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5α-Reductase-2 Gene Mutations in The Dominican Republic*

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ABSTRACT

Male pseudohermaphroditism due to 5α-reductase deficiency was clinically and biochemically described in a large Dominican kindred of 23 families with 38 affected subjects in 1974. Recently, the 5α-reductase-2 gene defect in the large Dominican kindred was found to be due to a single base substitution of thymidine (*T*GG) for cytosine (*C*GG) on exon 5 of the 5α-reductase-2 gene, causing a tryptophan replacement of arginine at amino acid 246 (R246W) of the enzyme.

In the present report, affected subjects from four additional Dominican families were studied to determine whether they carried the same 5α-reductase-2 gene defect as the large kindred, suggesting a common ancestry for the gene defect within this small country. Using single strand conformational polymorphism and DNA sequencing, two other mutations of the 5α-reductase-2 gene were found in affected subjects from two of the four families. A point mutation on exon 2 of the 5α-reductase-2 gene, in which substitution of adenine (GAC) for guanine (GGC) caused an aspartic acid replacement of glycine at amino acid 115 (G115D), was demonstrated in one of these families, and a substitution of adenine (AGT) for guanine (GGT) on exon 3 causing a serine replacement for glycine at amino acid 183 (G183S) was detected in the other family. Affected subjects from the two remaining families demonstrated the same exon 5 mutation of the 5α-reductase-2 gene as previously detected in the large Dominican kindred.

The phenotypic and biochemical characteristics of the male pseudohermaphrodites were similar regardless of the genetic defect, except that one affected subject (C-VI-2) with the same exon 5 mutation as the large Dominican kindred had much more facial and body hair. Thus, the identification of multiple mutations in the 5α-reductase-2 gene in male pseudohermaphrodites from the Dominican Republic demonstrates a lack of common ancestry, as had been previously postulated. (*J Clin Endocrinol Metab* **81:** 1730–1735, 1996)

MALE PSEUDOHERMAPHRODITISM is characterized by abnormal differentiation of male external genitalia in the presence of testicular tissue (1). Inherited deficiencies of androgen production or action may result in ambiguous genitalia (1, 2). One cause of male pseudohermaphroditism is a deficiency of the isoenzyme 5α-reductase-2, which converts testosterone to the more potent dihydrotestosterone (3, 4). This disorder has been well characterized from birth through adulthood in a large kindred of 29 interrelated families with 47 affected males from an isolated area of the Dominican Republic and is inherited as autosomal recessive (3, 5–11).

Molecular cloning techniques have identified two isoenzymes, 5α-reductase-1 and 5α-reductase-2, in the human, with distinct biochemical, genetic, and pharmacological properties (12–15). The isoenzyme 5α-reductase-2 is expressed during fetal development and predominates in mammalian reproductive tissues (16), suggesting an important role in reproductive tissue differentiation and development. Molecular genetic studies revealed multiple mutations of the 5α-reductase-2 gene in male pseudohermaphroditism due to 5α-reductase-2 deficiency throughout the world involving all five exons (17–19). Additionally, many affected subjects were found to be compound heterozygotes (17–19).

It has been previously shown that the gene defect in subjects from the large kindred in the Dominican Republic is due to a point mutation at exon 5 of the 5α-reductase-2 gene, where a single base substitution of thymidine (*T*GG) for cytosine (*C*GG) of the 5α-reductase-2 gene causes a tryptophan replacement for an arginine at amino acid 246 (R246W) (8). However, it is not known whether other families with male pseudohermaphrodites within this small country have the same mutation and, therefore, whether the gene defect in the Dominican Republic emanates from a common ancestor as has been postulated. Consequently, in this report, the 5α-reductase-2 gene of affected subjects from four additional Dominican families was studied.

Subjects and Methods

Subjects

Four male pseudohermaphrodites from four different families in the Dominican Republic (Table 1 and Figs. 1 and 2) and one carrier father

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TABLE 1. Summary of subjects with 5α-reductase-2 deficiency from the Dominican Republic

Subject	Family	Molecular abnormality			Androgens			Urinary steroid metabolites			
		Type	Location	Mutation	T (ng/dL)	DHT (ng/dL)	(T/DHT)	E/A	11OHE/11OHA	THB/5αTHB	THF/5αTHF
Homozygotes											
A-1	A	Missense	Exon 2	G→A,G115D	792	25	31	1.4	0.5	3.7	55
B-VII-1[a]	B	Missense	Exon 3	G→A,G183S							
C-VI-2	C	Missense	Exon 5	C→T,R246W	1053	18	59	3.3	2.0	8.0	18
D-1	D	Missense	Exon 5	C→T,R246W	896	17	53	3.5	1.1	4.8	18
Large kindred reference (n = 5; mean ± SD)		Missense	Exon 5	C→T,R246W	952 ± 139	27 ± 14	42 ± 20	5.4 ± 1.5	1.8 ± 1.0	4.6 ± 2.9	34 ± 12
Heterozygotes											
D-father	D	Missense	Exon 5	C→T,R246W	474	85	6	2.0	0.7	0.7	4.1
Normal male (mean ± SD)					582 ± 166	51 ± 21	12 ± 3	0.9 ± 0.4	0.4 ± 0.2	0.5 ± 0.2	1.1 ± 0.4

T, Testosterone; DHT, dihydrotestosterone; E/A, etiocholanolone/androsterone; 11OHE/11OHA, 11β-hydroxyetiocholanolone/11α-hydroxyandrosterone; THB/5αTHB, tetrahydrocorticosterone/5α-tetrahydrocorticosterone; THF/5αTHF, tetrahydrocortisol/5α-tetrahydrocortisol.

[a] Androgens and urinary steroid metabolites are omitted because this subject was castrated.

(Table 1) were studied. Two families whose pedigrees are depicted in Figs. 1 and 2 had more than one affected subject. Analysis of seven generations of family B identified four affected subjects; molecular genetic analyses of the 5α-reductase-2 gene were performed on subject B-VII-1 (Fig. 1). Figure 2 depicts seven generations of family C in which two affected subjects were identified. Molecular genetic analysis of the 5α-reductase-2 gene was performed on subject C-VI-2. Subject A-1, subject D-1, and his father (D-father) were also studied (Table 1).

All affected males had pseudovaginal perineoscrotal hypospadias. Five homozygous males with 5α-reductase-2 deficiency from the large Dominican kindred with known R246W exon 5 mutation (8) were compared to these subjects. Each of these subjects was clinically indistinguishable from one another and from the members of the large Dominican kindred (Table 1), except for patient (C-VI-2) from family C who had more facial and body hair than the affected males from the large Dominican kindred.

Methods

Plasma androgens and urinary steroid metabolites. Plasma testosterone and dihydrotestosterone were measured by RIA after separation by paper chromatography using a previously described method (5). Urinary steroid metabolite ratios, urinary etiocholanolone to androsterone, 11β-hydroxyetiocholanolone to 11β-hydroxyandrosterone, tetrahydrocortisol to 5α-tetrahydrocortisol, and tetrahydrocorticosterone to 5α-tetrahydrocorticosterone, were analyzed as methyloxime trimethylsilyl ether derivatives by capillary gas chromatography, as described previously (20, 21).

PCR and DNA sequencing

Blood was drawn into ethylenediamine tetraacetate (EDTA)-containing tubes, and genomic DNA from leukocytes was isolated as previously described (22). The concentrations of DNA were determined by ultraviolet absorbance. Exons 1–5 of the 5α-reductase-2 gene (15) were amplified by PCR using pairs of primers for each exon, as shown in Table 2. PCR amplification and labeling was carried out in 20 μL containing 0.1 μg genomic DNA, 1 μmol/L of each oligonucleotide primer, 50 μmol/L of each of four deoxyribonucleoside triphosphates, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 10 μCi [α-^{32}P]deoxy-ATP, and 2.5 U thermostable DNA polymerase (Perkin-Elmer, Norwalk, CT). The samples were heated at 94 C for 2 min, and then at 94 C for 1 min, at 70 C for 1 min for a total of 45 cycles; a final cycle consisted of 72 C for 10 min.

Amplification of exon DNA to be sequenced was carried out in a 50-μL reaction containing 0.1 μg genomic DNA, 1 μmol/L of each oligonucleotide primer, 200 μmol/L of each of four deoxyribonucleoside triphosphates, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 2.5 U thermostable DNA polymerase (Perkin-Elmer). The primers (Table 2) and thermocycle conditions were described above. Amplified DNA was separated by electrophoresis in 6% nondenaturing polyacrylamide gel and visualized by ethidium bromide staining. After excision of DNA bands, DNA was eluted, precipitated, and resuspended in TE buffer (pH 8.0). The purified DNA was sequenced by use of the fmol DNA sequencing kit (Promega, Madison, WI).

Single strand conformational polymorphism (SSCP) analysis

SSCP analysis was performed according to the method of Orita *et al.* (23, 24) with some modifications. Exon DNA, amplified and radiolabeled as described above, was diluted 1:6 in a solution containing 0.1% SDS and 10 mmol/L EDTA. Three microliters of the dilution were added to 3 μL formamide loading buffer (95% formamide, 50 mmol/L EDTA, and 0.05% each of xylene cyanol and bromophenol blue), then denatured at 100 C for 6 min and immediately cooled on ice. Four microliters of this sample were loaded onto a 6% nondenaturing polyacrylamide gel (acrylamide-bisacrylamide, 19:1) containing 10% glycerol and electropho-

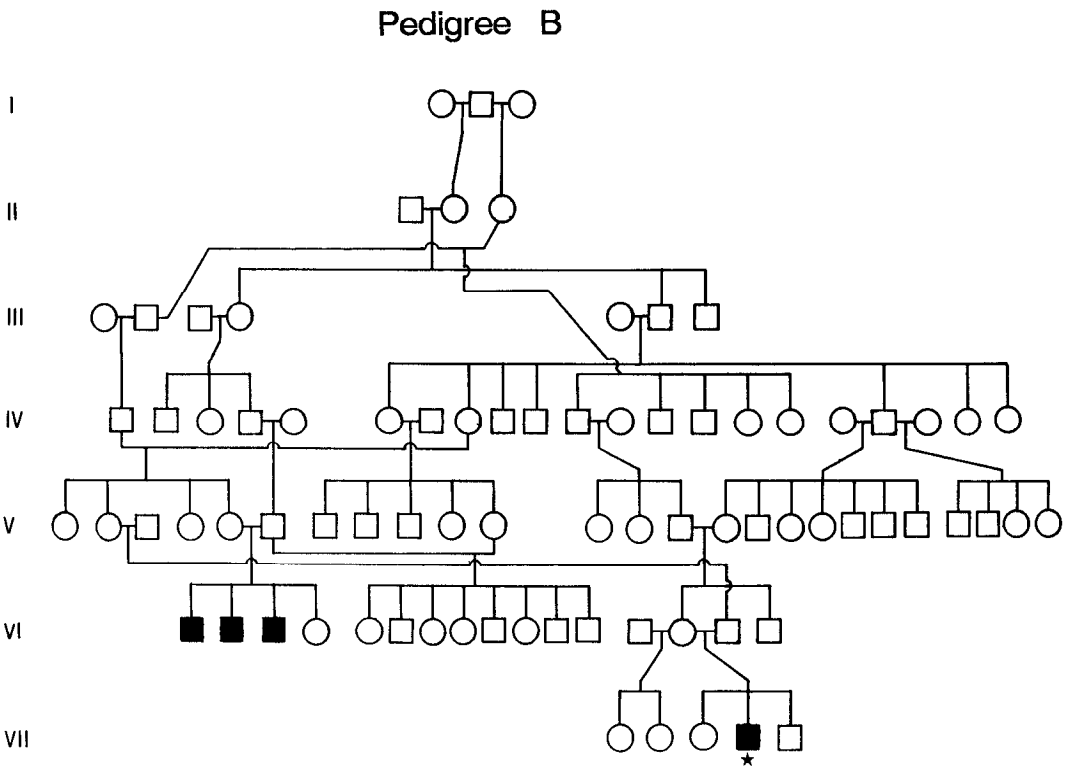

FIG. 1. Pedigree of family B from the Dominican Republic with 5α-reductase-2 deficiency. *Circles* and *squares* indicate females and males, respectively. *Solid squares* denote affected subjects. The subjects used for the present study are indicated by an *asterisk*. The *Roman numerals* indicate the generation.

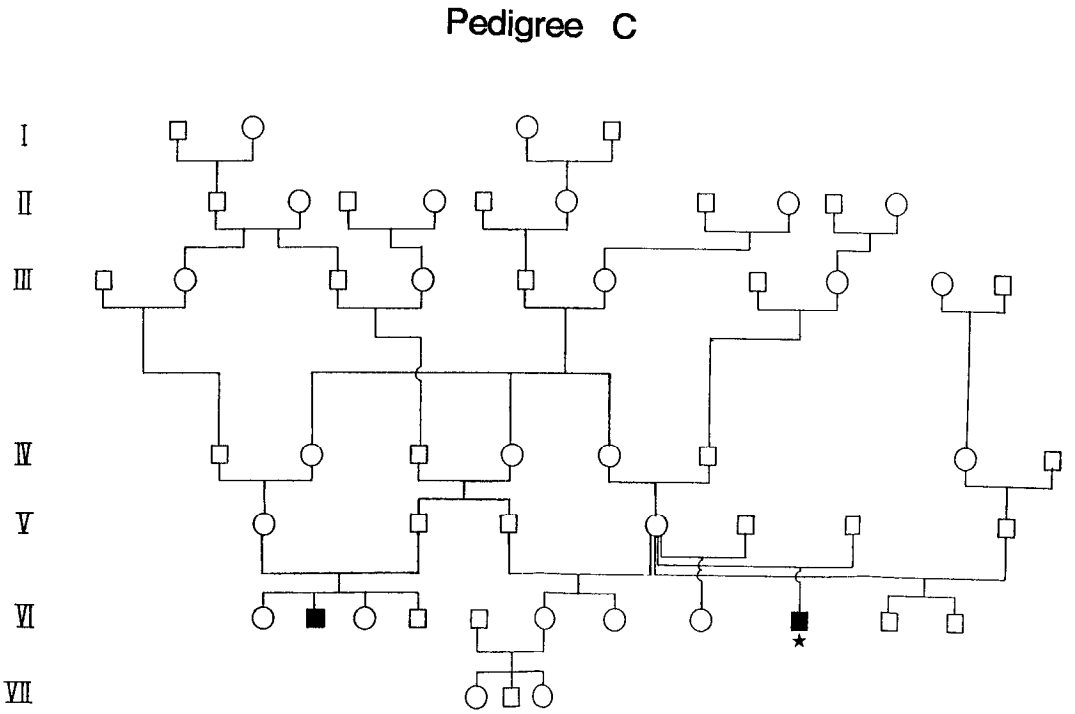

FIG. 2. Pedigree of family C. See Fig. 1 for details.

resed at 350 volts at room temperature overnight in 1 × TBE buffer (90 mmol/L Tris-borate, pH 8.3, and 4 mmol/L EDTA). An aliquot of sample was diluted in sucrose buffer (60% sucrose, 50 mmol/L EDTA, and 0.05% each of xylene cyanol and bromophenol blue) and loaded in an adjacent lane without denaturation to determine the position of migration of the double stranded DNA fragment. For SSCP analysis of exon 1, amplified and radiolabeled DNA was digested with *Bam*HI at 37 C for 1 h before electrophoresis. After electrophoresis, the gel was dried and exposed to Kodak X-Omat film (Eastman Kodak, Rochester, NY) at room temperature.

Results

In affected subject A-1 from A family (Table 1), a mutation in exon 2 of the 5α-reductase-2 gene was demonstrated by SSCP (Fig. 3A), where a single base substitution of adenine (GAC) for guanine (GGC) (Fig. 3B) resulted in an aspartic acid replacement of glycine at amino acid 115 (G115D; Table 1). Affected subject B-VII-1 from the B family (Table 1 and

TABLE 2. The oligonucleotides used in the analysis of the 5α-reductase-2 gene

Oligonucleotide	Location	Amplified target	Sequence 5′ → 3′
Exon 1-F	5′-Untranslated	Exon 1	5′-GCAGCGGCCACCGGCGAGGAACA-3′
Exon 1-R	Intron 1	Exon 1	5′-TGGACGCCGGGAGCAGGGCAGT-3′
Exon 2-F	Intron 1	Exon 2	5′-CAGTGAATCCTAACCTTTCCTCCC-3′
Exon 2-R	Intron 2	Exon 2	5′-TTGTTAGCTGGGAAGTAGGTGAG-3′
Exon 3-F	Intron 2	Exon 3	5′-AAGCACCACAATCTGGACACAT-3′
Exon 3-R	Intron 3	Exon 3	5′-CTCCAGGGAAGAGTGAGAGTCTG-3′
Exon 4-F	Intron 3	Exon 4	5′-CAATGATTGACCTTCCGATTCTTC-3′
Exon 4-R	Intron 4	Exon 4	5′-GTTTGGAGAAGAAGAAAGCTACGT-3′
Exon 5-F	Intron 4	Exon 5	5′-CAGGATCCGATCAGCCACTGCTCCATTATATTTA-3′
Exon 5-R	3′-Untranslated	Exon 5	5′-CGAAGCTTCATTGACAGTTTTCATCAGCATTGTGG-3′

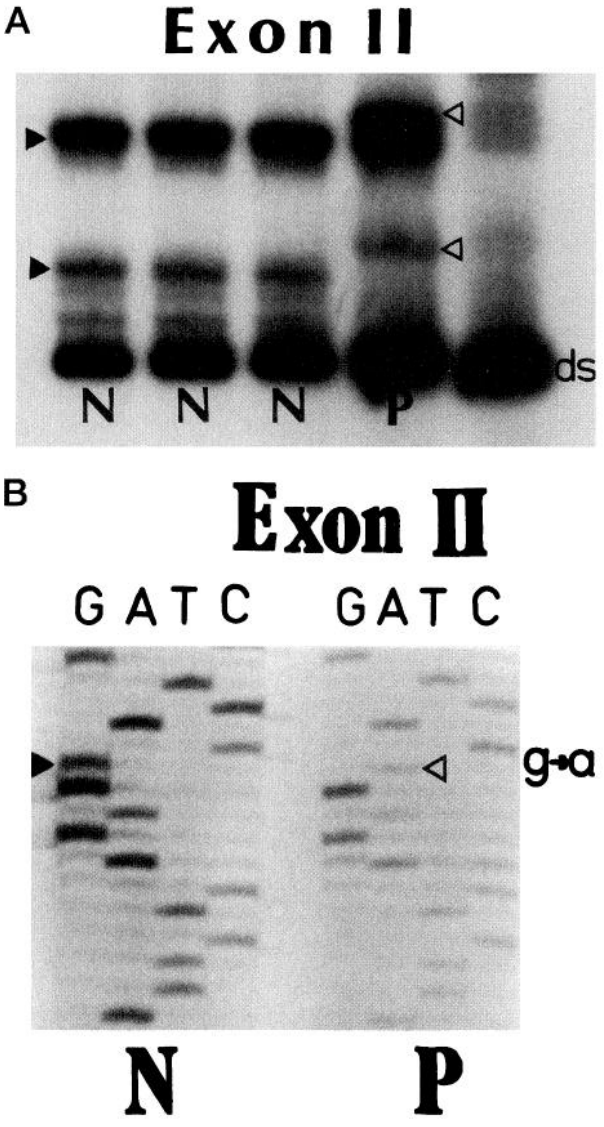

FIG. 3. Exon 2 mutation of the 5α-reductase-2 gene in the Dominican Republic detected by SSCP analysis (A) and DNA sequencing (B). In A, the *solid arrowheads* indicate the single stranded DNA bands of the normal subjects, and the *open arrowheads* indicate the single stranded DNA bands of the affected subject. The double stranded DNA is marked ds. In B, the *solid arrowhead* indicates a base G in the normal control sequence (N) that is substituted by a base A in the affected subject (P; A-1), as indicated by an *open arrowhead*.

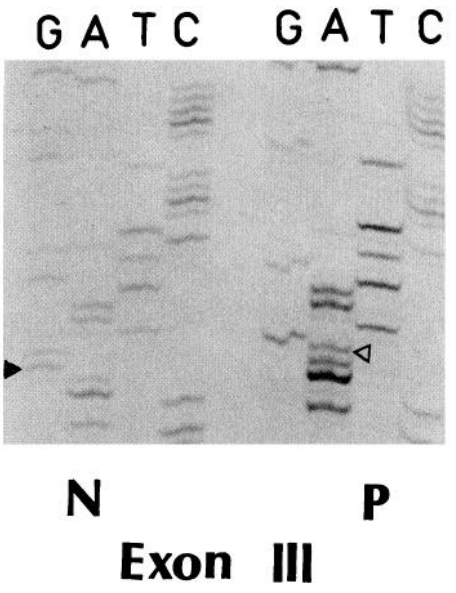

FIG. 4. Exon 3 mutation of the 5α-reductase-2 gene in the Dominican Republic by DNA sequencing. The *solid arrowhead* indicates a base G in a normal control subject that is substituted by a base A in the affected subject (B-VII-1) as indicated by an *open arrowhead*.

Fig. 1) had a mutation at exon 3 of the 5α-reductase-2 gene that was not detected by SSCP analysis (data not shown), but was identified by DNA sequencing with a single base substitution of adenine (*A*GT) for guanine (*G*GT; Fig. 4), resulting in a serine replacement of glycine at amino acid 183 (G183S). Affected subjects C-VI-2 and D-1 from the remaining two families (Table 1 and Fig. 2) had the same exon 5 mutation as the large Dominican kindred previously reported (8), involving a single base substitution of thymidine (*T*GG) for cytosine (*C*GG; Fig. 5), resulting in a tryptophan in place of an arginine at amino acid 246 (R246W). A heterozygous subject, D-father (Table 1), was demonstrated by SSCP analysis (Fig. 5A) and confirmed by DNA sequencing (data not shown).

The identified mutations of the 5α-reductase-2 gene in male pseudohermaphrodites from the Dominican Republic are summarized in Fig. 6.

Plasma androgen levels and urinary steroid metabolite ratios (Table 1) were consistent with the male pseudoher-

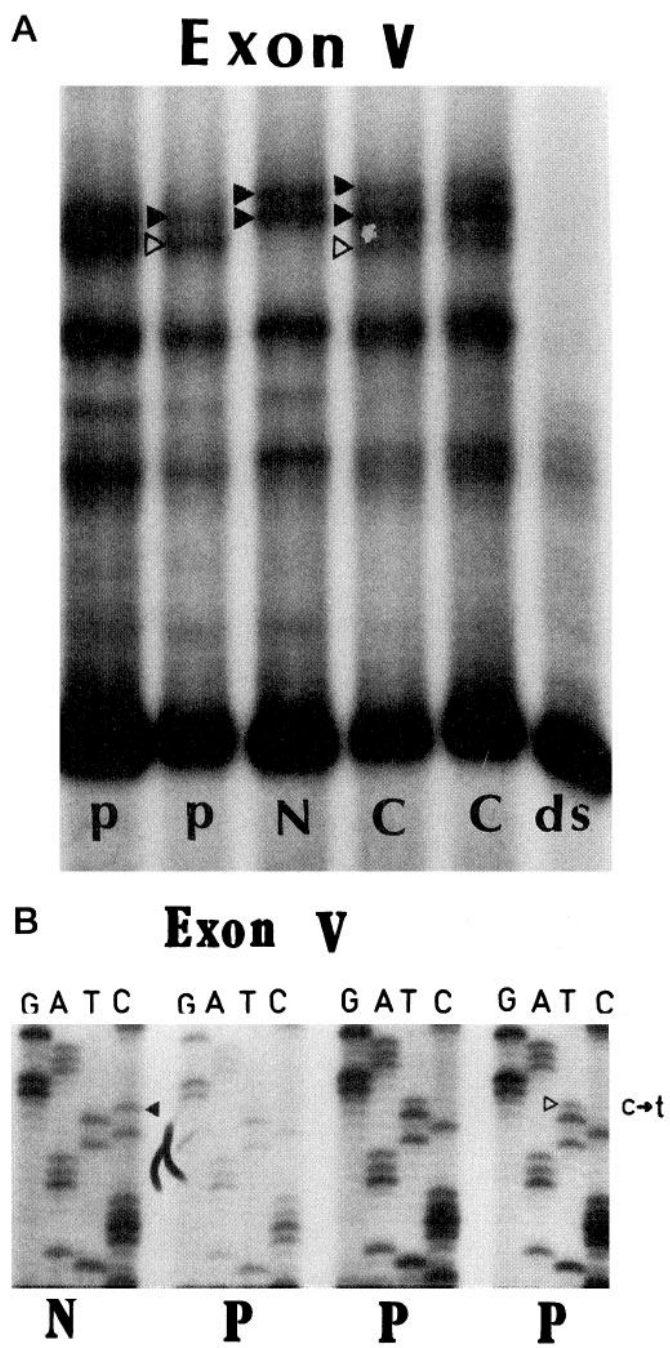

FIG. 5. Exon 5 mutation of the 5α-reductase-2 gene in the Dominican Republic by SSCP analysis (A) and DNA sequencing (B). In A, the SSCP pattern of a normal control (lane 3) is indicated by two *solid arrowheads*. The pattern in affected subjects was altered, as indicated by *solid and open arrowheads*. The pattern in carriers is a combination of the normal and affected patterns, as indicated by two *solid arrowheads* and one *open arrowheads*. The double stranded DNA is marked ds. An affected subject (lane 1) and a carrier (lane 5) from the large kindred (8) were used for comparison. Lane 2, The affected subject D-1; lane 4, his father. In B, the *solid arrowhead* indicates a base C in a normal control (N) that is substituted by a base T in the affected subjects (P), as indicated by an *open arrowhead*. The *left panel* represents partial sequences of exon 5 of the human 5α-reductase-2 gene in the normal control (panel 1), the affected subject D-1 (panel 2), the affected subject C-VI-2 (panel 3), and an affected subject from the large Dominican kindred (panel 4) (8).

maphroditism secondary to 5α-reductase-2 deficiency (3, 5–7, 9–11).

Discussion

Molecular genetic analysis of affected subjects with 5α-reductase-2 deficiency from four families was performed. The families were located in different towns in the Dominican Republic, apart from the large kindred previously reported (3). Additional gene defects in the 5α-reductase-2 gene were identified in affected subjects from this country. In affected subject A-1 from A family, a single base substitution of guanine (GGC) by adenine (GAC) on exon 2 was identified, which causes a replacement of glycine by aspartic acid at amino acid 115 (G115D) of the enzyme. In the B family, a single base substitution of adenine (AGT) for guanine (GGT) at exon 3 resulted in a serine replacement of glycine at amino acid 183 (G183S). These mutations were previously detected in a Mexican-American and a black Brazilian, respectively (17). Identification of the same mutation in different ethnic groups suggests that there may be hot spots in the 5α-reductase-2 gene where recurrent mutations occur. In two other families, a single base substitution of thymidine (TGG) for cytosine (CGG) on exon 5 of the 5α-reductase-2 gene, identical to that found in the large Dominican kindred, was detected (8).

The currently detected mutations in the 5α-reductase-2 gene from Dominican subjects were enzymatically characterized previously by *in vitro* mutation-transfection studies (8, 19). The replacement of arginine by tryptophan at amino acid 246 (R246W) on exon 5 of the 5α-reductase-2 gene resulted in decreased affinity of the enzyme for its cofactor, NADPH, a reduction of enzyme half-life, and residual enzyme activity of less than 5% (8). The serine to glycine replacement at amino acid 183 (G183S) of the enzyme due to a single base substitution of guanine (GGT) by adenine (AGT) on exon 3 resulted in a decreased affinity of the enzyme for the NADPH cofactor and an approximately 90% decrease in enzyme activity (19). The missense mutation on exon 2, which causes a replacement of glycine by aspartic acid at amino acid 115 (G115D) of the enzyme, produced a complete inactivation of the 5α-reductase-2 enzyme (18, 19). Despite differences in gene mutation and enzyme activity, no significant phenotypic or biochemical difference was discerned. Interestingly, an affected subject (C-VI-2) with the same exon 5 mutation as the large Dominican kindred had significantly more facial and body hair.

In summary, male pseudohermaphrodites with 5α-reductase-2 deficiency from different families in the Dominican Republic do not have the same defect in the 5α-reductase-2 gene and, therefore, do not share a common ancestry. To date, in this small country, which has a population similar to that of New York City and is smaller in size than the state of West Virginia, three different mutations of the 5α-reductase-2 gene have been identified.

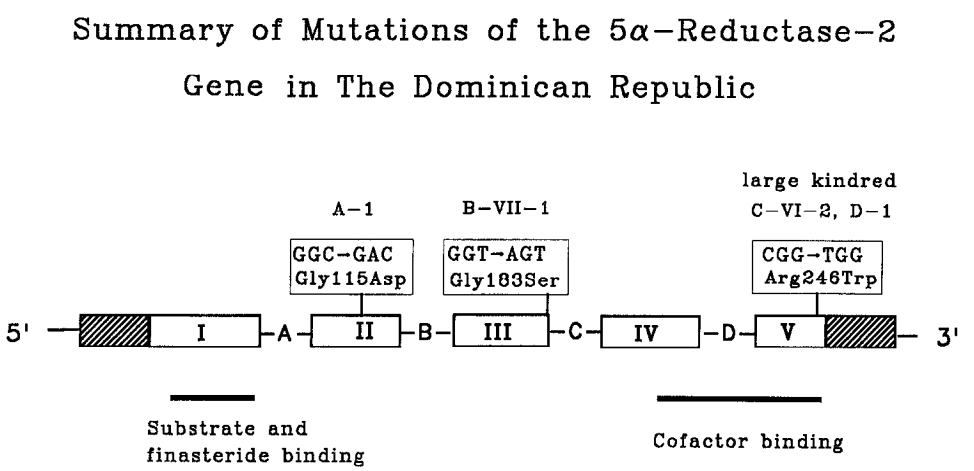

FIG. 6. A summary of the identified mutations in the 5α-reductase-2 gene of male pseudohermaproditism from the Dominican Republic. In the schematic representation of the human 5α-reductase-2 gene, exons are indicated by the *boxes* and labeled with *Roman numerals*; introns are indicated by the *lines* and labeled with *capital letters*. The *shaded boxes* denote the 5'- and 3'-untranslated regions. The specific mutations and subjects are also indicated. The large kindred indicates subjects from the large Dominican kindred previously described (8).

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