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A TURKISH FAMILY WITH 6.7 Kb DELETION ASSOCIATED WITH ISOLATED GROWTH HORMONE DEFICIENCY TYPE 1A*

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SUMMARY: Korkmaz KS, Sercan OH, Yazıcıoğlu MV, Sakızlı M, Darcan Ş, Büyükgebiz A. (Endocrinology Unit, Department of Pediatrics, and the Department of Medical Biology, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). A Turkish family with 6.7 kb deletion associated with isolated growth hormone deficiency type 1A. Turk J Pediatr 1997; 39: 99-104.

Familial growth hormone deficiency type 1A is an autosomal recessive disease caused by homogenous deletions of both alleles of growth hormone gene 1 (hGH1) in various patterns. The hGH1 gene deletion is an event that probably occurs between the 5' and 3' flanking regions by unequal recombination, and results in deletion of the hGH1 gene in different patterns. Deletions are mostly 6.7 kb and rarely 7.0 kb, 7.6 kb and 45 kb in size. A four-year-old girl diagnosed with growth hormone deficiency syndrome was send to us for further evaluation. DNA samples of the patient, her parents and controls were amplified by polymerase chain reaction (PCR); furthermore, restriction endonuclease analysis was done with Sma I enzyme and the patterns were evaluated. Our gel electrophoresis results show that the gene deletion pattern of the patient represents a homogenous 6.7 kb deletion, while her parents had a heterogeneous 6.7 kb deletion pattern. *Key words: growth hormone deficiency, 6.7 kb deletion, Turkish family.*

The human growth hormone (hGH) gene cluster has five very similar genes: GH1, CSHP1, CSH1, GH2 and CSH2[1-3]. The hGH1 gene and flanking sequences normally give three fragments when cut by EcoRI restriction enzyme[5]. Type 1A isolated growth hormone deficiency (IGHD), which was first described by Illig et al.[4], has an autosomal recessive mode of inheritance and presents a homozygous deletion of DNA containing the hGH1 structural gene[2,5]. IGHD type 1A patients are occasionally characterized by short stature at birth, hypoglycemia and severe growth retardation by the age of six months. The molecular basis of this autosomal recessive disorder depends on the type of deficiency, which is defined by different size deletions from 6.7 kb to 45 kb[1,2,5]. By using standard Southern-Blot and polymerase chain reaction (PCR) methods, 6.7, 7.0, 7.6 and recently a 45 kb gene deletion have been reported by different research groups[1,2,6,7]. We studied a Turkish family with phenotypically normal parents and one affected child.

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Material and Methods

The patient was admitted to the hospital at age four with the complaint of short stature. The delivery, peri and postnatal course were considered to be normal, and the parents were not related. The patient's birth height was 50 cm and weight was 3200 g. Her annual height increments were six cm between one and two years of age and 1.3 cm between two and three years of age. Her bone age was three years according to the Greulich and Pyle method, with a height of 76 cm (height SDS = 5.58), and a weight of 8.2 kg. The upper/lower ratio was 37.7/38.3 cm. Thyroid hormone values were normal. L-Dopa and insulin-induced hypoglycemia (ITT) tests revealed max 1.7 ng/ml GH response.

Preparation of Genomic DNA: Human lymphocyte DNA was isolated by using the CTAB (Cetyl trimethyl ammonium bromide)-DNA isolation method[8]. 150 µl whole blood was first treated with solution A (8% CTAB, 100 mM Tris-Cl pH 8.5, 50 mM EDTA pH 8.0, 1.5 M NaCl) and incubated at 68 °C for five minutes. Chloroform treatment was performed on the lystae, followed by centrifugation at 13.000 rpm in an Eppendorf centrifuge. The supernatant was transferred to a separate tube, and DNA was collected by adding five percent CTAB followed by a precipitation step using absolute ethanol. A second centrifugation at 13.000 rpm was performed. The pelleted DNA was washed with 1.2 M NaCl and a final precipitation using absolute ethanol was performed to recover the DNA for further analysis.

PCR and Restriction Fragment Analysis: PCR amplification of 100 ng template DNA was done using 1µM of each 5',GGATCCAGCCTCAAAGAGCTTAC and 3',GAATTCCCAGAGCCTTGA GCAATGGA hGH1 gene flanking sequence primers (Synthesized by GenoSys Biotech. Inc., Cambridge). A thermal profile (1 min at 94 °C for denaturation, 1 min at 65 °C for annealing, and 2 min at 72 °C for extension) of 30 cycles with an eight-minute initial denaturation at 94 °C and 10 min. at 72 °C for final extension periods was used, Each reaction mixture contained 10X reaction buffer (50 mM KCl, 100 mM TrisCl pH 8.3, 1.5 mM $MgCl_2$, % 0.001 [w/v] Gelatin), 200µM of each dNTP, and 2.5 Units Taq polymerase (Boehringer MN) Volume of in a 100 µl[9]. The DNA of the subject's and control DNA were amplifed and electrophoresed on two percent agarose gel to test the amplifications. The remaining PCR products were digested with Sma I restriction enzyme by adding 10 units of enzyme and 10X enzyme buffer to the amplified products, followed by an incubation period of two hours at 25 °C. Restriction fragments were run on a two-percent agarose gel electrophoresis (5 V/cm).

Results

When genomic DNA is digested by the Eco RI restriction enzyme, the hGH1 gene and its flanking sequences give three fragments. These three fragments are 4.7 kb, 2.6 kb and 4.9 kb in length, and are called R1, R2 and R3 respectively (5' → 3')[5]. The R1 and R3 regions contain a 2.24 kb fragment with 96 percent

homology. Due to this high homology, the same set of primers are used in the PCR amplification of these two regions. The amplified regions having different restriction sites are digested by different restriction enzymes. This knowledges help us to determine whether the tested material belongs to homozygous patients, heterozygous carriers or normal individuals. When the same set of primers are used, we get a 1900 bp amplification product from the R1 region and a 1921 bp amplification product from the R3 region. The 1900 bp R1 amplification product has restriction sites for Bg1 I and Hae II restriction enzymes, while the 1921 bp R3 amplification product contains two SmA I restriction sites[1]. In the 6.7 kb gene deletion, the sequences between the breaking points include the hGH1 gene within it. As a result of unequal recombination, when we use the same primers for the hR1 and R3 regions, we end up with a recombinant product resulting from fusion of the amplified sequences. This recombinant new sequence gives a 1918 bp amplification product, which contains the Bgl restriction site of the 1900 bp sequence and the Sma I restriction site of the 1921 bp sequence. Agarose gel electrophoresis of the PCR amplification products gives one band representing either the 1921 and 1900 bp fragments of the control or a 1918 bp band of a homogeneous patient. It is not possible to detect three bands so close on a two percent agarose gel. Thus, all three bands are detected at the same location (Fig. 1). The agarose gel electrophoresis of the Sma I digestion products showed that the controls gave four bands, size 1900, 761, 712 and 448 bp. Only the 1470 and 448 bp bands were seen in the patient (Fig. 2 lane 1). In the restriction fragment analysis, the parents were found to have an additional 1470 bp fragment in the control pattern. The parents were evaluated as heterozygous carries of this gene deletion (lanes 2 and 3, Fig. 2).

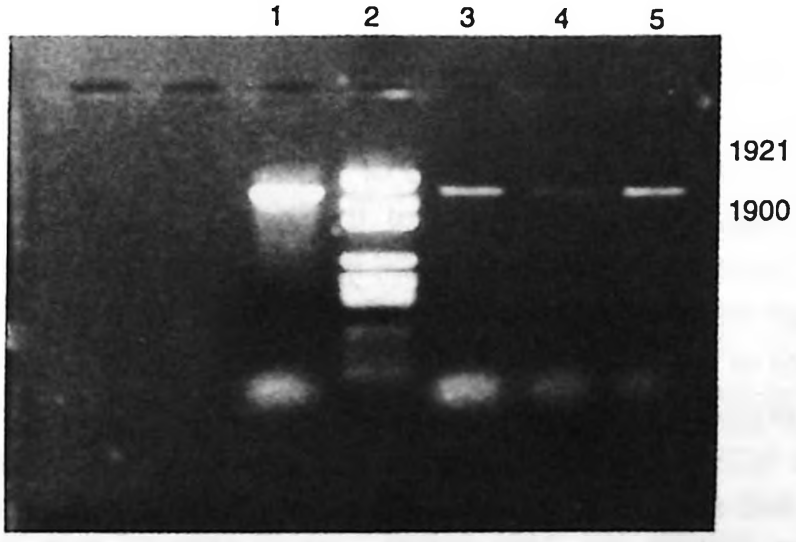

Fig. 1: Ethidium bromide-stained agarose gel electrophoresis patterns of amplified PCR products; lane 1 patient, lane 3 and 4 heterozygous parents, lane 5 control and lane 2 DNA marker (pGEM DNA marker-PROMEGA).

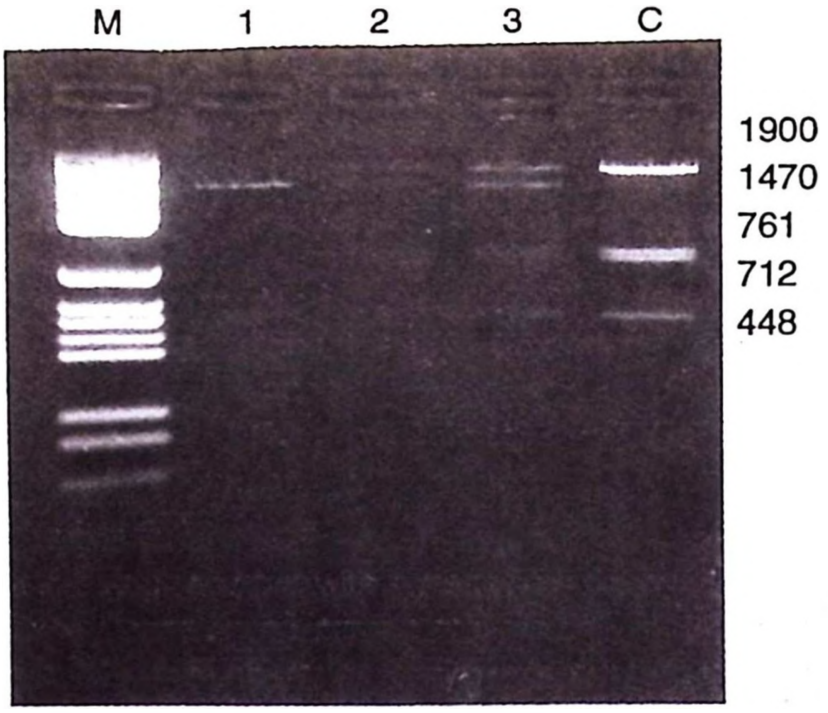

Fig. 2: The patterns of SmaI digested PCR amplification products in 2% agarose gel; lane M: pGEM 2Z DNA marker (PROMEGA). Lane 1 patient with 1470 and 448 bp fragments. Lane 2 and 3 parents with 1900, 1470, 761, 712 and 448 bp fragments. Lane 5 control DNA with fragments 1900, 761, 712, 448 bp in length.

Discussion

The clinical features of patients with IGHD type 1A have been described by Illig et al.[4] as short stature, early growth retardation, typical facial appearance and good response to an initial course of hGH treatment. PCR and restriction endonuclease analysis done from whole blood obtained from families with IGHD type 1A have shown that the affected patients have a deletion of DNA encompassing the hGH-1 gene that encodes hGH endogenously. Deletions are mostly 6.7 kb and in rare cases 7.0 kb or 7.6 kb in size[1]. Different families with the same size gene deletions present the same restriction fragment length polymorphism patterns within their hGH gene locus. This suggests that most deletions result from independent unequal recombination events[9, 10]. The molecular nature of the hGH[1] gene deletions of our patient and her parents were determined by analysis of the amplified PCR products including 5' and 3' flanking regions of the hGH1 gene. Philips et al.[5] reported that the amplified DNA fragments of 1900 and 1921 bp are in the EcoRI segments of the hgH1 gene, referred to as R1 and R3, respectively. The R1 and R3 sequences have extensive homology which includes a 1.38 kb segment which is found to be 96 percent homologous, Alu sequences 0.3 kb in length that are 86 percent homologous,

and 0.56 kb segments that are 88 percent homologous in 5' to 3' order[9, 11, 12]. The recombinational events resulting from these homologies might be the cause of the deletions of the hGH1 gene region of our subjects.

Unequal recombination of these homologous flanking regions yields fusion fragments or deletions as seen in the 6.7 kb hGH1 gene deletion. PCR amplification of the hGH flanking regions in our patient yielded a 1918 bp fragment having one Sma I site and gave a distinct Sma I restriction pattern of two fragments 1470, 448 (Fig. 2 lane 1), while controls gave 1900, 761, 712, and 448 bp fragments. The parents gave an extra 1470 bp band to the control pattern. This was evaluated as the parents being heterozygous for this gene deletion with one normal allele and one deleted allele.

We introduced a homozygous 6.7 kb hGH 1 gene deletion in a Turkish family in which both parents were heterozygous carriers. The method to detect the hGH gene deletions can mainly be divided into three steps: DNA isolation, restriction digestion of amplified products, and finally evaluation of RFLP patterns by agarose gel electrophoresis. These are rare cases and will provide further advances in our understanding of the nature of hGH gene deletions.

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