Mutations in HS6ST1 Cause Self-Limited Delayed Puberty in addition to Idiopathic Hypogonadotropic Hypogonadism

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Background - Puberty
Puberty is the normal developmental stage when reproductive capacity is attained. Disturbances of pubertal timing affect over 4% of the population. Deranged pubertal timing has significance for public health in view of the association between early or late puberty and an adverse cardiovascular, metabolic and cancer risk profile.1-3

Background – Puberty Genetics
The timing of pubertal onset has high heritability; 60-80% of variation is accounted for by genetic factors.4,5 Extensive family studies and twins have revealed that high or intermediate-impact variants will be enriched in a delayed puberty (DP) population at the extreme of normal pubertal timing (Figure 1).

Genetics of Delayed Puberty

Self-Limited DP
Condition of healthy individuals with pubertal onset delayed by more than 2 standard deviations.6,7 Repeatedly been shown to cluster in families, often with AD pattern8, but pathophysiology and genetic regulation remain unclear.9 Very limited number of rare, high impact genetic variants identified in families with both hypogonadotropic hypogonadism (HH) and DP7

Methods
Our cohort was collected from patients seen under specialist Paediatric care from Finland between 1982-2004. Cohort contains 403 affecteds from 170 families and their unaffected relatives (total of 910 individuals)

Whole Exome Sequencing Filtering Strategy

Whole exome sequencing was performed in 140 of the 170 families. Quality control filtering was performed to identify variants with a minor allele frequency (MAF) < 2.5% (Figure 2). Variants with MAF < 2.5% were then sorted by effect size and assessed for their biological relevance (Table 1).

Results
4 genes which passed rare variant burden testing included one gene known to cause HH: HS6ST1
1 pathogenic variant in 6 members of one family was validated

HS6ST1 mutations have been previously identified in up to 2% of patients with IHH7
HS6ST1 codes for an enzyme which modifies extracellular matrix components critical for normal neural branching

Assessment of sulphotransferase activity of HS6ST1 mutant protein

Mouse embryo studies show strong expression of HS6ST1 mRNA (in purple) from e11.5, mainly within the vomeronasal organ and olfactory epithelium (Fig. 5).

Conclusions
Mutations in HS6ST1 contribute to the phenotype of both HH and DP:
• Highly conserved, deleterious variant segregating perfectly in one family with DP from our cohort
• Mutant protein has reduced sulphotransferase activity in vitro
• Expression studies implicate role for HS6ST1 in developmental GnRH migration

Figure 1. Genetics of delayed puberty1

Figure 2. Our strategy for identification of new variants

Figure 3. Details of pathogenic variant p.Arg375Gln.
Clinical details of this family revealed them to have typical features of self-limited DP. The proband case was first investigated for growth delay at 12.8yrs, at which time his bone age was 11yrs. His sister’s age at menarche was 15yrs; both had normal birth weight and birth length. Their father and paternal uncle and aunt all had delayed puberty with delayed linear growth.

Figure 4. Assessment of the sulphotransferase activity of the HS6ST1 mutant protein.
After normalising the enzymatic activity to the densitometric measure of the bands, we showed reduced activity of the mutant protein compared to WT.

Figure 5. Expression pattern of Hs6st1 mRNA in mouse and human developing brain
In human 9pcw brains, Hs6st1 expression was similar to that observed in mouse, with GnRH neurons interspersed in the vomeronasal organ (VNO) and olfactory epithelium (OE), as well as in the nasal mesenchyme (NM) (panel A, at e14.5). In human 9pcw, brains, HS6ST1 expression pattern was similar to that observed in mouse, with GnRH neurons interspersed in an HS6ST1-positive NM (C&D, frontal sections). GnRH neurons are shown in brown (panels C&D). Sense probes resulted in no specific signal (mouse probe shown in panel B, human probe not shown). NS – nasal septum.

References

Table 1

<table>
<thead>
<tr>
<th>C#</th>
<th>VNO#</th>
<th>OE#</th>
<th>Sulphotransferase predicted deleterious</th>
<th>Rare variant Burden Testing Adjusted p value &gt; 0.025</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.01%</td>
<td>100</td>
<td>126,626</td>
<td>3 genes (HS6ST1, KAL1, G2) *</td>
<td>4 genes with p &lt; 0.025 prioritised</td>
</tr>
</tbody>
</table>

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