GROWTH HORMONE RECEPTOR 6Ω PSEUDOEXON ACTIVATION: A NOVEL CAUSE OF SEVERE GROWTH HORMONE INSENSITIVITY

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ESPE 2021
The 59th Annual Meeting Online
22-26 September 2021

Queen Mary University of London
Barts and The London

INTRODUCTION

Severe forms of Growth Hormone Insensitivity (GHI) are characterized by extreme short stature, dysmorphism and metabolic anomalies. They are classically caused by homozygous or compound heterozygous mutations of the Growth Hormone Receptor gene (*GHR*). Genetic analysis often focuses on the exonic regions of genes that encode the protein, rather than the non-coding regions. These seldom explored non-coding regions may harbour numerous disease-causing mutations that are not yet well recognised or understood.

AIMS

Identification of the genetic cause of growth failure in 3 'classical' GHI subjects. Assessment of identified novel 6Ω pseudoexon *GHR* variant.

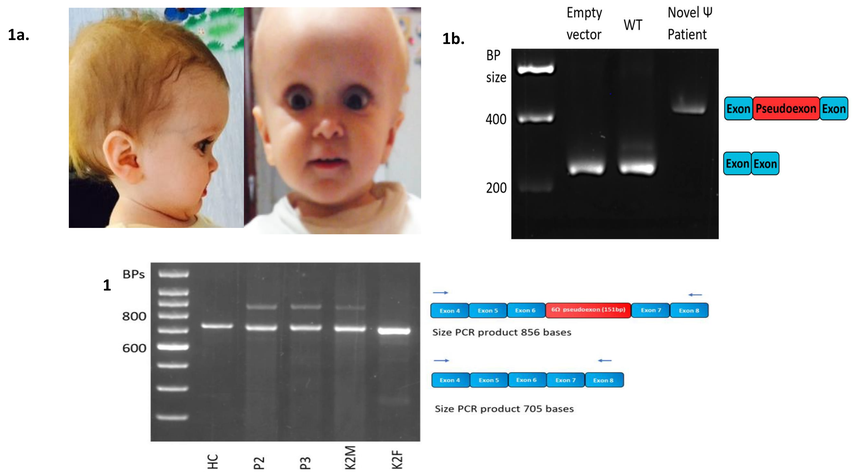
METHOD

A novel intronic *GHR* variant was identified using our GHI targeted whole genome custom gene panel. In vitro splicing assays were performed to confirm aberrant splicing. Patient fibroblast analysis was performed to determine the presence of *GHR* 6Ω pseudoexon in cDNA transcripts. A 6Ω pseudoexon *GHR* vector created by Gibson assembly enabled us to assess the functional consequence of the novel 6Ω pseudoexon inclusion.

RESULTS

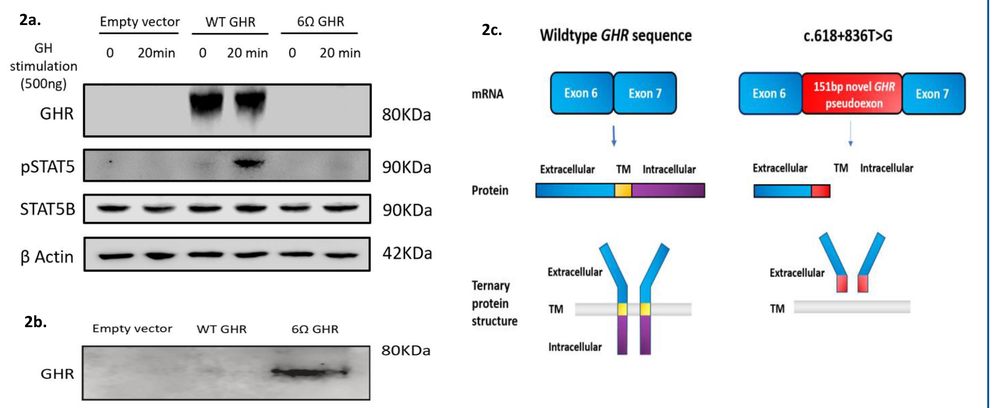
We identified a novel homozygous intronic *GHR* variant (g.5:42700940T>G, c.618+836T> G), 44bp downstream of the previously recognized intronic 6Ψ *GHR* pseudoexon mutation, in our index patient, patient 1 (**Figure 1a**). In the second kindred, two siblings were found to harbour this novel intronic 6Ω pseudoexon *GHR* variant in compound heterozygosity with the known *GHR* c.181C>T (R43X) mutation. In vitro splicing analysis confirmed inclusion of a 151bp mutant 6Ω pseudoexon not identified in wild-type constructs (**Figure 1b**). RT-PCR of patient fibroblasts demonstrated presence of the 6Ω pseudoexon transcript in the cDNA of patients 2 and 3 (**Figure 1c**). Our experiments using the 6Ω pseudoexon Gibson construct demonstrated diminished activation of STAT5B signalling following growth hormone stimulation and extracellular accumulation of the mutant GHR protein (**Figures 2a + b**). Inclusion of the 6Ω pseudoexon causes a frameshift resulting in a non-functional truncated GHR lacking the transmembrane and intracellular domains (**Figure 2c**).

Figure 1. Identification of novel *GHR* pseudoexon



1a shows the classic GHI phenotype of patient 1 with severe postnatal growth failure (height SDS -7.5 at 1.3yrs) and homozygous *GHR* mutation c.618+836T>G. **1b.** Electrophoresis gel following PCR amplification over the region of interest in the spliced product. BP, base pair; WT, Wildtype; Ψ, pseudoexon. Novel Ψ patient sample includes a 151bp pseudoexon in addition to the two exons of the exon trap vector. **1c.** RT-PCR of fibroblast cDNA; HC, Healthy Control; P2, Patient 2; P3, Patient 3; K2M, Kindred 2 Mother; K2F Kindred 2 Father.

Figure 2. Characterisation of 6Ω pseudoexon inclusion on GHR function



2a. Whole cell lysates from untreated or GH-stimulated HEK293 cells transfected with pcDNA3.1 empty vector, wild type (WT) *GHR* or 6Ω *GHR* mutant constructs. **2b.** Immunoblot analysis of conditioned media with anti-GHBP antibody from HEK293 cells transfected with the 6Ω *GHR* mutant construct showing extracellular accumulation of the truncated mutant 6Ω GHR protein. The inclusion of this 6Ω pseudoexon leads to frameshift of the GHR, as demonstrated in **2c**.

CONCLUSIONS

Novel *GHR* 6Ω pseudoexon inclusion results in loss of GHR function consistent with a severe GHI phenotype. This represents a novel mechanism of Growth Hormone Insensitivity and is the first deep intronic variant identified causing severe postnatal growth failure. The two kindreds originate from the same town in Campania, Southern Italy, implying common ancestry. Our findings highlight the importance of studying variation in deep intronic regions as a cause of monogenic disorders.

ACKNOWLEDGEMENTS

Thank you to the patients, their families and our funders. This work was supported by the 2018 ESPE Research Fellowship, sponsored by Novo Nordisk A/S, and also supported by Barts Charity and Sandoz Ltd UK.

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