A homozygous point mutation in the GH1 promoter (-161C>T) leads to reduced GH expression in siblings with isolated GH deficiency (IGHD)


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Abstract 0625

P1 - D1 - 136 - Growth

Introduction

The incidence of short stature due to GH deficiency is estimated between 1:4000 - 10000 live births. GH is codified by GH1, located at chromosome 17q23 within a cluster of five related genes: GH2, CSH1, CSH2 e CSHP1. GH1 expression is regulated by the interaction between the GH1 promoter region (pGH1) and transcriptional factors1,2 (Figure 1).

Clinical Features

Three siblings (2 boys) born from consanguineous parents were evaluated in the outpatient clinic due to short stature (Table 1). Their height at first visit ranged from -5.8 to -4.1 SDS (Figure 2).

Table 1. Patient’s GH response to ITT

<table>
<thead>
<tr>
<th>Patient II.1</th>
<th>Patient II.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childhood</td>
<td>Adulthood</td>
</tr>
<tr>
<td>Age</td>
<td>14,5y</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>26</td>
</tr>
<tr>
<td>GH Peak (ug/L)</td>
<td>3.5*</td>
</tr>
<tr>
<td>IGF-1 (ug/L)</td>
<td>&lt;18</td>
</tr>
<tr>
<td>IGFBP-3 (mg/L)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

pGH1 sequenced by Sanger revealed 2 rare variants shared by the patients in homozogous state that segregate in the family:

-161 C>T: rs41295015
-123 T>C: rs71651677

Variants in GH1, GHRHR and LCR were dismissed. Variant -161C>T would lead to loss of NF1 binding site while -123T>C would lead to loss of POU1F1 and gain of SP1 binding sites (Figure 3).

Molecular Analysis

Figure 4. Plasmid containing both variants led to decreased GH1 expression in homozygous state, but normal GH1 expression in heterozygous state compared to wild type plasmid.

Results

Figure 5. Plasmid containing pGH1 mutated for -161 nucleotide and wild type for -123 led to decreased GH1 expression compared to the wild type plasmid and even compared to the plasmid containing both variants, suggesting that only -161C>T is pathogenic. This result is in line with previous report3.

Discussion

Experiments have shown that variant -161C>T leads to reduced GH1 expression due to loss of interaction between pGH1 and transcriptional factors. As the variant -123T>C preserved pGH1-protein interaction in EMSA and the plasmid mutated for -161 nucleotide and wild type for -123 led to decreased GH1 expression compared to the plasmid containing both variants (which is in line with previous functional studies performed by Millar et al5), variant -123T>C is probably not related to IGHD in this family.

Conclusion

To our knowledge, mutation -161C>T is the first point mutation described in pGH1 that leads to IGHD in an autosomally recessive inheritance pattern with complete penetrance.

Aim

We aimed to perform functional studies to check the effect of variants -161C>T and -123T>C on the phenotype.

Material and Methods

In order to evaluate the effect of both variants in GH1 expression, we have performed a transient transfection into GH3 cell lineage using wild and mutated pGH1 region cloned upstream to the luciferase reporter gene. For the purpose of studying -161C>T mutation effect, pGH1 mutated for -161 nucleotide and wild type for -123 was obtained through mutagenesis. They were performed in triplicate at least three times. The DNA-protein interaction was tested through an electromobility shift assay (EMSA or gel shift) using GH3 nuclear extract or purified POU1F1 protein together with the wild and mutated probes for both variants.

References