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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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 factors[1,2] (Figure 1).

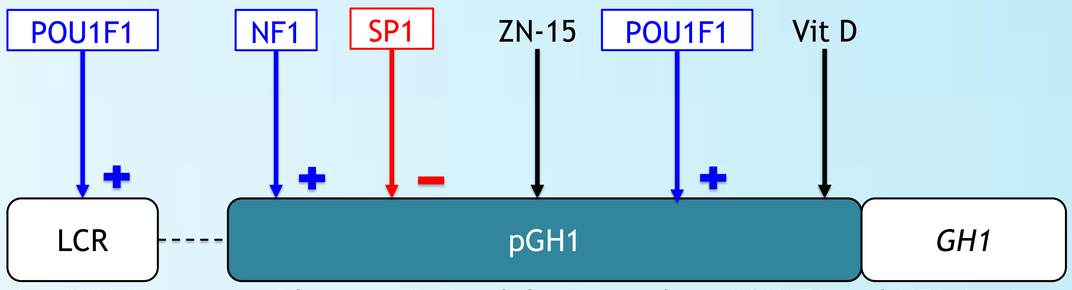


Figure 1. pGH1 interacts with transcriptional factors such as POU1F1 and NF1 (activators) or SP1 (repressor).

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).

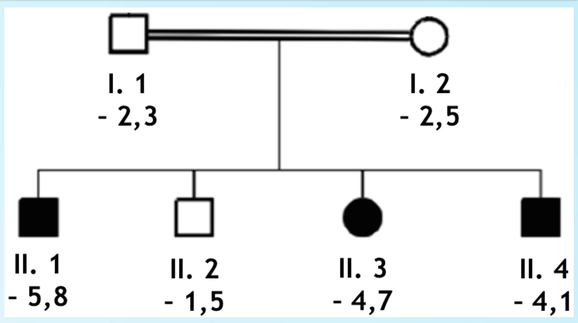


Figure 2. Heredogram: Autossomic Recessive Inheritance Pattern

Table 1. Patient's GH response to ITT

	Patient II.1		Patient II.4	
	Childhood	Adulthood	Childhood	Adulthood
Age	14,5y	32y	7,5y	24y
Glucose (mg/dL)	26	35	17	18
GH Peak (ug/L)	3.5*	2.5φ	2.1*	0.5φ
IGF-1 (ug/L)	-	<18	-	101
IGFBP-3 (mg/L)	-	0.7	-	2.4

*GH by polyclonal RIA φGH by monoclonal IFMA

Molecular Analysis

pGH1 sequenced by Sanger revealed 2 rare variants shared by the patients in homozygous 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).

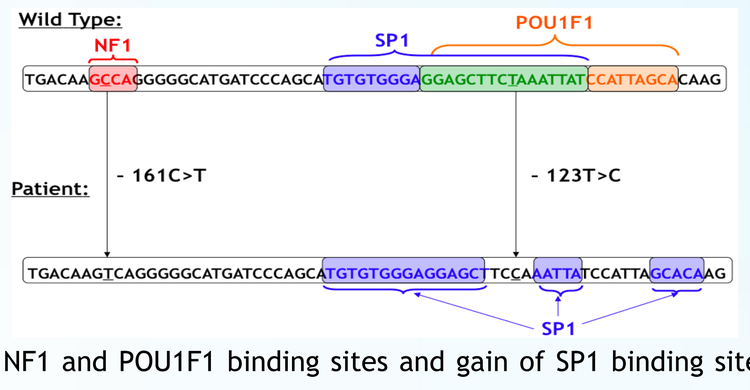


Figure 3. Loss of NF1 and POU1F1 binding sites and gain of SP1 binding site according to TESS website

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.

Results

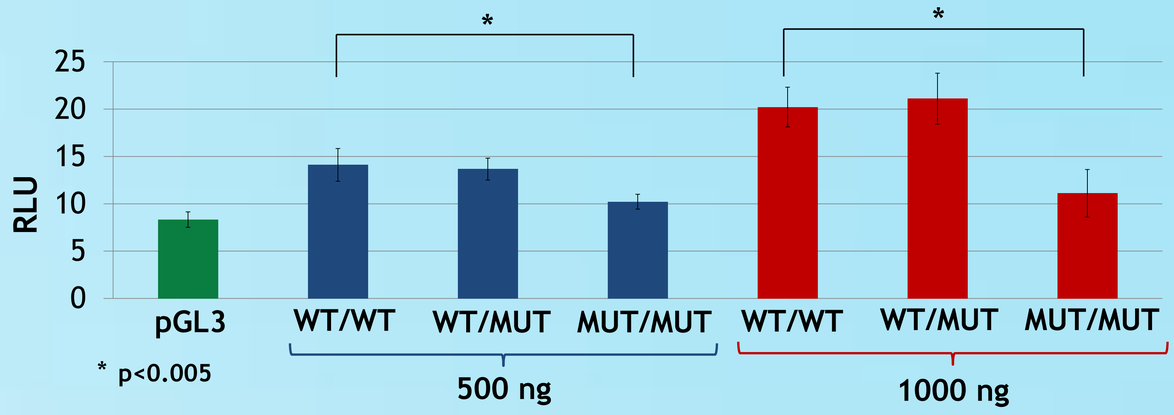


Figure 4. Plasmid containing both variants led to decreased *GH1* expression in homozygous state, but normal *GH1* expression in heterozygous state compared to wild type plasmid.

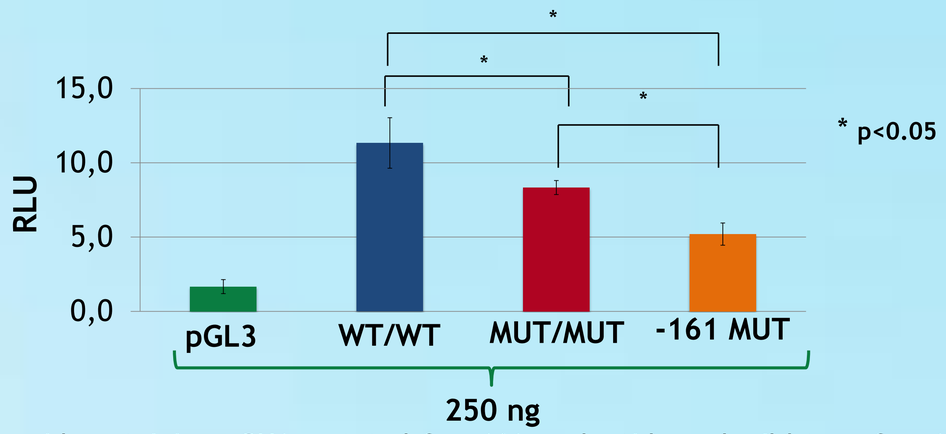
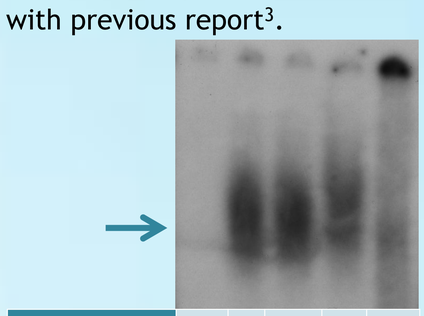
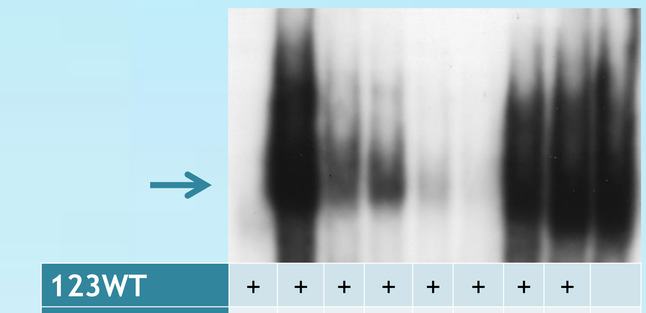


Figure 5. Plasmid containing pGH1 mutated for -161 nucleotide and wild type for -123 led to decreased *GH1* expression compared to the wild type plasmid and even compared to the plasmid containing both variants, suggesting that only -161C>T is pathogenic. This result is in line with previous report[3].



161WT	+	+	+	+
161MUT				+
GH3		+	+	+
Cold Probe			▲	

Figure 6. Variant -161C>T leads to loss of pGH1-GH3 nuclear extract interaction



123WT	+	+	+	+	+	+	+	+			
123MUT											+
GH3		+	+	+	+	+	+	+			+
Anti-Sp1									+	+	
Cold Probe			▲								

Figure 7. Variant -123T>C does not lead to loss of pGH1-GH3 nuclear extract interaction.

123WT	+	+	+	+	+	+	+	+	+	+	
123MUT											+
POU1F1		+	+	+	+	+	+	+	+	+	+
Anti-POU1F1			+								
Non-specific Antibody				+							
Cold Probe						▲					

Figure 8. Variant -123T>C does not lead to loss of pGH1-POU1F1 interaction.

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 al[3]), 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 autossomical recessive inheritance pattern with complete penetrance.

References

1. Horan et al 2003. Hum Mutat 21:408-423.
2. Giordano et al 2006. Mol Cell Endocrinol 249:51-57.
3. Millar et al 2003. Hum Mutat 21:424-440.