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Growth Hormone Deficiency Type II: Clinical and Molecular Evidence of Impaired Regulated GH Secretion Due to an Gln181Arg *GH-1* Gene Mutation

Maria Consolata Miletta[1], Andrée Eblé[1], Andrew Dauber[2], Ivo JP. Arnhold[3], Christa E. Flück[1], Amit V. Pandey[1]

[1]University Children's Hospital, Pediatric Endocrinology, Diabetology and Metabolism, Inselspital, CH-3010 Bern, Switzerland and Department of Clinical Research, University of Bern, 3010 Bern, Switzerland

[2]Cincinnati Center for Growth Disorders, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

[3]Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular LIM/42, Disciplina de Endocrinologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil

Authors have nothing to disclose

Introduction

A novel heterozygous missense mutation in the *GH-1* gene converting codon 181 from glutamine (Q) to arginine (R) was identified in a Brazilian girl (Figure 1). The index patient was referred for assessment of her severe short stature (-4.6 SDS) at a chronological age of 7yr 10 mo. The GH deficiency was confirmed by standard GH provocation tests, which revealed severely reduced GH and IGF-I concentrations. Genetic Analysis of the *GH-1* gene identified heterozygosity for p.Q181R mutation leading to the diagnosis of growth hormone deficiency type II (IGHD II).

Here, we describe the structure-function characterization of GH-Q181R by *in vitro* GH secretion studies as well as *in silico* mutagenesis and molecular dynamics simulations. Moreover, we performed a detailed structural analyses of the GH-Q181R mutant by generating recombinant *wt*-GH (wild type) and mutant GH protein in *Escherichia coli* (*E. coli*).

Pedigree / Clinical Data

Growth Hormone Deficiency type II

Category	Inheritance	GH RIA	Candidate gene	Status
IGHD type IA	Recessive	Absent	Human *GH-1*	Deletion/mutation, frameshift
IGHD type IB	Recessive	Low	Human *GH-1* GHRH GHRH-receptor	Splice-site mutations Unlikely Mutations
IGHD type II	Dominant	Low	Human *GH-1*	Splice-site mutations Splice enhancer/ missense mutations
IGHD type III	X-linked	Low	Unknown	

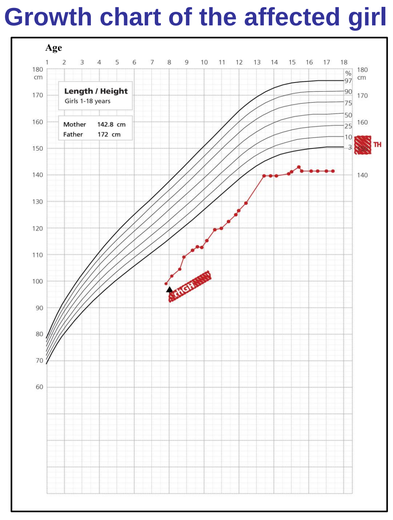
Patients with IGHD II

Highly variable clinical phenotype:

- Short stature (-2.5 SD score or less for chronological age)
- Delayed bone age (>2 years)
- Peak GH level less than 10 ng/ml after standard pharmacological stimulation test
- Low concentration of IGF-1

Figure 1.
Growth hormone deficiency classification

Growth chart of the affected girl



Birth: 2046 g (-0.1 SDS) and 50 cm (-0.9 SDS)

Normal psychomotor development

Severe growth retardation (-4.6 SDS)

Arginine stimulation test:

GH peak: 1.55 ng/ml

IGF-1 not measurable

Normal magnetic resonance imaging (MRI)

Genetic analysis of the *GH-1* gene identified heterozygosity for p.Q181R

IGHD II was diagnosed

Figure 2.
Growth charts of the affected girl
Adult height of mother and father, as well as adult target height are given. Percentiles are shown on the *extreme right*. The *solid circles* indicate the height measurements, the *open circles* the bone ages. The pointing up arrow indicates the beginning of rhGH treatment.

Results of Functional Analysis

Superimposition of wt-GH and GH-Q181R protein structural models

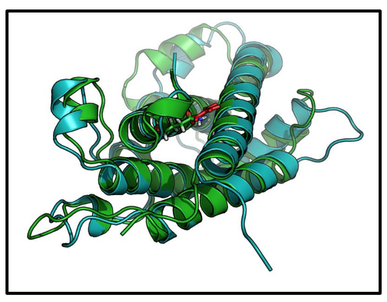


Figure 3: Structure of *wt*-GH (green) and mutant (cyan) hGH molecules superimposed on each other. The *wt* 181 residue (Gln) is in green and mutant (Arg) in red.
The large side chain of arginine protrudes towards the N terminus helix, getting in close proximity to form several hydrogen bonds and salt bridges.

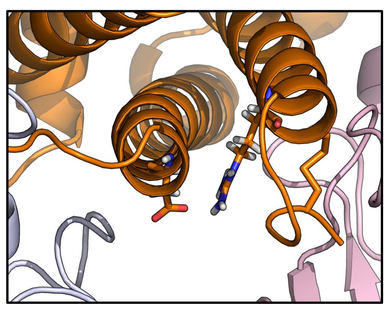


Figure 4: A close-up of the mutant GH-Q181R. The R181 residue (right) forms salt bridge with aspartate 11 residue (left) located at the N-terminus helix forming a highly rigid structure compared to wt-GH.

The Q181R mutation results in a drastic change in inter atomic contacts between the N and C terminus helices in hGH. Mutation on glutamine to arginine results in strong salt bridge formations between aspartate 11 on the N terminus helix and arginine 181 on the C terminus helix of the mutated molecules. These interactions are absent in WT hGH. This would result in a far more rigid hGH protein than the WT and may impact the binding with hGHR

Secretion of wt-GH and/or GH-Q181R in AtT-20 cells after forskolin stimulation

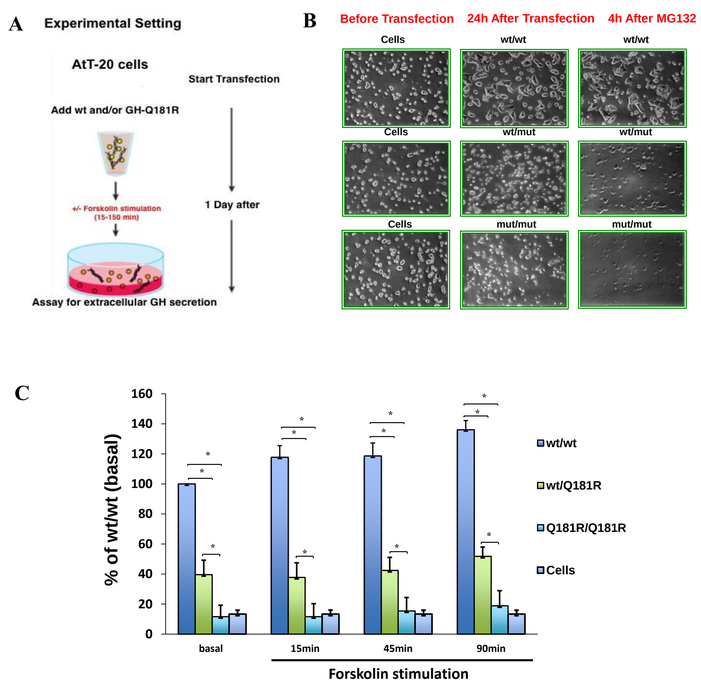


Figure 5. **A.** Cells were transfected either with *wt*-GH (*wt*-GH/*wt*-GH), GH-Q181R (Q181R/Q181R), or cotransfected with both *wt*-GH and GH-Q181R (*wt*-GH/Q181R). Twenty-four hours after transfection, AtT-20 cells were stimulated with 50 µM forskolin for 1.5h. Aliquots of culture medium were collected for GH measurement 0 to 90 min after stimulation.
B. Representative phase-contrast microscope pictures of cells transiently transfected with *wt*-GH and/or mutant and additionally treated for 4 h with MG132, a proteasome inhibitor.
C. The basal amount of GH measured in the medium of AtT-20 cells transfected with *wt*-GH (*wt/wt*) was arbitrarily set at 100% and the other measurements were compared against this. Results are given as the means ± SD of three independent experiments (n=3). *, P < 0.01.

Analysis of wt-GH and GH-Q181R stability by Fast Proteolysis assay (FASTpp) and thermofluor assay

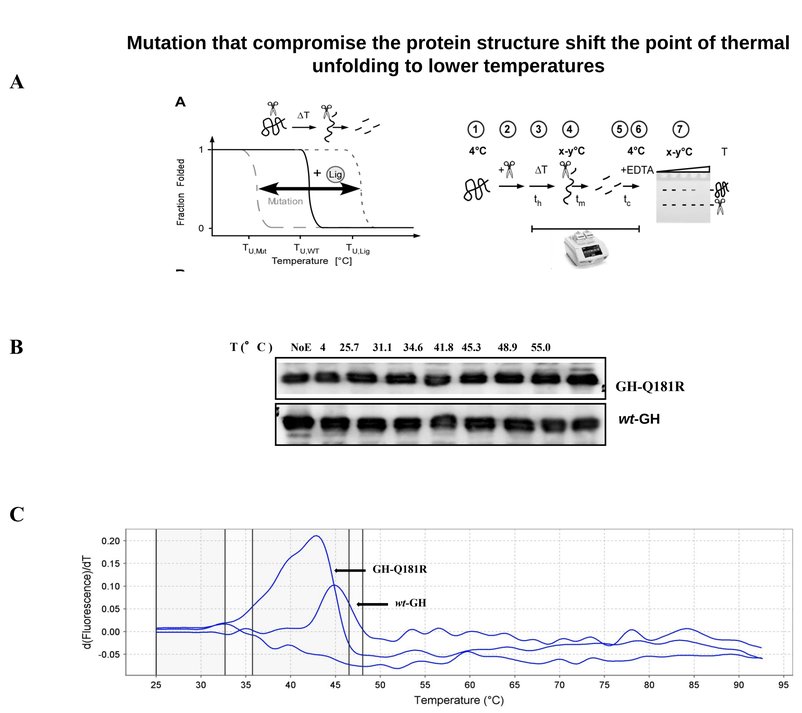


Figure 6. A. A schematic representation of Fastpp.
B. FASTpp of *wt*-GH and GH-Q181R analyzed by Western Blot. 0.02 mg/ml thermolysin was used to digest 0.40 mg/ml of *wt*-GH or GH-Q181R. This experiment was repeated at least three times and representative blots are shown.
C. Thermofluor profile of *wt*-GH and GH-Q181R. Data from Protein Thermal Shift™software show the derivative melt curves of *wt*-GH, and GH-Q181R (as indicated by the arrows). Data were collected at 2° C intervals from 25° C through 95° C on the 7500 Real-Time PCR System and analyzed using the Protein Thermal Shift™ Software.

Conclusions

- We found a heterozygous missense mutation, Q181R in the GH molecule in a Brazilian patient associated with severe short stature.
- *In silico* mutagenesis analysis revealed that the nature of the amino acid substitution (glutamine to arginine at position 181) in the GH molecule causes a drastic change in interatomic contacts between the N and C terminus helices in hGH and this might result in a far more rigid hGH protein than the *wt*-GH.
- No significative differences in intracellular GH folding, stability between *wt*-GH and GH-Q181R were found by functional characterization of the GH-Q181R purified and expressed in E. coli through FASTpp and the thermofluor assay.
- Significative differences between wt-GH and GH-Q181R were found by functional characterization of the GH-Q181R through secretion studies together with cell proliferation when transiently transfected cells were used.
- Our results show that specific analyses of any GH variant, despite the presence of obvious clinical features of IGHD type II (low peak GH secretion, low IGF-1 concentrations) may reveal novel mechanisms of secretory pathophysiology and hence, help explaining the range of clinical features associated to IGHD II patients.

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