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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.
- 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), rs10459592 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).

**Aim**
- To test the impact of the promoter SNPs rs1024531, rs3110697, rs10459592 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 multiple variables: age, gender, peak GH, 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.
- 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.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wald Chi-Square</th>
<th>p</th>
<th>Mean (95% CI) for Normalised 1st year Height Velocity SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GG</td>
</tr>
<tr>
<td>rs1024531</td>
<td>30.800</td>
<td>0.0001</td>
<td>0.78</td>
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<tr>
<td>GRB10</td>
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<td></td>
<td>GA</td>
</tr>
<tr>
<td>rs3110697</td>
<td>37.861</td>
<td>0.0003</td>
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<td>GG</td>
</tr>
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<td>rs10459592</td>
<td>36.665</td>
<td>0.0009</td>
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<td>CC</td>
</tr>
<tr>
<td>rs2888586</td>
<td>24.686</td>
<td>0.010</td>
<td>1.36</td>
</tr>
</tbody>
</table>

**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.