Background

Congenital hypogonadotropic hypogonadism (HH) without or with anosmia (Kallmann syndrome [KS]) is
• clinically and genetically heterogeneous disease with
• X-linked, recessive, oligogenic or dominant inheritance with variable penetrance
• Molecular genetic testing may prompt the treatment in adolescence

Aim: To identify causative variants in genes associated with HH in a cohort of 14 Slovenian patients.

Results (Table 1)

9 mutations in 6 genes identified in 9 out of 14 patients (64%), each of them carrying a single heterozygous mutation in a single gene.
• 3 variants were novel.
• Of the remaining 5 patients 4 were part of the pedigrees with multiple affected members, which suggests an unidentified genetic cause.

Table 1: Clinical & genetic characteristics of the cohort

<table>
<thead>
<tr>
<th>ID #</th>
<th>Sex</th>
<th>Age (y)</th>
<th>HH</th>
<th>Cryptorchid.</th>
<th>Additional phenotype</th>
<th>Family history</th>
<th>Gene</th>
<th>Mutation</th>
<th>dbSNP</th>
<th>MAF</th>
<th>PolyPhen</th>
<th>Sift</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>18</td>
<td>KS</td>
<td>NA</td>
<td>/</td>
<td>Fath DP</td>
<td>FGFR1</td>
<td>c.295T&gt;C, p.Trp99Arg</td>
<td>/</td>
<td></td>
<td>Delet.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>33</td>
<td>nHH</td>
<td>Bil</td>
<td>color blindness, miopia</td>
<td>/</td>
<td>GNRHR</td>
<td>c.317A&gt;G, p.Gln106Arg</td>
<td>rs104890343</td>
<td>0.01</td>
<td>Delet.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>58</td>
<td>nHH</td>
<td>NA</td>
<td>/</td>
<td>/</td>
<td>GNRHR</td>
<td>c.416G&gt;A, p.Arg139H</td>
<td>rs104890542</td>
<td></td>
<td>Delet.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>16</td>
<td>nHH</td>
<td>/</td>
<td>short stature</td>
<td>Parents DP</td>
<td>FGFR2</td>
<td>c.254G&gt;A, p.Arg85Glu</td>
<td>rs74315418</td>
<td>0.0006</td>
<td>Delet.</td>
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<tr>
<td>5</td>
<td>M</td>
<td>24</td>
<td>nHH</td>
<td>Bil</td>
<td>schizophrenia</td>
<td>Moth DP</td>
<td>PROK2</td>
<td>c.518T&gt;G, p.Leu173Arg</td>
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<tr>
<td>6</td>
<td>M</td>
<td>21</td>
<td>KS</td>
<td>R</td>
<td>aortic coarctation</td>
<td>Fath DP</td>
<td>PROK2</td>
<td>c.171_172 delITT, p.Leu57MetfsTer17</td>
<td>/</td>
<td></td>
<td>Frameshift, premature STOP</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>68</td>
<td>KS</td>
<td>NA</td>
<td>/</td>
<td>/</td>
<td>PROK2</td>
<td>c.171_172 delITT, p.Leu57MetfsTer17</td>
<td>/</td>
<td></td>
<td>Frameshift, premature STOP</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>22</td>
<td>KS</td>
<td>Bil</td>
<td>ASD prim, mitral valve cleft, kifoscoliosis, GERD, develop. delay, short stature, dysmorphic signs</td>
<td>/</td>
<td>CHD7</td>
<td>c.5095+1G&gt;T</td>
<td>/</td>
<td></td>
<td>Splice site mutation</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>19</td>
<td>KS</td>
<td>Bil</td>
<td>TGA, kifoscoliosis, short stature, dysmorphic signs</td>
<td>/</td>
<td>CHD7</td>
<td>c.7879C&gt;T, p.Arg2627*</td>
<td>rs104890542</td>
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<tr>
<td>10</td>
<td>M</td>
<td>39</td>
<td>KS</td>
<td>R</td>
<td>unilateral sensorineural deafness</td>
<td>Fath hearing loss</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td>STOP gain</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>35</td>
<td>KS</td>
<td>L</td>
<td>impaired glucose tolerance</td>
<td>Cousins, aunt KS</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>22</td>
<td>KS</td>
<td>Bil</td>
<td>equinovarus, depression, disorder</td>
<td>Moth hypoplasia, feast DP</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>37</td>
<td>KS</td>
<td>Bil</td>
<td>color blindness</td>
<td>Grandmother hypoplasia</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>25</td>
<td>KS</td>
<td>/</td>
<td>hypocalciuric hypercalcemia</td>
<td>Fath anosmia &amp; hypercalcemia</td>
<td>CASR</td>
<td>c.2383C&gt;T, p.Asp795Trp</td>
<td>rs104890542</td>
<td>0.0002</td>
<td>Delet.</td>
<td></td>
</tr>
</tbody>
</table>

Table legend: Green letters mark novel mutations, F-female, M-male, HH-hypogonadotropic hypogonadism, nHH-normosmic HH, KS-Kallmann syndrome, NA-not applicable, Bil—bilateral, R-right side, L-left side, ASD-atrium septum defect, GERB-gastroesophageal reflux disease, TGA-transposition of the great arteries, DP-delayed puberty

Patients and methods

14 subjects (13 males, 1 female) with HH (Table 1)
• Targeted next generation sequencing of genomic DNA isolated from peripheral blood
• TrueSight One sequencing Panel Kit on miSeq (Illumina) apparatus
• 24 genes analysed:
  • ANOS1, FGFR1, FGFR2, PROK2, PROKR2, WDR11, SOX10, GNRHR, GNRH1, TAC3, TACR3, KISS1R, CHD7, HS6ST1, NSMF, KISS1, LEP, LEPR, NROB1, SEMA3A, HESX1, SOX2, AXL, SOX10
• Analysis of results with illumina Variant Studio programme
• Identified mutations confirmed by Sanger sequencing
• Coverage of genes checked with Galaxy web tool
• In case of coverage <10x - Sanger sequencing

Conclusions

1. NGS enables fast and reliable identification of causal mutations in several genes related to HH simultaneously.
2. Presented subject group with HH was genetically very diverse and the results expand the spectrum of mutations implicated in HH.
3. By known genetic origins oligoestrogenic was not identified and variable penetrance demonstrated in some pedigrees remained unexplained.

Distribution of genetic causes in the cohort

- Unknown (36%)
- FGFR1 (7%)
- FGFR2 (7%)
- GNRHR (14%)
- PROKR2 (14%)
- CHD7 (14%)

Pedigree #1

Hypogonadotropic hypogonadism
Delayed puberty
Anosmia
Pedigree #13

Pedigree #14

Familial hypocalciuric hypercalcemia

SPECTRUM IN SLOVENIAN PATIENTS WITH HYPOGONADOTROPIC HYPOGONADISM

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The authors declare no conflicts of interests.