GHR transcript heterogeneity may explain the phenotypic variability in patients with homozygous *GHR* pseudoexon (6Ψ) mutation

S Chatterjee[1], SJ Rose[2], T Mushtaq[3], E Cottrell[1], AV Maharaj[1], J Williams[1], MO Savage[1], LA Metherell[1], HL Storr[1]

[1] Cenre for Endocrinology, William Harvey Research Institute, QMUL; [2] Birmingham Heartlands Hospital, Heart of England NHS Foundation Trust, Birmingham, UK;

[3]The Leeds Teaching Hospital NHS Trust, Leeds, UK.

Background and Objectives:

•*GHR* 6Ψ mutation leads to aberrant splicing of *GHR* gene with clinical and biochemical heterogeneity[1,2,3].

•We investigated whether phenotypic variability could be explained by transcript heterogeneity i.e. ratio of abnormal (6Ψ *GHR*) to normal (WT *GHR*) transcripts and/or the presence of concurrent defects in other short stature (SS) genes.

Methods:

•6Ψ *GHR* and WT *GHR* mRNA transcripts from four 6Ψ patients' fibroblasts (Patients 1-4) and 1 control subject were investigated by reverse-transcriptase PCR (RT-PCR) using intron skipping primers (Fig. 1).

•Transcripts (mean±SD) were quantified by qRT-PCR and double delta CT analysis (5 experimental repeats) and compared using ANOVA with Bonferroni correction.

•In eleven 6Ψ patients, 38 genes known to cause SS were analysed by targeted, gene panel sequencing.

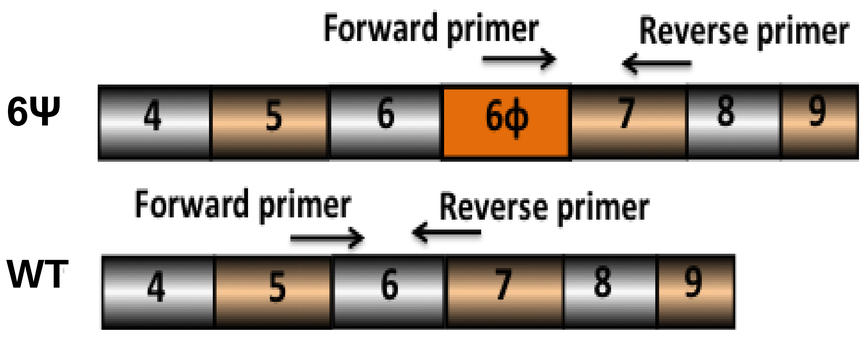


Fig. 1: Schematic diagram of intron-skipping primers

Results:

•WT transcript (193 bp) was present in control and the 6Ψ patients (Fig. 2a).

•6Ψ transcript (217 bp) was present in 6Ψ patients but absent in control (Fig. 2b).

•Relative 6Ψ transcript expression was significantly different amongst patients (1.0026±0.0035, 0.552±0.061, 1.003±0.18 and 0.40±0.069), p=0.017 between patients 2 and 4, all other p<0.001, except between patients 1 & 4 (Fig. 3)

•The mean 6Ψ:WT transcript ratios (39.17, 70.67, 46.87 and 29.44) correlated negatively with height SDS (R=-0.85, p <0.001) in 6Ψ patients (Fig. 4).

•Genetic analysis of eleven 6Ψ patients revealed 9 deleterious variants in 6 genes. However, there was no correlation between the number of gene hits and degree of short stature in 6Ψ patients.

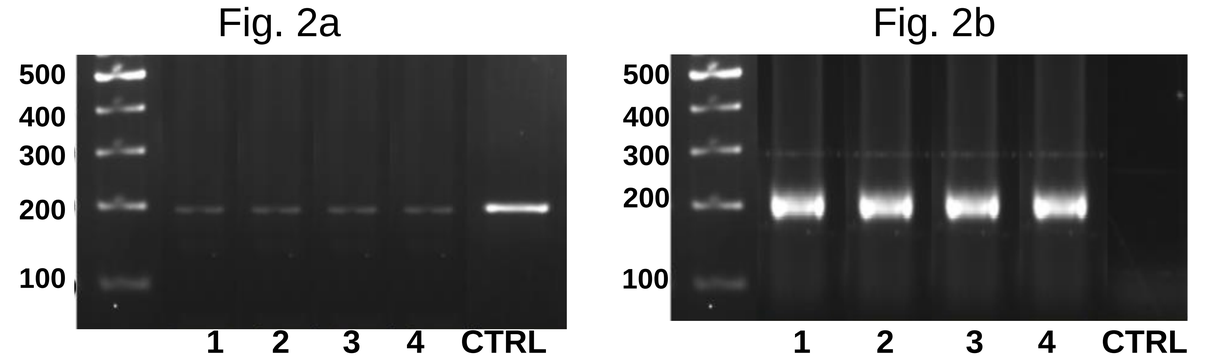
References:

1. Metherell *et al*. Pseudoexon activation as a novel mechanism for disease resulting in atypical growth-hormone insensitivity. Am J Hum Genet. 2001;69(3):641-646.
2. David *et al*. An intronic growth hormone receptor mutation causing activation of a pseudoexon is associated with a broad spectrum of growth hormone insensitivity phenotypes. J Clin Endocrinol Metab. 2007;92(2):655-659.
3. Chatterjee *et al*. Phenotypic spectrum and responses to recombinant human igf1 (rhigf1) therapy in patients with homozygous intronic pseudoexon growth hormone receptor mutation. Eur J Endocrinol. 2018;178(5):481-489.

b+tlc BARTS CHARITY

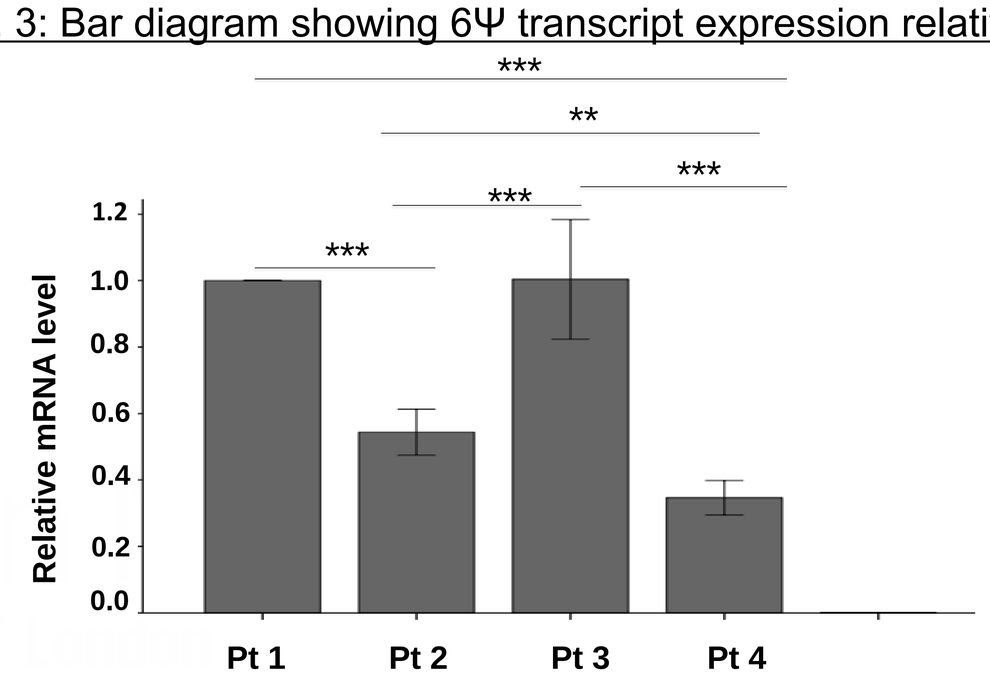
William Harvey Research Limited

Fig. 2a and 2b: 2% agarose gel showing WT and 6Ψ transcripts



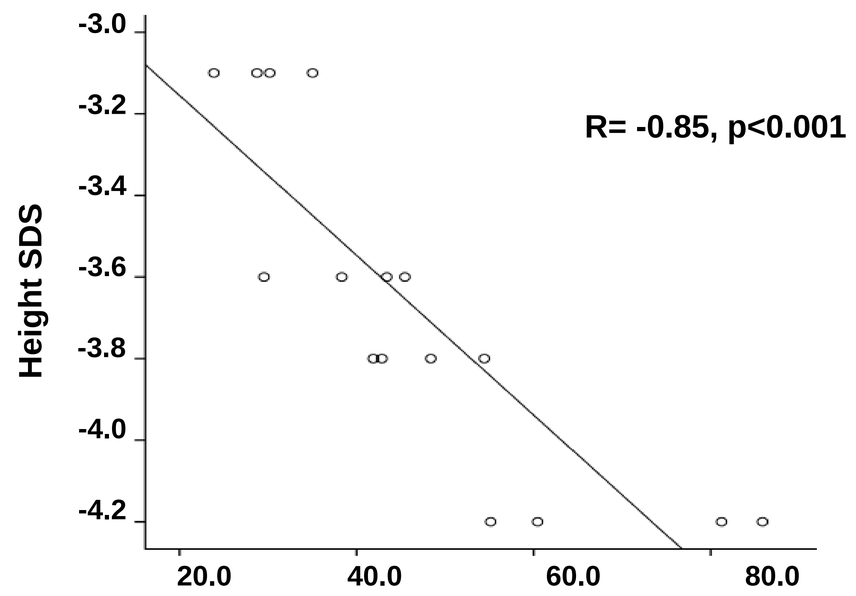
1, Patient 1 (Height (Ht) SDS -3.6); 2, Patient 2 (Ht SDS -4.2); 3, Patient 3 (Ht SDS -3.8); 4, Patient 4 (Ht SDS -3.1); CTRL, control.

Fig. 3: Bar diagram showing 6Ψ transcript expression relative to Pt 1



Pt, patient; ** p=0.017; ***p<0.001

Fig. 4: Scatter plot showing correlation between height SDS and mean 6Ψ: WT transcript ratios



R, pearson coefficient.

Conclusions:

• 6Ψ and WT *GHR* transcripts were identified in 6Ψ patients, with no 6Ψ transcript identified in the WT control.

• A higher 6Ψ:WT GHR transcript ratio correlates with the severity of short stature and thus may explain the phenotypic variability seen in 6Ψ patients.

•Genetic changes in a subset of SS genes do not account for the phenotypic variation.

•First report of transcript heterogeneity causing variable phenotype within an identical genetic mutation.