1 **LETTER TO THE EDITOR**

## Presence of *GH1* and absence of *GHRHR* gene mutations in a large cohort of Argentinian patients with severe short stature and isolated GH deficiency

The prevalence of *GH1 gene* alterations is around 20% in patients with severe isolated growth hormone deficiency (IGHD). Mutations in the *GHRHR* gene are recognized as a significant cause of familial IGHD, mainly in areas of the Indian subcontinent.[1]

This study was designed to identify, in the Argentinian population, known and new molecular alterations in the *GH1* gene in a cohort of 46 patients (41 nonconsanguineous pedigrees, 22 familial cases) with IGHD, and molecular alterations in the *GHRHR* gene in 12 familial index cases devoid of molecular abnormalities in the *GH1* gene.

The study was approved by the Ethics Committee of the Garrahan Pediatric Hospital.

Genomic DNA was extracted from blood according to standard procedures. The *GH1* gene was specifically PCR-amplified using sense and antisense primers corresponding to nucleotides 5101–5136 and the complement of nucleotides 7255–7226 (NG_011676.1). The coding sequence and flanking intronic regions of the *GHRHR* gene were PCR-amplified using specific primers.[2] The whole *GH1* and each *GHRHR* purified
2 fragment (Qia Quick PCR purification kit, Qiagen) were directly sequenced using BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems) and 3130 Genetic Analyzer capillary DNA sequencer (Applied Biosystems). The nucleotide sequences obtained were compared with those from Genebank accession numbers NG_011676.1 and NG_021416.1 for *GH1* and *GHRHR* genes, respectively. Gene deletions of *GH1* were detected by RFLP with the restriction enzymes Sma I, Bgl I or Hae II for the detection of 6·7, 7·0 and 7·6 deletions, as previously described.[3] Simulation tests were applied to identify the potential functional impact of newly found variants, using online tools such as sequence homology-based tool, SIFT (Sorting Intolerant from Tolerant; http://sift.jcvi.org/) version 2.0.6, the structure-based tool, PolyPhen2 (Polymorphism Phenotyping; http://genetics.bwh.harvard.edu/pph2) and mutation taster (http://www.mutationtaster.org/).

We identified formerly reported *GH1* gene mutations, in 9/41 (22%) nonconsanguineous pedigrees (13 patients), with a higher prevalence among familial cases (36·4%). Clinical information, laboratory findings and molecular studies in patients and progenitors with mutations in the *GH1* gene are shown in Table 1.

Analysis of *GH1 and GHRHR* genes revealed (i) *GH1* gene deletions in three patients from two nonconsanguineous pedigrees (Table 1). In the two unrelated families, we identified a 6·7-kb deletion in the *GH1* gene. Parents were heterozygous for the *GH1* gene deletion. (ii) *GH1* gene mutations in 10 patients from seven nonconsanguineous pedigrees and always in one progenitor of the six pedigrees studied (Table 1). We identified two previously described, heterozygous, missense mutations: p.Arg183His[4] in six pedigrees and p.Val110Ile in one family, described as a functional polymorphism with significantly reduced GH1 secretion.[5] (iii) *GH1* gene variants: Two novel missense variations in the *GH1* gene in two unrelated patients (p1, p2, Table 1). The nucleotide sequences of genomic DNA in p1 revealed a heterozygous novel variation in exon 2, substituting C for T at cDNA nucleotide position 55, resulting in a substitution of proline to serine at codon -7 (p.Pro-7Ser) of the GH1 protein. The nucleotide sequences of genomic DNA in p2 revealed a heterozygous novel variation in exon 4, substituting T for C at cDNA nucleotide position 305, resulting in the variation of leucine for proline at codon 102 (p.Leu102Pro) of the mature protein. His father had severe short stature (height SDS:-5·12) and was also found to be heterozygous for the same *GH1* gene variation. No allele carrying this variation was found in 60 control subjects (120 alleles) studied. As p.Leu102Pro affects a highly conserved amino acid of the GH1 protein and PolyPhen2 tool predicted it to be probably damaging, this novel variant might be deleterious for protein activity. On the contrary, p.Pro-7Ser does not affect a conserved residue and PolyPhen2 tool predicted that this variation was not deleterious for the GH1 protein, strongly suggesting a common polymorphism. Similar prediction results for both *GH1* novel gene variants using mutation taster and SIFT tools were obtained. (iv) The *GHRHR* gene, sequenced in 12 IGHD index cases with familial IGHD devoid of molecular abnormalities in the *GH1* gene, showed no molecular alteration.

The Garrahan Pediatric Hospital is a referral centre for the whole country, and, therefore, patients attending the hospital are a representative sample of the Argentinian population. To date, at least 58 mutations have been reported in the *GH1* gene associated with IGHD, including splice site and missense mutations and large deletions. In our cohort, type II IGHD was the commonest genetic form. In contrast to other populations, missense mutations instead of *GH1* splice site mutations were predominant. We have identified three mutations previously described in the *GH1* gene with a predominance of p.Arg183His (9/41 pedigrees, 22%). In agreement with previous reports in other populations, this percentage increased to 36·4%, considering familial cases only (8/22 pedigrees). In addition, in two unrelated patients, two novel heterozygous nonsynonymous exonic variants causing a change in the encoded amino acid (p.Pro-7Ser, p.Leu102Pro) were identified in the *GH1* gene. Functional studies could not been carried out. However, the functional impact was estimated according to (i) the presence of the *GH1* gene variant in our general population, (ii) analysis of the evolutionary conservation of the amino acids and (iii) application of simulation tests. Using this approach, it could be

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**Table 1.** Clinical data of patients and families with mutations in the *GH1* gene

| Patient | (A) | (B1) | (B2) | (C1) | (C2) | (D) | (E) | (F1) | (F2) | (G) | (H) | (I1) | (I2) | X ± SD | (1) | (2) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | Male | Male | Male | Male | Female | Female | Female | Male | Male | Male | Male | Male | Male | | Male | Male |
| Mutation | Del 6-7 | Del 6-7 | Del 6-7 | p.Arg | p.Arg | p.Arg | p.Arg | p.Arg | p.Arg | p.Arg | p.Arg | p.Val | p.Val | | p.Pro-7 | p.Leu |
| | kb | kb | kb | 183 His | 183 His | 183 His | 183 His | 183 His | 183 His | 183 His | 183 His | 110 Ile | 110 Ile | | Ser | 102 Pro |
| IGHD | IA | IA | IA | II | II | II | II | II | II | II | II | II | II | | | II |
| CA | 0·71 | 1·42 | 0·75 | 13·5 | 7 | 0·75 | 3·58 | 7·09 | 7·09 | 9·58 | 1·75 | 1·67 | 6·33 | | 11·50 | 12·75 |
| BA | 0·5 | 0·5 | ND | 11 | 5·30 | 0·50 | 1·50 | 2·6 | 2·6 | 7 | ND | 0·75 | 2·50 | | 5·50 | 9·50 |
| HSDS | −3·77 | −5·97 | −4·07 | −2·46 | −2·10 | −3·65 | −5·13 | −5·86 | −5·80 | −3·39 | −3·87 | −4·40 | −5·02 | −4·26 ± 1·24 | −2·58 | −2·79 |
| WSDS | −1·51 | −4·22 | −1·83 | −2·56 | −3·38 | −3·22 | −4·70 | −4·3 | −4·13 | −2·48 | −3·00 | −4·22 | −3·97 | −3·35±1·02 | −1·25 | −2·16 |
| F HSDS | 0·18 | −3·35 | −3·35 | 1·50 | 1·41 | −4·34 | −2·18 | −1·88 | −1·88 | −3·54 | −2·47 | −1·67 | −1·67 | −1·97 ± 1·84 | −2·64 | −5·12 |
| M HSDS | −1·84 | −2·90 | −2·90 | −2·57 | −2·57 | −2·10 | −3·05 | −2·16 | −2·16 | −2·90 | 0·67 | −1·73 | −1·73 | −2·06 ± 1·13 | −0·95 | −1·59 |
| GH μg/L | 0·05 | 0·10 | 0·05 | 1·99 | 3·06 | 1·24 | 2·87 | 5·00 | 2·00 | 1·80 | 1·20 | 0·97 | 1·20 | 1·94 ± 1·32 | 4·70 | 3·20 |
| IGF1SDS | −3·02) | −3·08 | −2·77 | −2·09 | −2·89 | <−3·73 | −2·91 | −3·43 | −3·39 | −2·21 | −2·31 | −2·31 | −4·73 | −2·99 ± 0·73 | −0·23 | −4·49 |
| IGFBP3 SDS | ND | <−3·50 | ND | <−2·63 | <−2·48 | 0·65 | 0·36 | ND | ND | ND | −2·01 | −2·20 | ND | | ND | ND |
| FDR Mut | M & F | M & F | M & F | M | M | F | M | ND | ND | F | F | M | M | | ND | F |
| | HT | HT | HT | | | | | | | | | | | | | |

Pedigrees are denoted by letters and siblings within a pedigree by numbers. Pedigrees of the new *GH1* variants found are denoted by numbers. X ± SD, mean ± standard deviation was calculated considering pedigrees (A) to (I).

CA, chronological age at diagnosis; BA, bone age at diagnosis; HSDS, height standard deviation score; WSDS, weight standard deviation score; GH (μg/l) corresponds to the maximum peak response to pharmacological GH stimulation tests; ND, not determined; FDR Mut, first-degree-relative mutation; F, father; M, mother; HT, heterozygous.

suggested that only the novel *GH1* gene variant p.Leu102Pro might be deleterious for protein activity. Interestingly, the father who carried this novel *GH1* gene variant has extremely short stature (Height SDS:-5·12), suggesting that the p.Leu102Pro *GH1* gene variant might encode an inactive protein causing a severe growth disorder in the father as well. Similarly to other reports in Caucasian populations, no mutations in the *GHRHR* gene were found[1].

In conclusion, we present the results of screening for mutations in *GH1* and *GHRHR* genes in a large cohort of Argentinian patients with IGHD. These suggest that the p.Arg183His mutation associated with the type II dominant form of IGHD might be relatively common. We also report a novel variation (p.Leu102Pro) with strong clinical and indirect data that this represents a loss-of-function mutation in the *GH1* gene.

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**Matias Juanes, Roxana Marino, Marta Ciaccio, Isabel Di Palma, Pablo Ramirez, Diana M. Warman, Valeria De Dona, Eduardo Chaler, Mercedes Maceiras, Marco A. Rivarola and Alicia Belgorosky**

*Endocrinology Service, Hospital de Pediatria Garrahan, Buenos Aires, Argentina*

*E-mail:abelgo@netizen.com.ar*

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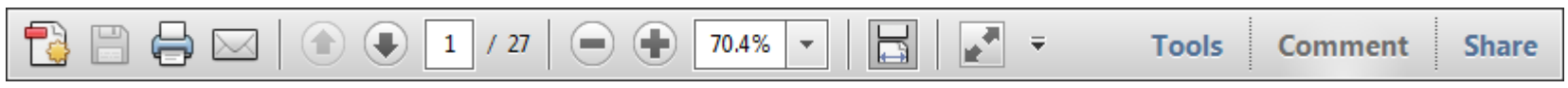


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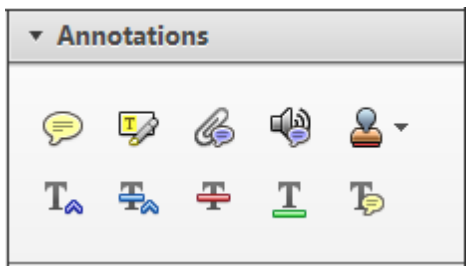


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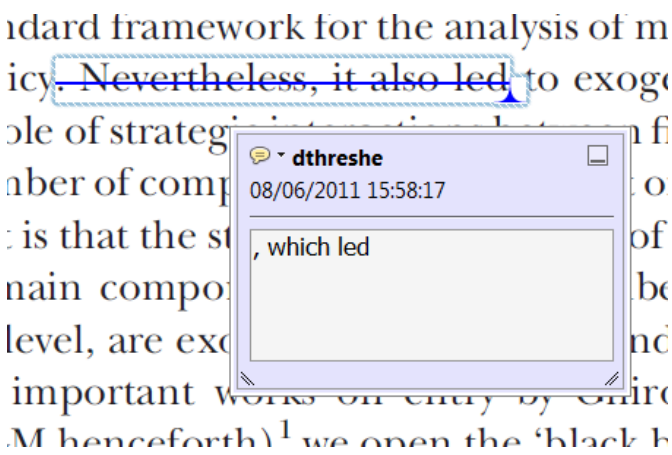


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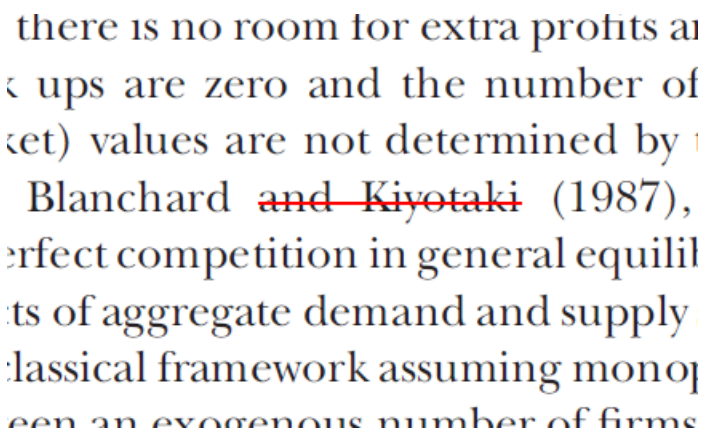

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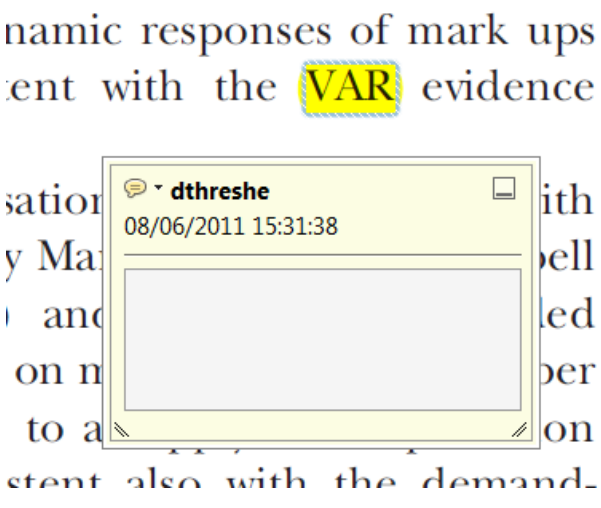


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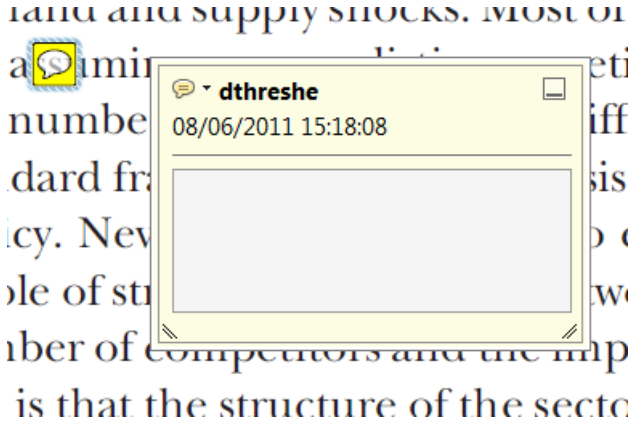

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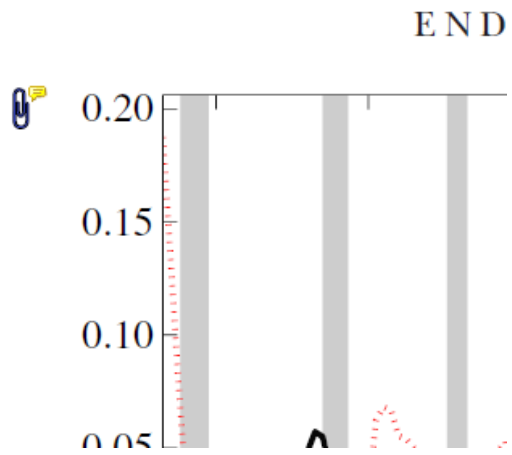


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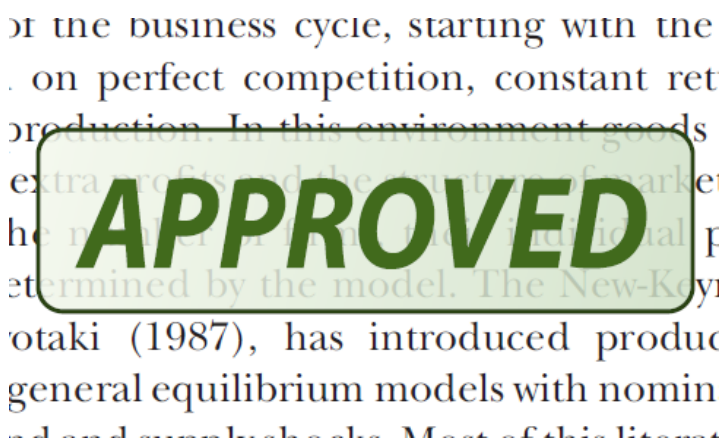


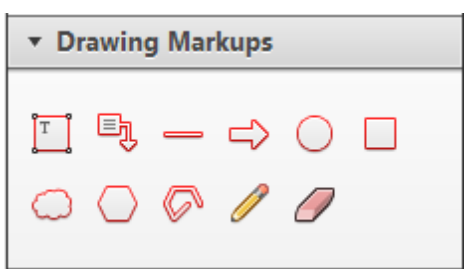


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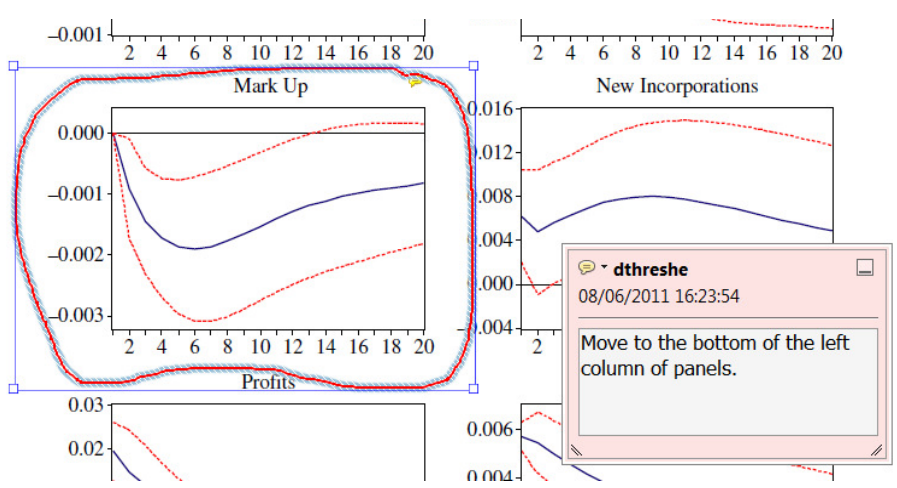


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