Important contribution of *GH*, *GHRHR* and *GHSR* mutations in isolated growth hormone deficiency with a normal location of the posterior pituitary –Functional characterization of new variants

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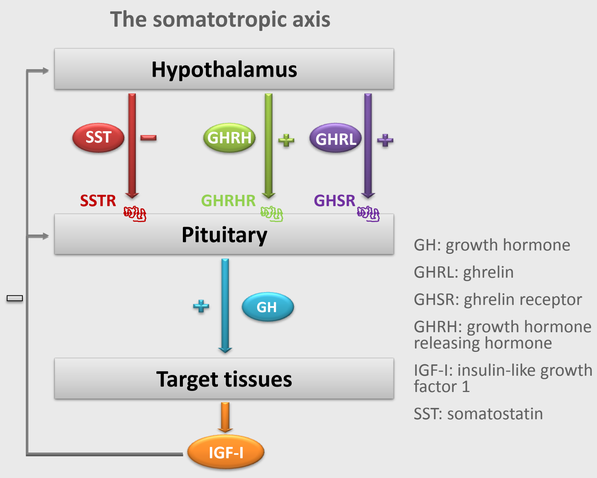
Introduction

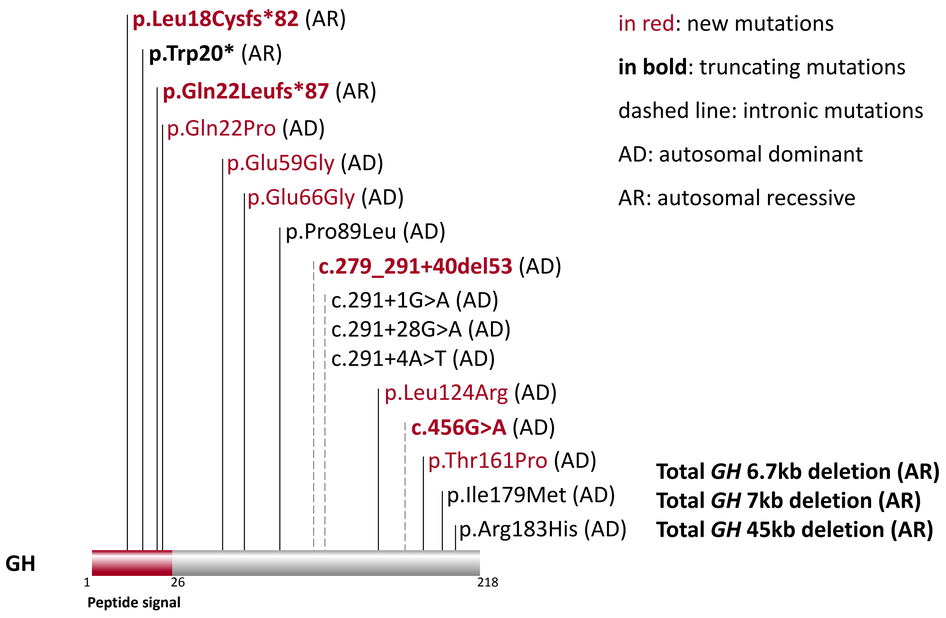
Growth is a complex phenomenon that depends on several factors including growth hormone (GH) secretion, the GH releasing hormone (GHRH) and its receptor (GHRHR), and ghrelin (GHRL) and its receptor (GHSR). Although *GH*, *GHRHR* and *GHSR* have been recognized as key etiologic factors in non-syndromic forms of isolated growth hormone deficiency (IGHD), a small number of mutations have been identified in this rare condition. Depending on the studies, GH and GHRHR defects would account for 6-12.5% and 0-6.7% of IGHD cases. So far, as for GHRHR and GHSR, very few functional studies have been performed in order to assess the consequences of the identified variants.



Objective

With the aim to assess the contribution of the *GH, GHRHR* and *GHSR* genes in the pathogenesis of IGHD, we screened for mutations all coding exons and flanking intronic sequences of these three genes by Sanger sequencing or Next Generation Sequencing in a large cohort of patients with a non-syndromic form of IGHD characterized by a small or normal anterior pituitary and an eutopic posterior pituitary.

1 *GH* variations



in red: new mutations

in bold: truncating mutations

dashed line: intronic mutations

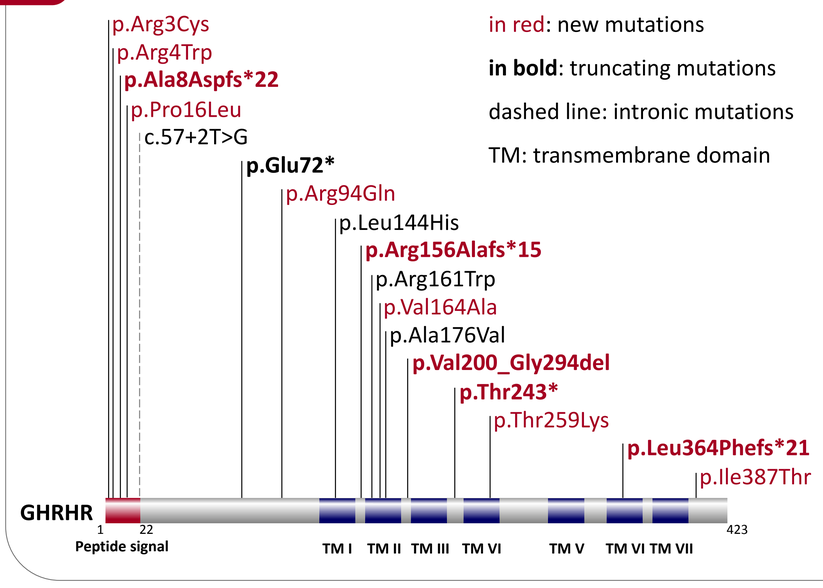
AD: autosomal dominant

AR: autosomal recessive

Total *GH* 6.7kb deletion (AR)
Total *GH* 7kb deletion (AR)
Total *GH* 45kb deletion (AR)

The *GH* gene was first analyzed in a total of 360 independent patients. Variations were identified in 40 patients (11%), 17 of them (17/40, 43%) representing familial forms of IGHD. These include 9 novel mutations, among which 2 frameshifts, 2 splicing defects and 5 missense mutations. Whole gene deletions and truncating mutations were associated with a recessive GH deficit; missense mutations and mutations affecting exon 3 splicing were associated with a dominant deficit.

2 *GHRHR* variations



in red: new mutations

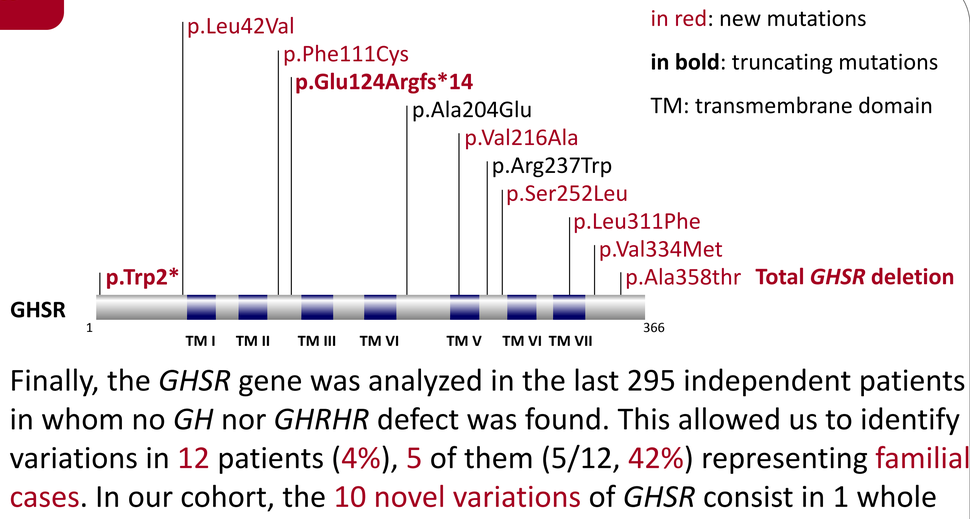
in bold: truncating mutations

dashed line: intronic mutations

TM: transmembrane domain

The *GHRHR* gene was subsequently analyzed in the remaining 320 independent patients in whom no *GH* defect was identified. This led to the identification of variations in 25 patients (8%), 8 of them (8/25, 32%) representing familial cases. The *GHRHR* mutation spectrum, which comprises 12 novel variations, consists of 6 truncating mutations, 1 splicing defect and 10 missense variations. Apart from the 3 peptide signal variations, all mutations were associated with recessive GH deficits.

3 *GHSR* variations



in red: new mutations

in bold: truncating mutations

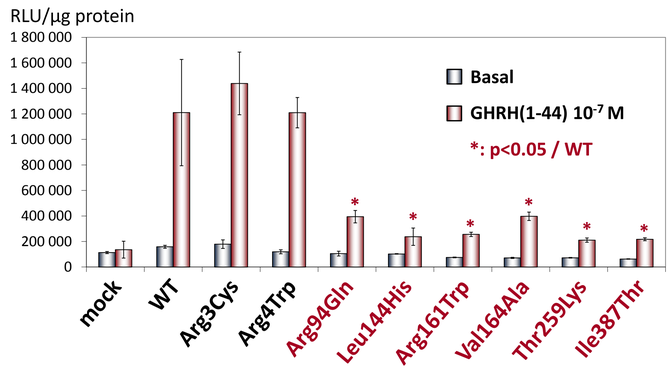
TM: transmembrane domain

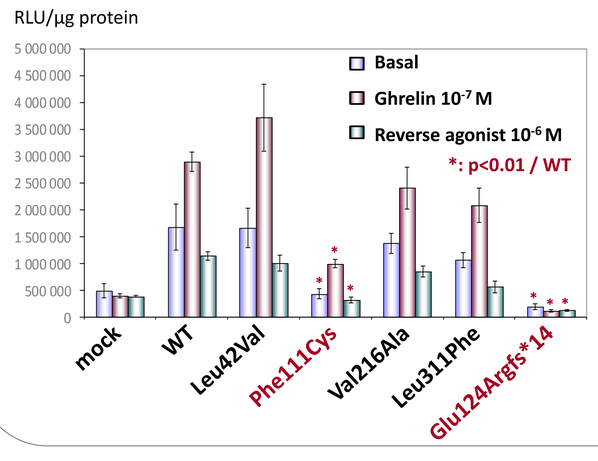
Total *GHSR* deletion

Finally, the *GHSR* gene was analyzed in the last 295 independent patients in whom no *GH* nor *GHRHR* defect was found. This allowed us to identify variations in 12 patients (4%), 5 of them (5/12, 42%) representing familial cases. In our cohort, the 10 novel variations of *GHSR* consist in 1 whole gene deletion, 2 truncating mutations and 7 missense mutations.

4 Signal transduction of GHRHR and GHSR variants

In vitro functional studies of the missense *GHRHR* mutations were performed to assess the GHRH response through a CRE-dependent luciferase assay. All the missense variations located in the mature protein show an impaired function ($p<0.05$). The signal peptide variants p.Arg3Cys and p.Arg4Trp are probably benign polymorphisms.

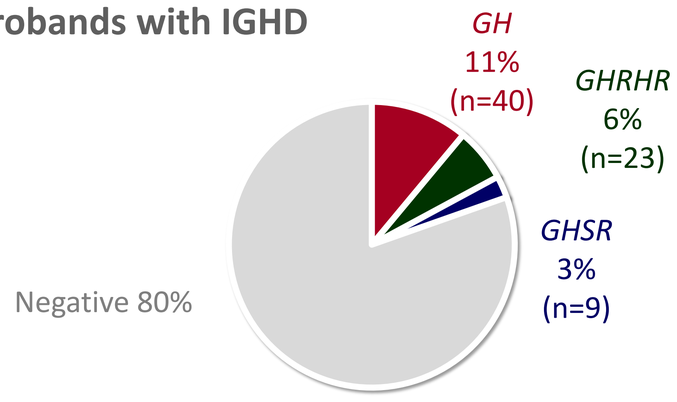




In addition to the previously assessed *GHSR* missense variations p.Ala204Glu and p.Arg237Trp, the p.Phe111Cys showed an impaired constitutive and ghrelin-induced activity (SRE-dependent luciferase assay, $p<0.01$). Missense variants p.Leu42Val, p.Val216Ala and p.Leu311Phe are likely to be rare polymorphisms.

5 *GH, GHRHR* and *GHSR* mutations account for 20% of IGHD

Deleterious or potentially deleterious mutations in 360 independent probands with IGHD



GH: growth hormone

GHRHR: growth hormone releasing hormone receptor

GHSR: growth hormone secretagogue receptor

Conclusion

Overall, this study performed in a large cohort of patients, which identified deleterious or potentially deleterious molecular defects in the *GH*, *GHRHR* or *GHSR* gene in 72 out of 360 independent patients (20%), reveals the importance of those three genes in the pathogenesis of non-syndromic IGHD with a normal location of the posterior pituitary. Noteworthy, up to 61% (43/72) of the patients with a *GH*, *GHRHR* or *GHSR* germline mutation represent sporadic cases.

Authors have no conflict of interest

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Growth: Mechanisms

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