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E180splice Mutation in the Growth Hormone Receptor Gene in a Chilean Family with Growth Hormone Insensitivity: A Probable Common Mediterranean Ancestor

Claudia Espinosa[1], Marcela Sjoberg[1], Teresa Salazar[2], Adrian Rodriguez[1], Fernando G. Cassorla[2], M. Veronica Mericq[2] and Pilar Carvallo[1]

[1]*Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile and* [2]*Instituto de Investigaciones Materno Infantil (IDIMI), Facultad de Medicina, Universidad de Chile, Santiago, Chile*

ABSTRACT

Mutations in the GH receptor gene have been identified as the cause of growth hormone insensitivity syndrome (GHIS), a rare autosomal recessive disorder. We studied the clinical and biochemical characteristics and the coding sequence and intron-exon boundaries of the GH receptor gene in a consanguineous family with severe short stature which consisted of two patients, their parents and five siblings. The two adolescents had heights of -4.7 and -5.5 SDS, respectively, with elevated growth hormone associated with low IGF-I, IGFBP-3 and GHBP concentrations. Molecular analysis of the GH receptor gene revealed a mutation in exon 6, present in both patients This mutation, E180 splice, has been previously described in an Ecuadorian cohort, and in one Israeli and six Brazilian patients. We determined the GH receptor haplotypes based on six polymorphic sites in intron 9. Co-segregation of the E180splice mutation with haplotype I was found in this family, compatible with a common Mediterranean ancestor, as shown for previous cases with the E180splice mutation described to date.

KEY WORDS

growth hormone receptor, growth hormone insensitivity, gene mutations, dwarfism

Reprint address:
Pilar Carvallo
Departamento de Biología Celular y Molecular
Facultad de Ciencias Biológicas
Pontificia Universidad Católica de Chile
Casilla 114-D, Santiago, Chile
e-mail pcarvallo@bio.puc.cl

INTRODUCTION

Growth hormone insensitivity syndrome (GHIS) was first described by Laron in 1966 in three Israeli siblings of Yemeni origin. This syndrome with autosomal recessive inheritance is characterized by resistance to growth hormone (GH) action[1]. Patients have typical signs of severe GH deficiency, such as growth failure, frontal bossing, central obesity, small hands and feet, fasting hypoglycemia, and low serum levels of insulin-like growth factor-I (IGF-I), but with elevated GH concentrations and unresponsiveness to exogenous GH administration[2-4]. This disorder is caused by GH receptor (GHR) defects, which interfere with GH binding or GHR signaling[2-4].

The human GHR is coded by a gene spanning at least 87 kb of chromosome 5, which consists of nine coding exons[5]. Exon 2 encodes the signal peptide, exons 3 to 7 the extracellular domain, exon 8 the transmembrane domain, and exon 9 and part of exon 10 the intracellular domain[5]. More than 40 mutations spanning the coding region and intron-exon boundaries of the GHR gene have been described in patients with GHIS[6]. All mutations are inherited in an autosomal recessive manner except for two heterozygous mutations involving the intracellular domain which have a dominant negative effect and result in a relatively mild phenotype[7,8]. A few mutations involve the transmembrane domain, and all the rest the extracellular domain[5,9].

Most mutations in GHIS are unique or have been described in families showing common ancestors through genetic association studies. One of these mutations, E180splice, has been reported among members of an inbred population from

Southern Ecuador, in Israeli patients of Yemeni origin and in six Brazilian patients[10-12]. This mutation creates a new donor splice site in exon 6, which eliminates eight amino acids in the GHR protein[10]. A probable common Mediterranean ancestor for the Ecuadorian cohort and the Israeli and Brazilian patients has been proposed after documenting that the E180splice mutation is associated with GHR haplotype I in these patients[10-12]. Haplotype I is one of the seven haplotypes defined by six SNPs (single nucleotide polymorphisms) present in the GHR gene intron 9, and is the most frequent among Mediterranean populations[13].

We report a similar E180splice mutation in two Chilean siblings born from two first cousins, which co-segregated with GHR haplotype I within the family, suggesting a common Mediterranean ancestor with the other kindreds reported.

FAMILY AND METHODS

The family

This study was approved by the Institutional Review Board of the "Hospital San Borja Arriarán" in Santiago, Chile. Written consent was obtained from each of the nine family members. Our index patient was a boy referred to us from southern Chile for evaluation of severe short stature at the age of 13 years. He had an immature face, with frontal bossing, depressed nasal bridge, no pubic or axillary hair, small penis (4 cm), and his testicular volume was 5 ml. At the time of his first evaluation, he had a height of 110 cm (-5.6 SD), weight 30.3 kg, body mass index (BMI) 25, an arm span of 113.5 cm, and an upper/lower segment ratio of 1.3. He had a history of early growth failure, with birth length 49 cm and birth weight 3.8 kg. Family history was positive for short stature in one older adult sister with a height of 137 cm (-5.5 SD), who also had delayed menarche at the age of 18 years, and a similar phenotype with a baseline GH of 8 ng/ml. During his first evaluation the proband had a bone age of 10 years, with normal prolactin and thyroid function. IGF-I was 34 ng/ml (normal value [NV] 66-427) and IGF binding protein-3 (IGFBP-3) of 1.2 mg/l (NV 2.3 ± 0.3). A GH stimulation test with clonidine (150 μg/m^2) was performed which showed a baseline GH of 10 ng/ml and a peak of 57 ng/ml. Thereafter, the patient underwent an IGF-I generation test performed with 0.1 U/kg/day of GH for 4 days, which showed no change in IGF-I or IGFBP-3 levels. At the age of 14 years 8 months he was evaluated for IGF-I therapy at the National Institutes of Health, Bethesda, MD, USA. At that time he had a height of 122.8 cm (-5.1 SD) with testicular volume of 8 ml (left) and 12 ml (right), genital Tanner stage 3, bone age of 12½ years and a pubertal response to a luteinizing hormone releasing hormone (LHRH) test (LH peak 38.5 mU/ml). He started therapy with leuprolide depot at a dose of 300 μg/kg/28 days for 6 months, followed by leuprolide depot associated with IGF-I 80 μg/kg/12 h during another period of 6 months. During therapy with IGF-I he improved his growth velocity to 7.6 cm/year compared to 2.5 cm/year previous to IGF-I therapy. He continued with IGF-I therapy until he was 17-11/12 years and his bone age had advanced to 14 years, reaching a final height of 148 cm (-3.93 SDS) 3 years later with a bone age of 17 years. During the time the patient was treated with combined IGF-I plus LHRHa, gonadotropins were suppressed but his testosterone levels remained elevated at approximately 200 ng/dl. The family's clinical and laboratory data are presented in Table 1.

Hormone assays

Serum GH was measured by a double antibody radioimmunoassay (RIA) with a sensitivity of 0.8 ng/ml and a within-assay and between-assay variability of 10% and 6.5%, respectively. GH was labeled using the lactoperoxidase method[14]. All reagents for GH RIA were donated by the National Hormone and Pituitary Program (human GH-I-3, Antihuman GH-2 antisera, human GH-RP). Serum IGF-I was measured by RIA after acid-ethanol extraction, using a reference standard purchased from Bachem (Torrance, CA) and an antiserum (NIH UB2-495) donated by the National Hormone and Pituitary Program (Rockville, MD). This assay has a within-assay variability of 7.5% and a between-assay variability of 11.1%[15]. Serum IGFBP-3 was measured by immunoradiometric assay (IRMA) with a commercial kit (DSL) with a

between-assay variability of 1.8% and a within-assay variability of 1.1%. The IGF-I and IGFBP-3 detection limits were 10 ng/ml and 0.05 mg/l, respectively. GH binding protein (GHBP) was determined by monoclonal RIA (Mab 263) showing within-assay variability of 11.4% and between-assay variability of 5.2%[16]. This assay incubates the patient's serum with ^{125}I-labeled GH plus a monoclonal antibody, Mab 263, against the GH receptor in the presence or absence of non-labeled GH. The soluble form of GH receptor (GHBP) binds to MAB 263 plus non-labeled GH to form a ternary complex anti-GHR–GHBP–^{125}I-hGH. Separation of bound/free forms of ^{125}I-hGH was performed using a second antibody (Antimouse IgG, whole molecule, Calbiochem 401210) and polyethylene glycol. The percent binding of GHBP from each patient is in reference to 100% binding of GHBP obtained from a pool of adult serum. Reference values for this method in our laboratory are published elsewhere[17].

The IGF-I generation test consisted of a baseline (day 1) blood sample for measurement of serum IGF-I level, followed by administration of GH at a dose of 0.1 U/kg/day sc from days 1 to 4. On day 5 we obtained a blood sample for the final serum IGF-I determination[18].

Molecular genetic study

DNA was isolated from peripheral leukocytes of each family member[19]. PCR amplification covering coding sequences and intron-exon boundaries of exons 2 to 9 was performed. Only the coding region of exon 10 was PCR amplified using three overlapping pairs of primers. PCR primers are shown in Figure 1. In exons 2, 4, 5, 6, 7, 8 and 9, PCR amplification involved an initial denaturation for 5 min at 95°C, followed by 28 cycles consisting of 1 min at 95°C, 1 min at 48°C, and 1 min at 72°C. Exon 3 was amplified with an initial denaturation of 2 min at 94°C, followed by 28 cycles involving a denaturation step of 30 s at 94°C, an annealing of 30 s at 55°C, and an extension period of 30 s at 72°C. The three overlapping fragments of the coding region of exon 10 were amplified with a touchdown PCR program including an initial denaturation of 2 min at 94°C, followed by three cycles consisting of 94°C, 30 s; 59°C, 30 s; 72°C, 30 s; another three cycles consisting of 94°C, 30 s; 57°C, 30 s; 72°C, 30 s; and finally 25 cycles including a denaturation step of 30 s at 94°C, annealing of 30 s at 55°C, and extension of 30 s at 72°C. All PCR amplification reactions ended with a final extension period of 10 min at 72°C. After PCR amplification, all DNA fragments were purified from agarose gels using Geneclean II (Bio 101 Inc., Vista, CA).

For single strand conformational analysis (SSCA), PCR products were submitted to electrophoresis on MDE (BioWhittaker, Inc., Rockland, ME) 0.5X in the presence of 10% glycerol, at 18°C, 6 W for 14 h, or in the absence of glycerol, at 18°C, 3 W for 13 h. The gels were silver stained after electrophoresis. Sequence analysis of exon 6 and intron 9 was performed by direct sequencing using the dsDNA Cycle Sequencing System (Life Technologies Inc., Gaithersburg, MD), and γ-^{32}P-ATP. Electrophoresis was carried out in 6% acrylamide/bisacrylamide (19:1) gels, in the presence of 8 M urea. Gels were dried and subjected to autoradiography for 24-48 h. The E180splice mutation was determined by restriction analysis of a PCR fragment from exon 6 with the enzyme Mnl I[12].

Haplotype determination in intron 9

Intron 9 haplotype determination for each family member was determined by PCR amplification of a fragment of 333 base-pairs (bp) containing intron 9 with the primers described in Figure 1. PCR products were purified and sequenced. Nucleotide changes were determined based on the reference sequence[13].

RESULTS

Clinical and hormonal evaluations of affected patients and other family members

Table 1 summarizes the clinical features of the two affected siblings, their parents and their five siblings. The parents were first cousins. All family members exhibited a Caucasian phenotype, with no clear evidence of Amerindian admixture. Obesity was present in the parents, the two patients and two non-affected siblings. The two affected siblings had

EXON	PRIMER SEQUENCE
2	Forward: TCTGCTTTTAATTGCTGGGCTTT Reverse: GAATACAGTTCAGTGTTGTTTCAA
3	Forward: TACACAGGGTCATATCAGATTG Reverse: CTATTCCAGTTACTACCATCCC
4	Forward: ATATGACTCACCTGATTTCATGC Reverse: TAGGTACATCCATGGAGAGGAA
5	Forward: GCTACAACATGATTTTTGGAACAA Reverse: CATTTATTTAGTCTAAAACTATGTCA
6	Forward: AATATTAAATTGTGTCTGTCTGTGT Reverse: GAAAGAAAAGTCAAAGTGTAAGGT
7	Forward: TTGAGTTGTTGACTCTTTGGCC Reverse: AACTGTTATATTGACAAAAGCC
8	Forward: GAAACTGTGCTTCAACTAGTC Reverse: AACGGTACAAAATACCAA
9	Forward: AGAATATGTAGCTTTTAAGATGTCA Reverse: GACAGGAGTCTTCAGGTGTTAA
10a	Forward: GAGTTTCTTTTCATAGATCTTC Reverse: TTAACCTCTGTGGCTGAG
10b	Forward: ACATGAGGGTACCTCAGA Reverse: CAGAAGTAGGCATTGTCC
10c	Forward: GGAAATGGTCTCACTCTG Reverse: CAAAAGAAAGGCTAAGGC
INTRON 9	Forward: CCCAGTTCCAGTTCCAAAGA Reverse: CACTGTGGAATTCGGGTTTA

Fig. 1: PCR primers used for amplification of coding sequences and intron-exon boundaries of exons 1-10 and intron 9.

the typical features of GHIS with frontal bossing, central obesity, small hands and feet, and decreased pubic and axillary hair. These features were not present in the other siblings. The unaffected family members had normal heights, providing no clinical evidence for partial defects in GH action. The two affected siblings had blue sclerae as has been reported previously in patients with GHIS from southern Ecuador[20], and normal intelligence and high-pitched voice. Body proportions were abnormal in both affected patients, with reduced arm span and increased upper/lower segment ratio. The affected sister had delayed menarche (18 years) compared to her mother (12 years) and the male proband had micropenis and delayed puberty, at the time of first evaluation, whereas his father had early puberty. Later, the affected boy developed spontaneous puberty and penis growth reached an appropriate size. Hormonal baseline assessments from all family members are shown in Table 2. As expected, serum IGF-I, IGFBP-3 and GHBP levels were low in the patients, but were normal in the rest of the family. Fasting insulin levels were elevated, independent of BMI in all family members, except for the affected siblings. The GHBP levels were normal in non-affected family members, according to age and pubertal status[17].

Molecular analysis of the *GHR* gene

SSCA for all coding exons was performed in the two affected siblings. This analysis revealed a band shift compared to the control for the PCR fragment corresponding to exon 6 in both patients (Fig. 2A, lanes M1 and M2). Exon 6 was sequenced in both patients and one control. As shown in Figure 2B, an A to G transition was detected in both patients. As reported previously, this mutation creates a new donor splice site within the exon, and generates a receptor which lacks eight amino acids. This mutation, E180splice, corresponds to the same mutation that was previously described in the Ecuadorian cohort, and the Israeli and Brazilian patients[10-12]. In order to determine the presence of this mutation in the rest of the family members, restriction analysis with the enzyme Mnl I was performed as described in **Methods**. Figure 3 shows the family pedigree and agarose electrophoresis with the restriction pattern. Under each lane, the presence (+) or the absence (–) of the mutation for both alleles is indicated. Both parents and four siblings are heterozygous for the mutation, whereas in one brother the mutation was absent from both alleles.

Considering that the Ecuadorian cohort, as well as most Chilean families, are of Spanish origin[20], we explored the possibility of a common ancestor for the E180splice mutation. As reported previously, this mutation is associated with haplotype I, from the GHR gene intron 9, in the Ecuadorian, Israeli and Brazilian patients described to date[10-12].

TABLE 1

Clinical characteristics of the family members

	Father	Mother	Patient 1	Patient 2	Sib 1	Sib 2	Sib 3	Sib 4	Sib 5
Age (years)	50	45	26	18	17	15	14	10	8
Height (SDS)	-1.6	-2.8	-3.9	-5.5	-0.5	0	-0.2	-1.4	-0.4
Body mass index (SDS)	4	3.4	3.0	2.6	2.7	1.6	2.9	0.9	1.6
Arm span (SDS)	-1.7	-3.7	-5.6	-4.4	-0.9	-0.4	-0.6	-2.2	-1.8
Sitting height (SDS)	-0.2	-3.9	-6.7	-2.8	-2.5	-0.1	-0.4	-1.9	-1.8
Head circumference (SDS)	2.5	-0.4	-1.8	0	0	0.4	0.2	-0.2	-0.4
Hand size (percentile)	2.5	-1	-2	0	1.5	2	1.5	0	1
Sclera (color)	N	blue	blue	blue	N	N	N	blue	blue
Menarche (years)	NA	12	18	NA	12	NA	NA	NA	NA
Pubic/axillary hair	+/+	+/+	+/–	+/–	+/+	+/+	+/+	–	–

SDS = standard deviation score for chronological age.

TABLE 2

Hormonal characteristics of the family members

	Father	Mother	Patient 1	Patient 2	Sib 1	Sib 2	Sib 3	Sib 4	Sib 5
Random GH (ng/ml)	0.8	1	10	8	1	1.2	0.8	0.8	1.2
IGF-I (ng/ml)	189	247	97	86	376	328	337	283	210
IGFBP-3 (mg/l)	4.5	4.3	1.9	1.4	3.5	4.2	4.5	4.4	4.5
GHBP (%RSB)	NR	86.7	21.9	24.0	51.4	45.3	NR	49.6	37.6
Insulin (mU/ml)	241.3	18.5	9.2	22	37.7	99.4	82.3	17.7	18.7

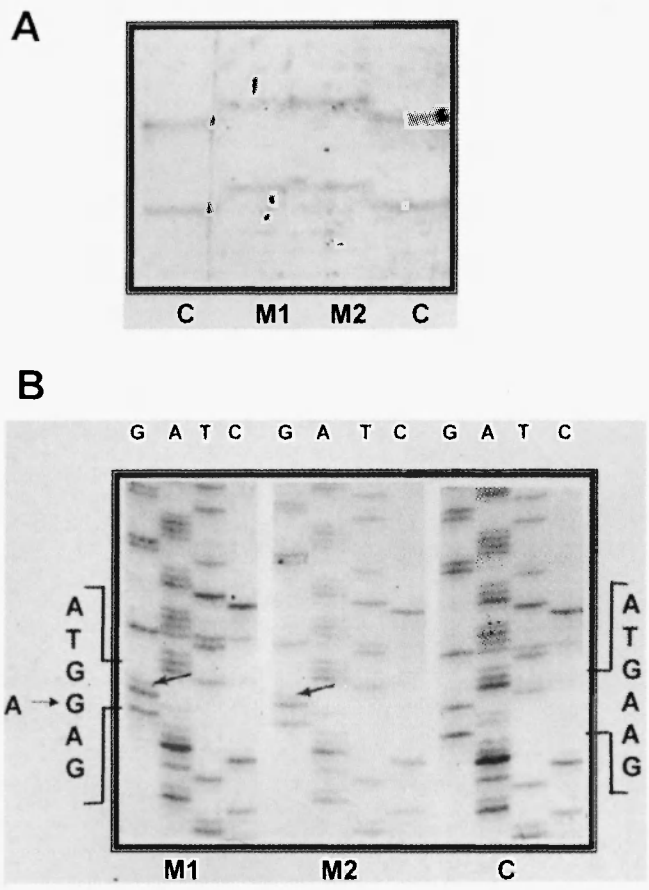

Fig. 2: **A.** SSCA gel electrophoresis from the two affected siblings with GHIS. Exon 6 from the GHR gene was PCR amplified, and the reaction product was submitted to electrophoresis as described in **Methods**. The gel was silver stained after electrophoresis. C: control DNA, M1: DNA from child 1, M2. DNA from child 2. **B.** Partial autoradiogram of the mutated and normal sequences from the GHR gene exon 6. Exon 6 was PCR amplified from the two patients; the products were purified and sequenced as described in **Methods**. An arrow indicates the nucleotide changes in lanes M1 and M2. C: control DNA, M1: DNA from child 1, M2: DNA from child 2.

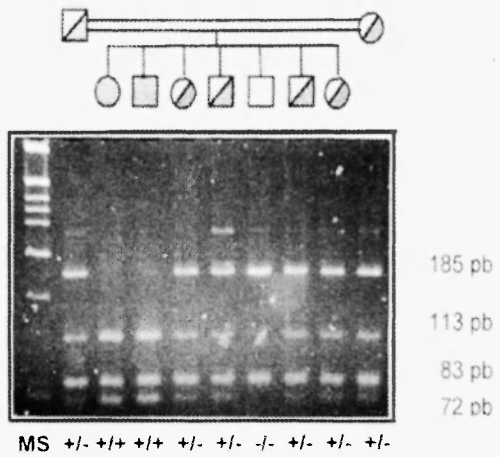

Fig. 3: Detection of the E180splice mutation through restriction digestion with Mnl I. A fragment of 268 base-pairs (bp) corresponding to exon 6 was PCR amplified from genomic DNA of each family member, and digested with the Mnl I enzyme. The reaction products were analyzed by electrophoresis on 2% agarose gels. The normal allele gives products of 185 bp and 83 bp, and the mutated allele gives products of 113 bp, 83 bp and 72 bp. The presence of the four digestion products indicates heterozygous siblings. MS: MW standard pBR322 digested with Hinf I. The remaining lanes correspond to each family member from the pedigree on top of the figure. +/-: heterozygous for the E180splice mutation, +/+: homozygous for the E180splice mutation, -/-: non-mutated.

We determined the haplotype present in the GHR gene in all family members, through direct sequencing of intron 9. As shown in Figure 4, both affected siblings are homozygous for haplotype I, indicating an association of this haplotype to the E180splice mutation. In addition, all the heterozygous siblings for the E180splice mutation present heterozygosity for haplotype I. The sibling not presenting the mutation has haplotypes II and III. Intron 9 haplotype frequencies were determined in a sample of 50 individuals (100 chromosomes) from the Chilean population, revealing the presence of four of the seven described haplotypes. Frequencies are haplotype I: 0.12; haplotype II: 0.59; haplotype III: 0.09; haplotype IV: 0.20.

DISCUSSION

We report two patients from a consanguineous Chilean family with clinical and biochemical features of GHIS who were found to bear the known mutation E180splice in the GHR gene. This mutation has been previously reported in a large Ecuadorian cohort, and in Israeli and Brazilian patients[10-12]. The family reported in this paper lives

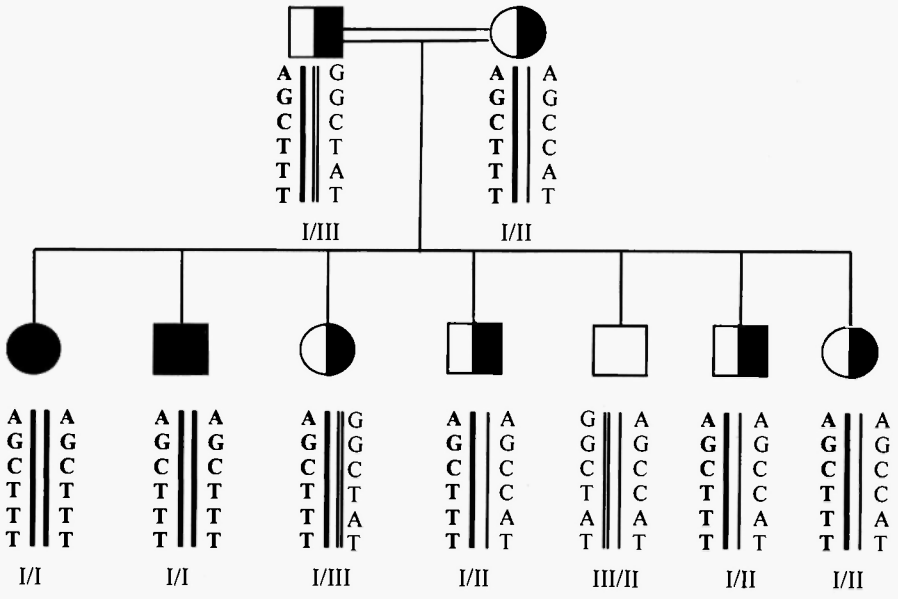

Fig. 4: Family pedigree showing intron 9 haplotype determination for each family member. ●: homozygous for the E180splice mutation, ◪: heterozygous, □: non-mutated.

in a small town in southern Chile. They did not have any information about their ancestors beyond their grandparents, who were born and lived in the same area. It is highly probable that this family has Spanish ancestors, due to the origin of their last names for four generations. Unfortunately, no other relatives were available for genetic study. Chile and Ecuador had a heavy Spanish immigration for at least three centuries, so a common ancestor for the Ecuadorian and Chilean families with GHIS is quite possible.

The determination of the intron 9 haplotype revealed a co-segregation of the E180splice mutation with haplotype I in the Chilean family, as described for the Ecuadorian cohort, and the Israeli and Brazilian patients. This finding led us to suggest a common ancestor, most probably Mediterranean, for the Ecuadorian cohort, and the Israeli and Brazilian patients already published[10-12], and the Chilean family. Haplotype I has been described as the most common haplotype among Mediterranean populations. For each of the seven haplotypes described[12], we determined the frequencies in a random population of 50 Chilean individuals (100 chromosomes). We concluded that haplotype II is the most frequent among Chilean individuals, at 59%. The relative low frequency of haplotype I in the Chilean population, and its consistent segregation with the E180splice in our family, supports, but does not prove, the hypothesis of a common Mediterranean ancestor.

In relation to a possible dose effect of the heterozygous expression of the E180splice mutation on stature or hormonal function in relatives of this family, we observed that all heterozygous members, except for the mother (-2.8 SDS), have a height within 2 SDS of the Chilean mean. Serum IGF-I, IGFBP-3, and GHBP concentrations were within the normal adult range in all family members (Table 2). As reported previously[21,22], the findings reported here suggest that there is no effect of heterozygosity for the E180splice mutation on stature or hormonal function. The GH insensitivity caused by this mutation is probably due to the deletion of eight amino acids which may produce protein misfolding and degradation[23]. Therefore, it is likely that the E180splice mutation is a functional null mutation. In contrast, mutations that lead to a stable mutant protein might have a dominant negative effect, leading to growth failure in heterozygous patients. Among other clinical features that have been observed in these patients are

blue sclerae and decreased androgen dependent hair. The latter was a very prominent finding in our family, but blue sclerae was observed in all members, irrespective of GHR state. We should mention that GHIS has been associated with a wide variation in clinical and biochemical phenotype[24], but this variation cannot be accounted for solely by disorders of the GHR gene, suggesting that other genetic and/or environmental factors may contribute to phenotype in GHIS.

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REFERENCES

1. Amselem S, Duquesnoy P, Attree O, Novelli G, Bousnina S, Postel-Vinay MC, Goossens M. Laron dwarfism and mutations of the growth hormone-receptor gene. N Engl J Med 1989; 321: 989-995.
2. Eshet R, Laron Z, Pertzelan A, Arnon R, Dintzman M. Defect of human growth hormone receptors in the liver of two patients with Laron-type dwarfism. Isr J Med Sci 1984; 20: 8-11.
3. Daughaday WH, Trivedi B. Absence of serum growth hormone binding protein in patients with growth hormone receptor deficiency (Laron dwarfism). Proc Natl Acad Sci USA 1987; 84: 4636-4640.
4. Buchanan CR, Maheshwari HG, Norman MR. Laron-type dwarfism with apparently normal high affinity serum growth hormone-binding protein. Clin Endocrinol (Oxf) 1991; 35: 179-185.
5. Godowski PJ, Leung DW, Meacham LR, Galgani JP, Hellmiss R, Keret R, Rotwein PS, Parks JS, Laron Z, Wood WI. Characterization of the human growth hormone receptor gene and demonstration of a partial gene deletion in two patients with Laron-type dwarfism. Proc Natl Acad Sci USA 1989; 86: 8083-8087.
6. Rosenbloom AL. Growth hormone insensitivity: physiologic and genetic basis, phenotype, and treatment. J Pediatr 1999; 135: 280-289.
7. Ayling RM, Ross R, Towner P, Von Laue S, Finidori J, Moutoussamy S, Buchanan CR, Clayton PE, Norman MR. A dominant-negative mutation of the growth hormone receptor causes familial short stature. Nat Genet 1997; 16: 13-14.
8. Iida K, Takahashi Y, Kaji H, Nose O, Okimura Y, Abe H, Chihara K. Growth hormone (GH) insensitivity syndrome with high serum GH-binding protein levels caused by a heterozygous splice site mutation of the GH receptor gene producing a lack of intracellular domain. J Clin Endocrinol Metab 1998; 83: 531-537.
9. Woods KA, Fraser NC, Postel-Vinay MC, Savage MO, Clark AJ. A homozygous splice site mutation affecting the intracellular domain of the growth hormone (GH) receptor resulting in Laron syndrome with elevated GH-binding protein. J Clin Endocrinol Metab 1996; 81: 1686-1690.
10. Berg MA, Guevara-Aguirre J, Rosenbloom AL, Rosenfeld RG, Francke U. Mutation creating a new splice site in the growth hormone receptor genes of 37 Ecuadorian patients with Laron syndrome. Hum Mutat 1992; 1: 24-32.
11. Berg MA, Peoples R, Perez JL, Guevara-Aguirre J, Rosenbloom AL, Laron Z, Milner RD, Francke U. Receptor mutations and haplotypes in growth hormone receptor deficiency: a global survey and identification of the Ecuadorian E180 splice mutation in an oriental Jewish patient. Acta Paediatr Suppl 1994; 339: 112-114.
12. Jorge AA, Menezes Filho HC, Soares Lins TS, Rondini Guedes D, Damiani D, Setian N, Prado Arnhold IJ, de Mendonca BB. Founder effect of E180 splice mutation in growth hormone receptor gene (GHR) identified in Brazilian patients with GH insensitivity. Arq Bras Endocrinol Metabol 2005; 49: 384-389.
13. Amselem S, Duquesnoy P, Duriez B, Dastot F, Sobrier ML, Valleix S, Goossens M. Spectrum of growth hormone receptor mutations and associated haplotypes in Laron syndrome. Hum Mol Genet 1993; 2: 355-359.
14. Rogol AD, Chrambach A. Radioiodinated human pituitary and amniotic fluid prolactins with preserved molecular integrity. Endocrinology 1975; 97: 406-417.
15. Garcia H, Henriquez C, Ugarte F, Fernandez E, Beas F, Iniguez G, Boric A, Pizarro F, Cassorla F. GH-IGF axis during catch up growth in small for gestational age (SGA) infants. J Pediatr Endocrinol Metab 1996; 9: 561-556.
16. Carlsson L, Mercado M, Baumann G, Stene M, Attie K, Reichert M, Albertsson-Wikland K, Dawson K, Wong WL. Assay systems for the growth hormone-binding protein. Proc Soc Exp Biol Med 1994; 206: 312-315.
17. Salazar T, Mericq V, Espinoza M, Iniguez G, Carvallo P, Cassorla F. Reference values of growth hormone binding protein (GHBP) for a normal pediatric population. Rev Med Chile 2001; 129: 382-389.
18. Merriam GR, Wachter KW. Algorithms for the study of episodic hormone secretion. Am J Physiol 1982; 243: E310-318.
19. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991; 19: 5444.
20. Rosenbloom AL, Guevara Aguirre J, Rosenfeld RG,

Fielder PJ. The little women of Loja: growth hormone receptor deficiency in an inbred population of southern Ecuador. N Engl J Med 1990; 323: 1367-1374.
21. Rosenbloom AL, Guevara-Aguirre J, Berg MR, Francke U. Stature in Ecuadorians heterozygous for growth hormone receptor gene E180 splice mutation does not differ from that of heterozygous normal relatives. J Clin Endocrinol Metab 1998; 83: 2373-2375.
22. Guevara-Aguirre J, Rosenbloom AL, Guevara-Aguirre M, Yariz K, Saavedra J, Baumbach L, Shuster J. Effects of heterozygosity for the E180 splice mutation causing growth hormone receptor deficiency in Ecuador on IGF-1, IGFBP-3 and stature. Growth Horm IGF Res 2007; 17: 261-264.
23. Francke U, Berg MA. Genetic heterogeneity in Laron syndrome. Acta Paediatr Suppl 1993; 391: 3-7.
24. Woods K, Dastot F, Preece M, Clark A, Postel-Vinay MC, Chatelain P, Ranke M, Rosenfeld R, Amselem S, Savage M. Phenotype:genotype relationship in growth hormone insensitivity syndrome. J Clin Endocrinol Metab 1997; 82: 3529-3535.