

Molecular Analysis of the *SRD5A2* in 46,XY Subjects with Incomplete Virilization: the P212R substitution of the steroid 5 α -Reductase-2 may constitute an Ancestral Founder Mutation in Mexican Patients

Short running head: Mutations of the *SRD5A2* gene

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ABSTRACT

Inactivating mutations of the *SRD5A2* gene result in steroid 5 α -reductase 2 deficiency, an autosomal recessive disorder expressed as a male-limited disorder of sex development.

Herein, genomic DNA was isolated from eleven new patients with apparent steroid 5 α -reductase 2 deficiency. Coding sequence abnormalities in *SRD5A2* were assessed by exon-specific polymerase chain reaction, single-stranded conformation polymorphism and direct sequencing. Likewise, enzymatic activity of the P212R gene variant of *SRD5A2* was assessed.

DNA analysis revealed mutations in all patients (G115D, R171S, N193S, E197D, G203S, P212R). Three individuals were compound heterozygotes; six were homozygotes and two more were single heterozygotes for *SRD5A2* mutations; remarkably, 40% of the mutant alleles (9/22) contained the gene variant P212R.

The results described in this study represent, along with our previous reports, the largest number of patients with steroid 5 α -reductase 2 deficiency belonging to non-related families. Regarding the frequency of the p.P212R mutation in our population and its presence throughout all of our country, it allows us to **hypothetized** that the presence of this mutation **may constitute** a founder gene effect.

Key words: 46, XY DSD, Steroid 5 α -reductase 2 deficiency, mutations, gene founder effect, genotype-phenotype correlation.

INTRODUCTION

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27 The 5 α -reductase type-2 isozyme, plays an essential role in development, growth and
28 differentiation of the prostate and male external genitalia. This isozyme, encoded by the 5 α -
29 reductase 2 (*SRD5A2*) gene, is responsible for the conversion of testosterone (T) to
30 dihydrotestosterone (DHT) during embryogenesis. It is known that inactivating mutations in the
31 *SRD5A2* result in steroid 5 α -reductase 2 deficiency (OMIM #264600), a rare autosomal recessive
32 disorder expressed as a male-limited disorder of sex development (46, XY DSD). Affected
33 individuals are 46,XY males who, at birth, generally present ambiguous external genitalia
34 characterized by perineoscrotal hypospadias with pseudovagina, microphallus, and
35 cryptorchidism (Imperato-McGinley et al, 1974; Wilson et al, 1993). Due to their phenotypic
36 appearance most of these patients are reared as females, although because the clinical spectrum is
37 so heterogeneous (ranging from a male phenotype with hypospadias to a female phenotype with
38 Wolffian structures) some affected subjects, who are sufficiently virilized at birth or in early infancy,
39 are reared as males (Vilchis et al, 2000; Hackel et al, 2005). During puberty virilization occurs and
40 without any therapeutic maneuver masculinization is frequently accompanied by a gender
41 identity change, from female to male (Méndez et al, 1995).

42 **Three large clusters of patients with 5 α -reductase type-2 deficiency have been**
43 **described worldwide: the Dominican Republic family involving more than 40 members, the**
44 **Turkish clusters of 12 subjects and the Sambia tribe in the New Guinea highlands. However, it**
45 **would be difficult to establish the real incidence of this entity due to the fact that the high**
46 **incidence of the disorder in these groups is probably due to a founder effect in geographic**
47 **isolates of people with a high coefficient of inbreeding (Griffin et al., 2001).**

48 The gene sequence of the *SRD5A2* shows that its structural organization consists of five
49 exons and four introns. In humans, this gene is located on chromosome 2p23 and encodes for a
50 protein of 254 amino acids (Andersson et al, 1991; Labrie et al, 1992). The wide distribution of
51 hydrophobic amino acids throughout the gene's sequence suggests the existence of at least four
52 putative transmembrane regions, as well as an androgen-binding domain at its amino-terminal end
53 (Griffin et al, 2001; Vilchis et al, 2008). The 5 α -reductase 2 enzyme (EC 1.3.99.5) catalyzes the
54 reduction of T to DHT (Labrie et al, 1992) and is expressed predominantly in androgen-
55 dependent tissues (Silver et al, 1994). To date, more than 50 different mutations
56 (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SRD5A2>) scattered throughout the five exons that
57 constitute the gene, have been described. The majority are missense mutations, although premature
58 stop codons and deletions have also been found, yielding nonfunctional or sub-functional proteins
59 (Canto et al, 1997; Vilchis et al, 2008).

60 Variability on phenotypic expression depends on the type of mutation and its effect on
61 enzymatic activity (Russell and Wilson, 1994). Overall, 60% of the mutations described are
62 homozygous and the remainder are compound heterozygous (Fernández-Cancio et al, 2004; Vilchis
63 et al, 2008). In addition in some cases the affected individuals present only one mutated allele
64 (Hackel et al, 2005; Nicoletti et al, 2005).

65 Here, we report the molecular findings regarding the analysis of the *SRD5A2* gene in **eleven**
66 Mexican-Mestizo patients, recruited **between 2001 and 2008** because of genital ambiguity,
67 characterized by incomplete virilization of various degrees. Additionally, we discuss the possibility
68 of a founder effect of the P212R substitution, due to the high prevalence of this mutation among
69 Mexican patients with this enzymatic disorder.

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SUBJECTS AND METHODS

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PATIENTS

75 **Eleven** cases of incomplete virilization, presenting clinical phenotypes of 5 α -reductase
76 deficiency were screened for mutations in the *SRD5A2* gene. **All individuals were of Mexican-**
77 **Mestizo ethnic origin (ethnic group that represents the general Mexican population) and from**
78 **different geographic locations (Mexico City, State of Mexico, State of Hidalgo, State of Puebla**
79 **and State of Veracruz).** All the patients were negative for consanguinity, except for patient #11 in
80 whom both parents were first degree cousins (Fig. 1A). Because of the latter, we also studied
81 **moleculary** patient's #11 family constituted by seven additional members (three phenotypical males,
82 and four phenotypical females). In all patients, the presumptive diagnosis was raised on the basis of
83 the clinical phenotype (presence of hypospadias, micropenis, cryptochid testes, lack of Mullerian
84 derivatives), endocrinological (T/DHT circulating levels; in some cases by determining the activity
85 of 5 α -reductase in cultured mutant fibroblasts) and genetic studies. In all the cases, the high
86 resolution G-banded karyotype was 46, XY. The most relevant clinical findings are shown in Table
87 1. The study was approved by the Institutional Ethical Committee for Research in Humans.

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METHODS

Mutation Detection

91 Genomic DNA was isolated from blood leukocytes by standard techniques (Sambrook and
92 Russell, 2001) and the complete coding region of the *SRD5A2* gene, including intron-exon
93 boundaries, was amplified. Coding sequence abnormalities were assessed by exon-specific
94 polymerase chain reaction (PCR), and single-stranded conformation polymorphism (SSCP) analysis,
95 followed by direct sequencing. PCR and SSCP analyses were carried out using specific primers, and

96 amplification conditions previously described (Vilchis et al, 1997; Chávez et al, 2000). PCR
97 fragments showing aberrant mobility patterns on SSCP were sequenced using the ThermoSequenase
98 ($[\alpha\text{-}^{33}\text{P}]$ ddNTP) Radiolabeled Terminator Cycle Sequencing Kit (USB Co., Cleveland, OH, USA)
99 as described elsewhere (Vilchis et al, 2000). In all cases, both strands were sequenced and
100 compared; the mutations were confirmed from at least two independent PCR amplification
101 reactions.

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103 *Site-directed mutagenesis*

104 The commercial vector *pCMV6-XL4* (OriGene Technologies Inc., Rockville, MD, USA),
105 containing the human *SRD5A2* cDNA (clone #SC119922), was used as template for P212R
106 construction. Expression of the mutant construct was tested by introducing the single base changes
107 (C→G) into the *pCMV6-SRD5A2* cDNAs, at nucleotide 635 (relative to the translation start site).
108 The mutant cDNA was created with the Gene Tailor Site-Directed Mutagenesis System (Invitrogen,
109 Life Technologies, Carlsbad CA, USA) according to the manufacturer's guidelines. PCR
110 amplifications were performed with preheating at 94°C for 3 min, followed by denaturation at 94°C
111 for 30 sec, annealing at 55°C for 30 sec and extension at 68°C for 8 min and for 20 cycles. The
112 primers 212F (forward) 5'-CCCTGGCCACTTGGTCCCTCCGAGCACTTG-3' and 212R
113 (reverse) 5'-GAGGGACCAAGTGGCCAGGGCATAGCCGAT-3', used to synthesize the mutant
114 sequence *pCMV6-SRD5A2/P212R*, were designed with the Primer X automated program
115 (<http://bioinformatics.org/primerx/documentation.html>). Nucleotide sequence in the final constructs
116 was confirmed by sequencing as described above.

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119 *Steroid 5 α -reductase activity*

120 Enzyme activity of the P212R gene variant of SRD5A2 (in homozygous or in heterozygous
121 state), was assessed at pH 5.5, using normal and mutant fibroblasts and HEK-293 cells transfected
122 with expression vectors containing wild-type or mutated cDNA. The conversion of [³H]T to
123 [³H]DHT was assayed in cellular sonicates from normal and mutant fibroblasts and from HEK-293
124 cells transfected with the normal or the mutated (P212R) SRD5A2. Transfection assays and 5 α -
125 reductase studies were carried out using the methods and incubation conditions previously described
126 (Vilchis et al., 2008). The results are expressed as pmol of DHT formed per hour; per milligram of
127 cell sonicate protein.

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RESULTS

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145 Abnormalities in the migration pattern were detected by SSCP analysis in exons 2, 3 and 4 of
146 the *SRD5A2* (data not shown). Regarding the family of patient #11, we found that the propiti
147 exhibited a band shift that suggested the presence of a mutant allele; subject II-1 presented a
148 mobility shift pattern similar to the normal control, whilst heterozygosity in the mother (I-2), as well
149 as in subjects (II-2-to-II-7), is pointed out by multiple bands indicating the presence of both normal
150 and mutant alleles (Fig. 1B).

151 The results of the direct sequencing of *SRD5A2* are shown in Table 2. **Additionally,**
152 sequence analyses demonstrated that the mother (I-2) and close relatives of patient #11 (subjects II-
153 2-to-II-7 were heterozygous for the P212R mutation (data not shown).

154 Because this particular mutation has been described exclusively in Mexican patients and
155 because it results in different phenotypes when occurring in either a homozygous or a heterozygous
156 form, it was of interest to determine its catalytic ability in both situations. *In vitro* assays carried out
157 using cultured fibroblasts of patients #2 (P212/212R) and #11 (212R), showed a null as well as a
158 diminished enzymatic activity, respectively. Under the same experimental conditions, the conversion
159 of [³H]T to DHT was consistently lower in mutant cells when compared to normal control cells
160 (Fig. 2A). Functional studies in transfected HEK-293 cells, confirmed that the p.P212R change
161 severely impairs the enzyme ability to synthesize DHT (Fig. 2B).

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DISCUSSION

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169 Steroid 5 α -reductase 2 deficiency is a rare autosomal recessive form of 46,XY disorder of
170 sex development caused by mutations in the *SRD5A2* gene, presenting at birth and expressing
171 variable degrees of undervirilization. Diverse mutations in this gene have been identified as
172 causative in patients with this disorder. Identical mutations have been found in individuals with
173 widely divergent geographic and ethnic backgrounds, suggesting the existence of mutational hot
174 spots in the gene. Likewise, identical mutations within the same ethnic group have been observed,
175 suggesting that they derive from a common ancestor, by a founder gene effect which is responsible
176 for the dissemination of genetic abnormalities (Canto et al, 1997; Hackel et al, 2005; Skordis et al,
177 2005; Baldinotti et al, 2008).

178 Due to the possibility of discordance between genotypic sex and sex of assignment, a correct
179 diagnosis is mandatory in early infancy and male sex assignment can be advisable in a large
180 proportion of these subjects, in order to assure a natural male pubertal development including
181 fertility in adulthood (Hughes et al, 2006). Unfortunately, several patients are not correctly
182 diagnosed, they are reared as females and gonadectomized in childhood or when virilization occurs
183 at puberty (Imperato-McGinley and Zhu, 2002; Hackel et al, 2005; Nicoletti et al, 2005). Because
184 the diagnosis of steroid 5 α -reductase 2 deficiency is sometimes difficult, due to its clinical
185 variability and cannot be ruled **out by the absence of an elevated T/DHT ratio, the finding of a**
186 **mutation confirms a clinical presumption (Hackel et al, 2005).**

187 In the present study we analyzed eleven Mexican-Mestizo patients with incomplete
188 virilization in whom deficiency of steroid 5 α -reductase 2 was demonstrated; in parallel, we
189 observed a high occurrence of the p.P212R substitution mutation in this particular ethnic group.

190 Of the twenty seven subjects with steroid 5 α -reductase 2 deficiency we have reported in
191 more than a decade, twelve of them have presented the p.P212R mutation, (eight homozygotes,
192 three compound heterozygotes and one heterozygote) (Canto et al, 1997; Vilchis et al, 1997;
193 Chávez et al, 2000; Vilchis et al, 2000; Vilchis et al, 2008; this study). This mutation **modified an**
194 **aminoacid that is conserved in different species** (i.e human, rat, mouse, dog, pig and bovine),
195 suggesting that proline located in position 212 of the protein is vital for enzymatic activity.
196 Strengthening the latter, *in vitro* site-directed mutagenesis experiments have confirmed a lack of
197 enzymatic activity **of the mutant protein (212R)** (Wigley et al, 1994); however, these experiments
198 **were performed only with the mutant protein in a homozygous state (212R)**. Since the p.P212R
199 mutation has been found only in our ethnic group and we have found patients with the **212R mutant**
200 **protein in a homozygous state, as well as one patient having a heterozygous state [the mutant**
201 **protein (212R) and the wild protein (P212)],** we also performed the site directed mutagenesis
202 experiments of both proteins. In these experiments, we confirmed that the mutant enzyme lacks
203 activity; furthermore, we found that the mutant enzyme **(212R)** together with the wild enzyme
204 **(P212)** present a reduction of the enzymatic activity by approximately 60% (Fig. 2).

205 **The finding presented by the family studied without phenotypic alterations is very**
206 **interesting. This** could be explained by the presence of a variable penetrance of the mutation,
207 depending on the genetic background affecting the level of *SRD5A2* expression in each case; on the
208 other hand, patient 2 (with a mutation in a heterozygous trait) could be presenting a defect in the
209 untranslated regulatory regions of the *SRD5A2* gene or within introns, **that might explain** the
210 phenotypic disorder. Further, there are several cases reported, in which a single mutant allele was
211 identified despite they had presented clinical features of the enzyme deficiency (Wilson et al, 1993;
212 Hackel et al, 2005; Nicoletti et al, 2005).

213 Interestingly, we have observed that 23 (~85%) of our 27 affected subjects, **had a**
214 homozygous or a heterozygous mutation within exon 4; two of them exhibiting a compound
215 heterozygous mutation (Table 3) (Vilchis et al., 2008; this study). In addition, there is evidence that
216 almost all mutations located between codons 197 and 230 in exon 4 render the enzyme to be
217 completely inactive (Wilson et al, 1993; Vilchis et al, 2008). Furthermore, residues 206-226 encode
218 for a stretch of 21 amino acids, which have been proposed as one of the four transmembrane
219 domains of the enzyme (<http://ca.expasy.org/uniprot/P31213>). Thus the recurrence of a mutation at
220 this particular site highlights the importance of the fourth transmembrane domain as a key region for
221 the correct functioning of 5 α -reductase type 2. Taken together, these observations provide further
222 evidence that exon 4 of *SRD5A2* may be a prone site for the presence of inactivating mutations in
223 this gene (Vilchis et al, 2008).

224 The high frequency of the p.P212R substitution **may be a prone site** to a founder gene
225 effect, since this substitution has been found in nineteen alleles from twenty seven subjects with
226 5 α -reductase type 2 deficiency from diverse geographic locations in Mexico (Table 3) (Canto et
227 al, 1997; Chávez et al, 2000; Vilchis et al, 2000; this study). In addition, this mutation was
228 originally reported in one Mexican-American subject with deficiency of this enzyme (Wilson et
229 al, 1993). It is noteworthy to mention that, until now, this mutation has been reported only among
230 patients with a Mexican-Mestizo ethnic origin, thus indicating the increased prevalence of this
231 mutation in this particular ethnic group.

232 In spite of the great number of mutations in *SRD5A2*, a reliable genotype-phenotype
233 correlation has not been established and subjects with the same molecular defect may exhibit
234 different phenotypes (Vilchis et al, 2008). However, homozygous patients with the p.P212R
235 mutation have always presented perineo-scrotal hypospadias, microphallus, cryptorchidism and a
236 female sex of rearing, thus demonstrating a genotype-phenotype correlation for this particular

237 mutation. In contrast, those patients presenting a compound heterozygous or a heterozygous
238 mutation observe a more wide phenotypic spectrum (Wilson et al, 1993; Russell and Wilson, 1994;
239 Wang et al, 2004).

240 Regarding the other mutations already described (i.e. p.E57Q, p.G85D, p.S210F), as well as
241 the P212X change; it is interesting to reaffirm that these gene variants have been identified
242 exclusively in Mexican patients. Recently, functional studies of site-directed mutagenesis
243 demonstrated that the mutants p.E57Q, p.S210F and p.P212X, have a deleterious effect on
244 enzymatic activity (Vilchis et al, 2008).

245 The remaining mutations found in our series (Table 3) have been previously identified in
246 other ethnic groups (Thigpen et al, 1992; Cai et al, 1996; Hackel et al, 2005; Sahakitrungruang et al,
247 2008). Several mutations are recurrent and reported in different populations (Thigpen et al, 1992;
248 Wilson et al, 1993; Mazen et al, 2003), while other mutations are highly spread in specific ethnic
249 groups (Griffin et al, 2001; Imperato-McGinley and Zhu, 2002), and their recurrence is probably the
250 result of a founder gene effect in people geographically isolated with a large coefficient of
251 inbreeding (Griffin et al, 2001; Imperato-McGinley and Zhu, 2002). Interestingly, consanguinity is
252 present in about one third of the affected patients and the family history is positive in about 40% of
253 the families (Wilson et al, 1993).

254 To our knowledge the results described in this study represent, along with our previous
255 reports (Canto et al, 1997; Vilchis et al, 1997; Chávez et al, 2000; Vilchis et al, 2000; Vilchis et al,
256 2008), the largest number of patients with steroid 5 α -reductase 2 deficiency belonging to non-related
257 families. Besides, **the presence of the P212R mutation in different non-related families, suggests**
258 **a possible founder gene origin of this mutation in the Mexican-Mestizo population.**
259 **Interestingly, this change (P212R) has not been described in other ethnic groups and neither in**
260 **control samples of Mexican-Mestizo population. Strengthened the importance of the proline**

261 **aminoacid (p.P212), *in vitro* site-directed mutagenesis experiments, demonstrated that this**
262 **mutation, affects the protein function in the heterozygous as well as in the homozygous state.**

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Table 1: Clinical findings in eleven patients with SRD5A2 deficiency

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Patient	Age (yrs)	Sex of rearing	Hypospadias	Cyptorchidism
1*	10	F	Perineo-scrotal	BILATERAL
2	4	M	Penoscrotal	NO
3*	8/12	NA	Perineo-scrotal	NO
4	1	M	Perineal	BILATERAL
5	1	M	Penoscrotal	NO
6	17	F	Perineo-scrotal	RIGHT TESTIS
7	3/12	NA	Penoscrotal	BILATERAL
8	17	F	Perineo-scrotal	NO
9	1	F	Perineo-scrotal	BILATERAL
10	13	F	Perineo-scrotal	BILATERAL
11	16	F	Perineo-scrotal	RIGHT TESTIS

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All patients had microphallus.

433

* = Patient with an affected sibling

434

NA = Not assigned

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Table 2: Molecular findings in eleven patients with SRD5A2 deficiency

Patient	Location	Nucleotide	Base Change	Amino acid change	Mutation
1	Exon 2	346	GGC → GAC	Gly → Asp	p.G115D
	Exon 4	635	CCA → CGA	Pro → Arg	p.P212R
2	Exon 4	635	CCA → CGA	Pro → Arg	p.P212R/wt*
3	Exon 4	635	CCA → CGA	Pro → Arg	p.P212R
4	Exon 4	606	GGC → AGC	Gly → Ser	p.G203S/wt*
5	Exon 4	606	GGC → AGC	Gly → Ser	p.G203S
	Exon 4	635	CCA → CGA	Pro → Arg	p.P212R
6	Exon 4	635	CCA → CGA	Pro → Arg	p.P212R
7	Exon 3	513	AGG → AGC	Arg → Ser	p.R171S
	Exon 4	679	CGA → TGA	Arg → Stop	p.R227X
8	Exon 4	578	AAT → AGT	Asn → Ser	p.N193S
9	Exon 4	635	CCA → CGA	Pro → Arg	p.P212R
10	Exon 4	591	GAG → GAT	Glu → Asp	p.E197D
11	Exon 4	635	CCA → CGA	Pro → Arg	p.P212R

441

442 * = Heterozygote

443 wt = wild type

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Table 3: Mexican patients with Steroid 5 α -Reductase 2 Deficiency

Numbers of Patients	Mutation	a.a. change	Protein	Exon	References
8	CCA-CGA	P212R	p.Pro212Arg	4	Canto <i>et al.</i> , 1997 Vilchis <i>et al.</i> , 2008 This study
1*	GCC-GAC	A207D	p.Ala207Asp	4	Canto <i>et al.</i> , 1997
1	GGC-GAC	G115D	p.Gly115Asp	2	Canto <i>et al.</i> , 1997
	GGC-AGC	G203S	p.Gly203Ser	4	
1	GGG-GGA	G34R	p.Gly34Arg	1	Canto <i>et al.</i> , 1997
1*	CGA-TGA	R227X	p.Arg227stop	4	Vilchis <i>et al.</i> , 1997
1	GAG-GAT	E197D	p.Glu197Asp	4	Chávez <i>et al.</i> , 2000
1	GAG-GAT	E197D	p.Glu197Asp	4	Chávez <i>et al.</i> , 2000
	CCA-CGA	P212R	p.Pro212Arg	4	
1	CGG-CAG	R246Q	p.Arg246Gln	4	Vilchis <i>et al.</i> , 2000
1	GGC-GAC	G115D	p.Gly115Asp	2	Vilchis <i>et al.</i> , 2000
1	GGC-GAC	G85D	p.Gly85Asp	1	Vilchis <i>et al.</i> , 2000
	GGC-GAC	G115D	p.Gly115Asp	2	
1	GGT-AGT	G183S	p.Gly183Ser	3	Vilchis <i>et al.</i> , 2008
	CCA-TGA	P212X	p.Pro212stop	4	
1	GGC-GAC	G115D	p.Gly115Asp	2	Vilchis <i>et al.</i> , 2008
	TCC-TTC	S210F	p.Ser210Phe	4	
1	GAG-CAG	E57Q	p.Glu57Gln	1	Vilchis <i>et al.</i> , 2008
	GGC-GAC	G85D	p.Gly85Asp	1	
1*	GGC-GAC	G115D	p.Gly115Asp	2	This study
	CCA-CGA	P212R	p.Pro212Arg	4	
1	GGC-AGC	G203S	p.Gly203Ser	4	This study
	CCA-CGA	P212R	p.Pro212Arg	4	
1*	CCA-CGA	P212R/Wt	p.Pro212Arg/N	4	This study
1	GGC-AGC	G203S/Wt	p.Gly203Ser/N	4	This study
1	AGG-AGC	R171S	p.Arg171Ser	3	This study
	CGA-TGA	R227X	p.Arg227stop	4	
1*	AAT-AGT	N193S	p.Asn193Ser	4	This study
1	GAG-GAT	E197D	p.Glu197Asp	4	This study

452 a.a= amino acid; Wt= wild type

453 *= Patient with an affected sibling

454 **FIGURE LEGENDS**

455 Fig 1: A) Pedigree and SSCP of exon 4 of the SRD5A2 gene from a family (P11) with deficiency of
456 5 α -reductase-2. The double line between I₂ and I₃ indicates consanguinity (first cousins). All but one
457 (II₁) of the relatives studied were carriers for the P212R mutation whereas the patient II₈ (arrow)
458 presented both alleles altered. B) Amplified DNA from the patient (P) mother (M) and affected
459 siblings (II₁-II₇) exhibits an abnormal migration pattern as compared with that of normal controls
460 (C).

461

462 Fig 2: *In vitro* activity of the P212R gene variant of SRD5A2. Enzyme activity was assessed at pH
463 5.5, using normal and mutant fibroblasts and HEK-293 cells transfected with expression vectors
464 containing wild-type or mutated cDNA. A) The conversion of [³H]T to [³H]DHT was assayed in
465 cellular sonicates from normal fibroblasts (N) or mutant fibroblasts from patient #2 (P212R/N) and
466 patient #11 (P212R), and B) from HEK-293 cells transfected with the normal (C) or the mutated
467 (P212R) SRD5A2. Assays were carried out for 15 min at 37 °C in the presence of 500 μ M of
468 NADPH as cofactor. The results are expressed as pmol of DHT formed per hour, per milligram of
469 cell sonicate protein.

Figure 1

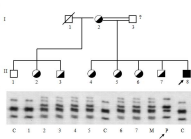


Figure 2

