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 genetically determined[4] – however, GWAS of age at menarche only account for 3.6 – 6.1% of variability[5]

We hypothesise that low-frequency, high or intermediate-impact variants will be enriched in a delayed puberty (DP) population at the extreme of normal pubertal timing (Figure 1).

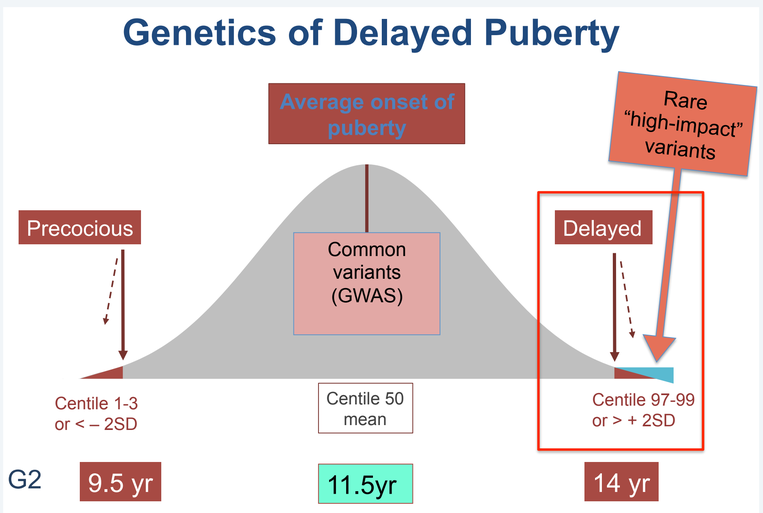

Figure 1. Genetics of delayed puberty(1). Studies range from GWAS of age at menarche in the general population, to discovery of rare, high impact mutations in a small number of genes causal in hypogonadotropic hypogonadism. Our strategy focused on discovery of important genetic regulators in a large cohort of families with significant DP. GWAS, genome wide association studies, SD, standard deviation

Self-Limited DP

Condition of healthy individuals with pubertal onset delayed by more than 2 standard deviations

Repeatedly been shown to cluster in families, often with AD pattern[6], but pathophysiology and genetic regulation remain unclear

Very limited number of rare, high impact genetic variants identified in families with both hypogonadotropic hypogonadism (HH) and DP[7]

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)

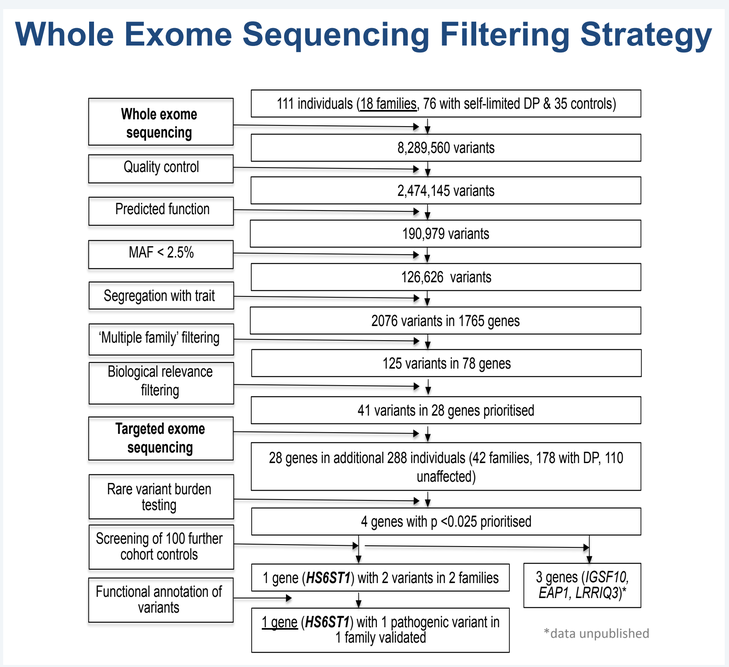

Figure 2. Our strategy for identification of new variants
Began by whole exome sequencing in our 7 most extensive families.
Variants returned were filtered by a classic bioinformatics pipeline looking for rare, deleterious variants that segregate with the delayed puberty trait in each family.
Then looked for genes with variants in more than one family, with possible biological relevance to the disease, including those 20-30 genes known to be determinants in HH.
Followed by targeted resequencing in top 28 candidate genes in a further 42 families from our cohort.

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

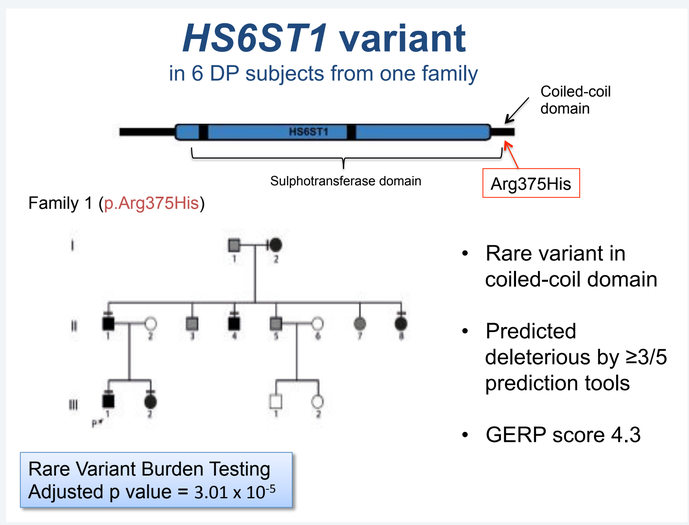

Figure 3. Details of pathogenic variant p.Arg375His.
Clinical details of this family revealed them to have typical features of self-limited DP. The proband case was first investigated for growth delay at 12.8yrs, at which time his bone age was 11yrs. His sister's age at menarche was 15yr; both had normal birth weight and birth length. Their father and paternal uncle and aunt all had delayed puberty with delayed linear growth.

HS6ST1 mutations have been previously identified in up to 2% of patients with IHH[7]

HS6ST1 codes for an enzyme which modifies extracellular matrix components critical for normal neural branching

Known to be required for the function of *FGFR1* and *KAL1* in vivo

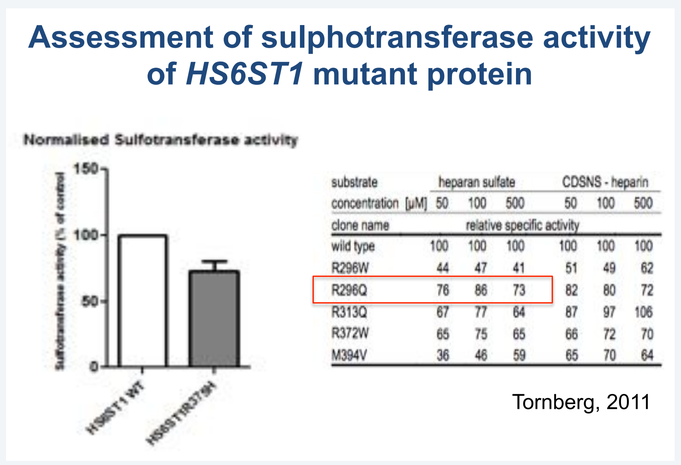

Figure 4. Assessment of the sulphotransferase activity of the HS6ST1 mutant protein.
After normalising the enzymatic activity to the densitometric measure of the bands, we showed reduced activity of the mutant protein compared to WT. Sulphotransferase activity was within the range of previously published[7] HS6ST1 mutations causing HH, towards the less deleterious end of the spectrum.

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

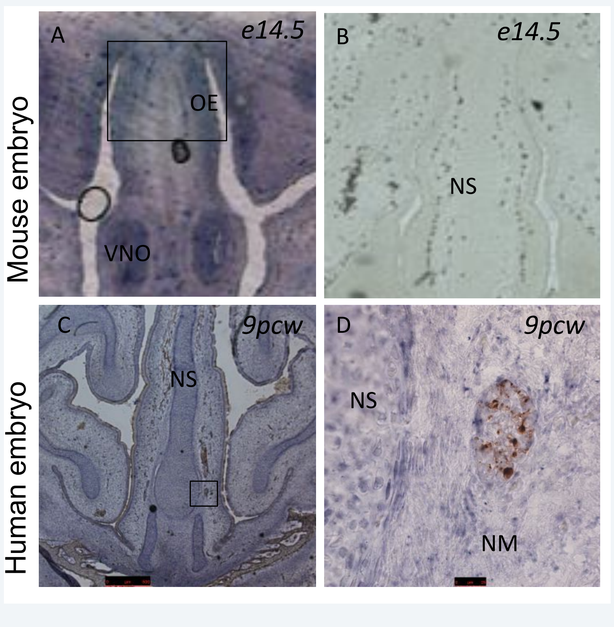

Figure 5. Expression pattern of Hs6st1 mRNA in mouse and human developing brain
Hs6st1 expression was observed from e11.5, and from e12 until e17.5 strong expression was seen in the vomeronasal organ (VNO) and olfactory epithelium (OE), as well as in the nasal mesenchyme (NM) (panel A, at e14.5).

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.

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
- Supports the hypothesis that defects in GnRH neuronal migration and development may result in self-limited DP
- To date, there has been limited overlap between the genetic basis for HH and DP demonstrated from our cohort

References

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