HHS Public Access

Author manuscript

Growth Horm IGF Res. Author manuscript; available in PMC 2021 February 01.

Published in final edited form as:

Growth Horm IGF Res. 2020 February ; 50: 23–26. doi:10.1016/j.ghir.2019.11.002.

p.R209H *GH1* variant challenges short stature assessment

Nora Sanguineti[1], **Debora Braslavsky**[1], **Paula A Scaglia**[1], **Ana Keselman**[1], **Maria G Ballerini**[1], **Maria G Ropelato**[1], **Sofia Suco**[1], **Sebastian Vishnopolska**[2], **Ariel J Berenstein**[3], **Héctor Jasper**[1], **Horacio M Domené**[1], **Rodolfo A Rey**[1], **Maria I Pérez Millán**[4], **Sally A Camper**[5], **Ignacio Bergadá**[1]

[1]División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Centro de Investigaciones Endocrinológicas 'Dr César Bergadá' (CEDIE) CONICET - FEI, Buenos Aires, Argentina.

[2]Department of Biological Chemistry (IQUIBICEN-UBA-CONICET), Faculty of Exact and Natural Sciences, University of Buenos Aires, Buenos Aires, Argentina.

[3]Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP), CONICET-GCBA, Laboratorio de Biología Molecular, División Patología, Hospital de Niños Ricardo Gutiérrez, C1425EFD Buenos Aires, Argentina.

[4]Institute of Biomedical Investigations (INBIOMED-UBA-CONICET), University of Buenos Aires, Buenos Aires, Argentina.

[5]Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA.

Introduction

Endocrine causes of short stature include defects in the growth hormone (GH)-insulin-like growth factor (IGF) system and main discrimination must be done between growth hormone deficiency (GHD) and GH insensitivity (GHI) [1]. Patients with GHD usually present severe postnatal short stature, low IGF-I and IGF binding protein-3 (IGFBP-3) levels, subnormal response to GH provocative tests (GHPT) and typical clinical features. GHI is generally characterized by pre- and/or postnatal growth retardation, low IGF-I levels, and normal or even increased circulating GH concentrations; additional features may vary according to its pathogenesis. Nevertheless, confirming the diagnosis of GHD and GHI is a challenge since clinical and biochemical features may overlap between these entities.

GHPT are a useful biochemical tool and have been the gold standard to confirm the diagnosis of GHD. Many limitations still exist such as weak grade of evidence for the cut-off values used and poor test specificity and reproducibility [2]. With the expansion of genomics, genetic evaluation has become an additional tool in aiding the process of obtaining a definitive diagnosis [3].

Declaration of Competing Interest

None of the authors has conflicts of interest to disclose.

Isolated GHD (IGHD) of genetic origin is mostly caused by *GH1* gene pathogenic variants, and less commonly by variants in GHRH receptor (*GHRHR*) or Ghrelin receptor (*GHSR*) genes [4–6]. *GH1* variants inherited with an autosomal dominant pattern are classified as type II IGHD. Most of them affect *GH1* splicing and result in exon 3 skipping, but missense mutations have also been described, usually with a milder phenotype [5,7].

The aim of our study was to describe marked variability in clinical and biochemical pattern due to a p.R209H *GH1* missense variant, previously described as p.R183H, in a large Argentinean pedigree, making the diagnosis of IGHD elusive.

Subjects and Methods

Auxological variables

Height, weight and head circumference were measured and expressed in SDS for age and sex, according to Argentinean reference growth charts [8]. Length and height were determined using an infantometer or a wall mounted stadiometer according to age.

Hormonal studies

Serum levels of GH, IGF-I and IGFBP-3 were determined by chemiluminescent immunometric assays (Immulite 2000, Siemens Healthcare Diagnostics, Llamberis, Gwynedd, UK). GH provocative tests were performed with arginine (0.5 g/kg) and clonidine (0.100 mg/m^2): GH was measured at baseline and at standard intervals (IS 98/574, Immulite). GHD was defined as a peak GH lower than 4.8 ng/ml in two GHPT [9]. Intra- and interassay coefficients of variation were <5.5% for all serum measurements. IGF-I generation test (IGFGT) was performed as follows: subcutaneous rhGH (0.23 mg/kg.day) administered daily for 7 days; IGF-I and IGFBP-3 serum concentrations were measured at baseline, 5th and 8th day. We considered a positive response the increment of IGF-I levels to the normal range for the patientś age, sex and pubertal status.

Subjects

We report a non-consanguineous pedigree spanning four generations (Figure 1, Table 1). Within this pedigree, four out of nine siblings (III.27, III.28, III.29, III.30) (2 females) were referred for short stature with median height of −3.8 SDS (range −2.60 to −4.02); none of them presented typical features of GHD. Biochemical assessment showed a normal GH response to GHPT (GH peaks ranged from 5.46 to 10.9 ng/ml), associated with low IGF-I (median −3.05; range −2.49 to −4.54 SDS) and low IGFBP-3 levels (median −2.10; range −1.04 to −2.95 SDS), thus resembling a pattern of GHI. Nevertheless, IGFGT revealed a median IGF-I increment of 3 times over basal (range 2.4 to 4.3 folds), which suggested adequate GH sensitivity. The remaining 5 siblings (III.23-III.26; III.31), aged 8 to 22 yrs. (4 females), had normal height (median height −0.40; range −1.05 to 0 SDS) and normal IGF-1 levels, while their parents had normal stature and normal-low IGF-I levels (Table 1).

Within the same pedigree, two children (IV.2, IV.3) presented classical IGHD: typical GHD phenotype with short stature (height −4.74 and −2.27 SDS, at 5.4 and 1.8 yrs., respectively), non-detectable IGF-I and low IGFBP-3 levels (−2.83 and −2.28 SDS, respectively).

Unexpectedly, GHPT (Arginine and Clonidine) performed in the first patient (IV.2) at 5.75 yrs. showed adequate GH response (GH peak 7.59 ng/ml). As his clinical features were strongly indicative of GHD, GHPT were repeated at 7.4 yrs., showing low GH response (GH peak of 3.62 ng/ml), that confirmed the diagnosis of GHD. The second patient (IV.3) showed an insufficient response to GHPT (GH peak 2.65 ng/ml) at 2.4 yrs. Brain MRI was normal in IV.2 and showed anterior pituitary hypoplasia (2.5 mm) in IV.3. Both responded adequately to recombinant human growth hormone treatment (rhGH, 0.17 mg/kg/wk; with a mean height increment of 1.4 SDS in the 1st yr of treatment) [10]. Patient IV.2ś mother presented with short stature. Patient IV.3ś parents and brother had normal stature, but the paternal grandmother (II.4) was severely short (134.6 cm, −4.3 SDS) with typical GHD clinical phenotype, undetectable IGF-I, and extremely low IGFBP-3 levels.

Molecular studies

Genomic DNA was isolated from peripheral venous blood by cetyltrimethylammonium bromide lysis buffer and chloroform-isoamyl alcohol extraction [11]. Patient IV.3 was included in a research protocol using a panel of 67 genes associated to combined pituitary hormone deficiency (CPHD) and IGHD in humans and mice [12]. In patients IV.2 and III.29, the whole coding sequence of *GH1* gene was PCR amplified [13] and sequenced in a 3500 Genetic Analyzer (Applied Biosystems™, Thermo Fisher Scientific). In patients III.27, III.28, III.30 and other family members (marked as sequenced in figure 1), *GH1* exon 5 was PCR amplified and sequenced. The following *GH1* reference sequences were used: NG_011676.1, NM_000515.4, NP_000506.2.

All subjects were informed of the purpose of the study and their written consent was obtained. The study was approved by the Ethics Committee of Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina.

Results and Discussion

Gene panel NGS analysis revealed a heterozygous c.626G>A transition in exon 5 of the *GH1* gene in GHD patient IV.3, which was confirmed by Sanger sequencing. This variant predicts the change of an arginine for a histidine in position 209 (p.R209H). Her father was heterozygous for the *GH1* p.R209H variant, and her paternal grandmother was homozygous for this variant.

Sanger sequencing revealed that GHD patient IV.2 and his mother were also heterozygous for p.R209H.

Data from the extended pedigree (III.27, III.28, III.29, III.30) suggested *GH1* as the initial candidate gene. The same pathogenic heterozygous *GH1* variant was identified in these four siblings and their father. Four of the non-affected siblings and their mother were negative for the variant (Table 1).

Autosomal dominant type II IGHD is caused by heterozygous *GH1* mutations, and these patients have variable clinical expression, including low but detectable serum GH levels, wide range of height deficit, and different pattern on brain MRI (normal or anterior pituitary

hypoplasia) [5,7,14,15]. The missense p.R209H variant reduces GH secretion due to defects in exocytosis of secretory granules, but without evidence of toxic effect on somatotrophs. Thus, the GH molecule remains fully bioactive but unable to reach its target receptor [16,17]. Hence, some type II IGHD patients may still produce and secrete GH at a reduced level and GHD can be time-dependent [18].

In the large pedigree described here, 10 individuals carried the same *GH1* p.R209H variant but presented variable clinical and biochemical phenotype. Firstly, two children presented the classical IGHD phenotype; the genetic disorder was inherited from a short mother in one case, and a normal stature father in the other. Additionally, four children carried the same *GH1* p.R209H variant and initially resembled a pattern of GHI: postnatal growth failure with minimal or absence of typical GHD phenotype, normal GHPT response and low basal IGF-I and IGFBP-3 levels. Thereafter, IGFGT was normal. They inherited the variant from the carrier father who achieved a normal adult height.

Homozygosity for p.R209H variant found in II.4 was unexpected. Unfortunately, we were not able to confirm whether the grandmother was hemizygous for the variant (one deleted together with one mutated allele). Thus, further studies are necessary to rule out the presence of hemizygosity.

These findings highlight the existence of a great variability within the clinical and biochemical phenotype associated to *GH1* p.R209H variant. Even more, the change in the response to GHPT suggests an evolving process in GH secretion [7].

We describe four siblings showing an elusive clinical and biochemical phenotype of GHD as they presented growth retardation and abnormal surrogates of GH action (IGF-I, IGFBP-3), but normal response to GHPT. In this subgroup of patients, GHPT failed to diagnose GHD.

Similar to other published cases, adult height in affected *GH1* p.R209H parents fell within a wide range of −1.0 to −4,5 SDS, showing that normal final height may be obtained, even with low-normal IGF-I levels [7,19]. If this could be due to a time-dependent deficiency or to other factors beyond GH, remains to be answered.

Conclusion

The identification of short stature disorders requires the integration of all clinical, biochemical and radiological data and it is critical to ascertain an extended family history. The wide spectrum of clinical and biochemical presentation in individuals carrying the p.R209H *GH1* mutation could hinder the diagnosis of GHD, therefore misleading the genetic diagnosis approach. We suggest considering *GH1* sequencing in children with short stature associated to low IGF-I and IGFBP-3 serum levels, even in the context of normal response to GHPT.

Acknowledgments

Funding

Funding was provided by National Institute of Health HD30428 (SAC); Agencia Nacional de Promoción Científica y Tecnológica PICT 2017-0002, PICT 2016-2913 (MIPM).

References

[1]. Allen DB, Cuttler L, Short Stature in Childhood — Challenges and Choices, N. Engl. J. Med 368 (13) (2013) 1220–28. [PubMed: 23534561]

[2]. Grimberg A, DiVall SA, Polychronakos C, et al., Guidelines for Growth Hormone and Insulin-Like Growth Factor-I Treatment in Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature, and Primary Insulin-Like Growth Factor-I Deficiency, Horm. Res. Paediatr 86 (2016) 361–397. [PubMed: 27884013]

[3]. Dauber A, Rosenfeld RG, Hirschhorn JN, Genetic Evaluation of Short Stature, J. Clin. Endocrinol. Metab 99 (9) (2014) 3080–3092. [PubMed: 24915122]

[4]. Mullis PE, Genetics of growth hormone deficiency. Endocrinol. Metab. Clin. North. Am 36 (17) (2007) 36–84.

[5]. Alatzoglou KS, Webb EA, Le Tissier P, Dattani MT, Isolated Growth Hormone Deficiency (GHD) in Childhood and Adolescence: Recent Advances, Endocrine Reviews 35 (2014) 376–432. [PubMed: 24450934]

[6]. Juanes M, Marino R, Ciaccio M et al., Presence of GH1 and absence of GHRHR gene mutations in a large cohort of Argentinian patients with severe short stature and isolated GH deficiency, Clinical Endocrinology 0 (2013) 1–2.

[7]. Hess O, Hujeirat Y, Wajnrajch MP, et al., Variable phenotypes in familial isolated growth hormone deficiency caused by a G6664A mutation in the GH-1 gene, J. Clin. Endocrinol. Metab 92 (2007) 4387–4393 [PubMed: 17785368]

[8]. Lejarraga H, del Pino M, Fano V, Caino S, Cole TJ, Growth references for weight and height for Argentinian girls and boys from birth to maturity: incorporation of data from the World Health Organisation from birth to 2 years and calculation of new percentiles and LMS values, Arch. Argent. Pediatr 107 (2009) 126–133. [PubMed: 19452084]

[9]. Chaler EA, Ballerini G, Lazzati JM et al., Cut-off values of serum growth hormone (GH) in pharmacological stimulation tests (PhT) evaluated in short-statured children using a chemiluminescent immunometric assay (ICMA) calibrated with the International Recombinant Human GH Standard 98/574. Clin. Chem. Lab. Med 51 (2013) 95–97.

[10]. Ranke MB, Lindberg A, Predicting growth in response to growth hormone treatment, Growth Hormone & IGF Research 19 (2009) 1–11. [PubMed: 18824380]

[11]. Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure J, Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation, Genome Res. 23 (5) (2013) 843–54. [PubMed: 23382536]

[12]. Pérez Millán MI, Vishnopolska SA, Daly AZ, et al., Next generation sequencing panel based on single molecule molecular inversion probes for detecting genetic variants in children with hypopituitarism, Mol. Genet. Genomic Med 6 (4) (2018) 514–525.

[13]. Moseley CT, Mullis PE, Prince MA, Phillips JA 3rd, An exon splice enhancer mutation causes autosomal dominant GH deficiency, J. Clin. Endocrinol. Metab 87(2) (2002) 847–52. [PubMed: 11836331]

[14]. Binder G, Nagel BH, B Ranke M, Mullis PE, Isolated GH deficiency (IGHD) type II: imaging of the pituitary gland by magnetic resonance reveals characteristic differences in comparison with severe IGHD of unknown origin, Eur. J. Endocrinol 147 (2002) 755–760. [PubMed: 12457450]

[15]. Alatzoglou KS, Turton JP, Kelberman D, et al., Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency, J. Clin. Endocrinol. Metab 94 (2009) 3191–3199. [PubMed: 19567534]

[16]. Deladoëy J, Stocker P, Mullis PE, Autosomal dominant GH deficiency due to an Arg183His GH-1 gene mutation: clinical and molecular evidence of impaired regulated GH secretion, J. Clin. Endocrinol. Metab 86 (2001) 3941–3947. [PubMed: 11502836]

[17]. Zhu YL, Conway-Campbell B, Waters MJ, Dannies PS, Prolonged retention after aggregation into secretory granules of human R183H-growth hormone (GH), a mutant that causes autosomal dominant GH deficiency type II, Endocrinology 143(11) (2002) 4243–8. [PubMed: 12399418]

[18]. Mullis PE, Deladoey J, Dannies PS, Molecular and cellular basis of isolated dominant-negative growth hormone deficiency, IGHD type II: insights on the secretory pathway of peptide hormones, Horm. Res 58(2) (2002) 53–66. [PubMed: 12207163]

[19]. Hamid R, Phillips JA 3rd, Holladay C, et al., A molecular basis for variation in clinical severity of isolated growth hormone deficiency type II, J. Clin. Endocrinol. Metab 94 (2009) 4728–4734. [PubMed: 19837935]

Highlights

- Growth hormone provocative tests have many limitations.
- Phenotype between GHD and GHI may overlap.
- Genetic evaluation is an additional tool towards a definitive diagnosis of GHD
- *GH1* p.R209H variant has markedly variable clinical and biochemical consequences.
- It is advisable to explore *GH1* in short children with low IGF-I and IGFBP-3, despite normal GHPT.

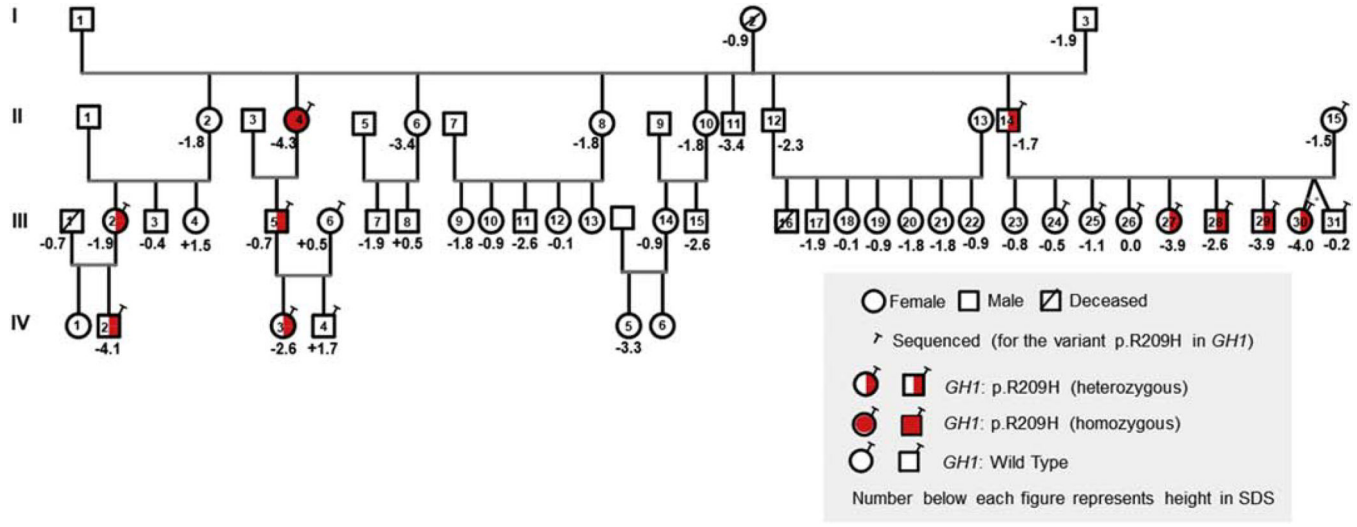

Figure 1.
Family Pedigree

Table 1.

Summary of the clinical and biochemical characteristics of the assessed family members

Patient n°	Age at presentation (yr)	Height (SDS)	IGF-I (ng/ml) (SDS or RR)[b]	IGFBP-3 (μg/ml) (SDS or RR)[b]	Basal GH (ng/ml)	GHPT Age (yr)	GH peak (ng/ml)	*GH1*
IV.2[a]	5.4	−4.7	ND	1.7 (−2.8)	0.2	5.8 7.4	7.6 3.6	p.R209H
IV.3[a]	1.8	−2.3	ND	1.6 (−2.3)	0.2	2.4	2.7	p.R209H
III.27	2.8	−3.9	29 (−3.2)	2.1 (−1.0)	1.7	5.9	7.6	p.R209H
III.28	9.8	−2.6	33 (−2.9)	1.9 (−2.1)	0.1	11	5.5[c]	p.R209H
III.29	2.1	−3.9	41 (−2.5)	1.9 (−2.1)	0.4	9.4	10.9	p.R209H
III.30	5.2	−4.0	26 (−4.5)	1.25 (−2.9)	1.2	8.3	8.5	p.R209H
III.1	39	−0.7	NA	NA	NA			NA
III.2	31	−1.9	NA	NA	NA			p.R209H
III.5	29	−0.7	NA	NA	NA			p.R209H
III.6	34	0.5	72 (115–307)	3.9 (3.5–7)	0.3			WT
IV.4	8.2	1.7	206 (1.9)	6.1 (3.4)	7.0			WT
II.4[a]	56	−4.3	ND	0.7 (3.4–6.9)	0.1			p.R209H
II.14	44	−1.7	64 (43–209)	1.8 (3.3–6.6)	<0.1			p.R209H
II.15	40	−1.5	75 (43–209)	3.9 (3.4–6.7)	0.1			WT
III.23	26	−0.8	NA	NA	NA			NA
III.24	22	−1.0	172 (107–367)	4.5 (3.4–7.8)	NA			WT
III.25	19	−1.1	175 (105–346)	4.2 (2.9–7.3)	3.6			WT
III.26	18	0.0	214 (176–429)	4.3 (3.1–7.9)	2.8			WT
III.31	8.3	−0.2	98 (−0.1)	3.1 (−0.6)	3.4			WT

ND = Not Detectable; **NA** = Not Available; **WT**: Wild Type.

a: Presence of classical GHD clinical features.

b: SDS was obtained from our local pediatric population values and kit-reference range (RR) was used for adults.

c: GH provocative Tests performed with priming (17 beta-estradiol).