Central Precocious Puberty Caused by Novel Mutations in the Promoter and 5'-UTR region of the Imprinted MKRN3 Gene

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Background

Central precocious puberty (CPP) is characterized by the premature activation of the hypothalamic-pituitary-gonadal axis due to the early activation of pulsatile Gonadotropin Releasing hormone (GnRH) secretion. CPP is clinically defined by the development of secondary sexual characteristics before the age of 8 years in girls and 9 years in boys and is associated with a range of clinical and biological implications. The most common genetic causes of CPP are the reported loss-of-function mutations in the MKRN3 gene. Although most of the studies describe loss-of-function mutations in the coding region of MKRN3 gene, defects in the regulatory regions of the gene were described only in two recent studies. In this study, we report four novel heterozygous mutations located in the proximal promoter and 5'-UTR regions of the MKRN3 gene.

1. Genetic Screening of CPP patients

- The promoter/5'-UTR region of the MKRN3 gene was screened in 73 index CPP girls
- Mutations in the coding sequence of the MKRN3, KISS1, KISS1R and DLK1 genes previously excluded

DNA sequencing analysis. Part of the sequencing electropherograms of the MKRN3 proximal promoter showing the novel heterozygous mutations identified. For each mutation the corresponding normal sequencing electropherograms is showed.

2. Clinical and laboratory findings

Clinical and laboratory characteristics for six girls with MKRN3 mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at referral (y)</th>
<th>Bone age</th>
<th>Stage of pubic hair</th>
<th>Stage of axillary hair</th>
<th>LHRH test FSH/LH (U/L)</th>
<th>MRI</th>
<th>Hearing impairment/cochlear implants/first cousin from father's side same clinical picture</th>
<th>Comments /Other symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>F</td>
<td>7.8</td>
<td>P2</td>
<td>11.0</td>
<td>11.5</td>
<td>8.9/9.4</td>
<td>Yes</td>
<td>No</td>
<td>No/Obesity-Insulin resistance</td>
</tr>
<tr>
<td>P2</td>
<td>F</td>
<td>8.3</td>
<td>P2</td>
<td>11.0</td>
<td>11.5</td>
<td>8.9/9.4</td>
<td>Yes</td>
<td>No</td>
<td>No/Obesity-Insulin resistance</td>
</tr>
<tr>
<td>P3</td>
<td>F</td>
<td>9.5</td>
<td>P3</td>
<td>12.0</td>
<td>14.0</td>
<td>14.1/18.8</td>
<td>Yes</td>
<td>Normal</td>
<td>No/Obesity-Insulin resistance</td>
</tr>
<tr>
<td>P4</td>
<td>F</td>
<td>7.8</td>
<td>P2</td>
<td>11.0</td>
<td>11.5</td>
<td>8.9/9.4</td>
<td>Yes</td>
<td>No</td>
<td>No/Obesity-Insulin resistance</td>
</tr>
<tr>
<td>P5</td>
<td>F</td>
<td>11.5</td>
<td>P3</td>
<td>12.0</td>
<td>14.0</td>
<td>14.1/18.8</td>
<td>Yes</td>
<td>Normal</td>
<td>No/Obesity-Insulin resistance</td>
</tr>
<tr>
<td>P6</td>
<td>F</td>
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<td>P3</td>
<td>12.0</td>
<td>14.0</td>
<td>14.1/18.8</td>
<td>Yes</td>
<td>Normal</td>
<td>No/Obesity-Insulin resistance</td>
</tr>
</tbody>
</table>

3. MKRN3 promoter/5'-UTR mutations reduce the promoter activity

A and B. shRNA knockdown efficiency on (A) RNA and (B) protein Mmr3 levels. GnRH expressing GN11 cells were treated with the indicated shRNA.

C. The MKRN3 promoter/5'-UTR mutations reduce the promoter activity in GN11 cells. The MKRN3 promoter reporter gene constructs containing the indicated MKRN3 mutations were transiently transfected in GN11 cells. Luciferase activities were calculated relative to the wild-type MKRN3 promoter reporter construct. Results are the average of three independent experiments with each sample assayed in triplicate.

4. In silico analyses of the novel MKRN3:g.+13C>T 5'-UTR mutation

MKRN3 5'-UTR in silico predictions

(A) and (B) predicted MKRN3 5'-UTR mRNA secondary structures showing the Minimum Free Energy (MFE) and centroid MFE secondary structure, respectively, for the wild-type and MKRN3:g.+13C>T mutant.

(C) Position of the Motif Ten Element (MTE) in relation with the MKRN3:g.+13C>T mutation. Black arrows indicate the position of the MKRN3:g.+13 nucleotide. The novel 5'-UTR MKRN3:g.+13C>T mutation would lead to the loss of a putative MTE binding site that can promote transcription by RNA polymerase II.

References/Acknowledgements


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