Gene expression profiles in growth hormone deficient (GHD) children relate peak GH levels to circadian clock, chromatin remodelling and WNT signalling pathways

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European Society for Paediatric Endocrinology (ESPE), Barcelona, Spain, 1-3 October 2015; Poster P2-394; Abstract 966

Background

- Growth Hormone (GH) deficiency is classically defined on the basis of a cut-off applied to the peak GH level during stimulation tests; a process with recognised limitations. Identifying the functional role of genes whose expression is associated with peak GH levels may help with our understanding and classification of GHD.

Objectives

- Identify patterns of gene expression (GE) related to peak GH levels and to describe the function, and regulation of these genes.

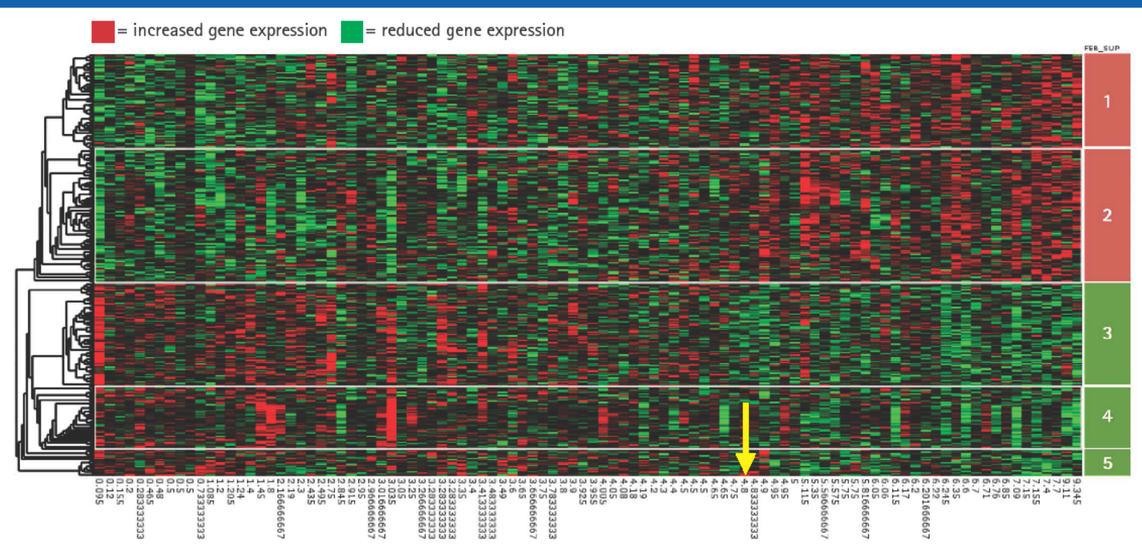
Methods

- Pre-pubertal children with GHD (n=98) were enrolled from the PREDICT study (NCT00256126) and childhood controls were obtained from available online datasets. GHD was defined at peak GH levels <10µg/ to two stimulation tests.
- Gene expression analysis was conducted on a library of gene expression datasets from normal children with age annotation collated from the NCBI Gene expression Omnibus (GEO) and EBI Arrayexpress databases (GSE9006, GSE26440 and TABM666). As the children in PREDICT were all prepubertal, GE profiles from normal children were removed if they were aged >11 which left 33 normal children.
- Whole blood GE was determined prior to GH treatment using Affymetrix U133v2 microarrays. GE was correlated with peak GH using rank regression [gender, ethnicity, age and body mass index (BMI) as co-variates].
- Network models were generated and the hierarchy of gene modules determined; upstream activity in the network model was assessed using causal network analysis.

Results

- Rank regression identified 347 probesets where expression correlated with peak GH concentrations: 188 positively and 159 negatively related (Rs > +/- 0.28, p<0.01). A heatmap of the genes identified by the rank regression plotted against peak GH (µg/l) as a continuous variable was generated (**Figure 1**).
- A point of inflexion between the +ve and -ve correlations is seen at around a peak GH of 4.8 µg/L (arrow on Figure 1). From the dendrogram 5 clusters of gene expression were identified – 2 related to genes where there is a positive correlation with peak GH and 3 related to genes where there was a negative correlation with peak GH.

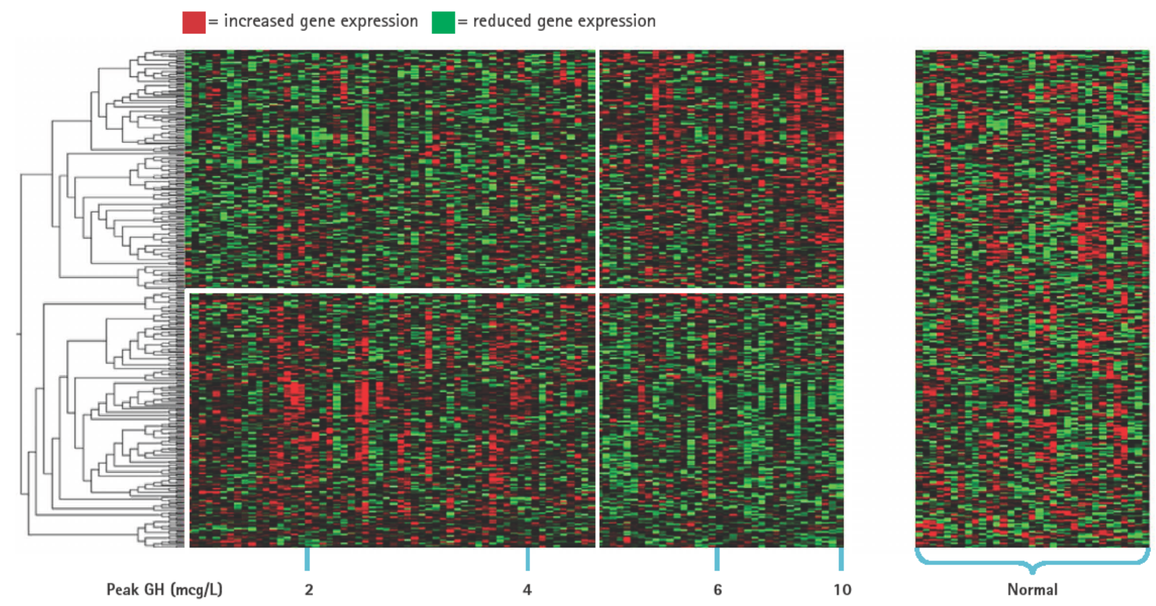
Figure 1. Identification of clusters of gene expression related to GHD severity



A Heat Map for the probesets identified by correlation with peak GH (n=347) (red = increased gene expression, green = reduced gene expression). Five distinct clusters of gene expression are identified via the dendrogram – two positively correlated with peak GH and three negatively correlated (labelled as boxes 1 -5, red = positively correlated, green = negatively correlated).

- The gene expression of the probesets identified by the rank regression model is displayed on a heatmap for both children with GHD and normal children in **Figure 2**. GE profiling demonstrated a clear difference in gene expression pattern between controls and patients even at peak GH levels between 6-10 µg/L.

Figure 2. Heatmap of gene expression for those probesets whose expression correlated with peak GH levels

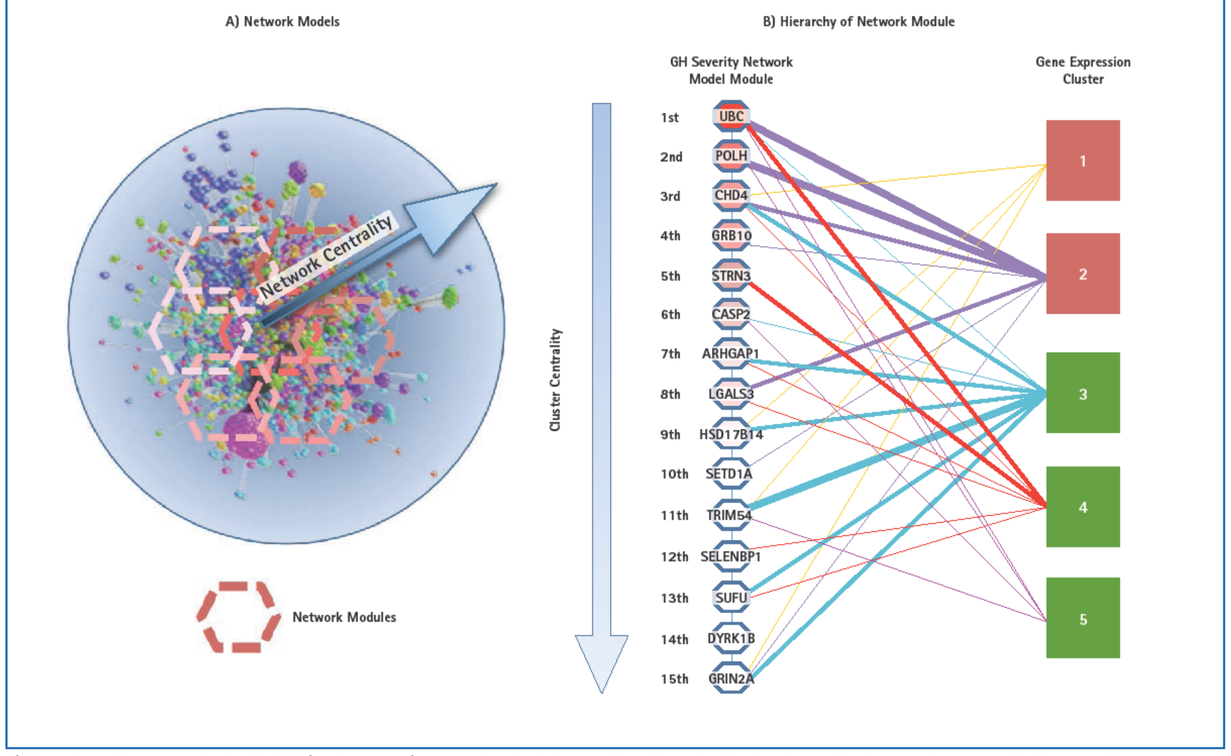


Normal children (n=33, age range 0-11 years of age, 18 male and 15 female) were combined with GHD patients (n=98), rank regression analysis was adjusted for gender and age as covariates, clusters of similar gene expression are identified using the Euclidean metric and marked using a dendrogram and white boxes. The distinction between normal subjects is marked by the break in the heatmap (red = increased gene expression, green = reduced gene expression); GHD as defined by a cut off level of 10 µg/L growth hormone as measured by provocation testing.

Network analysis

- Network modelling using the genes identified by the rank regression generated a model with 2427 nodes and 3604 links. Decomposition into a hierarchical modular structure revealed 43 network modules.
- Functionality was assessed on the top 15 modules as ranked by network centrality (**see Figure 3**). 5 of the 15 modules were related to circadian clock, 4 related to growth factor signalling, 3 related to DNA replication and repair and one module each related to Hedgehog signalling, WNT signalling, apoptosis, chromatin reorganisation, detoxification of reactive oxygen species and the cell cycle.
- Gene expression clusters identified using the heatmap derived via the rank regression analysis were overlapped with the network modules. This identified network modules where there was an enrichment of links with specific gene expression clusters. Gene cluster 1 linked to only one network module *(HSD17B14)* related to cell cycle while gene cluster 5 was also linked to only one network module – CASP2 related to apoptosis pathways.
- Gene cluster 2 associated with the 2nd, 3rd and 4th network modules related to Circadian Clock, chromatin organisation and growth factor signalling. Gene clusters 3 and 4 each linked to 4 network modules covering the whole spectrum of pathways identified except apoptosis.

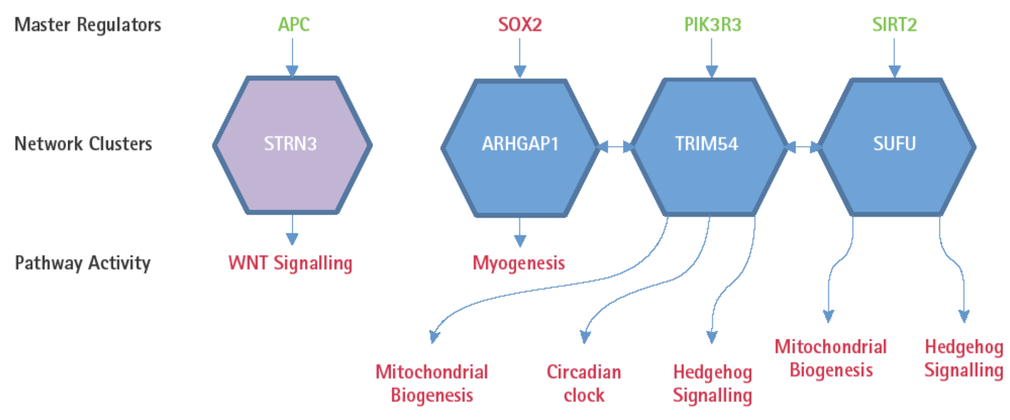
Figure 3. Network modelling of the overlap of gene expression between clinical markers



A) Network models generated using BioGRID (version 3.2.117) were analysed to define modules of functionally related genes. The "community structure" of these modules was assessed and ranked by their "centrality" score to form a hierarchy related to the biological action of the network. B) Community structure of modules within the network was assessed using the Moduland algorithm in Cytoscape 2.8.3. Hierarchy of the first ten network modules in each of the network models of gene expression overlap between clinical markers. Modules are shown as octagons labelled with the most central gene in the cluster and ranked by network centrality (1st through 15th). The five gene expression clusters labelled are those identified in Figure 1.

- Causal network analysis identified four elements within the network model which mapped to the 15 network modules (**see Figure 4**). Master regulators identified included *APC2* regulating the STRN3 network module related to apoptosis and gene cluster 4.
- *SOX2*, *PI3KR3* and *SIRT2* were identified as regulators of the *ARHGAP1*, *TRIM54* and *SUFU* network modules linked to gene clusters 3 and 4 affecting Hedgehog signalling, Circadian Clock, Mitochondrial biogenesis and myogenesis pathways.

Figure 4. Summary of predicted activity and regulators derived via causal network analysis for the network modules



The hierarchy of clusters of gene expression shown in figure 1 were mapped onto identified causal networks. Activity of pathways and master regulators are coloured red to show a positive correlation with the GHD severity or green where activity is negatively correlated.

Conclusions

- **This study has demonstrated the potential for gene expression profiling to aid in both the diagnosis and classification of GHD and in addition has identified the functions of the networks of genes related to peak GH concentrations along with their master regulators.**
- **Normal children appear to have a different pattern of GE to GHD children**
- **GE profiling identified a genomic signature of GHD functionally linked to circadian clock and growth factor signalling and regulated by PIK3R3, SIRT2 and APC2.**

Acknowledgments

The study was sponsored by Merck KGaA, Darmstadt, Germany. The authors would like to thank the patients and their families, investigators, co-investigators and the study teams at each of the participating centers and at Merck KGaA, Darmstadt, Germany.

Disclosures

AS, CDL, PCh, PCl, honoraria and research grants from Merck KGaA Darmstadt, Germany; EK is an employee of Merck KGaA, Darmstadt, Germany. PM declares no financial interest.