Short reports

Male pseudohermaphroditism resulting from a novel mutation in the human steroid 5α-reductase type 2 gene (SRD5A2)

R Anwar, S G Gilbey, J P New, A F Markham

Abstract

The enzyme steroid 5α-reductase, via NADPH, catalyses the conversion of testosterone to dihydrotestosterone, which is required for the embryonic differentiation of the external male genitalia and the prostate. An impairment of this reaction causes a form of male pseudohermaphroditism in which genetic males differentiate predominantly as phenotypic females. Molecular analysis of the 5α-reductase type 2 gene in a patient with confirmed biochemical 5α-reductase deficiency has resulted in the identification of a novel mutation, GAA to AAA, at codon 200. This mutation produces an amino acid change from glutamic acid to lysine, and may affect the ability of the enzyme to bind its co-factor.

(*J Clin Pathol: Mol Pathol* 1997;50:51–52)

Keywords: 5α-reductase; pseudohermaphroditism; testosterone; mutation.

Molecular Medicine Unit, Department of Medicine, University of Leeds, Clinical Sciences Building, St James's University Hospital, Leeds LS9 7TF

Correspondence to:
Dr R Anwar.
e-mail:
msjra@stjames.leeds.ac.uk

Accepted for publication
29 October 1996

The enzyme steroid 5α-reductase is an NADPH dependent protein that catalyses the conversion of testosterone into dihydrotestosterone, producing an androgen that is 50-fold more potent than testosterone.[1] Dihydrotestosterone is essential for formation of the male phenotype during embryogenesis (development of the male external genitalia, urethra and prostate), and for most androgen mediated events of male sexual maturation at puberty (growth of facial and body hair, maturation of the external genitalia).[2] The failure to convert testosterone into dihydrotestosterone leads to male pseudohermaphroditism in which 46, XY males have male internal urogenital tracts, but female external genitalia. The absence of dihydrotestosterone may also underlie other disorders of androgen metabolism.

Two isozymes of steroid 5α-reductase, type 1 and type 2, have been identified by cDNA cloning.[3] Mutations in the type 2 gene are responsible for the autosomal recessive genetic disease of 5α-reductase deficiency.[4]

The 5α-reductase-2 gene (SRD5A2) is located on chromosome 2p23, contains five exons separated by four introns and transcribes an mRNA of 2.437 kilobases.[5] The coding region of the mRNA is translated into a polypeptide of 254 amino acids. Here, we present the biochemical and molecular genetic analysis of 5α-reductase deficiency in a patient from the Kashmir region of Pakistan. The results show a novel sequence change within the co-factor binding region of the 5α-reductase-2 polypeptide.

Methods

PATIENT

The 26 year old 46, XY male was born in the UK of parents from Kashmir, Pakistan. His parents are related but are not first degree cousins. Two brothers are phenotypically normal. The patient was born with ambiguous genitalia and reared as a girl until early teenage years when increasingly male features prompted a re-assignment of sex. Extensive plastic surgery had been required to provide him with a satisfactory phallus. He was lost to follow up during his late teenage years but re-presented to our clinic when he was contemplating marriage. Examination showed ambiguous genitalia with palpable labial testes, poorly developed secondary sexual features, and mild bilateral gynaecomastia. Biochemical screening revealed a raised plasma testosterone to dihydrotestosterone ratio (12.7:0.45 nmol/l). Serum luteinising hormone, follicle stimulating hormone, prolactin, and oestradiol were all within the normal adult male range.

ISOLATION OF GENOMIC DNA

Ten millilitres of peripheral blood were obtained from the subjects, using EDTA as an anticoagulant. The peripheral blood mononuclear cells (PBMC) were separated from whole blood using lymphoprep (Nycoma Pharma AS, Birmingham, UK), according to the manufacturer's instructions. Genomic DNA was extracted from PBMCs as described by Sambrook *et al.*[6]

POLYMERASE CHAIN REACTIONS

Exons 1 to 5 were amplified individually via PCR using the oligonucleotides described by Thigpen *et al.*[7] The PCR mixture of 100 μl

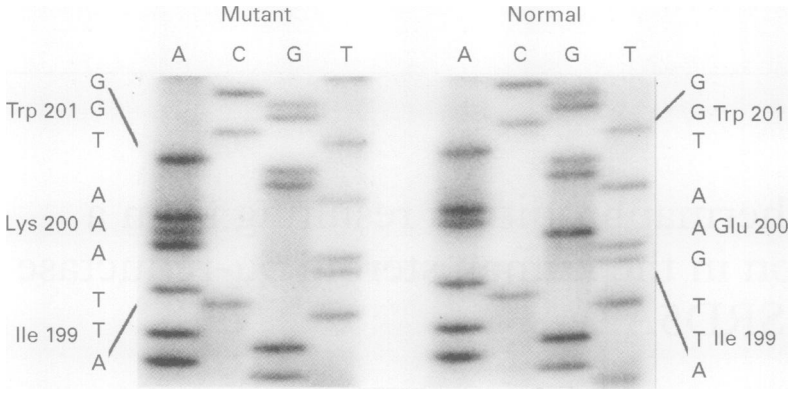

Figure 1 Sequence analysis of the 5α-reductase gene. Exon 4 of the 5α-reductase gene was amplified by PCR, the PCR product was purified and sequenced directly using the forward primer. This figure illustrates the G to A mutation, Glu200Lys, identified. The patient is homozygous for this sequence change.

contained 50 ng genomic DNA, 20 μM dNTPs, 1 μM each primer, 1 unit of Taq DNA polymerase (Promega, Southampton, UK), and reaction buffer (Promega). PCR conditions were as follows: 92°C for one minute, 66°C for one minute, and 72°C for one minute for 35 cycles. After a final extension step at 72°C for five minutes, 5 μl of the PCR product was analysed by agarose gel electrophoresis and ethidium bromide staining.

SEQUENCE ANALYSIS OF PCR PRODUCTS

PCR products were de-salted and purified from unincorporated nucleotides using Wizard DNA clean-up columns (Promega) following the manufacturer's protocol; 25–50 fmoles DNA was then used as template for direct sequencing using the Sequenase sequencing kit (USB) and γ^{32}P end-labelled amplimers.

Results

All five exons of the 5-α-reductase gene were amplified individually by PCR, and PCR products were sequenced directly using the amplimers.

Nucleotide sequence analysis of all the amplified exons showed that there was a sequence change from G to A at nucleotide 598 of the coding region, within exon 4 (fig 1). This translates to an amino acid change at codon 200 from glutamic acid to lysine, GAA to AAA. This patient was homozygous for this mutation, probably owing to consanguinity between his parents. The mother and the 11 year old male sibling were heterozygous for this Glu200Lys mutation. The father and 31 year old brother were not available for study.

Exon 4 was amplified from 124 unrelated normal subjects and sequenced directly using the amplimers. The Glu200Lys mutation was not detected in any of these subjects, confirming it is probably the mutation responsible for male pseudohermaphroditism in this family.

Discussion

This study describes the molecular genetic analysis of the 5α-reductase type 2 gene in a patient with male pseudohermaphroditism. A number of point mutations and deletions have been identified in the 5α-reductase-2 gene in different ethnic groups.[7] However, only two mutations have been described previously in this gene within the Pakistani ethnic group—a missense mutation G to A, Arg246Gln, in exon 5, and a G to T sequence change at the exon 4/intron 4 splice junction.[7] We report a novel mutation at codon 200, leading to an amino acid change from glutamic acid to lysine. This Glu200Lys mutation in the 5α-reductase-2 gene has not been observed in any other ethnic group studied so far.

The three-dimensional structure of the 5α-reductase-2 polypeptide has not yet been resolved. Thus, it is difficult to predict the possible functional domains within the various regions of the protein. Expression analysis of three mutations (Gly34Arg, Gly196Ser, and Arg246Trp) has indicated that the N-terminus of the polypeptide may be involved in steroid substrate binding, whereas the C-terminus, particularly the region between codons 196 and 246, is likely to play a role in the NADP(H) co-factor binding.[8] As glutamic acid has an acidic side chain whereas lysine has a longer basic side chain, the Glu200Lys mutation reported could alter the structure of the protein in this region such that it is unable to bind its co-factor and thus becomes inactive. It would be very interesting to express this Glu200Lys mutant in recombinant cells to study the effect of this mutation on the activity, half-life and therefore concentrations of the protein, and to gain a better understanding of the mode of action of this enzyme.

We thank our patient and his family for their co-operation and support. Work in our laboratories is supported by the Medical Research Council, Wellcome Trust, West Riding Medical Research Trust, and Yorkshire Cancer Research Campaign.

1 Wilson JD, Griffin JE, Russell DW. Steroid 5-alpha reductase-2 deficiency. *Endocr Rev* 1993;**14**:577–93.
2 Wilson JD. Metabolism of testicular androgens. In: Greep RO, Astwood EB, eds. *Handbook of physiology*. Vol 5. Section 7. Washington DC: American Physiological Society, 1975:491–508.
3 Russell DW, Wilson JD. Steroid 5-alpha-reductase—2 genes 2 enzymes. *Ann Rev Biochem* 1994;**63**:25–61.
4 Wigley WC, Prihoda JS, Mowszowicz I, Mendonca BB, New MI, Wilson JD, *et al.* Natural mutagenesis study of the human steroid 5α-reductase 2 isozyme. *Biochemistry* 1994; **33**:1265–70.
5 Labrie F, Sugimoto Y, Luu-The V, Simard J, Lachance Y, Bachvarov D, *et al.* Structure of human type II alpha-reductase gene. *Endocrinology* 1992;**131**:1571–3.
6 Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A laboratory manual.* 2nd edn. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989.
7 Thigpen AE, Davis DL, Milatovich A, Mendonca BB, Imperato-McGinley J, Griffin JE, *et al.* Molecular genetics of steroid 5α-reductase 2 deficiency. *J Clin Invest* 1992;**90**: 799–809.
8 Russell DW, Berman DM, Bryant JM, Cala KM, Davis DL, Landrum CP, *et al.* The molecular genetics of steroid 5α-reductases. *Recent Prog Horm Res* 1994;**49**:275–83.