Phenotypical, Biological, and Molecular Heterogeneity of 5α-Reductase Deficiency: An Extensive International Experience of 55 Patients

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Context: In 46,XY disorders of sex development, 5α-reductase deficiency is rare and is not usually the first-intention diagnosis in newborn ambiguous genitalia, contrary to partial androgen insensitivity syndrome. Yet the cause of ambiguous genitalia may guide sex assignment, and rapid, precise diagnosis of 5α-reductase deficiency is essential.

Objective: The aim of the study was to describe relevant data for clinical diagnosis, biological investigation, and molecular determination from 55 patients with srd5A2 mutations identified in our laboratory over 20 yr to improve early diagnosis.

Setting: The study was performed at Montpellier University Hospital.

Patients: We studied a cohort of 55 patients with srd5A2 gene mutations.

Main Outcome Measure(s): Genetic analysis of srd5A2 was conducted.

Results: Clitoromegaly (49.1%) and microphallus with various degrees of hypospadias (32.7%) were frequent phenotypes. Female external genitalia (7.3%) and isolated micropenis (3.6%) were rare. Seventy-two percent of patients were initially assigned to female gender; five of them (12.5%) switched to male sex in peripuberty. Over 72% of patients were considered for 5α-reductase deficiency diagnosis when the testosterone/dihydrotestosterone cutoff was 10. In 55 patients (with 20 having a history of consanguinity), we identified 33 different mutations. Five have never been reported: p.G32S, p.Y91H, p.G104E, p.F223S, and c.461delT. Homozygous mutations were present in 69.1% of cases, compound heterozygous mutations in 25.5%, and compound heterozygous mutations alone with the V89L polymorphism in 5.4%. Exons 1 and 4 were most affected, with 35.8 and 21.7% mutant alleles per exon, respectively.

Conclusions: In the largest cohort to date, we demonstrate a wide spectrum of phenotypes and biological profiles in patients with 5α-reductase deficiency, whatever their geographical or ethnic origins. **(*J Clin Endocrinol Metab* 96: 296–307, 2011)**

A rare form of the 46,XY disorders of sex development (DSD), 5α-reductase deficiency was first described in 1974 by Imperato-McGinley *et al.* (1) and Walsh *et al.* (2) in patients with pseudovaginal perineoscrotal hypospadias, microphallus, and cryptorchid testes. This undervirilization in the male is due to an alteration in the 5α-reductase type 2 gene (srd5A2), which encodes for 5α-reductase activity. Genetic and pharmacological approaches have demonstrated two isoenzymes in humans (designated as types 1 and 2) (3). Both isoenzyme genes

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Abbreviations: AR, Androgen receptor; DHT, dihydrotestosterone; DSD, disorders of sex development; hCG, human chorionic gonadotropin; PAIS, partial androgen insensitivity syndrome; T, testosterone.

contain five exons separated by four introns (4–6). The gene encoding for 5α-reductase-1 is located on chromosome 5p15 (4), whereas the gene encoding for 5α-reductase-2 is located in band p23 on chromosome 2 (4–6). The type 1 isoenzyme is not detectable in the fetus, is transiently expressed in newborn skin, and is permanently expressed in skin from the time of puberty (7). Type 2 is the predominant isoenzyme detectable in fetal genital skin, male accessory sex glands, and the prostate (7), and it has a higher affinity for steroid substrates, especially testosterone (T), than type 1 (8).

5α-Reductase type 2 deficiency impairs the conversion of T to its more active metabolite, dihydrotestosterone (DHT), which is required for the normal development of external genitalia, urethra, and prostate in the male fetus, whereas T plays a major role in the virilization of Wolffian ducts. Although T and DHT have specific roles during sex differentiation, their actions are mediated by the same androgen receptor (AR) (9).

The diagnosis of 5α-reductase deficiency is suspected in a newborn with ambiguous genitalia characterized by perineoscrotal hypospadias. In fact, the extent of undermasculinization of the genitalia has been reported to be quite variable in patients with 5α-reductase deficiency (6, 10–15). This variation may be related to residual enzyme activity, genetic background, or the action of 5α-reductase type 1. In the original report, several patients were initially raised as girls because of the female appearance of the genitalia at birth. However, at the onset of puberty and in the absence of therapeutic intervention, spontaneous virilization, both physical and psychological, occurred (16). This was due to a rise in serum T concentration and an increase in the activity of the 5α-reductase type I enzyme (7, 17).

The biological diagnosis of 5α-reductase deficiency is usually supported by an increase in the T/DHT ratio after human chorionic gonadotropin (hCG) stimulation testing (18). In some cases, however, the diagnosis cannot be ruled out by a lack of an elevated T/DHT ratio after hCG stimulation (11).

To date, several mutations distributed throughout the coding region of the srd5A2 gene have been identified (Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff: srd5A2 Gene: http://www.hgmd.cf.ac.uk). Furthermore, the most frequent polymorphism at exon 1, V89L (valine to leucine substitution), has been shown to decrease 5α-reductase type 2 activity by approximately 30% (20, 21).

Reports about 5α-reductase deficiency have generally been case reports of limited numbers of inbred subjects or patient clusters from the same geographical area and/or ethnic background. For this reason, srd5A2 mutations were initially thought to affect only specific and isolated populations such as Dominican (1, 22), Turkish (12, 23, 24), Mexican (25–27), Egyptian (28), or New Guinean (29) peoples, and this assumption may have masked the real incidence of the disease. Very few extensive studies are available (6), and our ongoing worldwide collaboration has allowed us to analyze for the first time 5α-reductase mutations from the European, Asian, African, and North American continents. This extensive experience may help to better collate the data on this defect and should highlight some of the clinical and biological characteristics that will allow clinicians to identify this DSD, thus facilitating the crucial decision of sex assignment.

In this study, we describe a large cohort of 55 patients with srd5A2 mutations. The mutations were identified in our laboratory, and some of the patients have been followed in our clinic over the last 20 yr. The analysis of these patients has provided data relevant to issues of clinical diagnosis, biological investigations, and molecular determination for early diagnosis.

Patients and Methods

Patients

We report the clinical, biological, and molecular data on 55 children with 46,XY DSD and incomplete virilization (such as

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complete female external genitalia, clitoromegaly without labial fusion, isolated micropenis, microphallus with various degrees of hypospadias, and cryptorchidism) due to 5α-reductase deficiency. In all cases, written informed consent for molecular analysis was obtained from the children's parents before study enrollment. The patients were from a variety of ethnic and geographical origins (see details in Tables 1, 2, and 3).

Genetic analysis

Molecular analysis of the srd5A2 gene was performed as previously reported (11): genomic DNA was extracted from peripheral blood leukocytes following the manufacturer's instructions (DNA QIAamp DNA blood Mini kit; QIAGEN, Courtaboeuf, France). Briefly, exons 1–5 of the srd5A2 gene were amplified by PCR, and direct sequencing was performed using the BigDye terminator v1.1 kit (Applied Biosystems, Courtaboeuf, France) and a ABI prism 310 Genetic-Analyzer (Applied Biosystems).

Endocrine investigation

DHT and T concentrations were assayed in each local laboratory, but in the majority of the cases radioimmunological methods are currently used.

Prediction of srd5A2 mutation effects

Amino acid substitutions were studied *in silico* to predict the effects. We performed *in silico* analysis using the SIFT (http://SIFT.jcvi.org) (30), Panther (http://www.pantherdb.org/tools/csnpScoreForm.jsp) (31), and Polyphen-1 (http://genetics.bwh.harvard.edu/pph/index.html) web software. These software packages predict the functional importance of amino acid substitutions. All the algorithms are based on the alignment of orthologous and/or paralogous protein sequences and/or structural constraints. The original protein sequences were obtained from the Ensembl and UniProt/Swiss-Prot databases.

Results

Subjects and family history

The clinical, biological, and genetic results of the 55 patients are summarized in Tables 1, 2 and 3.

The mean age at mutation identification was 7.6 ± 7.7 yr, ranging from the neonatal period to 30 yr. A history of consanguinity was reported in 20 cases (36.4%), although this parameter was not documented in one patient (adopted). The patients were from Egypt (n = 11), Turkey (n = 9), France (n = 8), Cyprus (n = 4), Canada (n = 2), India (n = 2), Morocco (n = 2), Poland (n = 2), Spain (n = 2), Tunisia (n = 2), Algeria (n = 1), Africa (n = 1), Belgium (n = 1), Cape Verde (n = 1), Italy (n = 1), Ivory Coast (n = 1), Laos/Caucasus region (n = 1), Mongolia (n = 1), Palestine (n = 1), Saudi Arabia (n = 1), and Vietnam (n = 1).

Phenotype

The phenotypical variability of the patients is presented in Fig. 1. Most patients presented with female external genitalia with clitoromegaly (27 of 55) or microphallus with various degrees of hypospadias (18 of 55), whereas the appearance of normal female genitalia (five of 55) or isolated micropenis (two of 55) was very rare. Forty of 55 cases (72.7%) were initially oriented toward female gender and 15 (27.3%) toward male gender. Among the patients oriented as females, a switch from female to male identity was requested by five patients (Fig. 2) in the peripubertal period. Eight of 55 cases were reported to have had bilateral gonadectomy. The mean age of gonadectomy was 7.4 yr (range, 13 months to 19 yr). In seven cases, gonadectomy had been performed before puberty or in the peripubertal period, whereas one patient had postpubertal gonadectomy.

Endocrine investigations

Biological investigations were performed in 51 of the 55 patients. Of this subset, baseline sex steroid concentrations were determined in 51 of 51 (100%) cases for T and 34 of 51 (66.6%) for DHT. T concentrations after an hCG stimulation test were available for 36 of 51 (70.6%), but DHT concentrations were available for only 25 of 51 (49%). The hCG test protocols were performed according to the local standardized procedures, and the T level increased in most cases.

The mean values for T, DHT, and the T/DHT ratio before and after hCG testing for each age group [newborns and very young infants (<1 yr), infants and children (1–11 yr), and adolescents and adults (>12 yr)] are presented in Fig. 3. The mean values of the T/DHT ratio at baseline and after hCG testing for the age groups were 9/25, 8.9/24.8, and 29.5/97.33, respectively. When a T/DHT ratio of 10 after hCG stimulation was set as the cutoff for diagnosing 5α-reductase deficiency, eight (72.7%) of the 11 newborns and young infants, eight (80%) of the 10 infants and children, and three (100%) of the three adolescents and adults had values above this cutoff.

5α-Reductase gene analysis

For 56.4% of the patients (31 of 55), the diagnosis of partial androgen insensitivity syndrome (PAIS) and the AR mutation were ruled out before srd5A2 was investigated, whereas srd5A2 was first evoked in 43.6% of the patients (24 of 55). In 55 patients, we identified 33 different mutations. Homozygous mutations were present in 38 of 55 (69.1%) patients (Table 1), compound heterozygous mutations in 14 of 55 (25.5%) (Table 2), and compound heterozygous mutations alone with the V89L polymorphism in three of 55 (5.4%) (Table 3). Of the 33 different mutations, five have never been described: p.G32S, p.Y91H (two patients), p.G104E, p.F223S, and c.461delT.

When the mutations were classified by exon, exons 1 and 4 were most affected, and the number of mutant alleles

TABLE 1. Main clinical, hormonal and molecular data of patients with 5αR type 2 homozygous mutations

Patient no.	Age at molecular diagnosis/age at hormonal evaluation	Ethnic group	Phenotype	Parental consanguinity	Sex of rearing
1	4 months	Egyptian	FEG + G bilateral in labioscrotal folds	Positive	F
2	5 months	Egyptian	MPH + penoscrotal hyp	Positive	F
3	4.8 yr	Egyptian	MPH + penoscrotal hyp	Positive	M
4	5.4 yr	Egyptian	MPH + penoscrotal hyp	Positive	M
5	16 yr	Egyptian	MPH + hyp	Positive	M
6	6 d	Moroccan	CM + G bilateral in labia majora + Gonadectomy at 15 months	Positive	F
7	18 yr	Turkish	CM + G bilateral in inguinal position + hyp perineoscrotal + pubertal virilization and primary amenorrhea	Positive	F
8	5 yr/3 d	Turkish	CM + G bilateral in labioscrotal folds	Negative	F
9	24 yr	Tunisian	CM + G bilateral in labioscrotal folds + hyp penoscrotal + primary amenorrhea	Positive	F to M at 17 yr
10	3 yr	Tunisian	CM + G bilateral in labia majora + gonadectomy at 13 months	Negative	F
11	10.5 yr	Cape Verdean	CM + right G in labia majora and left G in inguinal position	Negative	F to M at 13.5 yr
12	20 yr	Egyptian	MPH + G bilateral in scrotum + perineoscrotal hyp + virilization at puberty	Positive	F to M after puberty
13	2 yr	Egyptian	MPH + G bilateral in scrotum + perineoscrotal hyp	Positive	F
14	9 yr	Egyptian	MPH + G bilateral in the scrotum + perineoscrotal hyp	Positive	F to M
15	4 yr	Turkish	CM + G bilateral in labia majora	Negative	F
16	30 yr/3 yr	Palestinian	CM + G bilateral in inguinal position + gonadectomy at 11 yr	Positive	F
17	11 months	Saudi Arabian	CM + G bilateral in labia majora	Positive	M
18	1 month	Greek Chypriot	CM + G bilateral in inguinal position	Negative	F
19	4 yr	Greek Chypriot	CM + G bilateral in inguinal position	Negative	F
20	28 d	Moroccan	CM+ G bilateral in labia majora + gonadectomy at 8 yr	Negative	F
21	2 months	Belgian	CM + G bilateral in labia majora	Positive	F
22	10 yr	Algerian	CM + G bilateral in inguinal position + gonadectomy at 12 yr	Positive	F
23	9 yr	Spanish	CM + G bilateral in inguinal position	Negative	F
24	Neonatal	French	FEG + G bilateral in inguinal position	Negative	F
25	14 yr[a]	French	CM + G bilateral in labia majora	Negative	F
26	2 yr	Turkish	CM + G bilateral in inguinal position	Negative	F
27	2 yr	Turkish	CM + G bilateral in inguinal position	Positive	F
28	4 months	Egyptian	FEG + G bilateral in labioscrotal folds	Negative	F
29	14 yr	Egyptian	CM + early signs of pubertal virilization	Positive	F
30	18.5 yr	African	CM + G bilateral in inguinal position + primary amenorrhea + gonadectomy at 19 yr	Negative	F
31	10.8 yr	Turkish	MPH + G bilateral in inguinal position + perineoscrotal hyp	Positive	M
32	3 yr/10 d	Turkish	MPH + G bilateral in the scrotum + perineoscrotal hyp	Positive	M
33	2 yr	Vietnamese	MPH + G bilateral in the scrotum + glandular hyp	ND (adopted)	M
34	14 yr	Polish	CM + G bilateral in labioscrotal folds	Negative	F
35	9 months	Egyptian	FEG + G bilateral in labioscrotal folds	Negative	F
36	9 yr/22 months	Italian	CM + G bilateral in inguinal position + gonadectomy at 22 months	Negative	F
37	11 yr	Indian	MPH + G right in inguinal position and left in labioscrotal fold + perineoscrotal hyp	Negative	F to M
38	18 months	Indian	MPH + G bilateral in the scrotum + perineoscrotal hyp	Positive	M

Patients 12, 13 and 14 are members of the same family. Patients 24 and 25 are from the same family. Patients 31 and 32 are third cousins. F, Female; M, Male; FEG, female external genitalia; MP, micropenis; MPH, microphallus; G, gonads; hyp, hypospadias; CM, clitoromegaly; ND, not determined. T and DHT are expressed in nanomoles per liter.

[a] New mutation.

per exon was 35.8 and 21.7%, respectively. The frequency of mutant alleles by exons or introns is described in detail in Figs. 4 and 5.

To assess the potential deleterious effect of the amino acid change, the predicted functional consequences of the four new mutations, p.G32S, p.Y91H, p.G104E, and p.F223S, were assessed using the SIFT, Panther, and Polyphen-1 web software. The results indicated unanimously that p.Y91H and p.G104E placed these mutations in the "affected protein function" class, whereas for the variants p.G32S and p.F223S, a benign (Polyphen-1 and SIFT) or damaging (Panther) impact on protein function was predicted.

TABLE 1. Continued

Basal plasma T/T after hCG (nmol/liter)	Basal plasma DHT/DHT after hCG (nmol/liter)	Basal plasma T/DHT/ T/DHT ratio after hCG	srd5A2 mutation	Exon	Described
0.14/1.01	0.02/0.04	8.3/22.6	G34R	1	Previously (13)
0.04/0.87	ND	ND	G34R	1	
0.03/1.07	ND	ND	G34R	1	
0.03/1.38	0.03/0.04	1/35.2	G34R	1	
4.5/ND	0.25/ND	17.7/ND	G34R	1	
11.37/ND	0.35/ND	32.5/ND	c.122_123del	1	Previously (59)
22.4/ND	ND	ND	L55Q	1	Previously (12)
10.4/138.7	ND	ND	L55Q	1	This study
16.2/ND	0.9/ND	18/ND	Q56R	1	This study
2.6/97	1.2/2.4	2.2/40.4	Q56R	1	This study
24.3/ND	1.76/ND	13.7/ND	E57Q	1	This study
19.4/42.2	0.25/0.35	78/120	A62E	1	Previously (28)
0.34/10.8	0.05/0.33	6.8/33	A62E	1	
ND	ND	ND	A62E	1	
0.7/10	ND	ND	Y91H[a]	1	This study
1.4/4.1	ND	ND	Y91H[a]	1	This study
12.8/141.9	1/7.9	12.8/17.8	c.282–2A>G	2	This study
26.5/52.3	1.3/1.8	20/29	c.282–2A>G	2	Previously (14)[a]
ND	ND	ND	c.282–2A>G	2	Previously (14)
0.62/13.7	0.77/0.93	0.8/13.9	G115D	2	Previously (59)
9.4/39.6	0.5/1.1	18.8/36	G115D	2	This study
2.4/6.2	0.2/0.2	12/31	G115D	2	This study
2.4/9.1	4.8/6.6	0.5/1.4	Q126R	2	This study
ND	ND	ND	Q126R	2	Previously (11)
23/60	2.8/5.2	8.2/12.3	Q126R	2	
0.3/8.2	ND/ND	ND/ND	p.Met157del	3	This study
45/76	2.7/7.3	16.6/10.4	p.Met157del	3	Previously (23)
0.03/0.76	0.03/0.08	1/9	N160D	3	Previously (45)
0.8/3.2	0.02/0.02	40/160	N160D	3	Previously (45)[a]
21.5/ND	8.6/ND	2.5/ND	N193S	4	This study
3/23	1/1	3/23	G203S	4	This study
7.3/17	1.9/2.9	3.8/5.9	G203S	4	This study
2.4/ND	ND/ND	ND/ND	R227Q	4	This study
32.2/ND	ND	ND	H231R	4	Previously (11)
0.03/3.88	0.03/0.04	1/98.5	Y235F	5	Previously (45)
0.07/22.2	ND	ND	R246W	5	This study
22.8/27.7	0.59/0.76	37.3/36.4	R246Q	5	Previously (55)
9.46/16.4	0.8	11.3	R246Q	5	Previously (55)

Discussion

This report presents a large amount of data on srd5A2 gene mutations in populations with a variety of ethnic backgrounds and coming from several geographical areas. This range of data was collected only through the cooperation of a network of clinicians from around the world. To our knowledge, only one earlier study reported a large number of mutations in a range of populations (6), whereas most others have reported mutations only in specific ethnic groups (32). These results may broaden clinicians' understanding of this rare form of 46,XY DSD and familiarize them with the wide clinical, biological, and genetic spectrum of 5α-reductase deficiency.

Genotype/phenotype relationship

In the XY newborn with undervirilization and normal/high plasma T, PAIS is usually the first diagnosis evoked,

TABLE 2. Main clinical, hormonal, and molecular data of patients with 5αR type 2 compound heterozygous mutations

Patient no.	Age at molecular diagnosis/age at hormonal evaluation	Ethnic group	Phenotype	Parental consanguinity	Sex of rearing
39	15.7 yr	French	CM + primary amenorrhea + virilization	Negative	F
40	3 yr	Mongolian	CM + G bilateral in inguinal position	Negative	F
41	6.2 yr/6 months	Turkish	CM + G bilateral in inguinal position	Negative	F
42	13.5 yr/15 d	French	CM + bilateral G hernia + virilization	Negative	F
43	4 yr/2.5 yr	French	CM + G bilateral in inguinal position	Negative	F
44	6 yr	Spanish	CM + G bilateral in labia majora	Negative	F
45	18 yr/10 yr	Greek Chypriot	CM + G bilateral in labioscrotal folds +	Negative	F
46	Soon after birth	Greek Chypriot	CM + G bilateral in labioscrotal folds	Negative	F
47	16 yr	Polish	CM + G bilateral in labioscrotal folds	Negative	F
48	2 months	Ivorian	MP + single orifice + G bilateral in inguinal position	Negative	M
49	26 yr/2.5 yr	French	MPH + G bilateral in the scrotum + penoscrotal hyp	Negative	M
50	13 yr/at birth	French/Quebec	MPH + G bilateral in the scrotum + hyp	Negative	M
51	16 yr/1 yr	French/Quebec	FEG + G bilateral in labia majora + gonadectomy at 5 yr	Negative	F
52	9 d	French	MPH + G in the scrotum + scrotal hyp	Negative	M

Patients 50 and 51 are siblings. F, female; M, male; FEG, female external genitalia; MP, micropenis; hyp, hypospadias; CM, clitoromegaly; ND, not determined. T and DHT are expressed in nanomoles per liter.

[a] New mutation.

and this is confirmed in some cases by AR gene abnormality. However, we report that only 56% of patients were suspected of having PAIS/AR mutation, and this figure is probably low because we considered only a population with a srd5A2 mutation.

Our group and others have described the extremely variable clinical phenotypes of patients presenting an alteration in the 5α-reductase enzyme (6, 10–15, 23, 28). In this study, we confirmed various degrees of undervirilization in XY patients, ranging from total female appearance to clitoromegaly up to isolated micropenis or microphallus associated with various degrees of hypospadias. Nevertheless, our results clearly showed that clitoromegaly (~50%) and hypospadias associated with microphallus (~33%) were the most frequent phenotypes. The predominance of clitoromegaly may explain why we found a female sex of rearing in more than 72% of patients. In contrast, we reported only two patients presenting isolated micropenis. To our knowledge, this phenotype is very rare because it has been reported in only a very limited number of patients of Japanese (33), Vietnamese (34), and Pakistani origins, although this last was not confirmed by molecular analysis (35). Ng *et al.* (33) suggested that sufficient local DHT must have been produced to allow early virilization of the genitalia, including fusion of the labioscrotal folds to produce a normal urethra and spectrum.

The origin of the divergent phenotypes is not precisely known, but it has been acknowledged that a mutation of the srd5A2 gene can induce a range of effects, from complete loss of enzymatic activity to normal conversion of T into DHT, and a clear genotype-phenotype relationship has not been identified (36).

Moreover, the same mutation, such as p.G34R (13) or p.L55Q in this study, can also result in genotype/phenotype variability ranging from female phenotype to partially virilized external genitalia (microphallus and hypospadias). It is interesting to note that in two siblings

TABLE 2. Continued

Basal plasma T/T after hCG (nmol/liter)	Basal plasma DHT/DHT after hCG (nmol/liter)	Basal plasma T/DHT/ T/DHT ratio after hCG	Srd5A2 mutations	Exon	Described
23.2/ND	0.55/ND	42.1/ND	c.34delG/R246W	1/5	This study
0.24/ND	0.27/ND	0.9/ND	Q6X/R227Q	1/4	This study
0.4/25.4	0.8/0.8	0.5/30.6	L55Q/R171S	1/3	This study
2.1/ND	0.3/ND	7/ND	Q56R/C.548-2A>C	1/4	This study
0.4/25.6	0.4/0.9	1/28.4	G104E[a]/c.461delT[a]	2/3	This study
ND/ND	ND/ND	ND/ND	G115D/A207D	2/4	This study
3.8/16.3	0.93/3.4	4.08/4.79	c.282-2A>G/R171S	2/3	This study
19/23.7	1.4/1	13.5/23.7	c.282-2A>G/P181L	2/3	Previously (14)[a]
20.5/ND	ND	ND	N193S/?	4/?	Previously (11)
0.3/100	ND/ND	ND/ND	G196S/F223S[a]	4/4	This study
2.8/10.6	ND	ND	G203S/H231R	4/4	This study
1.9/9.8	ND	ND	G203S/H231R	4/4	This study
0.3/3.5	ND	ND	G203S/H231R	4/4	This study
6.9/24.9	1/4.3	7.3/5.8	A215V/X255S	4/5	Previously (59)

(patients 50 and 51) with the same compound heterozygous mutation (p.Q126R/p.G203S), we documented a difference in sex rearing, with one sibling having a normal clitoris and raised as female, and the second having microphallus and hypospadias and raised as male. To our knowledge, phenotype variability in two brothers with the same mutation was only reported twice before (37, 38). The lack of phenotype/genotype relationship for patients carrying the same mutation suggests that factors other than residual 5α-reductase enzyme activity, such as AR-mediated signal transduction activity, circulating and local concentrations of T *in utero*, or environmental factors, may contribute to the variable clinical expression of the disorder (13, 33, 39). It was nevertheless suggested that clinical expression and the severity of impaired enzyme function were correlated when functional analysis of mutations representing opposite clinical phenotypes (*i.e.* feminization of external genitalia *vs.* predominantly male development) was evaluated (6). This assumption was recently confirmed in four homozygous patients with p.P212R mutations by Vilchis *et al.* (32), who reported the same phenotype (perineoscrotal hypospadias, microphallus, cryptorchidism) and female sex of rearing, thus confirming a genotype-phenotype correlation when a complete lack of enzymatic activity is demonstrated (40).

In our study, the mean age at molecular diagnosis was 7.6 yr, but ages ranged from the neonatal period to 30 yr. When 5α-reductase enzyme deficiency is not diagnosed in newborns with DSD, it is generally diagnosed during puberty through clinical signs such as primary amenorrhea, a lack of breast development, and secondary virilization including voice deepening, muscle development, and male sex behavior, as reported here and by others (41- 43). The virilization coincides with the rise in serum T and 5α-reductase type 1 activity (6, 10). Delayed diagnosis of srd5A2 gene mutations may result in severe psychological suffering and sociocultural isolation, principally evident when a female to male gender identity switch is requested. This was the case for five patients in our series with Tunisian, Cape Verdean, Egyptian (n = 2), and Indian ethnic backgrounds,

TABLE 3. Main clinical, hormonal, and molecular data of patients with 5αR type 2 compound heterozygous mutation associated with a V89L polymorphism

Patient no.	Age at molecular diagnosis/age at hormonal evaluation	Ethnic group	Phenotype	Parental consanguinity	Sex of rearing
53	6 months/21d	Laotian F/Caucasian M	MPH + scrotal hyp	Negative	M
54	4 d	Turkish	MPH + perineoscrotal hyp	Negative	M
55	1.5 yr	French	MP + ectopic left testis	Negative	M

F, Female; M, male; MP, micropenis; MPH, microphallus; hyp, hypospadias; CM, clitoromegaly; ND, not determined. T and DHT are expressed in nanomoles per liter.

[a] New mutation.

and this was mainly observed during the peripubertal period. None of these patients had been castrated. It is clear that earlier determination of the disease would help to reduce these risks. Management might also be improved by percutaneous DHT to increase penile length (28) or surgical intervention. Greater familiarity with 5α-reductase deficiency among clinicians (pediatric endocrinologists, *etc.*), and better collaboration between clinicians and molecular diagnostics laboratories may reduce the delay in diagnosis.

Endocrine investigations

The classic hormonal profiles of these patients include normal or elevated T levels contrasting with low DHT levels. The T/DHT ratio after hCG stimulation is an indicator of 5α-reductase deficiency, and 10 is generally the cutoff (11, 13, 18, 28). In our study, more than 72% of patients presented a ratio above 10, confirming that this ratio is a good indicator and could easily be used for screening patients. Moreover, we demonstrated that the T/DHT ratio after hCG was reliable whatever the age group, but more discriminating than expected in adult patients. However, in certain clinical cases, srd5A2 mutations have been identified in patients with a nonsignificant increase in the plasma T/DHT ratio (11, 42, 44, 45). This may be related partly to the even more elevated basal T level (11), the activity of type 1 isoform 5α-reductase expressed in nongenital tissue (44), or the severity of the enzyme defect, suggesting that when this ratio is negative, the srd5A2 mutation should not be excluded and a suspected diagnosis should be systematically confirmed by molecular analysis. This was particularly well illustrated by patient 45 (c.282-2A>G>G/R171S), for whom the diagnosis of 5α-reductase deficiency was rejected on the basis of a normal T/DHT ratio. The mutation was found some years later, when we systematically screened for mutations in all patients presenting DSD with normal/high plasma T.

Molecular analysis

To date, more than 50 mutations have been documented (Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff: srd5A2 Gene: http://www.hgmd.cf.ac.uk), including single point defects, nonsense or splice-junction mutations and partial and total gene deletions (4, 10, 11, 23). We report 33 different mutations, five of which have never been reported: p.G32S, p.Y91H in two patients with different ethnic origins (Turkish and Palestinian), p.G104E, p.F223S, and c.461delT, which enlarge the molecular spectrum of the srd5A2 gene abnormalities. The functional study of these mutations regarding enzyme activity was not performed. However, to assess the deleterious effects of the four new substitutions, *in silico* predictions were performed and concluded to an affected function for the mutated enzymes for p.Y91H and p.G104E. Concerning p.G32S and p.F223S, their deleterious effect was less clear-cut. In patient 43, who had a deletion leading to a premature stop codon, it is conceivable that 5α-reductase activity was

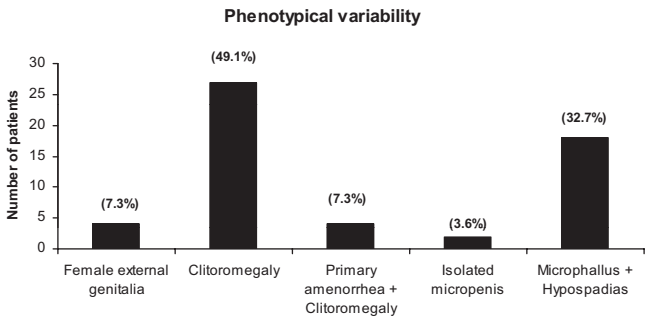

FIG. 1. Phenotypical variability of the 55 patients with 5α-reductase type 2 deficiency.

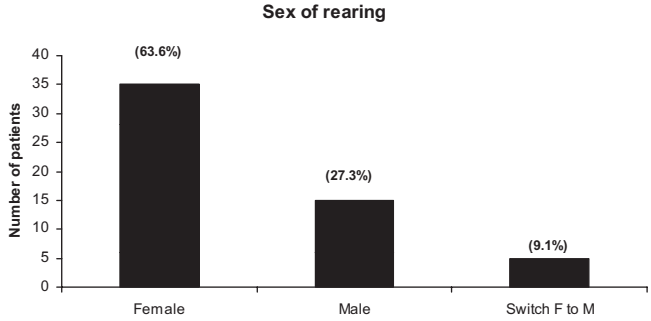

FIG. 2. Sex of rearing for the 55 patients with 5α-reductase type 2 deficiency.

TABLE 3. Continued

Basal plasma T/T after hCG (nmol/liter)	Basal plasma DHT/DHT after hCG (nmol/liter)	Basal plasma T/DHT/ T/DHT ratio after hCG	srd5A2 Mutations	Exon	Described
4.1/ND	1.1/ND	3.7/ND	V89L/R227Q	1/4	This study
4.4/ND	ND	ND	V89L/S14R	1/1	Previously (59)
2.2/ND	ND	ND	V89L/G32S[a]	1/1	This study

abolished. In our laboratory, these five gene abnormalities have never been identified in more than 200 control DNAs. Our results confirm the predominance of homozygous (69.1%) *vs.* compound heterozygous mutations, whether or not associated with the V89L polymorphism (30.9%), whereas deletions and disruptive mutations were relatively rare (four of 33) (6, 10). Moreover, the mutations reported in the literature seem to be spread throughout the srd5A2 gene, but we noted a predominance in exons 1 (35.8%) and 4 (21.7%), whereas exons 3 (11.3%) and 5 (9.4%) seemed to be relatively "preserved."

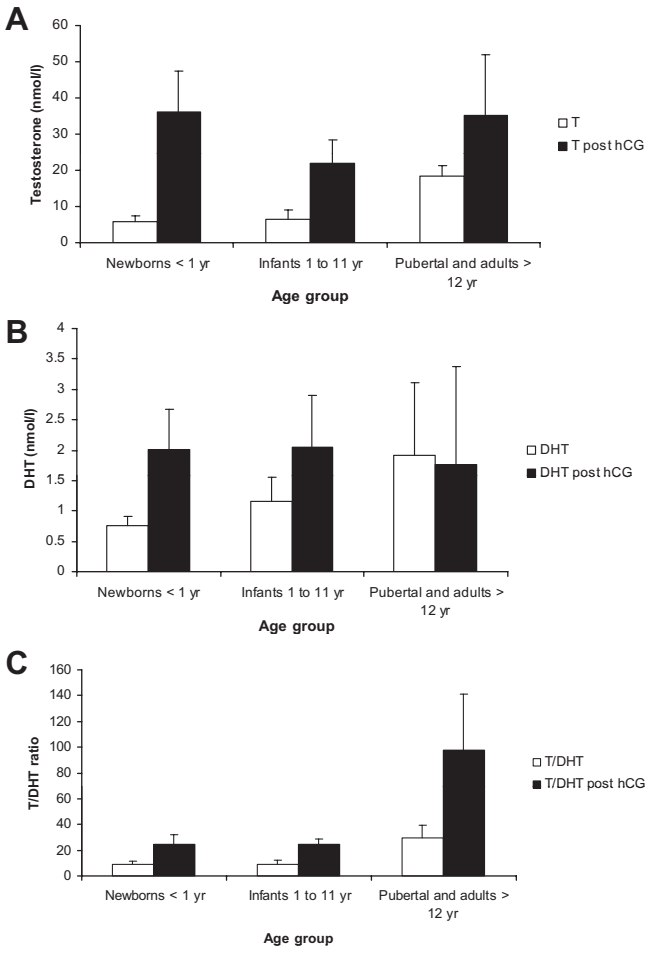

FIG. 3. Endocrine investigations in patients according to age group. A, T; B, DHT; and C, T/DHT.

Identical mutations have been found in individuals with widely divergent geographic and ethnic backgrounds, suggesting mutational hot spots such as for p.G115D, which we identified in Moroccan, Belgian, Spanish, and Algerian patients, or for p.Q126R, identified in Spanish and French patients. In homozygous or compound heterozygous forms, p.G115D mutations were also reported in Mexican (6, 25, 27), Dominican (22), and Spanish patients (46), and p.Q126R mutations in Brazilians with and without European or African origin (10, 37, 44, 47), Creole (6, 10), German (38), and Portuguese patients (10). Other hot spots of the srd5A2 gene may be p.G196S (6, 10, 38, 40, 44, 47–51), p.G203S (25, 52, 53), p.H231R (3, 6, 10, 38, 48, 54), and p.R246W (6, 22, 37, 46, 47). Still other mutations seem to be borderline between mutational hot spots and common ancestral mutations, like G34R, which is highly expressed in the Egyptian population (6, 13) and reported as well in Japanese (33), Mexican (25), and Sicilian (6) populations.

Similar mutations within the same ethnic group but in unrelated families have been described and are almost certainly derived from common ancestral mutations, reinforcing the founder-gene effect. These include A62E (28, 45) and N160 D (13) in Egyptians, Q56R in Tunisians, and R246Q in Indians (55), none of which have ever been reported in other populations. The p.Met157del deletion was found only in Turkish patients (23, 34). We report

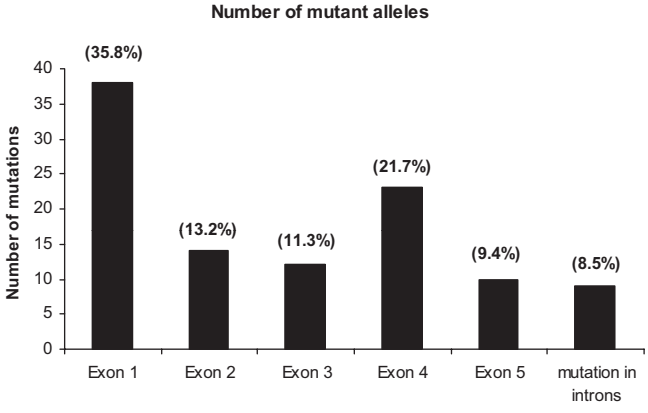

FIG. 4. Frequency of mutant alleles according to exon (the V89L polymorphism is not represented).

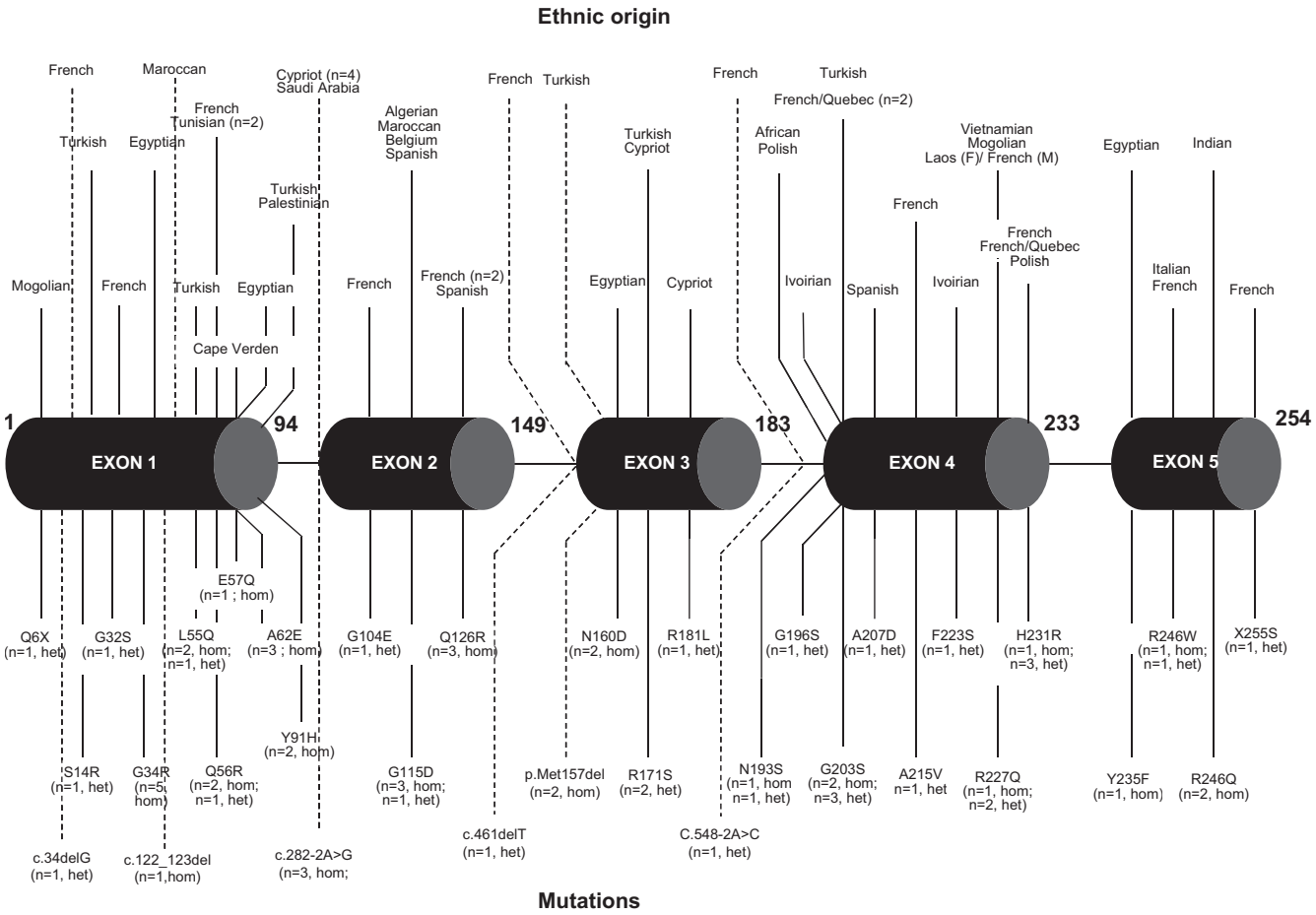

FIG. 5. Schematic representation of mutation localization and ethnic origin of the patients. hom, Homozygous; het, heterozygous; n, number of patients; F, female; M, male.

here a p.R227Q mutation in three patients with Vietnamese (p.R227Q/p.R227Q), Mongolian (p.Q6X/p.R227Q), and Laotian (p.V89L/R227Q) origins who presented male and female phenotypes. It is interesting to note that this mutation has only been reported in Asiatic patients of Chinese, Japanese, and Vietnamese origins (33, 34, 38, 52, 56–58). This may be the case for c.282-2A>G, which was mainly reported in Cypriot patients (14), although we also identified it in patients from Saudi Arabia, a country known for its high level of immigration.

We also identified three compound heterozygous mutations associated with the p.V89L polymorphism: p.V89L/p.S14R (59), p.V89L/p.R227Q, and p.V89L/p.G32S, which was not previously reported. These patients presented a male phenotype associated with micropenis and hypospadias. The transversion of valine to leucine at codon 89, the L-allele, was shown to reduce srd5A2 activity by approximately 30% compared with the V-variant in homozygous condition (20, 21). It was also reported that the p.V89L polymorphism indicates susceptibility for hypospadias (52, 60), which suggests that it may have an impact on the normal virilization of the external genitalia, although the degree of genital development does not seem exclusively dependent on 5α-reductase-2 (33). Moreover, the same frequencies in patients with micropenis and control males suggests that this polymorphism has no discernible effect on the development of micropenis, but constitutes probably one of the susceptibility factors for the development of androgen-related disorders (33) or environmental disruptors (19, 60).

As expected, our study demonstrated that this rare autosomal recessive disorder affects populations with a high rate of inbreeding, such as the Egyptian (13) and Turkish populations. In the current study, a history of consanguinity was documented in 36.4% of the patients. Our results corroborate those of Wilson *et al.* (10), who reported consanguinity in about one third of affected patients and a positive history in about 40% of the families. Interestingly, 5α-reductase deficiency was also identified in populations not considered at risk of inbreeding, such as Europeans (Belgian, French, Italian, Polish, and Spanish) or North Americans (Quebec).

In conclusion, this study of a large series underlines the very wide spectrum of phenotypes and biological profiles in patients with 5α-reductase deficiency. No genotype/phenotype relationship could be determined. Diagnosis is

oriented by a combination of criteria such as DSD, virilization at puberty, and a marked increase in the T/DHT ratio after hCG testing. However, because false negatives are possible, DNA sequencing of the entire srd5A2 gene is necessary, except when a mutation derived from common ancestral mutations is highly suspected. Clinicians should be better informed about this pathology so that diagnosis can be made more quickly because the choice of sex assignment is the most crucial decision in DSD.

Acknowledgments

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