**INTRODUCTION**

Growth hormone insensitivity (GHI) encompasses normal/elevated growth hormone (GH), low IGF-I levels and growth restriction. Heterozygous dominant negative (DN) variants located in the intracellular/transmembrane domain of the GH receptor (GHR) cause a non-classical GHI phenotype. Non-classical GHI is an emerging entity which is poorly characterised.

**AIM**

To characterise novel, naturally occurring GHR variants and improve our understanding of the physiology of human growth.

**METHOD**

- Two novel heterozygous GHR variants were identified in 2 GHI patients by our short stature whole genome panel.
- In vitro splicing assay was performed using an exon trap vector.
- Gibson assembly created GHR wild type (WT) and variant (MUT1 & 2) constructs.
- Additional WT and MUT constructs with either NanoLuc® Large BiT or Small BIT subunits were generated.
- These constructs were transfected into HEK293T cells and western blotting (WB) was performed.
- NanoBIT complementation assays allowed quantitative assessment of GHR dimerization.

**RESULTS**

Heterozygous GHR variants (c.876-15T>G (MUT 1) and c.902T>G (MUT 2) in intron 8/exon 9, respectively) were identified in 2 GHI subjects (Table 1). In vitro splicing assays confirmed both GHR variants activate the same alternative splice acceptor site resulting in abnormal splicing and exclusion of 26 base pairs of GHR exon 9 (Fig 1).

WB analysis confirmed the production of truncated MUT variants and reduced GH-induced STAT5B phosphorylation (Fig 2).

NanoBIT complementation assays showed increased luminescence readings of MUT:MUT and WT: MUT GHR homo/heterodimers compared to WT:WT homodimers suggesting increased cell surface expression of MUT:MUT and WT:MUT GHR receptor dimers (Fig 3).

**CONCLUSIONS**

Heterozygous defects in the intracellular domain of GHR should be considered in cases with a non-classical GHI phenotype. Our novel truncated GHR variants exert a dominant negative effect with blunted GHR signalling. The creation of NanoLuc®-GHR constructs provide a novel, innovative methodology for characterising the functional role of GHR variants.

**REFERENCES**


**ACKNOWLEDGEMENTS**

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**NOVEL DOMINANT NEGATIVE GH RECEPTOR VARIANTS PROVIDE IMPORTANT INSIGHTS INTO GH RECEPTOR PHYSIOLOGY**

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**Table 1: Novel genetic variants in GHR identified in patients with growth hormone insensitivity**

<table>
<thead>
<tr>
<th>GHR Gene Variant</th>
<th>Clinical Features</th>
<th>Gnomad %</th>
<th>CADD</th>
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</thead>
<tbody>
<tr>
<td>c.876-15T&gt;G</td>
<td>Relative macrocephaly, disproportionate short stature, pre- and post-natal growth restriction (BW SDS -2.4, height SDS -3.2, aged 16 yr). High/normal GHBP levels.</td>
<td>0.029</td>
<td>&lt;10</td>
</tr>
<tr>
<td>c.902T&gt;G</td>
<td>No dysmorphic features, normal BW (SDS 0.2), PGR, height SDS -2.7 aged 15 yr, GHI (high GH ST 2.8µg/L, low IGF-1 &lt;25µg/ml; SDS -3.0). Extremely high GHBP levels.</td>
<td>Novel</td>
<td>27.7</td>
</tr>
</tbody>
</table>

**Figure 1:** Cell electrophoresis of PCR cDNA splicing products from GHR exon trap assays. Lane 1: 250bp empty vector (EV), representing the two exons of the exon trap (ET) vector. Lane 2: 320bp wildtype sequence (WT). Lanes 3 & 4: A smaller 294bp band was detected in both patients (P1 & P2) consistent with the mutant GHR exon 9, which leads to a frameshift (confirmed by Sanger sequencing).

**Figure 2:** Western Blot of HEK293T cells transfected with pCON3.1, GHI Wild Type (WT), GHR variants (M1; c.876-15T>G, M2; c.902T>G) and co-transfected with WT and variant constructs in a 1:1 ratio. A: Unstimulated whole cell lysates B: Whole cell lysates stimulated with rhGH at 100ng/ml for 20 minutes

**Figure 3:** NanoBIT complementation assays representing receptor homo/heterodimerization. A: Luminescence comparison for GHR WT and MUT1 hetero/homodimers pre and post rhGH stimulation, B: Luminescence comparison for WT and MUT2 (* p < 0.05; ** p< 0.01; *** p< 0.001)