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

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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 pseudexon GHR variant.

METHOD

A novel intronic 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 pseudexon in cDNA transcripts. A 6Ω pseudexon GHR vector created by Gibson assembly enabled us to assess the functional consequence of the novel 6Ω pseudexon inclusion.

RESULTS

We identified a novel homozygous intronic GHR variant (g.542700940T>G, c.618+836>T-G), 44bp downstream of the previously recognized intronic 6Ψ GHR pseudexon mutation, in our index patient, patient 1 (Figure 1a). In the second kindred, two siblings were found to harbour this novel intronic 6Ω GHR pseudexon 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 pseudexon not identified in wild-type controls (Figure 1b). RT-PCR of fibroblasts demonstrated presence of the 6Ω pseudexon transcript in the cDNA of patients 2 and 3 (Figure 1c). Our experiments using the 6Ω pseudexon 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 pseudexon causes a frameshift resulting in a non-functional truncated GHR lacking the transmembrane and intracellular domains (Figure 2c).

Figure 1. Identification of novel GHR pseudexon

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+836>T-G. 1b. Electrophoresis gel following PCR amplification of the region of interest in the spliced product. BP, base pair; WT, Wildtype; Ψ, pseudexon. Novel Ψ patient sample includes a 151bp pseudexon 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; KDM, Kindred 2 Mother; K2F, Kindred 2 Father.

Figure 2. Characterisation of 6Ω pseudexon 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 pseudexon leads to frameshift of the GHR, as demonstrated in 2c.

CONCLUSIONS

Novel GHR 6Ω pseudexon 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.

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