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

Maria Consolata Miletta¹, André Eblé¹, Andrew Dauber², Ivo JP. Arnhold³, Christa E. Flick³, Amit V. Pandey⁴

¹University Children’s Hospital, Pediatric Endocrinology, Diabetology and Metabolism, Inselspital, CH-3010 Bern, Switzerland and ²Department of Clinical Research, University of Bern, 3010 Bern, Switzerland

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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 7 yr 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).

Results of Functional Analysis

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

Figure 3. Structure of wtGH (green) and mutant (cyan) GH4 molecules superimposed on each other. The wt 181 residue (Gln) is in green and mutated (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 mutant (right) forms salt bridge with aspartate 11 residue (left) located at the N terminal helix forming a rigid high structure compared to wt-GH.

The GH/Q181R mutation results in a drastic change in inter atomic contacts between the N and C terminus helices in IGH4. Mutation on glutamine to arginine results in strong salt bridge formations between aspartate 11 on the N terminal helix and arginine 181 on the C terminal helix of the mutated molecule. These interactions are absent in WT-GH. This would result in a far more rigid GH protein than the WT and may impact the binding with target.

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 IGH4 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.
- Significant 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.

Mutation that compromise the protein structure shift the point of thermal unfolding to lower temperatures

Figure 5. A. Cells were transfected either with, wtGH (wtGH/wtGH), GH/Q181R (GH/Q181R/GH/Q181R), or cotransfected with both wtGH and GH/Q181R (wtGH/GH/Q181R). Twenty-four hours after transfection, AIT20 cells were stimulated with 50 µM forskolin for 1.5h. Aliquots of culture medium were collected for GH measurement 0 to 60 min after stimulation.

B. Representative phase-contrast microscopic pictures of cells transiently transfected with hGH 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 AIT20 cells transfected with wtGH (wtGH) was slightly altered at 100% and the other measurements were compared against this. Results are shown as the means ± SD of three independent experiments (n=3), *P<0.05.

Figure 6. A, B. A schematic representation of Fastpp.

B, FASTpp of wtGH and GH181R analyzed by Western Blot. 0.02 mg/ml thermolin was used to digest 0.4 mg/ml of wtGH or GH181R. This experiment was repeated at least three times and representative blots are shown.

C. Thermofluor profile of wGH/GH/Q181R. Data from Protein Thermal Shift™ software show the derivative melt curves of wtGH and GH/Q181R (as indicated by the arrow). Data were collected at 2°C min intervals from 25°C to 90°C on the 7502 Real-Time PCR System and analyzed using the Protein Thermal Shift™ Software.