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

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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 (MUT 1 & 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**).

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

GHR Gene Variant	Clinical Features	Gnomad %	CADD
c.876-15T>G (Patient 1)	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.	0.029	<10
c.902T>G (Patient 2)	No dysmorphic features, normal BW (SDS 0.2). PGR, height SDS -2.7 aged 15 yr, GHI (high GH 57.5µg/L, low IGF-1 <25ng/ml; SDS -3.0). Extremely high GHBP levels.	Novel	27.7

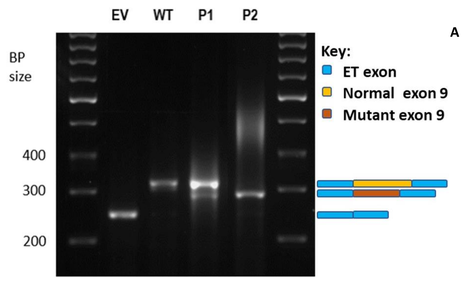

Figure 1*:* **Gel electrophoresis of PCR cDNA splicing products from *GHR* exon trap assays. Lane 1:** 250bp empty vector (EV), representing the two exons of the exon trap (ET) vector. **Lane 2:** 320bp wildtype sequence (WT), **Lanes 3 & 4**: A smaller 294bp band was detected in both patients (P1 &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

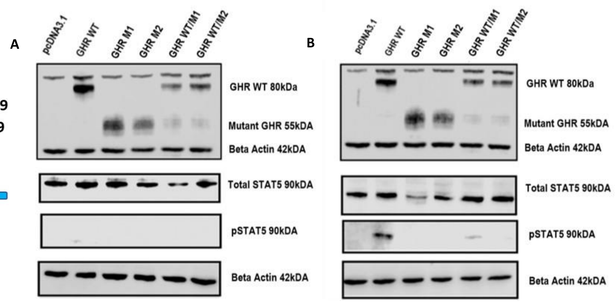

Figure 2*:* **Western Blot of HEK293T cells transfected with pcDNA3.1, *GHR* Wild Type (WT), *GHR* variants (M1; c.876-15T>G, M2; c.902T>G) and co-transfected with WT and variant constructs in a 1:1 ratio. A**: Unstimulated whole cell lysates **B**: Whole cell lysates stimulated with rhGH at 500ng/ml for 20 minutes

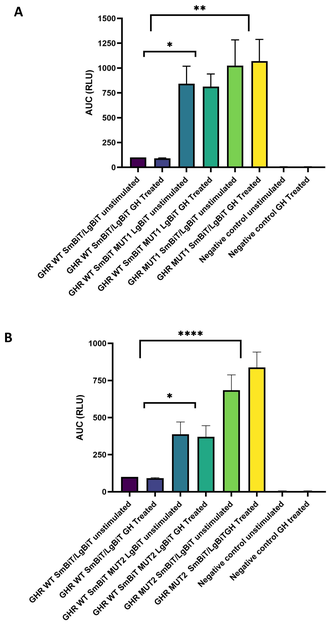

Figure 3: **NanoBiT complementation assays representing receptor homo/hetrodimerization. A**: Luminescence comparison for GHR WT and MUT1 hetero/homodimers pre and post rhGH stimulation, **B**: Luminescence comparison for WT and MUT2 (* p <0.05; ** p< 0.01; ****p < 0.0001)

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.

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