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

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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.

Methods
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.

POLYMERASE CHAIN REACTIONS
Exons 1 to 5 were amplified individually via PCR using the oligonucleotides described by Thigpen et al. 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.

Results
All five exons of the 5α-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. 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. 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 NAD(P)H co-factor binding. 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.