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CASE REPORT

Study of the family of a patient with male-limited precocious puberty (MPP) due to T1193C transition in exon 11 of LH receptor gene

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ABSTRACT. Molecular diagnostics of the LHR gene was conducted in a 5-year-old boy with clinical symptoms and hormonal profile typical of precocious puberty. His parents and 4 sisters were also diagnosed. Single-strand conformation polymorphism analysis under temperature gradient conditions (Multitemperature SSCP) of 3 overlapping fragments of exon 11 of LHR gene revealed a mutation in the fragment spanning nucleotides 1072 to 1804. This mutation was found in the patient, in his mother and in his 4 sisters, and was confirmed by digestion with the use of restriction enzyme Bbr CI. Direct sequencing revealed a heterozygous T1193C transition in the DNA fragment of the patient and in one of the alleles of his mother's and sister's DNA. This mutation causes Met398Thr substitution in the second transmembrane helix and results in a constitutive activation of LH receptor. This is the second identical mutation detected in Poland and one of the 7 identified so far in the world population.

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INTRODUCTION

The LH receptor (LHR) is a member of the family of glycoprotein hormone receptors, which are involved in hormone dependent signal transduction through coupling with guanine nucleotide binding regulatory proteins (G proteins) (1). The LHR is composed of three domains: extracellular domain (ED), involved in ligand binding; transmembrane domain (TD) consisting of 7 helices connected by loops; and intracellular domain (ID) (2). Binding of the ligand causes conformational changes of the receptor, leading to dissociation of the α-subunit of the G protein and activation of adenyl cyclase. Although the molecular mechanism of constitutive activation of the receptor is largely unknown (3, 4), these mutations result in an increase of intracellular cyclic AMP (cAMP) concentration, leading to subsequent transactivation of (CYP1A1) and (CYP17) genes. Protein products of these genes: cytochrome P4501A1 and cytochrome P450c17 catalyse reactions that limit the rate of T synthesis in Leydig cells. Fourteen different types of missense mutations causing constitutive activation of the receptor were described. Eight of these mutations are localized in the hot spot region of the 6th transmembrane helix, encoding amino acids 571 through 581 (5-9), whereas two are situated in the third cytoplasmic loop at positions 564 and 568 (7, 10). It has been shown that these 2 regions are involved in direct interaction with G protein during signal transduction. Single mutations were detected in a highly conserved region of the first helix (position 373) and in the second (position 398), third (position 457) and fifth (position 542) helices (7,11-13) (Fig. 1). This reflects an important role of interhelical interaction of TM VI with TM V and TM VII in the ligand-independent activation of LHR (14, 15). It has been demonstrated that constitutive activation of LHR is a principal cause of gonadotropin-independent male-limited precocious puberty (MPP) (8). This autosomal dominant disease exists in familial and spo-

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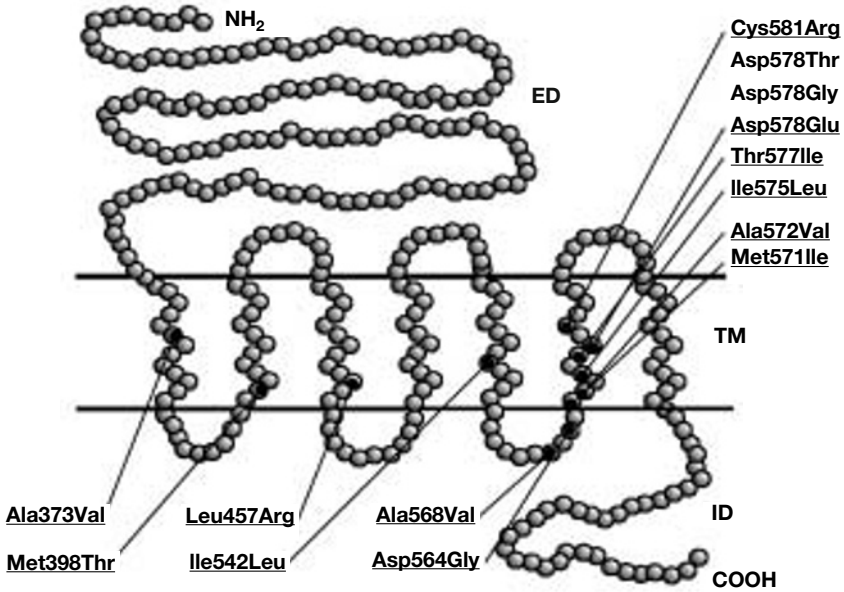

Fig. 1 - *Localization of mutations in lutropin receptor (LHR). Mutations responsible for constitutive activation of the receptor are marked with arrows. ED: extracellular domain; TM: transmembrane domain; ID: intracellular domain.*

radic forms and manifests in male with development of secondary sexual characteristics caused by excessive formation of T (16).

DESCRIPTION OF THE CASE

Physical examination of the patient at the age of 5 and 4/12 yr, revealed accelerated growth (height age, 8 and 3/12 yr), slightly more developed musculature, facial acne and a deep voice. Pubarche – Tanner III, axillarche – Tanner I. Testes were enlarged and smooth. The volume according to Prader's scale was: left - 10 ml, right 8- ml, and penis length was 11 cm. Bone age, determined according to Greulich-Pyle, was 8 yr and corresponded to the height age. The GnRH test showed prepubertal gonadotropin response. Basal and stimulated levels of LH and FSH were 0.37 and 0.96 mU/ml and 0.59 and 1.14 mU/ml respectively. Basal serum T concentration was 2.72 ng/ml (norm for the prepubertal is less than 1 ng/ml) and did not rise after GnRH stimulation. Serum hCG level did not exceed normal range. Oestradiol levels were within the prepubertal range. Serum 17α-hydroxyprogesterone and DHEA concentration, as well as urinary excretion of 17-ketosteroids was normal. Serum free cortisol level was normal and the result of the dexamethasone test was within the normal range. Body imaging methods revealed no evidence of tumors of the central nervous system, chest, mediastinum, retroperitoneal space, liver, adrenal gland and testes. On the basis of clinical examination and hormonal profile, male-limited precocious puberty was diagnosed.

There had been no history of early sexual development in other family members. The patient's father did not report any symptoms of precocious puberty. In the patient's mother, menarche occurred at the age of 14. Four sisters of the patient did not show any signs of androgen excess.

METHODS

Genomic DNA was isolated from peripheral blood leukocytes and was used as a template to amplify three overlapping fragments of exon 11 of LHR gene, spanning nucleotides 1072 to 1804. PCR was performed under the following conditions: initial denaturation (3 min 94 C), 35 cycles, each involving: denaturation (20 s 94 C), annealing (45 s 56 C), elongation (45 s 72 C) and terminal extension (7 min 72 C). Three sets of primers were designed to perform single strand conformation polymorphism (SSCP) analysis:

1F 5'GGCTCTTTTCTTCTTCTAATTG3'; 1R 5'GTTACTGTTGAATAAGAGGTACT3'; 2F 5'TATCTGCTGCTCATAGCC3'; 2R5'GACCTTCATGTAATTGCTG3'; 3F 5'GTCCATGACTTCCTTAGGG3'; 3R 5'GCAGGTTATATGTTACCTGGGC3'. The SSCP analysis was conducted in 8% (1:49) acrylamide/bisacrylamide gel containing 7% glycerol. The conformers were separated by electrophoresis (300 V for 5 h) at room temperature and at 4 C. Protean II ix Cell (BIO-RAD, U.S.A.) was used. Identical set of SSCP analyses was

performed on the same gel using newly designed Multitemperature SSCP (MSSCP) System (Kucharczyk, Poland), which allows the control and change of gel temperature during electrophoresis (30 min, 30 C; 30 min, 15 C; 30 min, 5 C) at 30 W.
In order to detect mutation the fragment generated with primers 1R and 1F was also digested with restriction endonuclease BbrCI (Roche Diagnostics, U.S.A.).
The PCR products harbouring the mutation were purified and sequenced in both directions using an ALFexpress Sequence Analyser (Amersham-LKB, Sweden).

RESULTS

Molecular diagnostics of LHR gene was conducted in a boy with typical symptoms of MPP, as well as his parents and four sisters. Three fragments of exon 11 of LHR gene, spanning nucleotides 1072 to 1804, which encode the main part of transmembrane domain of LHR, including part of the first and the second through 6th helices, were screened for the presence of mutations. The SSCP analysis performed under standard conditions did not reveal any mutation in the amplified fragments, including the fragment generated with primers 1R and 1F (Fig. 2A). However, analysis of the same fragments under different temperature conditions produced during electrophoresis (multiple temperature SSCP), revealed additional bands the DNA fragments generated with primers 1R and 1F (Fig. 2B). These bands were found in the patient, in his mother and in his four sisters, while the banding patterns in the DNA samples of his father and in a healthy individual were the same.

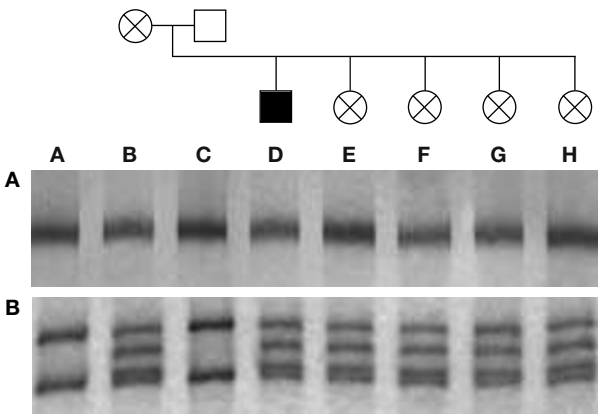

Fig. 2 - *Single-strand conformation polymorphism (SSCP) analysis of exon 11 of LHR gene. Amplified fragments of DNA, harboring nucleotides 1072-1288, were subjected to SSCP analysis under standard conditions (A), and to temperature gradient SSCP analysis (B). The analysis was conducted in 8% polyacrylamide gel containing 7% glycerol under conditions described in the Methods section. Lane A, healthy individual; lane B, patient's mother; lane C, patient's father; lane D, patient, lanes E-H, patient's sisters.*

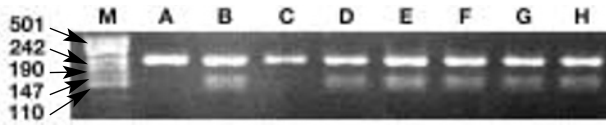

Fig. 3 - *Restriction digestion analysis of the 1072-1288 fragment of exon 11 of LHR gene. The DNA samples of the amplified fragment of exon 11, harboring nucleotides 1072-1288, were digested with restriction endonuclease Bbr CI as described in the Methods section. Lane A, healthy individual; lane B, patient's mother; lane C, patient's father; lane D, patient, lanes E-H, patient's sisters.*

Since this mutation generates a novel restriction site for endonuclease Bbr CI, the amplified fragment harbouring the mutation was digested with restriction enzyme Bbr CI. Electrophoretic analysis of the digestion products (Fig. 3) demonstrated that the patient as well as his mother and all 4 sisters were heterozygous for the mutation. Direct sequencing revealed a T1193C transition in the patient (Fig. 4B) and in one of the alleles of his mother and four sisters, who showed abnormal banding pattern in temperature gradient SSCP analysis (Fig. 4D through H). In his father (Fig. 4C) and in a healthy individual (Fig. 4A), no abnormalities in the sequence of the entire fragment were found. Genotyping of grandparents has not been possible. This mutation causes Met398Thr substitution in the second transmembrane helix of LHR.

DISCUSSION

Our patient demonstrated the phenotype typical of precocious puberty: androgen excess resulting in male secondary sex characteristics: accelerated growth and abnormal bone age. Characteristic hormonal profile: high serum T, normal 17α-hydroxyprogesterone and DHEA concentration and normal urinary excretion of 17–ketosteroids. This allowed us to exclude congenital adrenal hyperplasia and adrenal tumor, while normal values of hCG excluded hCG-dependent pseudopuberty. On the basis of clinical examination and hormonal profile, male-limited precocious puberty was diagnosed.
A T1193C transition was demonstrated in the patient upon examination of an amplified DNA fragment of exon 11 of LHR gene. Multiple tempera-

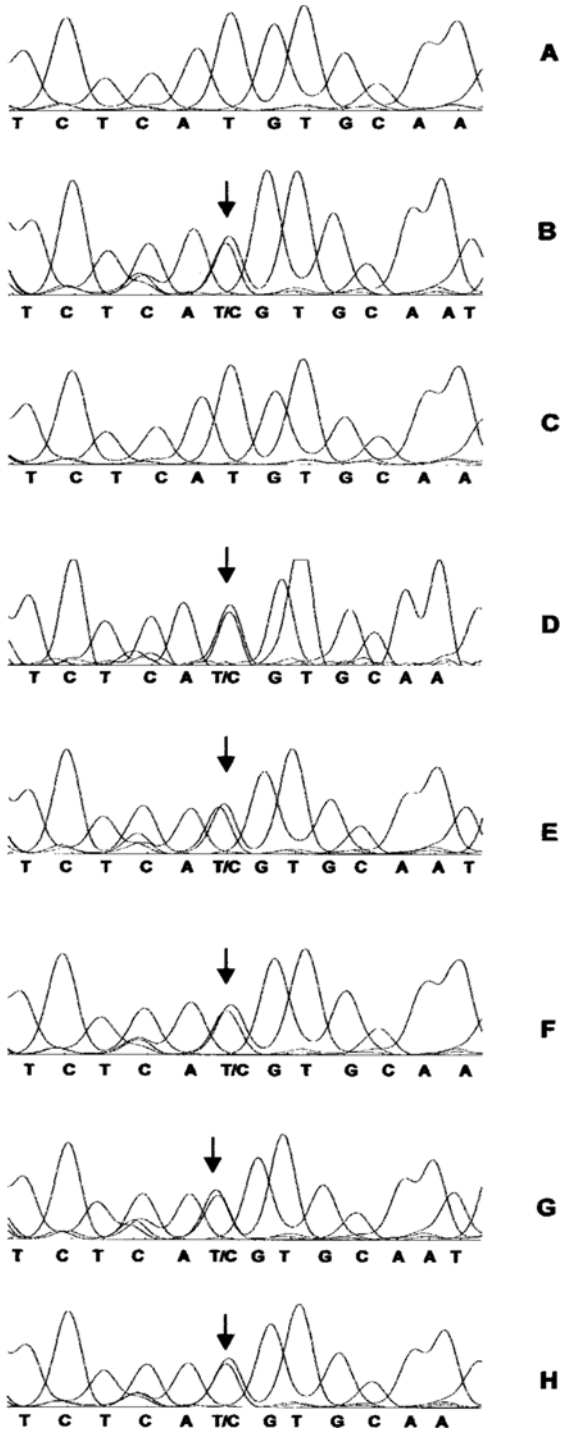

Fig. 4 - *Sequence analysis of the 1072-1288 fragment of exon 11 of LHR gene. Fragment of exon 11 of LHR gene harboring nucleotides 1072-1288 was amplified and subjected to direct sequencing as described in the Methods section. T1193C transition is marked by the arrows. Lane A, healthy individual; lane B, patient's mother; lane C, patient's father; lane D, patient, lanes E-H, patient's sisters.*

ture SSCP analysis with the use of DNA POINTER, as well as restriction digestion and sequence analysis, clearly demonstrated heterozygocity for this mutation in his mother and four sisters. The patient's father did not reveal any abnormalities in the LHR gene. This mutation causes Met398Thr substitution in the 2nd transmembrane helix and results in a constitutive activation of LH receptor as earlier reported (12, 17).

Our study permitted to change the initial diagnosis from the sporadic to the familial form of MPP and establish that this mutation was maternally inherited. The heterozygous T1193C transition in exon 11 of the LHR gene has previously been described in both sporadic (12) and familial (18) forms of MPP.

The investigated family was one of the largest ever described amongst MPP families and consisted of the patient and 5 asymptomatic carriers of the mutation (mother and 4 sisters). It has been established that the female carriers of the disease do not suffer from androgen excess, since T produced from D4 in granulosa cells of the ovary could be converted to 17β-E2 in these cells, thus preventing an increase in blood T and the consequences of androgen excess. This concept is consistent with the observation that hCG-secreting tumors cause puberty only in males but not in females (19).

In our report, the second Polish family with the diagnosis of MPP was presented. In the first family (20), the same heterozygous T1193C transition was evidenced. This transition was also maternally inherited. However, in that case, the mutation was probably transmitted to the patient's mother from her father, who might have been the founder of the mutation. It is most probable that the same mutation in both families occurred *de novo*. However, the existence of a common ancestor of the two families has not been excluded, although no direct relation between two families has been demonstrated.

In the U.S.A., the most common form of MPP is due to Asp578Gly substitution in LHR and is responsible for over 90% of the familial forms of MPP. Furthermore, this abnormality may also appear *de novo* and result in a sporadic form of MPP (21). Interestingly, this mutation was not detected in the population of Europe, although in this area an almost complete spectrum of mutations causing MPP was observed - the most common being Ile542Leu (9 cases) (22).

In both sporadic and familial forms of MPP, Met398Thr substitution was detected in Germany (2 cases) and the U.K. (2 cases) (21, 18). Single cases were described in the Netherlands and Italy (12, 21). The

only non-European country where this mutation has been found is Japan (17). Our two families with male-limited precocious puberty triggered by Met398Thr substitution add to the short list of cases of the familial form of MPP and were the only ones described in Eastern Europe. The presence of mutation in the patient's 4 sisters opens the possibility of spreading this type of mutation in the country of Poland.

ACKNOWLEDGEMENTS

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