ACTN3 R577X Genotypes Associate with Class II and Deep Bite Malocclusions

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Genotyping & Masseter Gene expression using GWAS candidate genes
So far we have identified 28 significant genetic associations for malocclusion, including: HDAC4, KAT6B, MYO1H, MYO1C, ACTN2, ACTN3, ENPP1, ATP2A2, NLRK1, PPPTCC, RUNX2, GABRA6, CACN2A2D1, IL1B, IL1R2, ILE, IL8, CCL2, CCL4, CCL26, CCL4, CXCL12, and Nodal Pathway genes Nodal, Lfetf, Nodal-modulators Nomo-1, 2, 3, MiR15B/16, and Pitx2.

1. IGF1 Growth pathway

2. Nodal Pathway development of skeletal & muscular structures of 1st Brachial Arch

Asymmetric Nodal expression in the node, and signaling in the left Lateral Plate Mesoderm.

ACTN3 Associates with Athleticism and Muscle Size

577RR, 577RX= (+) α-actinin-3
577XX= (-) α-actinin-3

Athleticism by R577X Genotype

Lateral Cephalograms

What is the association of ACTN2 and ACTN3 expression and musculoskeletal malocclusion phenotypes in masseter muscle of orthognathic surgery patients?

>340 orthognathic surgeries/year
• Including mandibular bilateral split ramus osteotomy

"High Throughput" clinic for collection of clinical & biological samples
Materials and Methods

- Genotyping
  - 2 SNPs
    - rs1815739 (the 577X SNP)
    - rs678397 (no known functional consequences)
- Fiber type and RT-PCR analysis of muscle
- Immunostaining with antibodies specific for myosin heavy chain (MyHC) isoforms
  - Type I, type hybrid, type IIA and/or IIX, neonatal, and atrial
- Quantification of Actinin mRNA
  - ACTN2 and ACTN3 were quantified by TaqMan® quantitative real time PCR (qRT-PCR)

**Masseter Muscle Fiber Types Change with ACTN3 Genotypes**

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Area %</th>
<th>Occupancy %</th>
</tr>
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<tbody>
<tr>
<td>Type I</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>Type II</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Type IIA</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Type IIX</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Type II hybrid</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Type I hybrid</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

**TaqMan RT-PCR Quantification**

- Actinin Expression in Masseter Muscle of Orthognathic Surgery Subjects

<table>
<thead>
<tr>
<th>ACTN2</th>
<th>ACTN3</th>
</tr>
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<tbody>
<tr>
<td>P = 0.780</td>
<td>* P = 0.001</td>
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</table>

**Conclusions**

1. ACTN3 expression varied more than ACTN2, with relative amounts of α-actinin-3 decreased in open bite and skeletal Class II.
2. Decreased α-actinin-3 associated with smaller type II fiber diameter.
3. ACTN3 mRNA expression decreased to almost undetectable with 577XX genotype, while ACTN2 expression levels remained unchanged, suggesting α-actinin-2 may not compensate for loss of α-actinin-3.
4. Actn3 knockout mice have significant decreases in bone mineral density. Further absence of α-actinin-3 in bone resulted in increased expression of Enpp1, a negative regulator of mineralization. These relationships may be important to the development of Class II malocclusions in humans and merit further investigation.
Future Directions

**Actn3 KO Reductions in Mandibular Length**

<table>
<thead>
<tr>
<th>KO</th>
<th>YIT</th>
</tr>
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<tbody>
<tr>
<td>n=4</td>
<td>n=4</td>
</tr>
<tr>
<td>Length</td>
<td>Width</td>
</tr>
<tr>
<td>25.60</td>
<td>11.85</td>
</tr>
<tr>
<td>25.60</td>
<td>11.85</td>
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</tbody>
</table>

Future Directions: Gene microarray comparing mandibular gene expression in Actn3 KO, The GIAN Consortium GWAS as a priori genes of interest

**References**


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**THANK YOU!**

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