

GROWTH HORMONE RECEPTOR GENE POLYMORPHISM. SPONTANEOUS CATCH UP GROWTH IN SMALL FOR GESTATIONAL AGE PATIENTS

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Abstract The growth hormone receptor (GHR) mediates the effect of growth hormone (GH) on linear growth and metabolism. In humans, it exists as two isoforms differing by the retention or exclusion of exon 3; a full-length GHR isoform (GHRfl) and the exon 3-deleted isoform (GHRd3). The genotypic frequency of this polymorphism was analyzed in several studies and in different human populations. However scarce information in Argentinean population is available. Associations between GHRd3 and growth have been reported previously. Some studies have shown that the presence of GHRd3 polymorphism might be a potential variant that improves growth response to recombinant human GH (rhGH) therapy in patients born small for gestational age (SGA), among others. However, over the years the results have been controversial and inconclusive. Based on this, it would be proposed that variants at the genomic level are not completely reflected at the mRNA level. Our aim was to evaluate the genotypic frequencies (%) of the GHR gene polymorphism (GHRfl/GHRfl; GHRfl/GHRd3; GHRd3/GHRd3) in normal Argentinean population (n = 94) and SGA patients (n = 65), and the expression of these polymorphisms at mRNA level in the fetal side of placenta tissues was analyzed. In addition, their association with spontaneous postnatal catch-up growth in SGA patients was also evaluated. In this study, we show a significant increment of compensatory growth in small for gestational age children (SGA) associated to the presence of the GHRd3 allele polymorphism. In addition, the expression of *GHR* in healthy placentas revealed that no alternative splicing mechanism occurs.

Key words: isoforms, polymorphism, growth hormone receptor gene

Resumen *Polimorfismo del gen del receptor de la hormona de crecimiento. Crecimiento postnatal espontáneo en niños pequeños para la edad gestacional.* El receptor de la hormona de crecimiento (GHR) media la acción de la hormona de crecimiento (GH) en el crecimiento lineal y el metabolismo. En los seres humanos, existen dos isoformas que difieren en la retención (GHRfl) o exclusión del exón 3 (GHRd3). La frecuencia genotípica de este polimorfismo fue analizada en varios estudios y en diferentes poblaciones. Sin embargo, la información disponible en la población argentina es escasa. Se ha reportado anteriormente asociación entre el polimorfismo GHRd3 y el crecimiento. Varios estudios han demostrado que la presencia del polimorfismo GHRd3 podría mejorar, en pacientes nacidos pequeños para la edad gestacional, entre otros, la respuesta a la terapia con GH humana recombinante (rhGH). Sin embargo, a lo largo de los años los resultados han sido controvertidos y no concluyentes. En base a esto, se propondría que las variantes a nivel genómico no se reflejan completamente a nivel del ARNm. Nuestro objetivo fue evaluar la frecuencia genotípica de los polimorfismos del gen del GHR (GHRfl/GHRfl; GHRfl/GHRd3; GHRd3/GHRd3) en la población argentina normal (n = 94) y en niños pequeños para la edad gestacional (n = 65), y se analizó la expresión de estos polimorfismos a nivel de ARNm en la porción fetal de placentas sanas. Además, se evaluó la asociación de este polimorfismo con el crecimiento postnatal espontáneo en pacientes pequeños para la edad gestacional. En este estudio, mostramos un incremento significativo del crecimiento compensatorio en niños pequeños para la edad gestacional asociado a la presencia del polimorfismo del alelo GHRd3. Además, los ensayos de expresión de *GHR* en placentas sanas revelaron que no se produciría ningún mecanismo de *splicing* alternativo.

Palabras clave: isoformas, polimorfismo, receptor de hormona de crecimiento

KEY POINTS

- The growth hormone receptor exists as two isoforms differing by the retention or exclusion of exon 3. Associations between GHRd3 and growth have been reported.
- In this study the genotypic frequency of GHR gene polymorphism in our population was 48% for the homozygous GHRfl genotype, 38% for the heterozygous genotype and 14% for the homozygous GHRd3 genotype.
- A significant increment of compensatory growth in SGA children was found associated to the presence of the GHRd3 allele polymorphism.
- GHR expression in healthy placentas revealed that no splicing mechanism occurs.

The growth hormone receptor (GHR) mediates the effect of growth hormone (GH) on linear growth and metabolism. In humans, GH is secreted by the pituitary gland and acts directly on GHR in various cell types generating an increase in the expression of insulin-like growth factor type I (IGF1) and other GH-dependent genes. The growth hormone receptor (*GHR*) gene is located on the short arm of chromosome 5 (p13.1-p12) and consists of 9 coding exons (II to X) that expands at least 87kb and 1 non-coding exon (I).

The GHR protein comprise of a large extracellular domain involved in GH binding and GHR dimerization (exons III to VII), a single transmembrane domain that anchors the receptor to the cell surface (exon VIII), and an intracellular domain involved in GH signaling (exons IX and X)¹ In humans, the GHR protein is composed by 638 amino acids and exists as two isoforms of GHR differing by the retention or exclusion of exon 3; a full-length GHR isoform (GHRfl) and the exon 3-deleted isoform (GHRd3). The genomic deletion of exon 3 determines the loss of a 22 amino acids sequence in the N-terminal portion of the extracellular domain (residues 7-28) with the loss of a potential glycosylation site and the substitution of alanine to aspartic acid at position 6, with changes in size charge and hydrofobicity² This common polymorphic variant in exon 3 has been reported in the general population³ In addition, associations between GHRd3 and growth have also been reported⁴⁻⁸

The genotypic frequency of this polymorphism was analyzed in several studies and in different human populations. Frequencies of 1-26% in homozygosis and 18-58% in heterozygosis were found depending on the population under study. Although there are significant variations between different populations, in all cases it is a frequent variant⁹⁻¹² However scarce information in Argentinean population is available.

Since its discovery until today this polymorphism has been widely studied, it is found in a highly conserved site in different mammalian species and consists of the deletion of a complete exon without affecting the function of the

protein or its affinity for GH. These characteristics suggest that it may play an important role in adaptive mechanisms, growth and response to GH treatment.

Some studies have shown that the presence of GHRd3 polymorphism at least in one allele might be a potential variant that improves growth response to recombinant human GH (rhGH) therapy, in patients born small for gestational age (SGA), among others¹³⁻¹⁶. However, over the years the results have been controversial and inconclusive. Based on this, it would be proposed that variants at the genomic level are not completely reflected at the mRNA level, and for this reason, the results obtained are so controversial.

During pregnancy, the placental growth hormone (PGH) becomes the predominant GH and influences placental and fetal growth through GHR signaling pathway in the placenta as well as in maternal tissues. Therefore, it could be proposed that differential expression of placental *GHR* at mRNA in comparison to DNA levels might occurred.

In this study, the genotypic frequencies (%) of the GHR gene polymorphism (GHRfl/GHRfl; GHRfl/GHRd3; GHRd3/GHRd3) in normal Argentinean population and SGA patients, and the expression of these polymorphisms at mRNA level in the fetal side of placenta tissues were analyzed. In addition, their association with spontaneous postnatal catch-up growth in SGA patients was also evaluated.

Materials and methods

Sixty five SGA patients and ninety four children born with adequate weight and/or body length for gestational age (AGA) were evaluated. At the beginning of the study all patients were over 4 years old and in all cases, data were obtained at birth and at the age of the first assessment. All patients in whom chromosomal diseases, malformative syndromes, disproportionate short stature, known severe chronic diseases and/or treatments that may affect adrenal function and/or hypothalamic-hypophysis-gonadal axis were excluded from this study. Patients were assessed for disproportion by measuring sitting height and using sitting height/height ratio along with head circumference^{17, 18}.

AGA patients were assessed to obtain the genotypic frequencies in our population. The auxological data of SGA patients are summarized in Table 1. Height and weight were measured with standard equipment. Anthropometric standard deviation score (SDS) was calculated on the basis of an Argentinean reference population¹⁹. This study was approved by the Ethics Committee of the Garrahan Pediatric Hospital. Written informed consent for the study was obtained from all patients or patients' parents or tutors.

Genomic DNA was extracted from peripheral blood leukocytes by standard procedures. The identification of both GHRfl and GHRd3 alleles were analyzed by multiplex PCR assay previously described³ using one sense primer G1 (5'-TGT-GCTGGTCTGGGTCTG-3'), and two anti-sense primers G2 (5'-AGTCGTCCTGGGACAGA-3') and G3 (5'-CCTGGAT-TAACACTTGACACTC-3'). The PCR products obtained were analyzed by electrophoresis on a 2% agarose gel stained

TABLE 1.– Clinical and auxological data of small for gestational age patients

N	Genotype	
	GHRfl/fl 41	GHRd3/- 24
Gestational age (weeks)	36 ± 3	37 ± 4
Weight at birth (SDS)	-1.7 ± 0.9	-2.0 ± 0.9
Body length at birth (SDS)	-2 ± 1	-2 ± 1
Mid parental height (SDS)	-1.1 ± 0.9	-0.6 ± 1.3
Chronological age (years)	7 ± 2	8 ± 2
Weight (SDS)	-1.9 ± 0.9	-1.9 ± 1.2
Height (SDS)	-2.9 (-2.9;-2.0)	-1.9 (-2.5;-1.6)
Body mass index (SDS)	-0.6 ± 1.2	-0.7 ± 1.6
Compensatory growth (%)	19.5	54.2*
ΔHeight (SDS) N = 42	-0.5 ± 0.2	+0.4 ± 0.4*

GHRfl/fl: homozygous full length; *GHRd3/-*: at least one allele that lacks exon 3; SDS: standard deviation score

Values are expressed as (mean ± standard deviation) or median (25-75th percentile) as appropriate by distribution

*p-value < 0.05

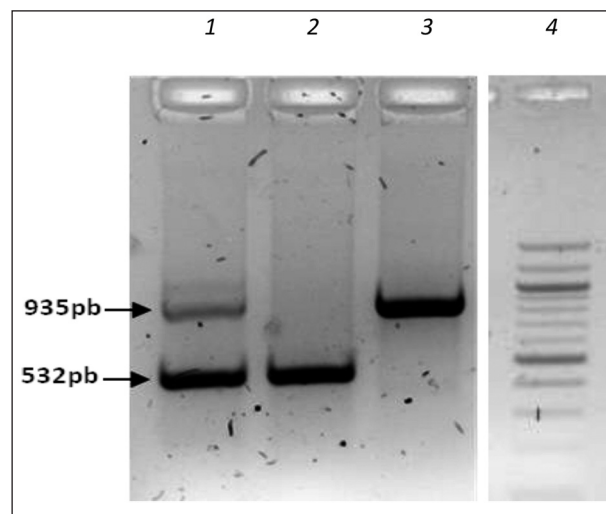
with ethidium bromide. The presence of a band of 935 bp, product of the amplification with G1 and G3, represents the allele *GHRfl* and a fragment of 532 bp represents the allele *GHRd3* resulting from the amplification of G1 and G2 (Fig. 1).

To assess the relationship between genotypic variants of the *GHR* gene and size at birth in children born with SGA, the genotypes of all patients were evaluated along with clinical and auxological data obtained at birth (gestational age, weight and body length). All children with a history of intrauterine growth retardation found during pregnancy monitoring or born with a weight below -2SDS for their gestational age and gender were considered SGA. When gestational age was not available, a newborn with a birth weight of less than 2.5 kg was considered SGA.

To assess the relationship of *GHR* gene polymorphism to post-natal growth in SGA children, a complete physical examination was performed by evaluating anthropometric data at an age greater than 4 years and comparing it with data obtained at birth. Those patients who have reached height >-2 SDS without treatment, were defined as spontaneous catch-up group.

To assess *GHR* gene expression, the presence of exon 3 of the *GHR* gene in mRNA was analyzed in placental samples by an RT-PCR technique previously described²⁰. Samples were taken from the fetal portion of nine placentas. From each sample, DNA and RNA were extracted. For RNA extraction, the samples were crushed and processed using Trizol (TRI reagent, Sigma-Aldrich, Missouri, United States) according to the manufacturer's recommendations. RNA concentration was determined by measuring absorbance at 260 nm and purity by evaluating the 260/280 ratio. The obtained RNA was retrotranscribed into copy DNA (cDNA) by RT-PCR using specific primers for *GHR* located in exons 2 and 10 and reverse transcriptase enzyme Superscript III (Thermo Fisher Scientific, Massachusetts, United States). To test for the presence of exon 3 in those transcripts, a PCR amplification was subsequently performed using primers located in exons 2 and 5²⁰. Amplified products were analyzed by electrophoresis on a 2% agarose gel and visualized after ethidium bromide stain-

Fig. 1.– Genotyping of polymorphisms GHR



(1) *GHRfl/d3* (2) *GHRd3/d3* (3) *GHRfl/fl* (4) size marker

Multiplex PCR products run on electrophoresis 2% agarose gel stained with ethidium bromide show *GHRfl* allele as a band of approximately 935bp and *GHRd3* by 532bp band.

ing. The presence of a band of 361 bp represents the *GHRfl* allele and a fragment of 295 bp represents the *GHRd3* allele (Fig. 2). DNA was extracted with a commercial extraction kit (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions.

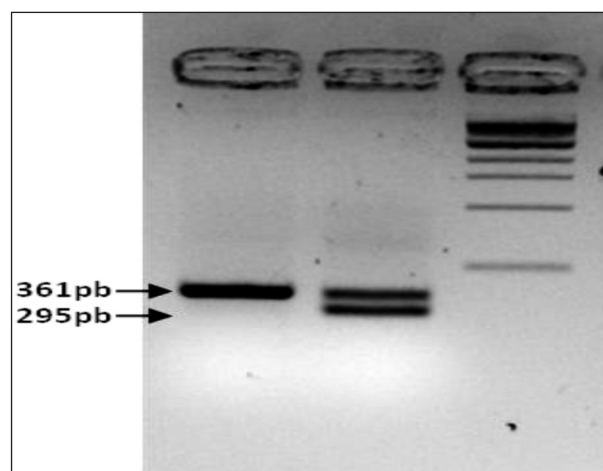
In the statistical analysis, Fisher's exact test was performed to compare the auxological data of SGA patients. A Chi-squared test for the comparison of proportions was used to compare the distribution of genotypic frequencies (%) of the *GHR* gene polymorphism (*GHRfl/GHRfl*; *GHRfl/GHRd3*; *GHRd3/GHRd3*) among populations. The level of significance

was set at $p < 0.05$. All statistical analyses were performed using MedCalc Statistical Software version 19.4.1 (MedCalc Software Ltd, Ostend, Belgium; <https://www.medcalc.org>; 2020).

Results

In order to determine the genotypic frequency of *GHR* gene polymorphism, 94 healthy AGA patients were genotyped. The frequency in our population was 48% for the homozygous GHRfl genotype, 38% for the heterozygous genotype and 14% for the homozygous GHRd3 genotype.

Fig. 2.– Expression of both isoforms of GHR in placenta



(1) GHRfl/fl (2) GHRfl/d3(3) size marker

The 361bp and the 295bp products correspond to the GHRfl transcript and the GHRd3 isoform, respectively.

No deviation from the Hardy–Weinberg equilibrium was observed. The results obtained are detailed in Table 2. Data for other populations were also included and the genotypic frequencies of the *GHR* gene polymorphism (GHRfl/GHRfl; GHRfl/GHRd3; GHRd3/GHRd3) were compared.

In general, the frequencies of the *GHR* gene polymorphism in our study were similar to those of the Caucasian and Latin American countries previously reported in the literature. With regard to Spanish population, significant differences were found in the frequencies of the homozygous GHRfl and heterozygous genotypes compared to the Argentinean population.

The frequencies of the genotypes homozygous and heterozygous GHRfl (GHRfl/GHRfl; GHRfl/GHRd3) were similar to those reported previously in Argentine in pediatric population. On the other hand, the genotypic frequency of the homozygous GHRd3 (GHRd3/GHRd3) is significantly lower in the study published by Ballerini et al⁹.

However, the genotype distribution in our subjects differed from the pattern observed in control subjects from Asia, which present a frequency significant increased for the homozygous GHRfl genotype and reduced for the homozygous GHRd3 genotype.

The studies revealed that the frequency of the homozygous GHRd3 genotype was high among the Caucasian and Latin American population, but low in Asian populations.

The 65 SGA patients studied were separated into two groups according to their genotype for *GHR* polymorphism: homozygous for the GHRfl allele and carriers of at least one GHRd3 allele. With regard to prenatal growth,

TABLE 2.– Distribution of genotypic frequencies (%) of GHRd3 polymorphism in different populations

Population	N	Frequency (%)			Reference
		GHRfl/GHRfl	GHRfl /GHRd3	GHRd3/ GHRd3	
This study	94	48	38	14	
Argentina	192	60 (0.0552)	34 (0.5069)	6 (0.0239)*	(9)
Turkey	477	35 (0.0172)*	39 (0.8558)	26 (0.0129)*	(11)
Korea	81	81 (0.001)*	18 (0.0037)*	1 (0.0016)*	(12)
Spain	289	27 (0.002)*	58 (0.007)*	15 (0.8125)	(10)
Germany	62	45 (0.7141)	40 (0.8025)	15 (0.8622)	(11)
Switzerland	211	48 (1.0000)	44 (0.3280)	8 (0.1050)	(11)
England	100	53 (0.4875)	40 (0.7759)	7 (0.1112)	(11)
France	150	58 (0.1279)	33 (0.4262)	9 (0.2240)	(3)
Canada	368	53 (0.3870)	36 (0.7144)	11 (0.4183)	(11)
Brazil	105	54 (0.3991)	36 (0.7710)	10 (0.3853)	(11)
Venezuela	50	56 (0.3623)	30 (0.3403)	14 (1.0000)	(11)

GHRfl/fl: homozygous full length; GHRfl/d3: heterozygous GHRd3/d3: homozygous exon 3-deleted

A Chi-squared test was used to compare the frequency of the *GHR* gene polymorphism from our population and others and p-values are shown in parenthesis and *when significant differences were found p-value < 0.05

no differences were found in anthropometric data at birth between the patient groups; however, the proportion of SGA patients that achieved spontaneous catch-up growth was greater in those with at least one GHRd3 allele as shown in Table 1.

Consistent with this finding, the difference in height between the time of assessment and body length at birth (Δ height) was higher for GHRd3 carriers. Other auxological parameters were not different among carriers of the different genotypes.

We evaluated the mRNA expression of the different isoforms of the GHR in placental samples. Nine placentas from the AGA group were genotyped, five were homozygous for the GHRfl allele and four were heterozygous. At the end of the study, no placenta was genotyped as homozygous for the GHRd3 allele. For all samples tested, RNA was extracted and both transcripts were evaluated. In all cases, the transcripts evidenced are perfectly consistent with the genotype obtained, indicating that the tissue evaluated would express all the isoforms present in the patient's genome.

Discussion

The genotypic frequency of GHRd3 polymorphism in our population resembles the results obtained by different groups in other populations worldwide, as shown in Table 2. We have observed lower frequency of homozygous GHRd3 genotype than the homozygous GHRfl and the heterozygous genotype in AGA ones. The distribution found in our healthy AGA group is similar to those found in studies evaluating control subjects of European ancestry and differs from those studies analyzing subjects of Asian ancestry.

In Spain, compared to other studies conducted in Europe, the reported frequency of homozygous GHRfl genotype was slightly lower and the heterozygous genotype higher. Perhaps it could be due to the fact that it is a territory with a complex demographic history and it is the only one among European regions that was under Arab rule for centuries.

With regard to the previously frequencies published in Argentina, the difference found in the frequency of homozygous GHRd3 genotype may be due to the fact that the Garrahan Hospital is a national hospital and receives patients not only from the city of Buenos Aires, but also 85% of patients are from the interior of the country, making it more representative of the Argentinean population.

Although no differences were observed in prenatal growth, a significant variation was observed in postnatal spontaneous growth. The latter is in agreement with the study by Wegmann et al., which shows a modest but significant effect of the GHRd3 allele on spontaneous postnatal growth in SGA children²¹, thus reinforcing the

hypothesis that SGA children carrying at least one GHRd3 allele perform compensatory growth more frequently than those homozygous for the GHRfl allele.

In terms of prenatal growth, the inconsistency with the results obtained by several authors in previous publications^{5, 22, 23} could be due to the fact that our sample size is small and the number of homozygous individuals for the GHRd3 allele was insufficient. On the other hand, it should be noted that much of the controversy observed over the years regarding the influence of this polymorphism could be because to the great heterogeneity of the populations in which the studies have been conducted. Therefore the study should be continued by increasing the number of individuals, but it would also be appropriate to thoroughly review the study population in search of factors that may generate heterogeneity in the results.

In contrast to a previously report²⁴ in healthy placenta tissues, no splicing mechanism in *GHR* expression occurs.

Although the results do not show any difference, it would be advisable to continue studying it and increase the number of samples analyzed in order to obtain a more representative population, as this could explain the differences found in other studies.

In this regard, in 2003, Pantel et al. studied the expression of both GHR isoforms in lymphocytes and fibroblasts in one family and the results in both cell types were identical²⁰. While in this case no differences were observed between genotype and expression of *GHR*, in 2018, Espinosa et al. studied the expression of both GHR isoforms in adipose tissue of 17 obese patients heterozygous for GHRd3 polymorphism. This study demonstrate that all expressed the GHRfl allele, but only 6 expressed the GHRd3 allele and these last ones had higher levels of glycosylated hemoglobin. Thus, Espinosa et al. showed that patients with the same genotype for GHRd3 may present differences in the level of expression of this polymorphism and this may be reflected in the phenotype of the patients²⁵.

After the study by Dos Santos et al., where a 30% increase in GHR activity was reported⁴, much interest was aroused about the physiological and therapeutic implications that this variant could have. Therefore, during these last two decades, numerous studies focused on investigating the effect on numerous conditions in which GH is involved. However, today we are faced with more questions than answers about the actual impact that the presence of the GHRd3 allele may have on different clinical variants. While there is a physiological basis and *in vitro* trials to support the hypotheses surrounding this polymorphism, it is obvious that the effect is less evident in the clinical setting and, while some clues exist, the underlying molecular mechanism is not yet fully elucidated. The difficulty presented in describing the relationship of the polymorphism could be due to the fact that the clinical features analyzed (birth weight, compensatory growth in

SGA children, age of pubertal onset, hydrocarbon metabolism, etc.) correspond to phenotypic features influenced by multiple factors (genetic, environmental, epigenetic, etc.), making it very complex to study the effect that a polymorphism could have on a single gene.

The small study population is a limitation. Nevertheless, there are very few previous studies investigating the expression of the different isoforms on tissues. This is the main strength of this study.

Even though there are difficulties in analyzing the effect of the GHRd3 allele polymorphism on different clinical features, in this study a significant increment of compensatory growth in SGA children was found associated to the presence of the GHRd3 allele polymorphism. In addition, the influence by multiple genetic, environmental and epigenetic factors should also be considered. Finally, more in-depth studies will be needed about the molecular mechanism involved, such as the dimerization of the receptor, its rotation and the activation of the transduction pathways, as well as clinical studies with a design and number of individuals that will allow the phenomenon to be clarified.

In summary, a significant increment of compensatory growth in SGA children associated to the presence of the GHRd3 allele polymorphism was found. In addition, *GHR* expression in healthy placentas revealed that no splicing mechanism occurs.

Conflict of interests: None to declare

References

- David A, Hwa V, Metherell LA, et al. Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. *Endocr Rev* 2011; 32: 472-97.
- Filopanti M, Giavoli C, Grotoli S, et al. The exon 3-deleted growth hormone receptor: Molecular and functional characterization and impact on GH/IGF-I axis in physiological and pathological conditions. *J Endocrinol Invest* 2011; 34: 861-8.
- Pantel J, Machinis K, Sobrier ML, Duquesnoy P, Goossens M, Amselem S. Species-specific alternative splice mimicry at the growth hormone receptor locus revealed by the lineage of retroelements during primate evolution. *J Biol Chem* 2000; 275: 18664-9.
- Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, Bougnères P. A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. *Nat Genet* 2004; 36: 720-4.
- Jensen R, Vielwerth S, Larsen T, Greisen G, Leffers H, Juul A. The presence of the d3-Growth hormone receptor polymorphism is negatively associated with fetal growth but positively associated with postnatal growth in healthy subjects. *J Clin Endocrinol Metab* 2007; 92: 2758-63.
- Sorensen K, Aksglaede L, Petersen JH, Leffers H, Juul A. The exon 3 deleted growth hormone receptor gene is associated with small birth size and early pubertal onset in healthy boys. *J Clin Endocrinol Metab* 2010; 95: 2819-26.
- Binder G, Baur F, Schweizer R, Ranke MB. The d3-growth hormone (GH) receptor polymorphism is associated with increased responsiveness to GH in Turner syndrome and short small-for-gestational-age children. *J Clin Endocrinol Metab* 2005; 91: 659-64.
- Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ. Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. *J Clin Endocrinol Metab* 2006; 91: 1076-80.
- Ballerini M, Domené HM, Scaglia P, et al. Association of serum components of the GH-IGFs-IGFBPs system with GHR-exon 3 polymorphism in normal and idiopathic short stature children. *Growth Horm IGF Res* 2013; 23: 229-36.
- Audi L, Esteban C, Carrascosa A, et al. Exon 3-deleted/full-length growth hormone receptor polymorphism genotype frequencies in Spanish short small-for-gestational-age (SGA) children and adolescents (n=247) and in an adult control population (n=289) show increased fl/fl in short SGA. *J Clin Endocrinol Metab* 2006; 91: 5038-43.
- Del Águila C, Ortiz C, Yarlequé M, Bellido C, Zaldivar M, Falen J. Polimorfismo GHRD3 del gen del receptor de la hormona de crecimiento en niños peruanos con talla baja idiopática. *Rev Peru Med Exp Salud Publica*. 2016; 33: 45-50.
- Ko JM, Park JY, Yoo H. Common exon 3 polymorphism of the GH receptor (GHR) gene and effect of GH therapy on growth in Korean children with idiopathic short stature (ISS). *Clin Endocrinol (Oxf)* 2009; 70: 82-7.
- Saenger P, Reiter E. Genetic factors associated with small for gestational age birth and the use of human growth hormone in treating the disorder. *Int J Pediatr Endocrinol* 2012; 2012: 12.
- Wassenaar MJ, Dekkers OM, Pereira AM, et al. Impact of the exon 3-deleted growth hormone (GH) receptor polymorphism on baseline height and the growth response to recombinant human GH therapy in GH-deficient (GHD) and non-GHD children with short stature: A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2009; 94: 3721-30.
- Ráz B, Janner M, Petkovic V, et al. Influence of growth hormone (GH) receptor deletion of exon 3 and full-length isoforms on GH response and final height in patients with severe GH. Deficiency. *J Clin Endocrinol Metab* 2008; 93: 974-80.
- Valsesia A, Chatelain P, Stevens A, et al. GH deficiency status combined with GH receptor polymorphism affects response to GH in children. *Eur J Endocrinol* 2015; 73: 777-89.
- Collett-Solberg P, Ambler G, Backeljauw PF, et al. Diagnosis, genetics, and therapy of short stature in children: a growth hormone research society international perspective. *Horm Res Paediatr* 2019; 92:1-14.
- Comité Nacional de Crecimiento y Desarrollo. Guía para la Evaluación del Crecimiento Físico. Sociedad Argentina de Pediatría, 2013, p 9-137. En: <https://www.sap.org.ar/docs/profesionales/percentilos/completo.pdf>, consultado septiembre 2020.
- Lejarraga H, del Pino M, Fano V, Caino S, Cole TJ. Referencias de peso y estatura desde el nacimiento hasta la madurez para niñas y niños argentinos: Incorporación de datos de la OMS de 0 a 2 años, recálculo de percentilos para obtención de valores LMS. *Arch Argent Pediatr* 2009; 107: 126-33.
- Pantel J, Grulich-Henn J, Bettendorf M, Strasburger CJ, Heinrich U, Amselem S. Heterozygous nonsense mutation in exon 3 of the growth hormone receptor (GHR) in

- severe GH insensitivity (Laron syndrome) and the issue of the origin and function of the GHRd3 isoform. *J Clin Endocrinol Metab* 2003; 88: 1705-10.
21. Wegmann M, Thankamony A, Roched E, et al. The exon3-deleted growth hormone receptor gene polymorphism (d3-GHR) is associated with insulin and spontaneous growth in short SGA children (NESGAS). *Growth Horm IGF Res* 2017; 35: 45-51.
 22. Setia S, Sridhar MG. Changes in GH / IGF-1 Axis in intra-uterine growth retardation. *Horm Metab Res* 2009; 41: 791-8.
 23. Padidela R, Bryan S, Abu-Amero S, et al. The growth hormone receptor gene deleted for exon three (GHRd3) polymorphism is associated with birth and placental weight. *Clin Endocrinol (Oxf)* 2012; 76: 236-40.
 24. Urbanek M, MacLeod JN, Cooke NE, Liebhaber SA. Expression of a human growth hormone (hGH) receptor isoform predicted by tissue-specific alternative splicing of exon 3 of the hGH receptor gene transcript. *Mol Endocrinol* 1992; 6: 179-87.
 25. Espinosa E, Salame L, Marrero-Rodriguez D, et al. Expression of the growth hormone receptor isoforms and its correlation with the metabolic profile in morbidly obese subjects. *Endocrine* 2019; 63: 573-81.

Holmes: [...] It is a capital mistake to theorise before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts. [...]

Holmes: [...] Es un error capital teorizar antes de tener datos. Insensiblemente uno comienza a distorsionar los hechos para que encajen con las teorías, en vez de que las teorías encajen con los hechos. [...]

Arthur Conan Doyle (1859-1930)

A Scandal in Bohemia. The Original Illustrated "Strand". Sherlock Holmes. The Complete Facsimile Edition. Ware (UK); Wordsworth, 1989; p 119