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

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Background and Objectives:
• GHR 6Ψ mutation leads to aberrant splicing of GHR gene with clinical and biochemical heterogeneity.
• 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.

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.

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.

References:
2. David et al. An intrinsic 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.

Fig. 1: Schematic diagram of intron-skipping primers
Fig. 2a and 2b: 2% agarose gel showing WT and 6Ψ transcripts
Fig. 3: Bar diagram showing 6Ψ transcript expression relative to Pt 1
Fig. 4: Scatter plot showing correlation between height SDS and mean 6Ψ:WT transcript ratios

Rc -0.85, p<0.001

CTRL
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.