif22, Lcrf1l, Mdm2, Mtm1, Mym5, Myh6, Myh7, Nasp, Ncapg, Ndc80, Nptx1, Pdela, Pr4, Pitx2, Rad5lap1, Ras1ib, Rrm2, Sgpl1, Skal, Sclé40a1, Smc6, Smyd1, Spc24, Spc25, Spc41, Srl, Tauc3, Tceal7, Tgfr2, Tmbs4, Tk1, Tmfs111, Tnnmd, Traip, Tnn, Tyma, Vwa2</td>
<td>90 of 123 genes, 73.17 %</td>
<td>13370 of 25000 annotated genes, 53.48 %</td>
</tr>
</tbody>
</table>
**Fgf9**

**Gene Detail**

**Symbol**
Fgf9

**Name**
fibroblast growth factor 9

**ID**
MGI:104723

**Synonyms**
Eks, egl a activating factor

**Feature Type**
protein coding gene

**Genetic Map**
Chromosome 14
30.51 cm, cytoband D
Detailed Genetic Map ± 1 cM

**Mapping data**

**Sequence Map**
Chr14:5807547-58112337 bp, + strand
From VEGA annotation of GRCh38

**Mammalian homology**
human; rat; cattle; chimpanzee; dog, domestic  
(Mammalian Orthology)

**Comparative Map** (Mouse/Human Fgf9 ± 2 cM)

**Protein SuperFamily**
fibroblast growth factor

**Gene Tree**
Fgf9

**Human ortholog**
Fgf9 fibroblast growth factor 9 (glia-activating factor) NCBI Gene ID 2254
Human Synonyms: GAF, HBFG-9, SVN53
Human Chr1:22245215-22278640 bp, + strand Reference GRCh37.p2 Primary Assembly
Human Diseases Associated with Human FGF9 (1)

**Alleles and phenotypes**
All alleles(1) : Targeted(6) Chemically induced(1)

Homozygotes for a targeted null mutation exhibit reduced size, pulmonary hypoplasia, cardiac dilation, impaired tastes development resulting in male-to-female sex reversal, abnormal retina, and neonatal lethality.

**Gene Ontology (GO) classifications**

**Process**
angiogenesis, cardiac left ventricle morphogenesis, ...

**Component**
basement membrane, cytoplasm, ...

**Function**
fibroblast growth factor receptor binding, growth factor activity, ...

**External Resources**
FuncBase

**Expression**

**Literature Summary** (113 records)

**Data Summary**
Results (172), Tissues (223), Images (46)

**Thelher Stages**
15, 16, 17, 18, 19, 20, 21, 22, 22, 24, 25, 26

**Assay Type**
RNA in situ

**Results**
167
Discovery of a novel TGF-β signaling mechanism

Target genes

Iwata et al., JCI 2012
TGF-β-mediated epithelial-mesenchymal interactions and palatal muscle development

Cleft soft palate

K14-Cre;Tgfbr2^{fl/fl}

Cleft palate

Hard palate

Soft palate

TVP

LVP
**TGF-β signaling mechanism in patients with TGF-β receptor mutations**

Loeys-Dietz syndrome

(*TGF-βRII or TGF-βRI mutations*)

(Marfan’s syndrome type II)

What is the TGF-β signaling mechanism in patients with TGF-βRII mutations?

TGF-β receptor mutations and cleft palate

Loeys et al., 2005, *Nature Genetics* 37, 275-281
Why is the soft palate important?

- Speech
- Hearing
- Swallowing

Cleft soft palate, risk for speech problems, middle ear infection, and difficulties in swallowing
Functional Significance of Soft Palate Muscles
Functional separation of the digestive and respiratory passages

The Soft Palate
Clinical problems:
Following the surgical correction of mis-oriented LVP muscle, these patients still have difficulty with precise pronunciation. Why?
1. Intrinsic functional defects in soft palate muscles (fast/slow fibers)?
2. Decreased volume of muscles?
3. Molecular regulation of muscles in soft palate is still defective.
Anterior

Posterior

Ptr; Pterygoid plate
Hn; Hamular notch
TVP; Tensor veli palatini muscle
LVP; Levator veli palatini muscle
TGF-β signal is required in the MEE cells for soft palate development.
Muscles of the soft palate analyzed by microCT

A

B

Side view

Bottom view

Control

Tgfbr2^{fl/fl};K14-Cre

Hard palate

Tongue

Control

Tgfbr2^{fl/fl};K14-Cre

Volume (mm$^3$)

Control

Tgfbr2^{fl/fl};K14-Cre
Muscle volume in $Tgfbr2^{fl/fl};K14-Cre$ mice

**Control**

**$Tgfbr2^{fl/fl};K14-Cre$**

Serial coronal sections for the LVP muscle (E18.5)

- Isolation of muscle fibers on each section
- Volume analysis

**E18.5 LVP area $\times 10^3$ mm$^2$**

Control: 60% reduction in volume

*
Cell proliferation defect in the muscles of $Tgfbr2^{fl/fl};K14$-Cre mice

$Tgfbr2^{fl/fl}$

$Tgfbr2^{fl/fl};K14$-Cre

BrdU positive cells (%)
Muscle development

Myoblast

Progenitors → Commitment to differentiation → Fusion into myotube → Maturation of myofiber

Myofiber
Muscle development defects in *Tgfbr2* mutant model

Control

Cleft soft palate

*Tgfbr2*^{fl/fl};K14-Cre
How does TGF-β mediated tissue-tissue interaction control muscle development in the soft palate?

K14-Cre;Tgfbr2^{fl/fl}
Microarray analysis

1.5-fold, <5% false discovery rate (FDR)

Wild-type (WT)  \( Tgfbr2^{fl/fl};K14-Cre \) (CKO)

<table>
<thead>
<tr>
<th>AFFY_ID</th>
<th>Symbol</th>
<th>( Tgfbr2^{fl/fl};Wnt1/Tgfbr2^{fl/fl} )</th>
<th>FDR</th>
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<tbody>
<tr>
<td>1420360_at</td>
<td>Dkk1</td>
<td>2.415</td>
<td>0.0448</td>
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<tr>
<td>1425447_at</td>
<td>Dkk4</td>
<td>5.602</td>
<td>0.0103</td>
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</table>

E15.5 Posterior palate

549 probe sets representing transcripts that were differentially expressed

(WT Post vs. CKO Post)
Control  |  K14-Cre;Tgfbr2^{fl/fl}  
---|---
Dkk1  |  Dkk4

E14.5

Color code:  
- MEE

Coronal view

Intraoral view

Secondary palate

Primary palate
Identification of TGF-β downstream molecules altered in the soft palate of \( Tgfbr2^{fl/fl};K14-Cre \) mice

In \( K14Cre;Tgfbr2^{fl/fl} \) mice, there is persistence of MEE cells. There is also persistence of DKK1 expression in the MEE and palatal mesenchyme.
Wnt11 expression in the palate

WNT11 acts as a directional cue to organize the elongation of early muscle fibers
Rescue of the cell proliferation defect in the soft palate of $Tgfbr2^{fl/fl};K14-Cre$ mice

A

$Tgfbr2^{fl/fl};K14-Cre$

Control  DKK1/4 NAb

B

$Tgfbr2^{fl/fl}$  $Tgfbr2^{fl/fl};K14-Cre$

Control  end-IWR1  Control

BrdU positive cells (%)

*
### LOPAC Library Screen Summary Statistics

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<td>7 (+2 DMSO)</td>
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<td>13.6 ± 2.6</td>
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<td>Control IC_{50} (μM)</td>
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National Center for Advancing Translational Sciences, NIH
Summary

1. Loss of \textit{Tgfbr2} in the palatal epithelium results in myogenic progenitor cell proliferation, differentiation and muscle fiber orientation defects. There is reduced soft palate muscle mass in \textit{Tgfbr2}^{fl/fl};\textit{K14-Cre} mice.

2. \textit{Dkk1} and \textit{Dkk4}, negative regulators of WNT/\(\beta\)-catenin signaling, are up-regulated in \textit{Tgfbr2}^{fl/fl};\textit{K14-Cre} mice and may be responsible for disrupting epithelial-mesenchymal interactions and causing muscle defects in the soft palate.

3. \textit{Tgfbr2}^{fl/fl};\textit{K14-Cre} mice can serve as an animal model to investigate tissue-tissue interactions in regulating palatal muscle formation.
Acknowledgements

CCMB USC

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