Novel variant in *GNRHR* gene regulatory region in a pedigree with maternally inherited precocious puberty

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Background

1. Gonadotropin releasing hormone (GnRH) and its receptor (GnRHR) - central regulators of puberty.
2. Loss-of-function mutations of the GnRH-GnRHR signaling pathway are associated with congenital hypogonadotropic hypogonadism, but no mutations were reported so far in patients with central precocious puberty (CPP).
3. Animal data demonstrate the importance of microRNAs (control mRNA stability and translation) in pubertal timing regulation.[1,2]

Aim: To identify genetic causes of maternally inherited CPP.

Patients and methods

- 8 family trios affected with maternaly inherited CPP (Figure 1, Table 1) from 2 unrelated populations (Izraeli and Slovene)
- **Whole genome sequencing**
  - Genetic variants with coverage >10x were retained and analyzed with Variant Studio 3.0 software
  - Family trio approach
  - Autosomal dominant model
  - MAF<0,2%
  - Synonimous changes filtered
- **Unbiased analysis** – Search for overlapping genes with rare variants in coding regions + 5' and 3' UTR
- **Targeted analysis -** 398 genes associated with the age at menarche[3]
  - coding and regulatory regions and copy number variations, sorted by a C score (Combined Annotation Dependent Depletion (CADD))[4]

In vitro functional analysis of *GNRHR* NM_000406.2:c.*1509G>A variant (Figure 2)

- A 465 nt long fragment of the hGnRHR 3' UTR (centered on the mutation) was inserted into the 3' UTR of the firefly luciferase cloned into pGL3 control.
- Mouse gonadotrope-derived cell line (LbêtaT2) were transfected
- After 24 h actinomycine D was added to block the transcription
- The luciferase activity was measured after 17 to 48 hours.
- The efficiency of the transcription was controlled by using a co-transfected vector encoding the renilla luciferase.
- The assay was performed in quadruplicates and using two different cell batches.

Results

- The average coverage of each genome was 38x and around 3.8M SNVs and InDels, 5k SVs and 600 copy number variants were detected on average per single sample.
- By unbiased analysis rare coding variants were identified in both populations in 6 genes (Figure 3), but *ZNF717* variants were not confirmed by Sanger.
- Targeted analysis ~ 200 rare coding and non-coding variants/pedigree
- Pedigrees #4 - a variant in 3'untranslated region (UTR) region of *GNRHR* gene segregating with CPP, NM_000406.2:c.*1509G>A, not present in gnomAD database, confirmed by Sanger.
- In vitro functional analysis demonstrated nonsignificant difference in decrease of firefly luciferase activity between wild type and mutant (Figure 4).

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Conclusions

1. The common cause of maternaly inherited CPP in the 8 affected pedigrees was not identified.
2. The hypothesis that deranged regulation of *GNRHR* mRNA decay influences pubertal timing in the pedigree #4 was not confirmed.
3. The cause of familial CPP in the presented pedigree remains to be determined and may be in the other regulatory regions, epigenetic or in a gene not known to be associated with pubertal timing in the general population.

Figure 1: Pedigrees

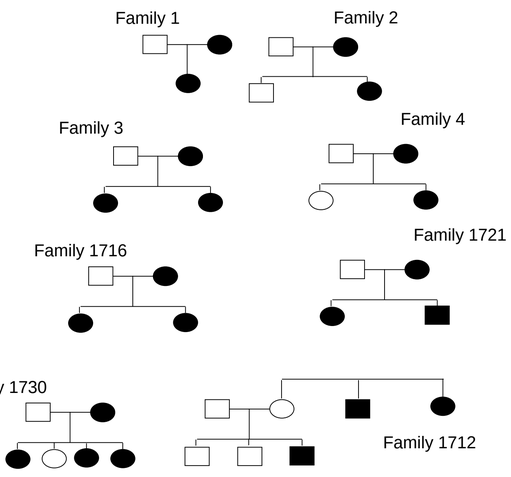


Table 1: Clinical characteristics

	Patient 1	Patient 2	Patient 3	Patient 4
~ age at onset	7,5 y	6	6	6
Age at evaluation	8,56 y	6,84	6,85	6,30
Pubertal stage	B2, P2-3, A1	B2, P2 (progression)	B3, P3, A2	B2-3, P2
Bone age SDS	+0,65	+4,51	+3,5	+3,37
LH basal [IU/L]	0,64	<0,1	0,35	0,3
LH peak [IU/L]	8,63	9,23	30,6	6,13
Growth spurt	Yes	Yes	Yes	Uncertain
Height SDS	1,32	3,20	3,99	1,86
BMI SDS	2,44	1,88	1,34	-0,03
Maternal menarche	9 y	10	10	9
Paternal puberty	normal	normal	normal	early
Other affected family members	/	/	sister	/

Figure 2: Shematic representation of a functional assay - *GNRHR* mRNA decay by luciferase reporter assay

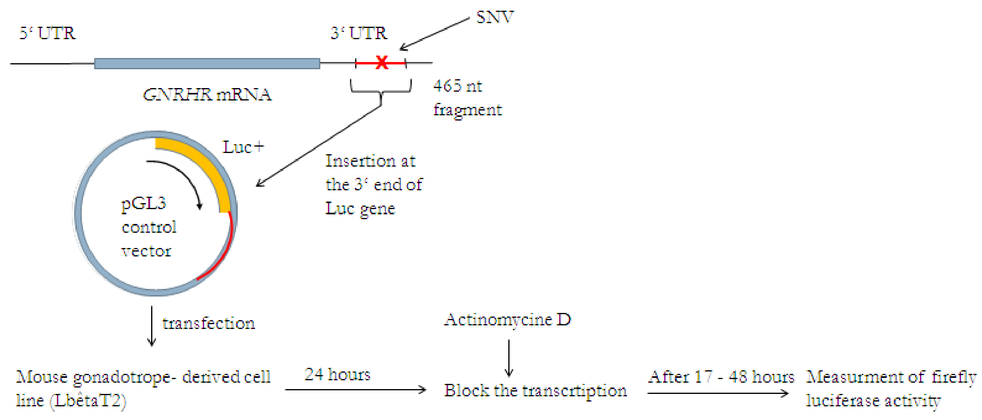


Figure 3: Overlapping genes in Izraeli and Slovenian pedigrees

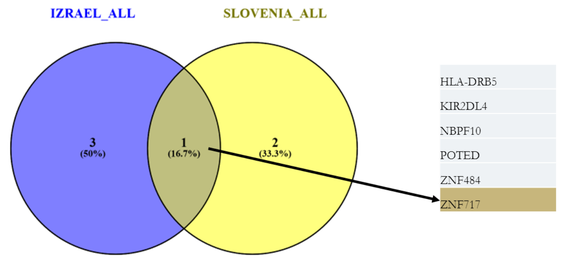
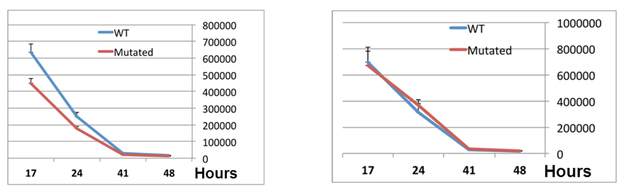


Figure 4: Nonsignificant difference in decrease of firefly luciferase activity between the wild type and the mutant using two different cell batches



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