Molecular Defects of the Growth Hormone Receptor Gene, Including a New Mutation, in Laron Syndrome Patients in Israel: Relationship between Defects and Ethnic Groups

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Abstract

Background: Laron Syndrome, first described in Israel, is a form of dwarfism similar to isolated growth hormone deficiency caused by molecular defects in the GH receptor gene.

Objective: To characterize the molecular defects of the GH-R in Laron syndrome patients followed in our clinic.

Methods: Of the 63 patients in the cohort, we investigated 31 patients and 32 relatives belonging to several ethnic origins. Molecular analysis of the GH-R gene was performed using the single strand conformation polymorphism and DNA sequencing techniques.

Results: Eleven molecular defects including a novel mutation were found. Twenty-two patients carried mutations in the extracellular domain, one in the transmembrane domain, and 3 siblings with typical Laron syndrome presented a normal GH-R. Of interest are, on one hand, different mutations within the same ethnic groups: W-15X and 5, 6 exon deletion in Jewish-Iraqis, and E180 splice and 5, 6 exon deletion in Jewish-Moroccans; and on the other hand, identical findings in patients from distinct regions: the 785-1 G to T mutation in an Israeli-Druze and a Peruvian patient. A polymorphism in exon 6, Gly168Gly, was found in 15 probands. One typical Laron patient from Greece was heterozygous for R43X in exon 4 and heterozygous for Gly168Gly. In addition, a novel mutation in exon 5: substitution of T to G replacing tyrosine 86 for aspartic acid (Y86D) is described.

Conclusions: This study demonstrates: a) an increased focal incidence of Laron syndrome in different ethnic groups from our area with a high incidence of consanguinity; and b) a relationship between molecular defects of the GH-R, ethnic group and geographic area.

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Laron Syndrome (LS [OMIM 262500]), also known as primary growth hormone resistance or insensitivity, is a rare autosomal recessive disorder, first described in 1966 in Oriental Jews [1]. Its clinical characteristics are indistinguishable from untreated patients with isolated GH deficiency [2]. Its main characteristics are dwarfism and progressive obesity. The pathology of this syndrome was found to reside in defects of the GH-R [3] or in post-receptor pathways [4]. The clinical and laboratory aspects of the disease were recently reviewed [5].

The molecular defects vary from multi-exon deletions [6], to nonsense, missense and splice site mutations. To date, 53 valid mutations in the human GH-R have been found in patients with LS [5]. The majority of defects exists in the extracellular domain – exons 3-7 [7], few in the transmembrane – exon 8 [8, 9] and intracellular domain – exons 9-10 [10,11]. All the defects abolish GH signal transmission regardless of the location of the defect. The majority of LS patients are of Mediterranean and Middle-Eastern origin or their descendents. A large genetic isolate has been reported from Ecuador, believed to be of Spanish-Jewish ethnicity [12], and smaller ones in Turkey [13] and in the Bahamas, the latter of Anglo-Saxon descent [14]. In the past only a few patients of the large Israeli cohort were genetically examined. The aim of the present study was to map the molecular defects in the GH-R of as many patients as possible from our large cohort, who belong to various ethnic groups. Some of the patients are from the same family.

Subjects and Methods

The study cohort

Sixty-three patients with classical Laron syndrome are being followed in our clinic. Thirty-one patients, children and adults, agreed to participate in the study: a) 26 patients from Israel, belonging to 15 families (Jews from Iraq, Iran, Afghanistan, Morocco and Yemen, and Druze and Palestinian-Arabs) and 22 of their first-degree relatives; and b) 5 patients from abroad, of Peruvian, Italian, Greek, Iranian and Malthusian origin, with their 10 first-degree family members.

Consanguinity was found in nine families: three Jewish-Iraqi families, one Jewish-Afghani, one Jewish-Yemenite, two Palestinian-Arab families, one Druze and one of Iranian origin.

Each patient and family members signed an informed consent form (in the case of children, the signature of the parents was given). Blood samples for DNA isolation were drawn; DNA samples from the Italian family and blood spots on Guthrie paper of the

* This investigation was part of the requirement towards the MSc degree.

GH = growth hormone
GH-R = growth hormone receptor

LS = Laron syndrome

Peruvian family were mail delivered by their physicians. Transformed lymphocytes were stored for future studies. The study was approved by The Hospital Ethics Committee.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes, using standard procedure. DNA from dry blood spots on Guthrie paper was extracted by rapid processing [15].

DNA amplification

Exons 2-10 (including exon-intron boundaries) of the GH-R gene were each amplified by polymerase chain reaction using intronic primers as previously reported [16]. Amplification reactions contained 1 µl of genomic DNA, 1xPCR buffer including 2–3 mM $MgCl_2$, 250 µM dNTPs, 7 pmol of each primer and 0.5 units Taq polymerase (GibcoBRL, UK). PCR conditions included initial denaturation at 9°C for 4 minutes, 36 cycles of 94°C for 45 seconds; annealing temperature differed for each exon from 48 to 62°C for 1 min, 72°C for 1 min, with final elongation at 72°C for 10 min. Amplified products were electrophoresed on 2% agarose gel to test for contamination and fragment size.

Table 1. Mutations of the GH-R found in Laron syndrome patients studied in Israel

Patient no.*	Ethnic group	Mutation (location) nucleotide change	G168G**	Zygosity / consanguinity
1	Jewish-Iranian	230delT (exon 7) del T at 744	+	Homozygous / No
2	Jewish-Iranian / Turkish	230delT / exon 5 del	+	Compound heterozygous / No
3a, 3b	Jewish-Iraqi	W-15X (signal	–,–	Homozygous / Yes
4		peptide)*** G→A at 83	–	Homozygous / No
5a, 5b	Jewish-Iraqi	5, 6 exon deletion	+,?	Homozygous / No
6	Jewish-Iraqi / Moroccan	5, 6 exon deletion	?	Homozygous / Yes
7a, 7b	Jewish-Afghani	5, 6 exon deletion	?,?	Homozygous / Yes
8			?	Homozygous / No
9	Jewish-Moroccan	5, 6 exon deletion	+	Homozygous / Yes
10	Jewish-Moroccan	E180 splice (intron 6) A→G at 594	+	Homozygous / No
11a, 11b, 11c	Jewish-Yemenite	R217X (exon 7)	+,+	Homozygous / Yes
12a, 12b		C→T at 703	+,+	Homozygous / Yes
13a, 13b, 13c, 13d	Palestinian Arab	R217X (exon 7)	–,– –,–	Homozygous / Yes
14a, 14b, 14c	Palestinian Arab	Post-receptor defect	–,– , –	? / Yes
15	Druze	785-1 G to T (intron 7) G→T at 785-1	+	Homozygous / Yes
16	Peruvian	785-1 G to T (intron 7)	+	Homozygous / No
17	Malthusian	R43X (exon 4) C→T at 181	–	Homozygous / No
18	Greek-Anatolian	R43X / normal	+/–	Heterozygous / No
19	Italian	L141X (exon 6) T→A at 476	+	Homozygous / No
20	Iranian	Y86D (exon 5) T→G at 310	+	Homozygous / Yes

* Each number represents a family; a,b,c,d indicate siblings/cousins of the same family.

** G168 polymorphism: (+) positive, (–) negative, (?) not detected.

*** Carrying a second homozygous mutation R211H (exon 7).

Analysis of single strand conformation Polymorphism

SSCP analysis was performed for all PCR products to detect aberrant bands. Samples were loaded onto 0.6xMDE gel (AT Biochem, USA). The gels were silver stained and marked.

DNA sequencing

Samples that presented an abnormal pattern were subjected to DNA sequence analysis on a capillary electrophoresis-based analyzer (ABI PRISM 3700 DNA Analyzer, Perkin-Elmer, Applied Biosystems, Hitachi, Japan). Sequencing was performed on both the sense and the antisense strands.

Results

Table 1 presents the molecular defects found in the GH-R in our cohort of patients, arranged according to ethnic group. We found three novel mutations of which two were previously reported [16] and the third is described here for the first time.

Twenty-two of the patients had negative serum GH binding protein and carried mutations in the extracellular domain of the receptor. One patient (Pt. 15) had very high GHBP levels and had a mutation in the transmembrane domain. Three siblings (Pts.14a, b, c) of Palestinian-Arab origin, with typical LS features, had normal serum GHBP with no molecular defect in the GH-R gene. They had been diagnosed as having a post-receptor defect [4] so far uncharacterized.

In the Israeli population six molecular defects were found. Seven Jewish patients of Iraqi, Afghani and Moroccan origin had 5, 6 exon deletion. Two of Jewish-Iranian origin had 230del T in exon 7. One of them was compound heterozygous, carrying also deletion of exon 5. The mutation R217X was detected in two related Jewish-Yemenite families and in a Palestinian-Arab family. A mutation in the

PCR = polymerase chain reaction

SSCP = single strand conformation polymorphism

GHBP = GH binding protein

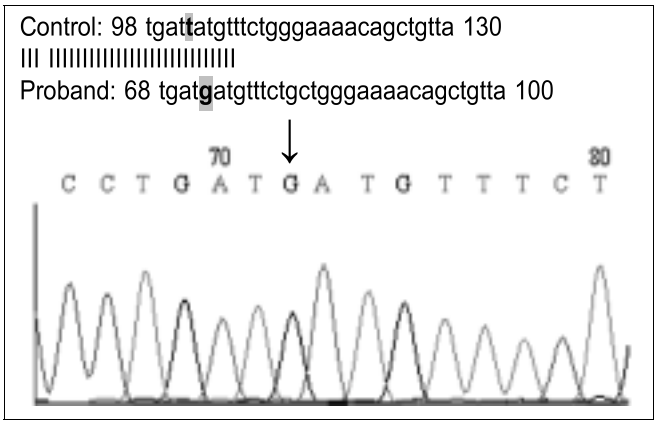

Figure 1. Direct sequence analysis of exon 5 of Laron syndrome patient originating from Iran (Pt. 20). Alignment between normal (control) sequence and the proband's revealed a T to G substitution, replacing tyrosine (TAT) for aspartic acid (GAT).

transmembrane domain, 785-1 G to T (intron 7), was found in one Druze and one Peruvian patient [17]. The E180 splice mutation in exon 6, known as "the Ecuadorian mutation," was found in a Jewish-Moroccan patient [18]. In the patient from Malta a homozygous mutation R43X (exon 4) was found. The Greek patient was heterozygous for R43X and heterozygous for a polymorphic substitution in exon 6, Gly168Gly. Despite sequencing all the exons of the GH-R in this patient we cannot exclude an additional defect [19]. In three Jewish-Iraqi patients we found W-15X (in the signal peptide), and in an Italian patient the L141X (exon 6) [16].

A novel missense mutation was detected in a boy of Iranian origin. Direct sequencing revealed in exon 5 a homozygous T to G substitution in codon 86, replacing tyrosine with aspartic acid (Y86D) [Figure 1].

A polymorphism in exon 6 (mentioned above), resulting from a silent nucleotide change in glycine 168 (Gly168Gly), was found in 15 probands and in normal control subjects.

Discussion

To date 53 mutations in the GH-R have been described including those in the present report, which describes a novel mutation. The correlation between molecular defects and ethnic origin reveals some interesting inter-relationships, indicating that the same mutation is found in adjacent countries such as Iraq-Turkey and Iran-Afghanistan and even in a population with different religions, such as the Jewish-Yemenites and the Palestinian-Arabs. This is exemplified by finding the same molecular defect – 5, 6 exon deletion – in a Jewish-Moroccan proband (Pt.9), in an ethnically mixed Jewish-Iraqi/Moroccan patient (Pt. 6), and in Jewish patients originating from Iraq and Afghanistan. This defect is part of the non-contiguous 3, 5 and 6 exon deletion (GHR Del-3, 5, 6) which was the first defect reported in Laron syndrome [6]. Exon 4 is syntenic and co-linear with the other GH-R exons and the normal intron-exon splice sites of exons 2, 4 and 7 were retained and used [20]. Furthermore, exon 3 deletion is not a disease-causing mutation but a polymorphism [21].

In two of the patients with the 5, 6 exon deletion (Pts. 5a, 9) we detected the Gly168Gly polymorphism in exon 6. This may indicate that they are of the same haplotype and, carrying the same mutation, could be the result of the population migration and movement between North Africa and Asia (along the "old silk and spice road").

Another interesting finding is the identity of the mutation E180 splice in the patients from Ecuador and our Jewish-Moroccan patient, which supports the hypothesis that the Ecuadorian patients are descendents of Jewish *conversos* fleeing the Spanish inquisition [18,22]. It is questionable whether the same relation applies to the finding of 785-1G to T in a Druze family and a Peruvian patient [17] also carrying Gly168Gly polymorphism.

In our cohort we found the R217X mutation in Jewish-Yemenite patients and in Palestinian-Arab patients. Of note is that, unlike the Jewish patients, the Arab patients we investigated did not carry the Gly168Gly polymorphism.

R43X was found in two of our patients who originated in two areas of the Mediterranean (Malta and Greece). The Greek patient was heterozygous for R43X and heterozygous for Gly168Gly; nevertheless, she presented the clinical and biochemical features of Laron syndrome [19].

The nonsense mutations R217X and R43X have already been described in several reports in several unrelated patients from various areas [7,23,24]. These mutations are CpG dinucleotide "hot-spots" [7]. They also are recurrent because they are on different haplotype backgrounds in different populations [18]. The finding of the hot-spot mutations R217X and R43X in patients originating from different geographic areas with close contact (Africa, Yemen, Middle East and Mediterranean countries) raises the possibility of a common ancestor.

The novel missense mutation Y86D in exon 5 first described in this study is likely a disease causing mutation. In favor is the fact that tyrosine 86 is located in one of the two cysteine-rich regions of the human GH-R that is highly conserved through evolution and is part of a consensus sequence among the members of the cytokine receptor superfamily [25].

Despite the fact that the LS patients investigated belong to several ethnic groups and revealed a variety of mutations in the GH-R, all had the typical appearance of the classical syndrome phenotype [5] resulting from insulin-growth factor-I deficiency.

In conclusion, this study demonstrates: a) an increased focal incidence of Laron syndrome in different ethnic groups from our area resulting from a high incidence of consanguinity; and b) a correlation between molecular defect of the GH-R, ethnic group and geographic area.

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Capsule

Reconstituting prion disease in mice

The prion hypothesis postulates the existence of infectious proteins capable of propagating disease. Legname et al. present evidence that a novel strain of prion disease can be induced in mice injected with recombinant prion proteins. Brain extracts from these mice could then be used to infect other mice to cause a neuropathologic disorder distinct from other known strains of prion disease. Recombinant mouse prion protein (recMoPrP) produced in *Escherichia coli* was polymerized into amyloid fibrils. Fibrils consisting of recMoPrP(89–230) were inoculated intracerebrally into transgenic (Tg) mice expressing MoPrP(89–231). The mice developed neurologic dysfunction between 380 and 660 days after inoculation. Brain extracts showed protease-resistant PrP by Western blotting; these extracts transmitted disease to wild-type FVB mice and Tg mice overexpressing PrP, with incubation times of 150 and 90 days, respectively. Neuropathologic findings suggest that a novel prion strain was created. These results provide evidence that prions are infectious proteins.

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