

Identification of constitutively activating mutation of the luteinising hormone receptor in a family with male limited gonadotrophin independent precocious puberty (testotoxicosis)

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Abstract

A family of male limited gonadotrophin independent precocious puberty was examined for activating mutation of the LH receptor. A transition of A to G in nucleotide 1733 of the human LH receptor gene was identified in all affected males and in an unaffected carrier female. The mutation was shown by identifying a new restriction site created by the mutation. This mutation appears to be a common feature of the disorder, as it has been reported previously in unrelated families. Therefore, the presence of this new restriction site can serve as a diagnostic tool in males at risk before the onset of symptoms, as well as identifying carrier females.

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of advanced skeletal maturation, raised serum testosterone (2.86 ng/ml, normal <0.2 ng/ml for prepubertal boys), and suppressed serum LH and FSH.

His father (IV.1), now 37 years old, had early puberty, starting before his third birthday. He grew rapidly and reached his final height of 1.65 m at the age of 12 years. The proband's older brother (V.2), now 11 years old, started to experience pubertal maturation at the age of 21 months. The evaluation showed advanced skeletal maturation, raised serum testosterone (2.7 ng/ml), and suppressed (prepubertal) serum LH and FSH in response to exogenous GnRH. The proband's relatives, III.1 and II.1, were both said to have "early puberty" beginning at a similar age to the father and reaching their adult stature before their 12th birthday.

Method

Blood (5 ml) was collected from each available member of the family (generations IV and V) and placed in heparinised test tubes. Genomic DNA was isolated from the whole blood of each patient using Stratagene DNA extraction kit.¹⁰ DNA fragments encoding amino acid residues 441 to 594 of the human LH receptor¹¹ was amplified by polymerase chain reaction (PCR). For PCR amplification, CACT-GCTGGCTTTTTCACTGTATT and TGA-AGGCAGCTGAGATGGCAAAA were used as upstream and downstream primers, respectively. The reaction mixture consisted of 25 pmol of each primer and 2.5 U of *Taq* polymerase (Boehringer Mannheim Biochemicals) and approximately 1 µg of the genomic DNA in a buffer consisting of 10 mmol/l Tris HCl, pH 8.3, 50 mmol/l KCl, 0.001% gelatin, 2.0 mmol/l MgCl₂, and 200 µmol/l each deoxynucleotide triphosphate in a final volume of 100 µl. PCR conditions were five minutes at 95°C for denaturation, followed by 30 cycles of one minute at 57°C, 30 seconds at 72°C, and one minute at 95°C and final extension for three minutes at 72°C. The PCR product was subjected to phenol:chloroform extraction followed by ethanol precipitation and the precipitate was dried in a vacuum desiccator. The size of the PCR amplified DNA corresponded to 464 bp when examined after electrophoresis on agarose gels. A previous study has identified a transition of A to G of nucleotide 1733 of the LH receptor cDNA thereby creating a new

Male limited gonadotrophin independent precocious puberty is a disorder characterised by the onset of pubertal development before the third birthday of the affected male.¹⁻⁶ Testosterone production in these young boys is raised comparable to the adult level. Serum levels of LH and FSH are nearly undetectable even following exogenous GnRH stimulation. This results in advanced skeletal maturation and short adult stature secondary to early closure of the epiphyses.

Testosterone production by the testes is driven by the receptor mediated activation of the adenylate cyclase by LH leading to increased production of cyclic AMP.⁷ Previous reports have identified a mutation in the LH receptor gene in affected families.^{8,9} We describe an unrelated family with three affected males and a carrier female with this disorder who has the same mutation. This method also identifies carriers and may serve as a useful tool in identifying subjects carrying this mutation before the onset of symptoms.

Materials and methods

SUBJECTS

The family pedigree is depicted in the figure. The proband (V.1), now 5 years old, was followed prospectively for precocious puberty because of the family history. At the age of 22 months, onset of pubic hair growth, enlarging testicles and phallus size led to the finding

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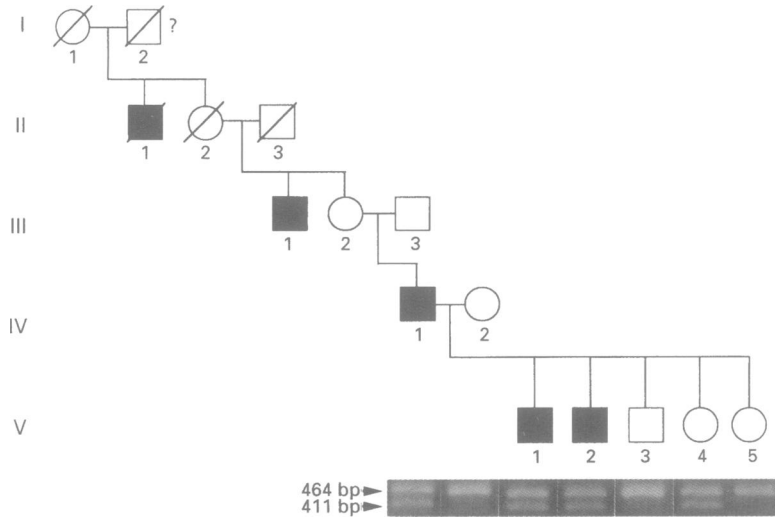
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Pedigree of a family with FMPP and restriction analysis of DNA with *MspI*. Affected males are shown as solid squares. It is unknown which of the subjects in I is the carrier. DNA fragments encoding the fourth to sixth transmembrane regions (464 bp) of the LH/hCG receptor were amplified by PCR as described under Methods. The PCR products were digested with *MspI* and subjected to agarose gel electrophoresis. An A to G transition at nucleotide 1733 of the human LH receptor gene creates the recognition site for *MspI* (C/CGG). PCR products from DNA isolated from unaffected subjects (464 bp) will not be hydrolysed by *MspI*, but the mutant allele will be cleaved into two fragments of 411 and 53 bp. DNA from subjects who are heterozygous for the mutation will yield both intact and digested fragments. The figure shows only 464 and 411 bp bands

restriction site (C/CGG) for *MspI*.^{8,9} Thus, the PCR product was treated with 10 units of *MspI* at 37°C for 20 hours to examine whether such a mutation exists in these patients. The digest was then subjected to electrophoresis on 2% agarose, at 80 V on a minigel for 1.5 hours. The ethidium bromide bound bands were visualised under ultraviolet light.

Results and discussion

The results of the restriction digestion from each patient from generation IV and V are shown at the bottom of the figure. The PCR amplified DNA from the unaffected subject appeared as a single 464 bp band. The DNA from the affected subjects would be expected to yield two bands, a 411 bp band in addition to the 464 bp corresponding to the normal allele. The 53 bp band corresponding to the remaining DNA product was also visible on the gels in DNA samples treated with *MspI* from the affected subjects, but was less intense as compared to the larger DNA band. That portion of the gel is not shown in the figure. The father (IV·1) and the two affected boys (V·1 and V·2) each showed two bands corresponding to 464 bp and 411 bp suggesting that they carried a normal allele and a mutant allele. The mother (IV·2) and the unaffected sibs (V·3 and V·5) showed only the normal allele. One of the unaffected daughters (V·4) showed both 464 bp and 411 bp bands corresponding to the normal and mutant alleles

suggesting that she is a carrier of the gene possessing the mutation.

Previous studies have shown that mutation of asp 578 to glycine on the sixth transmembrane helix of the LH receptor makes it constitutively active so that adenylate cyclase is activated in the absence of the ligand, LH.^{8,9} Thus, the carriers of this mutation have the potential to produce higher levels of testosterone even before the maturation of the hypothalamic-pituitary gonadal axis. Since we were able to locate the mutation at the expected place in the genomic DNA, we did not examine the possibility of the existence of other mutations in these patients. The family pedigree and the DNA restriction pattern support the notion that transmission of the disorder occurs as an autosomal dominant trait. Although mutations were thought to result in loss of function, the activating mutation as reported here belongs to an emerging and enlarging group of disorders.¹² Such mutations occur on the receptor itself as is the case for LH receptor in this family or stimulatory guanine nucleotide regulatory proteins (Gs_s) as in the case of McCune Albright syndrome.¹² This independent study confirms and extends previously reported findings in an unrelated family and suggests that this technique can be a useful tool in identifying carriers in the population at risk as well as a rapid screen for affected males before the onset of the symptoms.

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