

Search for abstract title, authors etc.



RFC14.4

[◀ Prev \(hrp0092RFC14.3\)](#)

[Next ▶ \(hrp0092RFC14.5\)](#)

[⬆ Table of contents \(/hrp/0092/\)](#)

[Cite \(/cite\)](#)

ESPE Abstracts (2019) **92** RFC14.4

GHR Transcript Heterogeneity May Explain the Phenotypic Variability in Patients with Homozygous GHR Pseudoexon (6ψ) Mutation

Sumana Chatterjee¹, Steven J Rose², Talat Mushtaq³, Emily Cottrell¹, Avinaash V Maharaj¹, Jack Williams¹, Martin O Savage¹, Loiuise A Metherell¹, Helen L Storr¹

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

S Chatterjee¹, SJ Rose², T Mushtaq³, E Cottrell¹, AV Maharaj¹, J Williams¹, MO Savage¹, LA Metherell¹, HL Storr¹

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

³The 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.

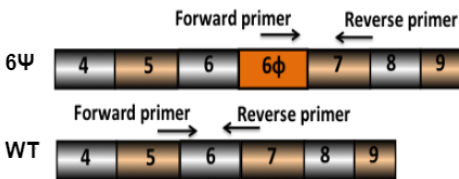


Fig. 1: Schematic diagram of intron-skipping primers

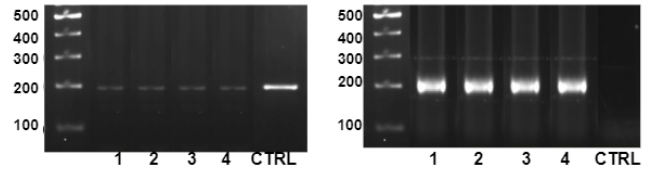
Results:

- 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:

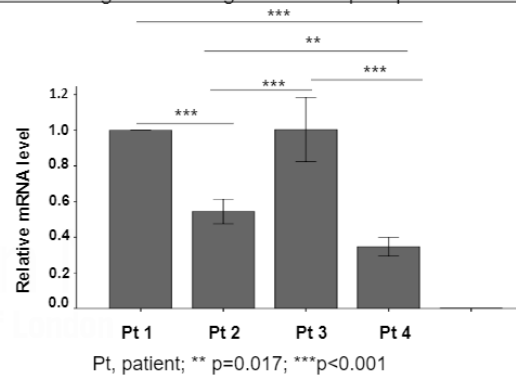
1. Metherell *et al.* Pseudoexon activation as a novel mechanism for disease resulting in atypical growth-hormone insensitivity. *Am J Hum Genet.* 2001;69(3):641-646.
2. David *et al.* An intronic growth hormone receptor mutation causing activation of a pseudoexon is associated with a broad spectrum 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.

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



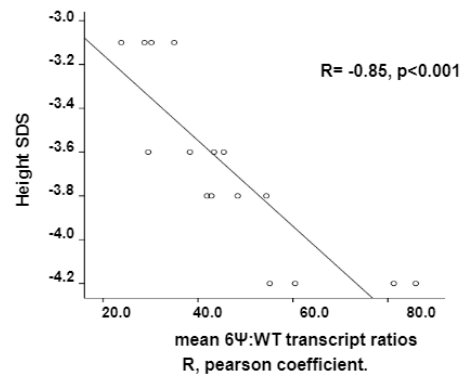
1, Patient 1 (Height (Ht) SDS -3.6); 2, Patient 2 (Ht SDS -4.2); 3, Patient 3 (Ht SDS -3.8); 4, Patient 4 (Ht SDS -3.1); CTRL, control.

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



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.

¹Centre for Endocrinology, William Harvey Research Institute, Queen Mary University London, London, United Kingdom. ²Birmingham Heartland Hospital, Heart of England NHS Foundation Trust, Birmingham, United Kingdom. ³The Leeds Teaching Hospital NHS Trust, Leeds, United Kingdom

Objectives: The homozygous *GHR* pseudoexon (6 ψ) mutation leads to aberrant splicing of the *GHR* gene with clinical and biochemical heterogeneity. We investigated whether the phenotypic variability could be explained by transcript heterogeneity i.e. ratio of abnormal (6 ψ GHR) to normal (WT GHR) transcripts and/or the presence of concurrent defects in other short stature (SS) genes.

Methods: 6 ψ GHR and WT GHR mRNA transcripts from 4 6 ψ patients' fibroblasts (height SDS -3.6, -3.4, -4.2 and -3.8) and 1 control subject were investigated by reverse-transcriptase PCR (RT-PCR) using intron skipping primers. Transcripts (mean \pm SD) were quantified by qRT-PCR and double delta CT analysis (5 experimental repeats) and compared using ANOVA with Bonferroni correction. In eleven 6 ψ patients, 63 genes known to cause SS were analysed by targeted sequencing.

Results: RT-PCR confirmed the presence of WT transcript (193 bp) in 6 ψ patients and control. 6 ψ transcript (217 bp) was seen in all 4 6 ψ patients but not control. Direct sequencing verified predicted mRNA sequences. 6 ψ transcript expression was significantly different amongst patients (1 ± 0 , 0.334 ± 0.032 , 0.549 ± 0.005 , 0.960 ± 0.071) p values <0.001, except between patients 1 & 4. The mean 6 ψ :WT transcript ratios (40.33, 29.69, 72.74, 47.39) correlated negatively with height SDS ($R=-0.96$, p value <0.01) in all 4 6 ψ patients.

Genetic analysis of 11 6 ψ 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 individual 6 ψ patients.

Conclusion: Varying amounts of 6 ψ and WT GHR transcripts were identified in 6 ψ patients, with no 6 ψ transcript identified in the control. A higher 6 ψ :WT GHR transcript ratio correlates with the severity of short stature and thus may explain the phenotypic variability. Genetic changes in other known SS genes do not appear to account for the phenotypic variation.

This Volume



58th Annual ESPE (<https://www.eurospe.org/meetings/2019/espe2019>)

Vienna, Austria

19 - 21 Sep 2019

European Society for Paediatric Endocrinology (<http://www.eurospe.org/>)

[Browse other volumes \(/browse/issues?GroupBy=series#espe-annual-meeting\)](/browse/issues?GroupBy=series#espe-annual-meeting)

[Table of contents \(/hrp/0092/\)](/hrp/0092/)

[Scientific Programme \(programme\)](#)

[ePosters \(eposters\)](#)

Article tools

[Attach ePoster to your abstract \(/eposter-instructions\)](#)

[CiteULike](#)

[Email this article to a colleague](#)

[Facebook](#)

twitter

Print

Select Language | ▼ | Disclaimer (/disclaimer)

My recent searches

metherell storr chatterjee (/search/search.aspx?q=metherell storr chatterjee) (3 mins ago)

pseudoexon (/search/search.aspx?q=pseudoexon) (3 mins ago)

metherell (/search/search.aspx?q=metherell) (1 mins ago)

metherell and (DC.Date contains date(1980-1-01~~2020-5-31)) and (Volume contains 92) (/search/search.aspx?q=metherell and (DC.Date contains date(1980-1-01~~2020-5-31)) and (Volume contains 92)) (<1 min ago)

metherell and (DC.Creator contains metherell) and (DC.Date contains date(1980-1-01~~2020-5-31)) and (Volume contains 92) (/search/search.aspx?q=metherell and (DC.Creator contains metherell) and (DC.Date contains date(1980-1-01~~2020-5-31)) and (Volume contains 92)) (<1 min ago)

My recently viewed abstracts

SGPL1 Deficiency Leads to Downregulation of Key Enzymes Within the Steroidogenic Pathway (/hrp/0092/hrp0092fc13.5) (9 mins ago)

GHR Transcript Heterogeneity May Explain the Phenotypic Variability in Patients with Homozygous GHR Pseudoexon (6ψ) Mutation (/hrp/0092/hrp0092rfc14.4) (<1 min ago)

Authors

Chatterjee Sumana

Rose Steven J

Mushtaq Talat

Cottrell Emily

Maharaj Avinaash V

Williams Jack

Savage Martin O

Metherell Loiuise A

Storr Helen L

(<http://www.bioscientifica.com>)

ESPE Abstracts

© BioScientifica 2020 (<http://www.bioscientifica.com>) | Privacy policy (<http://www.bioscientifica.com/privacy/>) | Cookie settings

BiosciAbstracts

(<http://www.biosciabstracts.com>) Bioscientifica Abstracts is the gateway to a series of products that provide a permanent, citable record of abstracts for biomedical and life science conferences.

Find out more (<http://www.biosciabstracts.com>)