Turkish Journal of Endocrinology and Metabolism, (2002) 1 : 13-19 **REVIEW**

Steroid 5 α-reductase type-2 Gene Mutations in the Turkish Population

Selçuk Can

Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, American Hospital, Istanbul, Turkey

5α-reductase deficiency results from reduced conversion of testosterone to the more potent androgen, dihydrotestosterone. Alterations in 5 α-reductase type-2 gene are responsible from the syndrome. Four distinct mutations are reported to cause 5 α-reductase deficiency in the Turkish population: Δ M157, G196S, L55Q and Δ P251 plus frameshift. Genetic diversity of the observed mutations disproves a founder effect for the whole country. A common ancestor may only be responsible for cases that live or migrate from isolated geographic areas in Turkey. The presence of high rate of consanguinity in affected subjects mandates a public education campaign against intrafamily-marriages. With the identification of etiological molecular genetic defects, genetic counselling and prenatal diagnosis should be offered to affected individuals.

Key words: 5α-reductase deficiency, male pseudohermaphroditism, 5 α-reductase type-2 mutations

Two steroid hormones, testosterone (T) and dihydrotestosterone (DHT) mediate virilization in mammals. Both hormones bind to androgen receptor and activate androgen responsive DNA elements. The conversion of testosterone into dihydrotestosterone by steroid 5α-reductase is a key step in androgen action, and is essential both for formation of male phenotype during embryogenesis and for androgen-mediated growth of tissues such as prostate (1). Identification of an inborn error of male phenotypic sexual differentiation termed steroid 5α-reductase deficiency confirmed the androgenic importance of DHT (2).

Male pseudohermaphroditism due to 5α-reductase deficiency

5α-reductase deficiency is an autosomal recessive inherited disorder that was first characterized by Imperato-McGinley and coworkers in subjects with pseudovaginal perineoscrotal hypospadias from the Dominican Republic (2). Further studies identified two large pedigrees from Turkey and New Guinea (3,4). Numerous individual cases have also been reported from all over the world (5-7). 46 XY individuals with 5α-reductase deficiency have striking ambiguity of the genitalia with a clitoral-like phallus, severely bifid scrotum, pseudovaginal perineoscrotal hypospadias and a rudimentary prostate. Genital ambiguity may show variation in affected individuals manifesting as a blind vaginal pouch opening into urethra in the most severe form or a penile urethra at the other end of the clinical spectrum (1,2). Cryptorchidism is common. Testes may be located in the abdomen but are usually in the inguinal canal or scrotum. Wolffian duct differentiation is complete in 5α-reductase deficiency with the existence of seminal vesicles, vasa deferentia, epididymides and ejaculatory ducts. Due to rising testosterone levels at puberty, 5α-reductase deficient patients have an increase in muscle mass, hyper-pigmentation and rugation of the scrotum, deepening of the voice, phallic elongation and male pattern hair distribution on the face and body (2). Their

Correspondence address:

Selçuk Can
American Hospital
Güzelbahçe Sok. No: 20
80200 Nişantaşı, Istanbul
E-mail: selcukcan@doruk.net.tr

sperm count is low. Fertility may be achieved with the aid of in vitro fertilization techniques otherwise they are usually infertile (8). Two patients who had predominantly male phenotype naturally fathered children (9). Plasma testosterone levels are elevated and DHT levels are decreased in 5α-reductase deficiency. Plasma T/DHT ratio is elevated (2).

Virilization at puberty, absence of gynecomastia and an elevated T/DHT ratio suggest 5α-reductase deficiency in adults or adolescents. Diagnosis is not straightforward during infancy or childhood. Clinical decisions about gender assignment, gonadectomy and androgen treatment for microphallus must be made early in life. Therefore, an accurate diagnosis during infancy is required to understand the capacity of testicular androgen production and sensitivities of genital tissues to the action of androgens. These two factors predict the potential for virilization at puberty. Measurements of T and DHT in serum after hCG stimulation, with determination of T/DHT ratios are required to establish the diagnosis. Assessment of urinary excretion of 5α/5β-steroid metabolites as well as measurement of 5α-reductase enzyme activity in cultured genital skin fibroblasts are ancillary investigations. All of these parameters are highly variable in prepubertal children and interpretation may be difficult. Therefore, it is crucial to perform molecular genetic analysis as an additional means for an accurate diagnosis (3,10).

Phenotypes may vary widely in patients with 5α-reductase type-2 gene mutations spanning the whole range from completely female to normal male without distinctive clinical signs of the disease (2-7, 9-11). Hence steroid 5α-reductase deficiency should be considered not only in sex reversed patients with female or ambiguous phenotypes but also in those with mild symptoms of undermasculinization such as hypospadias or micropenis.

Steroid 5 α-reductase isoenzymes and genes

Steroid 5α-reductase is a microsomal enzyme that reduces the double bond at the 4-5 position of C19 and C21 steroids. The enzyme uses NADPH as a cofactor and is expressed in prostate, seminal vesicles, epididymis, liver, hair follicles, sebaceous glands and skin of the external genitalia (12). Its major role in male physiology is to reduce testosterone, to the more potent androgen dihydrotestosterone DHT binds to the androgen receptor more avidly than T and DHT-androgen receptor complex is more efficiently transformed to the DNA binding state than is the T-receptor complex (13).

There are two isoenzymes of steroid 5α-reductase with different properties and tissue distribution. Complementary cDNAs have been isolated for two human 5α-reductase isoenzymes with distinct biochemical, genetic and pharmacological features (Table 1). Steroid 5α-reductase-type-1 is a 259 amino acid long protein with a molecular weight of 29562 Da. There are hydrophobic amino acids attached to its primary structure suggesting that the enzyme is an intrinsic membrane protein embedded in the lipid bilayer, not a transmembrane protein (12). Therefore, it has been very difficult to solubilize and purify the enzyme until molecular genetic studies predict its amino acid sequence. Optimal pH of type-1 isoenzyme is neutral to basic (pH 6-8.5). RNA blot hybridization experiments show that type-1 isoenzyme is expressed at low levels in prostate and localized to peripheral tissues like nongenital skin, liver, lung, kidney, intestine and brain (12). Overactivity of type-1 isoenzyme may be the most important factor for hirsutism in women and variation of its expression in ethnic groups is thought to be responsible for different facial and body hair content among different races (13).

The gene encoding type-1 isoenzyme has been designated as steroid 5α-reductase type-1 gene 5α-reductase type-1 gene is located on the short arm of chromosome 5 band 15. The gene consists of 5 exons and 4 introns. Genetic analyses excluded the type 1 locus as the responsible gene in eight unrelated families with 5α-reductase deficiency (14).

Table 1. Comparison of human steroid 5α-reductase isoenzymes

	Type-1	Type-2
Chromosome	5p15	2p23
Gene structure	5 exons, 4 introns	5 exons, 4 introns
5α-reductase deficiency	Normal	Mutated
Size	259 amino acids	254 amino acids
pH optima	Neutral to basic	Acidic
Expression in prostate	Low	High
Inhibition by finasteride	Ki ≥300 nM	Ki= 3-5 nM

Steroid 5α-reductase type-2 enzyme is a hydrophobic protein of 254 amino acids with a molecular weight of 28398 Da. It demonstrates an acidic pH optimum (pH 5.0) and is expressed in prostate, male internal genital structures and genital skin fibroblasts (12). Type-2 enzyme is sensitive to inhibition by finasteride (15). The gene encoding type-2 isoenzyme has been termed steroid 5α-reductase type-2 gene. Type-2 gene contains five exons that are 352, 164, 102, 151 and 1695 basepairs long, respectively. Coding sequences of type-1 and 2 genes demonstrate 50% homology. Exons of 5α-reductase type-2 gene are separated by four introns of >29, 2.3, 2.0 and 3.0 kilobases. Type-2 gene has been localized to chromosome 2 band p23 by somatic cell hybrid mapping and chromosomal in situ hybridization techniques (16). Andersson et al (17) reported a complete deletion of 5α-reductase type-2 gene in male pseudohermaphrodites from the Simbari Anga linguistic group in the Highlands of Papua New Guinea, proving the critical role of type-2 enzyme in determining the fate of the bipotential anlage of the mammalian external genitalia. Different chromosomal locations of type-1 and -2 genes are consistent with their divergence at the DNA sequence level. The two genes have similar architectures (5 exons/4 introns). The positions at which introns interrupt the exons are identical between them (14,16). These findings suggest that they arose as a consequence of an ancient duplication event and subsequently evolved separate physiological roles.

Genetic mutations leading to subtle abnormalities in 5α-reductase enzyme may underlie some forms of commonly encountered urogenital birth defects in males such as hypospadias or microphallus (18). Knowledge of 5α-reductase gene structure offers an opportunity to study frequent endocrine disorders, like prostate cancer, benign prostatic hyperplasia, acne, seborrhea and androgenic alopecia. The availability of DNA probes for both genes will allow the definition of 5α-reductase activity in these common disorders.

5α-reductase type-2 Gene Mutations in the Turkish Population

Molecular genetic analysis for point mutations, deletions or insertions has become a widespread diagnostic approach for a variety of diseases. In 1998, we characterized the molecular genetic defect causing 5α-reductase deficiency in one of the world's largest pedigrees with Turkish male pseudohermaphrodites (3). Our approach to molecular genetic diagnosis will be outlined and 5α-reductase type 2 mutations in the Turkish population will be reviewed.

After defining the phenotypic characteristics of a male pseudohermaphrodite, a clinical diagnosis pointing to a defect in androgen synthesis or action must be established. The culprit gene defect must be studied based on the information obtained from clinical and hormonal data. Initially, individual exons of the suspected gene defect must be amplified by polymerase chain reaction (PCR). The selected oligonucleotides (primers) must allow assessment of complete exonic sequence, including exon-intron boundaries. For the study of 5α-reductase deficiency, DNA sequences at the boundaries of exon 1 through 5 are used to design primers. A thermocycler program is identified to amplify all exons of the gene. DNA fragments of expected size are detected with each primer pair. In our study, screening of each exon for the etiologic mutation was performed by SSCP analysis (3). This method was first described by Orita and coworkers in 1989 and is based on the principle of identical electrophoretic mobility of identical single stranded DNA molecules (19). The wild type and mutant DNA are not identical; therefore they have different mobility on a gel. SSCP technique has sensitivity close to 100% if stringent conditions are used. This feature permits accurate identification of normal, homozygous and heterozygous individuals. The drawback of this method is the requirement of radioactive nucleotides (α-^{32}P dATP) during the experiment with its problems of availability and high cost. Hiort and coworkers successfully used denaturing gradient gel electrophoresis (DGGE) to screen for mutations (10). This method requires no radioactivity. Experience in molecular genetics is needed to accurately perform DGGE. When SSCP or DGGE finds an abnormal exon, the mutation should be characterized by DNA sequencing. An alternative approach is the sequencing of the whole gene without employing screening. This is a more costly way to detect mutations. Above mentioned methods are used for searching unknown mutations. If the causative mutation is already known, restriction

length fragment polymorphisms (RLFP), an easier and faster method can be utilized to identify patients, carriers and normals. The last step of the molecular genetic approach is the determination of the functional significance of the mutation. This is achieved by site-directed mutagenesis and invitro transfection analysis. Mechanisms that adversely alter the enzyme function must be delineated prior to genetic counseling or prenatal diagnosis. Localization or type of mutation may be responsible for severity of enzyme dysfunction. A structural change within the enzyme results in a major dysfunction, as evidenced by the severe phenotypic abnormalities and elevated T/DHT ratio found in 5α-reductase deficient male pseudohermaphrodites.

Four distinct mutations were reported to cause 5α-reductase deficiency in the Turkish population (Table 2). Boudon and coworkers (20) was the first to identify the molecular genetic defect in a 5α-reductase deficient Turkish patient. Clinical features included cliteromegaly, urogenital sinus with a blind vagina and high inguinal cryptorchidism. 5α-reductase activity was diminished in cultured genital skin fibroblasts. The parents were first cousins. Direct DNA sequencing without employing simple screening techniques identified a previously unreported mutation. A homozygous (AAT) deletion of nucleotides 468-470 in exon 3 was responsible for a methionine deletion at amino acid position 157. This deletion did not create a frameshift in the reading sequence. In vitro transfection analysis was not performed; therefore it is not possible to ascertain the functional significance of the mutation at the protein level. Genetic polymorphism is unlikely because the methionine residue at position 157 is conserved in both type-1 and type-2 human and rat 5α-reductase genes. The demonstration of diminished 5α-reductase activity in the patients' genital skin fibroblasts provided indirect evidence for the pathological role of ΔM157 mutation.

Table 2. 5α-reductase type-2 mutations in the Turkish population

Type	Exon	Mutation	Mechanism	Reference
Deletion	Exon 3	ΔM157	Not determined	20-22
Missense	Exon 4	G196S	Altered NADPH K_m	21
Missense	Exon 1	L55Q	Altered testosterone K_m	10,24
Deletion,	Exon 5	ΔP251, frameshift	Altered NADPH K_m	3

Sinnecker and coworkers reported four Turkish patients with 5α-reductase deficiency (21,22). Two of the patients had a female phenotype and the same ΔM157 mutation reported by Boudon et al (20). The third and fourth patients of Dr. Sinnecker's study (21) were infants with a predominantly male phenotype: penoscrotal hypospadias, chordee, micropenis (1 cm), and bifid scrotum. There was no vaginal rudiment in genitography and genitoscopy in the third subject. T/DHT ratio was slightly elevated in the third subject and was normal in the fourth proving that a normal T/DHT ratio does not exclude 5α-reductase deficiency. DNA analysis showed a missense mutation in exon 4. Guanine at nucleotide position 586 was replaced with adenine causing substitution of glycine (GGT) by serine (AGT) at amino acid position 196 (G196S). The subjects who had the same mutations were not relatives. It is striking that in this German report of 9 patients, all four Turkish patients had consanguineous parents, whereas none of the German subjects had consanguinity in their family (21).

G196S mutation had been previously reported in a Greek-American and three Swedish subjects with predominantly male phenotypes (5,9). In contrast, a Brazilian patient with G196S mutation had a female phenotype (23). In vitro expression analysis showed that G196S mutation reduced the affinity of the enzyme for NADPH, but did not alter the apparent Km for testosterone (6). The mutant enzyme harboring G196S mutation showed slightly more basic pH optimum (5.2) in contrast to pH optimum (4.9) of the wild type enzyme. In transfection assay the mutant enzyme had 8% of the activity of the wild type enzyme.

Hiort and colleagues (10) identified a point mutation in exon 1 of the 5α-reductase type-2 gene from a Turkish infant who lived in Germany. A homozygous thymidine to adenine substitution (GTC to GAC) at 167th nucleotide position caused substitution of leucine (L) to glutamine (Q) at amino acid position 55 (L55Q). The clinical features included clitoral enlargement and fusion of the labia minora and an elevated T/DHT ratio. The parents were consanguineous. This mutation was reported before from a Jordanian patient studied in New York, USA. This affected individual was a compound heterozygote carrying L55Q mutation on one allele and Q56R mutation on the other

allele (13). In vitro transfection analysis showed that L55Q mutation caused an almost complete loss of enzymatic function (6). In 1996, L55Q mutation was reported again from a large Lebanese pedigree with eight male pseudohermaphrodites studied in Israel (7). The fourth report of L55Q mutation is from Turkey by Akar and coworkers in 2000 (24). Six male pseudohermaphrodites and 3 females, a total of 9 homozygous subjects and 31 heterozygous individuals were identified from this Turkish pedigree. The authors used restriction fragment length polymorphism assay utilizing PstI enzyme for rapid screening of L55Q mutation (24). Sequencing analysis of individual exons was not performed in that study.

We have identified a unique molecular genetic defect in the largest 5α-reductase deficient Turkish pedigree with seven male pseudohermaphrodites (3). Plasma androgen levels and urinary steroid metabolite ratios were consistent with 5α-reductase deficiency. Some of the male pseudohermaphrodites had penoscrotal hypospadias as a variation in clinical expression different from the index case who had perineoscrotal hypospadias and a pseudo-vagina. A novel feature of this particular kindred is the coexistence of another androgen biosynthesis defect, 17β-hydroxysteroid dehydrogenase deficiency. SSCP analysis of the 5α-reductase type-2 gene of the proband indicated that only the migration pattern of the fifth exon was abnormal. DNA sequencing (Figure 1) characterized the etiologic mutation. A single base (adenine) at 753rd nucleotide position was deleted in affected subjects, causing a frameshift mutation at amino acid position 251, with the addition of 23 amino acids to the carboxyl-terminal of this normally 254 amino acid isoenzyme. We have performed site directed mutagenesis and invitro expression analysis to prove the pathogenic role of the identified mutation. After introducing an adenine deletion at the 251st amino acid position, both the wild type and mutant enzyme were expressed in CV1 cells. Enzymatic activity was determined in cell homogenates. The conversion of [^{14}C] T to DHT was completely lost in the mutant isoenzyme (only 0.5 % of the activity of the wild type) and was the same as that in the nontransfected or mock-transfected controls. Reverse transcriptase-PCR (RT-PCR) analysis of messenger ribonucleic acid (mRNA) from transfected cells was performed to document that

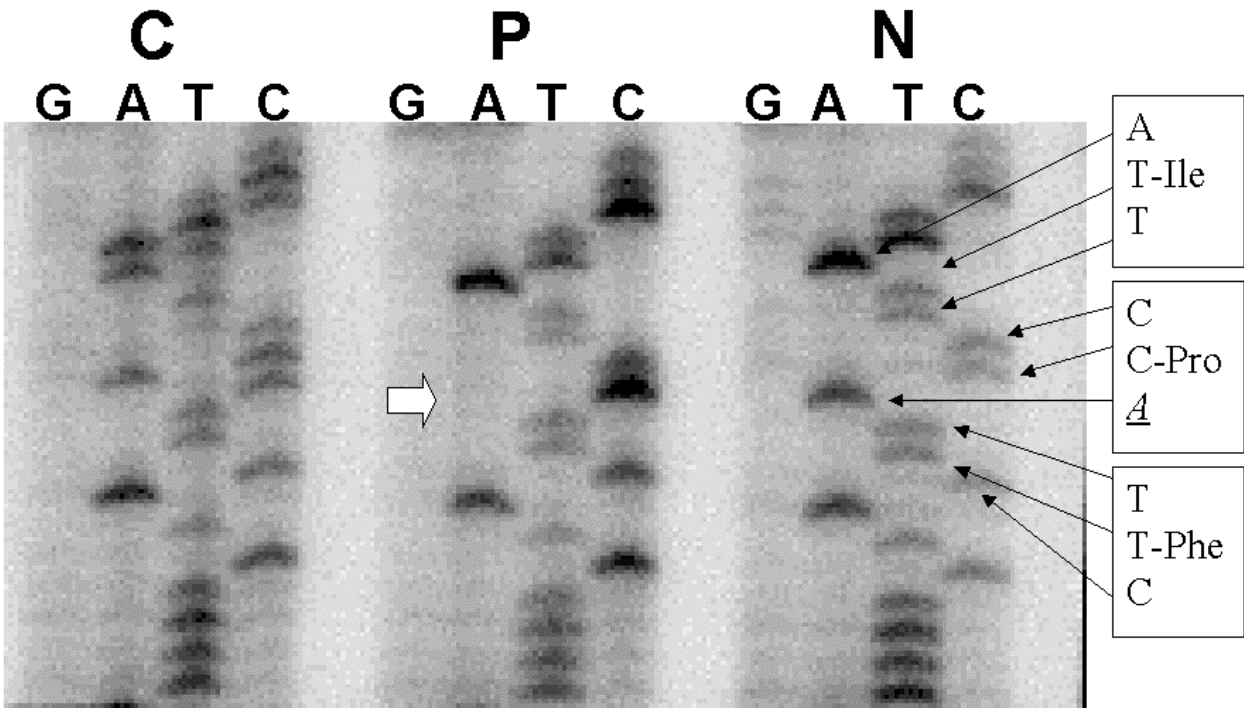

Figure 1. Representative DNA sequencing of exon 5 of steroid 5α-reductase type-2 gene in male pseudohermaphrodites from the Turkish kindred. N: normal, P: patient, C: carrier. Black arrows indicate normal sequence. At 251st amino acid position, normal sequence is CCA (proline). The white arrow indicates that adenine (A) in the normal sequence is deleted in the patient. The new sequence CCT also codes for proline, but causes a frame shift with the addition of 23 amino acids in the carboxyl-terminal portion of the enzyme.

both the mutant and wild type genes were expressed in sufficient amounts and there were no technical problems with the assay. RT-PCR showed that the level of mutant steroid 5α-reductase type 2 mRNA was almost equal to the level of wild-type mRNA during the transfection analysis, proving the adequacy of the experiment. In summary, this carboxyl-terminal mutation resulted in complete loss of enzymatic activity without altering the level of gene expression. The mutation impaired NADPH binding and demonstrated the functional significance of the carboxyl terminal portion of the enzyme (3). This particular mutation has not been reported from another population before.

Molecular genetic studies revealed multiple mutations involving all five exons of the 5α-reductase type 2 gene throughout the world (3-7,9,13,17). In toto, 42 mutations included 33 amino acid substitutions, two splice junction alterations, two premature stop codons, a large and four small deletions. Over half of the affected individuals were true homozygotes, whereas 40% were compound heterozygotes. Identical mutations are present in different ethnic groups suggesting the possibility of mutational hotspots in the gene. All substitution mutations occur in amino acids that are conserved amongst the sequenced 5α-reductase proteins, disproving random DNA polymorphisms. Other mutations delete a large or small portion of the gene, alter conserved splice junction sites (for example, 725 + 1, G→T) or cause a premature stop codon (R227X). R246W mutation is responsible for 5α-reductase deficiency in the Dominican kindred in whom the syndrome was first described (25).

Up to date, mutations spanning exon 1,3,4,5 of the 5α-reductase type-2 gene were reported in Turkish subjects. These mutations are not clustered in one area of the gene, suggesting genetic diversity of the disease in the Turkish population. L55Q mutation was reported from other populations in the Middle East area, previous Ottoman Empire territory. G196S mutation was reported in Turkish, Greek, Brazilian, and Swedish subjects. This site is conserved among the 5α-reductase isoenzymes and represents a mutational hotspot in the gene. ΔM157 mutation was observed in three different Turkish families. ΔP251 is unique to a particular Turkish kindred (3). The versatility of the mutations rejects the hypothesis of a founder effect for the whole Turkish population. Studies in the Turkish Republics of the former Soviet Union will be of interest to strengthen this view. The consanguineous marriages together with the lack of adverse expression of the gene defect in women contribute greatly to the dissemination of the defect in isolated communities. The finding of the same mutation in Turkish patients who live in different countries suggest there may be a common ancestor for those particular patients. Close cooperation among investigators is needed to document if patients with recurrent mutations are relatives. The high rate of consanguinity among Turkish 5α-reductase deficient patients prompts a public education campaign against marriages within the same family. Identification of molecular genetic defects provides an opportunity to offer genetic counseling and prenatal diagnosis to affected communities.

References

1. Imperato-McGinley J, Peterson RE. Male pseudohermaphroditism: complexities of male phenotypic development. *Am J Med* **61:** 251-272, 1976.
2. Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE. Steroid 5a-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science* **291:** 944-949, 1974.
3. Can S, Zhu YS, Cai L-Q, Ling Q, Katz MD, Akgun S, Shackleton CHL, Imperato-McGinley J. The identification of 5α-reductase-2 and 17β-hydroxysteroid dehydrogenase-3 gene defects in male pseudohermaphrodites from a Turkish kindred. *J Clin Endocrinol Metab* **83:** 560-569, 1998.
4. Imperato-McGinley J, Miller M, Wilson JD, Peterson RE, Shackleton C, Gajdusek DC. A cluster of male pseudohermaphrodites with 5α-reductase deficiency in Papua New Guinea. *Clin Endocrinol* **34:** 293-298, 1991.
5. Thigpen AE, Davis DL, Milatovich A, Mendonca BB, Imperato-McGinley J, Griffin JE, Francke U, Wilson JD, Russell DW. Molecular genetics of steroid 5a-reductase-2 deficiency. *J Clin Invest* **90:** 799-809, 1992.
6. Wigley WC, Prihoda JS, Mowszowicz I, Mendonca BB, New MI, Wilson JD, Russell DW. Natural mutagenesis study of the human steroid 5a-reductase 2 isoenzyme. *Biochemistry* **33:** 1265-1270, 1994.
7. Hochberg Z, Chayen R, Reiss N, Falik Z, Makler A, Munichor M, Farkas A, Goldfarb H, Ohana N, Hiort O. Clinical, biochemical, and genetic findings in a large pedigree of male and female patients with 5α-reductase 2 deficiency. *J Clin Endocrinol Metab* **81:** 2821-2827, 1996.
8. Katz MD, Kligman I, Cai LQ, Zhu YS, Fratianni CM, Zervoudakis I, Rosenwaks Z, Imperato-McGinley J. Paternity by intrauterine insemination with sperm from a

man with 5alpha-reductase-2 deficiency. *N Engl J Med* **336:** 994-997, 1997.

9. Nordenskjold A, Ivarsson SA. Molecular characterization of 5α-reductase type 2 deficiency and fertility in a Swedish family. *J Clin Endocrinol Metab* **83:** 3236-3238, 1998.
10. Hiort O, Sinnecker GHG, Willenbring H, Lehners A, Zollner A, Struve D. Nonisotopic single strand conformation analysis of the 5α-reductase type 2 gene for the diagnosis of 5a-reductase deficiency. *J Clin Endocrinol Metab* **81:** 3415-3418, 1996.
11. Ng WK, Taylor NF, Hughes IA, Taylor J, Ransley PG, Grant DB. 5α-reductase deficiency without hypospadias. *Arch Dis Child* **65:** 1166-1167, 1990.
12. Normington K, Russel DW. Tissue distribution and kinetic characteristics of rat steroid 5α-reductase isoenzymes. *J Biol Chem* **267:** 19548-19554, 1992.
13. Wilson JD, Griffin JE, Russel DW. Steroid 5α-reductase 2 deficiency. *Endocrine Reviews* **14:** 577-593, 1993.
14. Jenkins EP, Andersson S, Imperato-McGinley J, Wilson JD, Russell DW. Genetic and pharmacological evidence for more than one human steroid 5α-reductase. *J Clin Invest* **89:** 293-300, 1992.
15. Andersson S, Russell DW. Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases. *Proc Natl Acad Sci USA* **87:** 3640-3644, 1990.
16. Labrie F, Sugimoto Y, Luu-The V, Simard J, Lachance Y, Bachvarov D, Leblanc G, Durocher F, Paquet N. Structure of human type II 5α-reductase gene. *Endocrinology* **131:** 1571-1573, 1992.
17. Andersson S, Berman DM, Jenkins EP, Russell D. Deletion of steroid 5α-reductase 2 gene in male pseudohermaphroditism. *Nature* **354:** 159-161, 1994.
18. Silver RI, Russell DW. 5alpha-reductase type 2 mutations are present in some boys with isolated hypospadias. *J Urol* **162:** 1142-1145, 1999.
19. Orita M, Iwanaha H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci USA* **86:** 2766-2770, 1989.
20. Boudon C, Lobaccaro JM, Lumbroso S, Ogur G, Ocal G, Belon C, Sultan C. A new deletion of the 5α-reductase type 2 gene in a Turkish family with 5α-reductase deficiency. *Clin Endocrinol* **43:** 183-188, 1995.
21. Sinnecker GHG, Hiort O, Dibbelt L, Albers N, Dörr HG, Hauss H, Heinrich U, Hemminghaus M, Hoepffner W, Holder M, Schnabel D, Kruse K. Phenotypic classification of male pseudohermaphroditism due to steroid 5α-reductase 2 deficiency. *Am J Med Genet* **63:** 223-230, 1996.
22. Walden U, Rauch R, Hiort O, Sinnecker GH, Dorr HG. Diagnosis of 5alpha-reductase deficiency in a teenage Turkish girl. *J Pediatr Adolesc Gynecol* **11:** 39-42, 1998.
23. Caldas Ferraz LP, Guerra GJr, Matias Baptista MT, Maciel-Guerra AT, Hackel C. Detection of Gly-196-Ser mutation in 5alpha-reductase type II gene in a Brazilian patient with female assignment and behaviour. *J Pediatr Endocrinol Metab* **11:** 465-466, 1998
24. Akar N, Öcal G, Harmancı K, Duman T, Adıyaman P, Berberoğlu M, Uysal AR. A large 5-alpha reductase 2 deficiency (5A-SR2D) family with Leu 55 Gln mutation from Turkey. (Abstract) *J Endocrinol Invest* **23(Suppl 7):** 69, 2000.
25. Thigpen AE, Davis DL, Gautier T, Imperato-McGinley J, Russell DW. Brief report: The molecular basis of steroid 5α-reductase deficiency in a large Dominican kindred. *N Engl J Med* **327:** 1216-1219, 1992.