Screening of mutations in idiopathic hypogonadotropic hypogonadism using a targeted next-generation sequencing approach

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Background

To date at least 30 genes are known to be associated with idiopathic hypogonadotropic hypogonadism (IHH). Analysis of all these gene candidates by Sanger sequencing would be expensive, labor-intensive and time-consuming. Recent introduction of next-generation sequencing (NGS) enables simultaneos analysis of multiple gene targets making it an attractive approach in such conditions as IHH.

Objective

To study the spectrum of molecular defects in IHH using a targeted NGS approach.

Subjects and Methods

25 patients with IHH (males, n=23; females, n=2) were studied.
5 subjects showed features of Kallmann syndrome (KS), 20 were normosmic.
'Hypogonadotropic hypogonadism panel' genes were sequenced using a custom Ion Ampliseq gene panel and PGM semiconductor sequencer (Ion Torrent).
Bioinformatic analysis was performed using Torrent Suite (Ion Torrent) and ANNOVAR* (annovar.openbioinformatics.org) software packages. Non-synonymous sequence variants were rated as "probably pathogenic" if they had allele frequency less than 1% and pathogenic *ljb* database scores.

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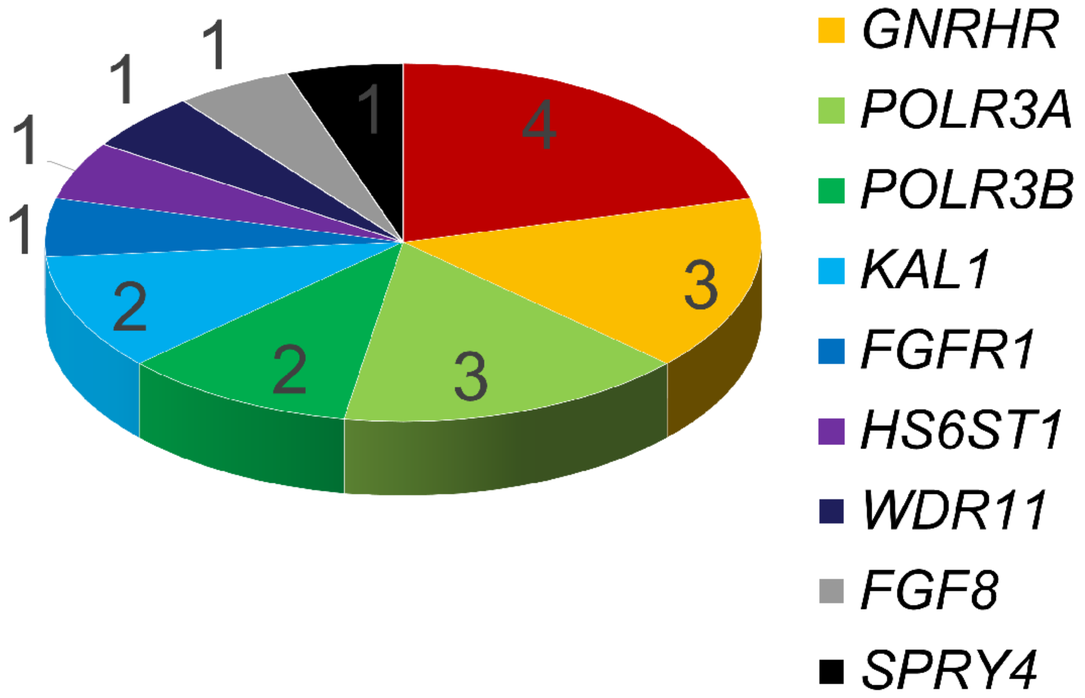
Results

21 heterozygous pathogenic or probably pathogenic mutations were found in 13 of 25 patients (52%). Mutations were identified in 4 of 5 KS cases, and in 9 of 20 subjects with normosmic IHH. 9 of 21 mutations were novel.
In 2 patients mutations were found in more than 2 genes.

Patient	Affected genes
№1 (with KS)	*HS6ST1, WDR11, POLR3A*
№2 (with nIHH)	*KAL1, HS6ST1, POLR3B*

Distribution of mutations



Conclusion

The results confirm predominance of mutations associated with defects of development and migration of GnRH neurons. The targeted NGS method can be successfully used for differential diagnosis of IHH

References:
* Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from next-generation sequencing data Nucleic Acids Research, 38:e164, 2010

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