Substitution of M398T in the Second Transmembrane Helix of the LH Receptor in a Patient with Familial Male-Limited Precocious Puberty

MONIKA IGNACAK, MACIEJ HILCZER*, JANUSZ ZARZYCKI* AND WIESLAW H. TRZECIAK

Department of Physiological Chemistry, University of Medical Sciences, 60-781 Poznan, Poland
Department of Pediatric Endocrinology, Polish Mothers Health Institute, 93-338 Lodz, Poland

Abstract. Familial male-limited precocious puberty (MPP) is described in a 10 year old patient with typical symptoms of the disease. Sequence analysis of genomic DNA clearly demonstrated a heterozygous T1 193C transition in exon 11 of the LH receptor (LHR) gene, which results in M398T substitution in the second transmembrane helix of the protein product of this gene. The same mutation was found in the patient's mother and in her brother. The grandmother and the relatives of the patient’s father were free of the mutation. The boy was successfully treated with inhibitors of steroid biosynthesis and androgen antagonists. It is suggested that this mutation caused constitutive activation of the LHR, which results in excessive formation of androgens in Leydig cells and is responsible for the symptoms of precocious puberty in this patient. This is the second case of the familial form of MPP that was maternally inherited.

Key words: Male sexual precocity, LHR, M398T, Substitution

FAMILIAL male-limited precocious puberty (MPP), also called testotoxicosis, is a gonadotropin-independent sexual precocity in an autosomal dominant, male-limited pattern. The symptoms of this disease include rapid virilization (usually at the age of 1 to 4 years), accompanied by growth acceleration connected with premature epiphyseal maturation and adult short stature. Testosterone production is raised to the adult level. Serum levels of LH and FSH are barely detectable, even following exogenous GnRH stimulation [1]. Testotoxicosis is due to the constitutive activation of the lutropin receptor (LHR) and results in excessive synthesis of androgens in Leydig cells [2].

The LH receptor (LHR) consists of N-terminal extracellular domain, seven transmembrane helices, joined by extra- as well as intracellular loops, and the C-terminal intracellular domain [3]. Under physiological conditions puberty is initiated when LH, produced in the anterior pituitary, binds to the LHR in Leydig cells to increase intracellular cyclic adenosine monophosphate (cAMP) concentration and the activity of cAMP-dependent protein kinase A. This promotes androgen synthesis and initiates sexual maturation [4, 5].

It has been reported that missense mutations of the LHR gene are responsible for the amino acid substitutions in the protein product of the gene that results in a constitutive activation of the receptor and cAMP-mediated stimulation of androgen formation in the gonad [2]. Most of the mutations of the LHR gene were located in the sixth transmembrane helix and in the third intracellular loop [2, 6–10]. Other mutation are located in the first [11], second [12–14], third [15] and fifth [16] transmembrane helix.

The T1193C transition results in substitution of
Met for Thr (M398T) in the second transmembrane helix of the LHR. In the present report, we describe a case of MPP caused by M398T mutation which was transmitted maternally.

**Case Report**

Our patient was the only child of healthy non-consanguineous parents. There had been no history of early sexual development in other family members. The boy had presented a progressive increase in the size of testes, penis and the occurrence of pubic hair at the age of 5. At the first clinical examinations (5 years and 3 months), pubescence was determined as stage III, according to Tanner's classification. According to Prader the volumes of the testes evaluated amounted to 15 ml for the right and 12 ml for the left testis. The boy's height was 118.8 cm, SDS +1.78 and the bone age was 6.5 years. Serum gonadotropin concentration was barely detectable and total testosterone concentration was elevated up to an adult level [4.9 ng/ml]. In the GnRH test, a flat curve of gonadotropin secretion was observed, suggesting a premature pseudopuberty. Prior to the first clinical examination the patient had not been hormonally treated and the anamnesis was without any significance. Histological examination of the testes revealed the features of premature development of seminiferous epithelium, with the development of spermatids and an enlargement of the interstitial gland.

Following one year of treatment with ketoconazole, the patient's height increased to 25.8 cm, SDS +1.81 and the bone age was 9 years. However, the therapy was temporarily discontinued because of elevated serum aminotransferase activities and was restored only after 6 months. In addition to treatment with ketoconazole, the patient was subsequently medicated with spironolactone and testolactone, followed by cyproterone acetate (100 mg/daily). This treatment has still been continued. On a recent examination, at the age of 10.4 years, the boy's height increased to 159.6 cm, SDS +3.24 and the bone age was 15.5 years.

None of the relatives exhibited any abnormalities in sexual development. In particular, the patient's mother had regular menses. There were no abnormalities in the growth of her brother who carried the same mutation. He also did not exhibit any signs of androgen excess and he had reached puberty no earlier than his healthy colleagues.

**Methods**

Leukocyte DNA was obtained from the patient, his mother, her brother, his grandmother, as well as from the parents of the boy's father and from their daughter. Two fragments of exon 11 of the LHR gene (nucleotides 1072–1288 and 1471–1804), encoding part of the first and the entire second transmembrane helix, most of the fourth, the entire fifth and sixth transmembrane helices, respectively were subjected to amplification by PCR followed by direct sequencing. The primers used were: 1F 5'GGC TCTTTTCTTTCTCTAATTG3'; 1R 5'GTTACTGTGTTGATAAGAGGTACT3' and 2F 5'GTCCATGACTTCCCTAGGG3' and 2R 5'GCAGGTTATATGTTCCTGGGC3'. The conditions of the reaction were: initial denaturation (95°C, 5 min) and 35 cycles, each involving denaturation (95°C, 30 sec); annealing (56°C, 30 sec); and elongation (72°C, 30 sec). The last cycle was followed by final elongation (72°C, 5 min). The PCR products were purified and sequenced from both directions on an ALFexpress Sequence Analyser (Amersham-LKB, USA).

**Results and Discussion**

We examined a Polish patient with typical symptoms of MPP without any family history of the disease. Two fragments of exon 11 of the LHR were analyzed. There were no sequence abnormalities...
detected in the fragment encoding part of the fourth, the entire fifth and sixth helices (nucleotides 1471-1804). However, sequence analysis of the amplification product, derived from the fragment encoding part of the first and the entire second transmembrane helix of the LHR gene (nucleotides 1072-1288), revealed a heterozygous transition T1193C (Fig. 2A). The same mutation was detected in the patient’s mother (Fig. 2B) and her brother (Fig. 2C). Sequence analysis of the same fragment in the patient’s grandmother (Fig. 2D) did not reveal any abnormalities. Although it was impossible to diagnose the patient’s father, this mutation was not found in the father’s parents or in his sister.

The T1193C transition in exon 11 of the LHR gene has previously been described [12–14] in both sporadic and familial forms of testotoxicosis. However, only in one case was this mutation maternally inherited [14] and our case was the second reported familial form of testotoxicosis.

Kraaij et al. [12] and Yano et al. [13] investigated the effects of this mutation on the receptor function. In the cells transfected with recombinant plasmid harboring the mutated receptor, they independently showed an increase in the basal cAMP production, compared to the same cells transfected with recombinant plasmid containing the normal receptor.

We suggest that the heterozygous T1193C transition in exon 11, which caused substitution of Met\textsuperscript{398} for Thr in the second transmembrane helix, is responsible for the symptoms of the disease. It remains to be determined how the Met\textsuperscript{398} activates LHR.

The mutation described herein was maternally inherited, and was probably transmitted to mother by her father, who did not exhibit symptoms of precocious puberty, or else these symptoms were overlooked in childhood. The same mutation was also found in his son who did not exhibit any abnormalities of sexual maturation. Interestingly, Yano et al. [13] and Evans et al. [14] independently described the same mutation in male individuals asymptomatic for MPP. At present, no reasonable explanation can be offered why the same mutation causes MPP in one individual and does not affect sexual maturation in the other, unless we assume that

Fig. 2. Sequence analysis of the DNA fragment of exon 11 of the LHR gene, encoding the transmembrane helix. A—patient, B—his mother, C—her brother, D—grandmother. The T1193C transition is marked by arrow.
this was a conditional mutation subject to other yet undefined genetic or environmental factors.

The female carriers of the disease do not suffer from androgen excess. It is possible that testosterone produced from androstendione in the granulosa cells of the ovary could be converted to 17β-estradiol in these cells thus preventing an increase in blood testosterone and the consequences of androgen excess.

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