P-2-398

The in vitro Functional Analysis of Promoter Single Nucleotide Polymorphisms Associated with Growth Hormone (GH) Response in Children with GH Deficiency

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Background

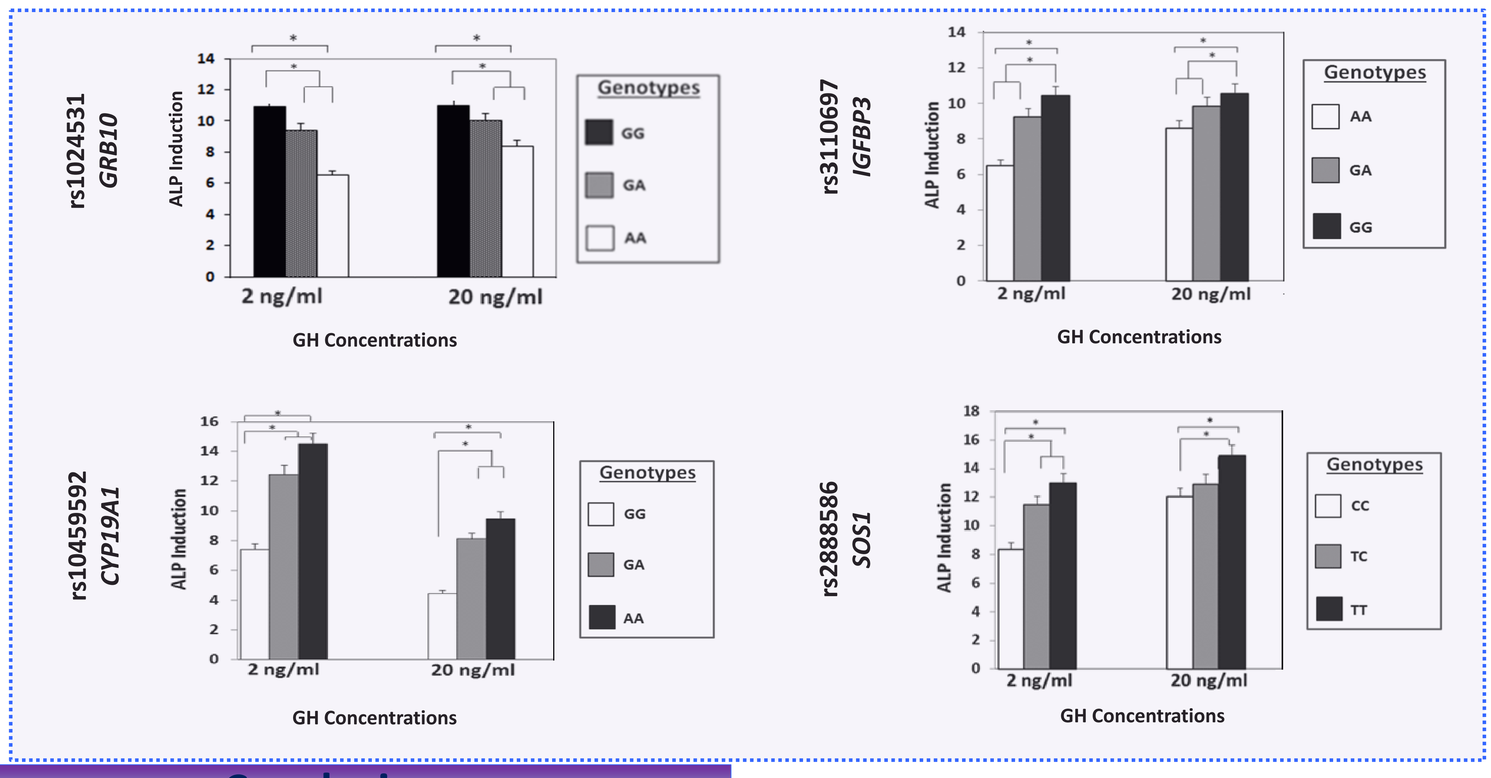
- The PREDICT study was a pharmacogenomic assessment of response to Growth Hormone (GH) therapy in children with GH deficiency
- It identified an association between response to GH therapy (defined as 1st year Height Velocity) with 22 single nucleotide polymorphisms (SNPs) located in 14 genes involved in growth pathways and oestrogen production[1]
- We selected four SNPs for detailed analysis of *in vitro* activity associated with increased growth response: rs1024531 in *GRB10* (A/G, genotype A ↑HV), rs3110697 in *IGFBP3* (G/A, genotype G ↑HV), rs1045992 in *CYP19A1* (G/A, genotype A ↑HV) and rs2888586 in *SOS1* (C/T, genotype T ↑HV)
- At baseline, the alleles related to better clinical outcome were significantly associated with greater transcriptional activity for *IGFBP3* and *GRB10* (p=0.003) and lower transcriptional activity for *SOS1* and *CYP19A1* (p<0.05)[2]

Aim

- To test the impact of the promoter SNPs rs1024531, rs3110697, rs1045992 and rs2888586 on growth response and on transcriptional activity in an *in vitro* cell system for each genotype

Material and Methods

- A generalized linear mode (GLM) was performed to assess the effect of carrying each allele on growth response (1st year height velocity SDS, HV) corrected for mutliple variables: age gender, GH peak, GH dose
- Each SNP with surrounding 500 bp fragment of the promoter sequence was cloned into a plasmid containing secreted alkaline phosphatase (ALP) as a reporter gene and transfected into human MCF-7 cell lines
- Transcriptional activity of each construct was evaluated by ALP induction and assessed at baseline and after 24 hours of GH stimulation (range: 2 and 20 ng/ml)



Results

- The GLM showed significant differences in 1st year HV for each SNP (**Table 1**)
- After GH stimulation, the alleles associated with better growth response had higher transcriptional activity at all GH concentrations for *IGFBP3*, *SOS1* and *CYP19A1.* For *GRB10*, the allele associated with better GH response had a significantly lower transcriptional activity at both GH stimulations (**Figure 1**)

Table 1 – Normalised 1st year height velocity SDS by genotype for the four SNPs where we have assessed transcriptional activity in this study. Height velocity was calculated by generalised linear model correcting for age, gender, peak GH to simulation testing and GH dose.

	Wald Chi-Square	P	Mean (95% CI) for Normalised 1st year Height Velocity SDS		
Genotype			GG	GA	AA
rs1024531 GRB10	30.800	0.0001	0.78 (-0.15 – 1.72)	2.40 (2.07 – 2.73)	3.12 (2.78 – 3.43)
Genotype			AA	GA	GG
rs3110697 IGFBP3	37.861	0.0003	0.98 (0.30 – 1.57)	2.64 (2.21 – 3.06)	2.68 (2.22 – 3.10)
Genotype			GG	GA	AA
rs10459592 CYP19A1	36.665	0.0009	1.86 (1.30 – 2.43)	1.43 (1.06 – 1.81)	2.99 (2.46 – 3.52)
Genotype			CC	TC	TT
rs2888586 SOS1	24.686	0.010	1.36 (0.86 – 1.86)	2.07 (1.67 – 2.46)	2.86 (2.30 – 3.42)

Figure 1 – Transcriptional activity assessed using Alkaline Phosphatase reporter for four SNPs associated with response to GH therapy. Higher transcriptional activity across all concentrations of GH is seen for the genotype associated with better growth response for all SNPs assessed except GRB10 where lower transcriptional activity was seen with higher growth response

Conclusions

- These results show that for the promoter SNPs, associated with growth response in the PREDICT study, genotype affects transcriptional activity
- Increased transcriptional activity of *CYP19A1, IGFBP3* and *SOS1 in vitro* is associated with increased height velocity *in vivo.* For *GRB10*, a negative regulator of IGF-I signalling and growth, decreased transcriptional activity was associated with increased height velocity

[1] Clayton P et al. Eur J Endocrinol 2013
[2] De Leonibus C et al. Horm Res Paediatr 2014

ESPE 2015 398-P2
GH and IGF Physiology
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DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: ESPE 2015 BARCELONA