Substitution of Arginine-839 by Cysteine or Histidine in the Androgen Receptor Causes Different Receptor Phenotypes in Cultured Cells and Coordinate Clinical Androgen Resistance

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Abstract

We aim to correlate point mutations in the androgen receptor gene with receptor phenotypes and with clinical phenotypes of androgen resistance. In two families, the external genitalia were predominantly female at birth, and sex-of-rearing has been female. Their androgen receptor mutation changed arginine-839 to histidine. In a third family, the external genitalia were predominantly male at birth, and sex-of-rearing has been male: their codon 839 has mutated to cysteine. In genital skin fibroblasts, both mutant receptors have a normal androgen-binding capacity, but they differ in selected indices of decreased affinity for 5α-dihydrotestosterone or two synthetic androgens. In transiently cotransfected androgen-treated COS-1 cells, both mutant receptors transactivate a reporter gene subnormally. The His-839 mutant is less active than its partner, primarily because its androgen-binding activity is more unstable during prolonged exposure to androgen. Adoption of a nonbinding state explains a part of this instability. In four other steroid receptors, another dibasic amino acid, lysine, occupies the position of arginine-839 in the androgen receptor. Androgen receptors with histidine or cysteine at position 839 are distinctively dysfunctional and appear to cause different clinical degrees of androgen resistance. (J. Clin. Invest. 1994, 94:546–554.) Key words: androgen ∙ receptor ∙ mutation ∙ steroid ∙ resistance

Introduction

The androgen receptor (AR)1 protein is an androgen-sensitive transcription factor. Androgen binding enables its DNA-binding domain to recognize particular regulatory sequences of DNA (androgen-response elements) and thereby to increase or decrease the expression of certain target genes. In 1974 (1) several laboratories (2, 3) began to characterize the androgen-binding activities in the genital skin fibroblasts (GSF) of 46,XY humans with various clinical expressions of androgen resistance (insensitivity), on the presumption that they had mutant ARs. More recently, the cloning of the AR cDNA (4–7) and the application of derivative molecular techniques have permitted us (8–14) and others (15–27) to try to correlate specific germline lesions in the androgen-binding region of X-linked AR gene with various dysfunctional behaviors of the AR and with their clinical consequences for the affected subjects. The ultimate aim of such correlative studies is to elucidate the stereochemistry that imparts androgen-binding specificity to an AR and the contribution of an androgenic ligand to the transcriptional regulatory attributes of an A–R complex. In this paper, we describe three families that have alternative, single-nucleotide, missense mutations at the Arg-8392 codon in exon 7 of the androgen-binding domain of the AR gene (Fig. 1).

Methods

Subjects and their families. 4308, the index subject in a black-American family, was described previously (28). She was born with a single perineal urogenital orifice flanked by labioscrotal folds. With the aid of corrective surgery, she and an affected maternal aunt were reared as females. The properties of the specific androgen-binding activity in 4308’s GSF (28) are recalled, as appropriate, below.

A.E.L. and K.E.L. are maternal half-sisters in a second black-American family, historically unrelated to the one above. A.E.L. was brought to the St. Christopher’s Hospital for Children at age 2 wk because of a right groin mass. There was posterior labial fusion and questionable clitoromegaly. The karyotype was 46,XY. At age 4 mo, vaginography revealed a short, blind vagina, and extirpative surgery revealed histologically normal testes, epididymes, and proximal vasa deferentia. K.E.L. was born 6 yr later with a 1.5-cm clitoris, posterior labial fusion, and masses in the right labium majus and left groin. Vaginography revealed a short, blind vagina. The karyotype was 46,XY. At age 8 mo, basal serum testosterone was 197 ng/dl; after 4,000 IU of human chorionic gonadotropin daily for 3 d, it rose to 980 ng/dl. Bilateral orchietomy revealed normal testicular histology. The abnormalities of the specific androgen-binding activity in the GSF of these two half-sister subjects are presented here for the first time.

335002 and 333203 are brothers in a white-Canadian family. 335002 was born with cryptorchidism and penile hypospadias; each required

1. Abbreviations used in this paper: AI, androgen insensitivity; AR, androgen receptor; DHT, [1,2,4,5,6,7-3H]5α-dihydrotestosterone; GSF, genital skin fibroblasts; h, human; k, nonequilibrium rate constant of dissociation; Kd, (apparent) equilibrium dissociation constant; MB, [17α-methyl-3H]mibolerone; MMTV.GH, mouse mammary tumor virus.growth hormone; MT, [17α-methyl-3H]methyltrienolone; pCMV.β-gal, cytomegalovirus promoter. β-galactosidase reporter gene construct; T, testosterone.

staged surgical correction. Bilateral gynecomastia appeared at 12 yr and
required reduction mammoplasty. Pubic and axillary hair appeared at
age 14. At 22 yr, he had no facial or chest hair; pubic hair was Tanner
stage 3. The right testis was 25 ml; the left was 10 ml. He is potent.
Between 20 and 23 yr of age, repeated serum total testosterone (T)
determinations varied from 72 to 180 nM (normal: 10–35) while his non—sex hormone–binding globulin (free T) levels varied from
8.3 to 31 nM (normal: 3–13). On these occasions, his free T varied from
12 to 15% of the total serum T. In normal males, the fraction of
total T that is not bound to sex hormone–binding globulin is ~30%.
The values for luteinizing hormone (LH) varied from 16 to 23 U/liter
(normal: 2–15), and those for follicle-stimulating hormone (FSH) have
been 15 on two occasions (normal: 4–18). After 3 d of abstinence, a
0.5-ml sample of semen contained 11.8 million sperm/ml; 10% had
normal motility, but 90% had normal morphology. 333203 was born
3.5 yr later with a nonhypospadiac microphalpos and bilateral cryptorchidism that needed several orchidopexy procedures. Bilateral gynecomasia required mammoplasty at age 14. At 19 yr he had no facial or chest hair; axillary hair was plentiful; pubic hair was Tanner stage 4. The penis was only 3 cm long. The testes were 8 ml bilaterally. At 20 yr, when his total serum T was 74 nM, the free was 12 nM, 17% of the
total; the LH level was 21 U/liter (normal: 5–25), and the FSH was
29 U/liter (normal: 4–18). The affected brothers have three affected
maternal first cousins once removed, two of whom are siblings. Two
were born with penile chordee; one had microphalpos with hypospadias
at the penile base. All three had a nonbifid scrotum and have been
reared as males with supportive surgery.

Androgen-binding activity in cultured cells. For GSF, specific andro-
gen-binding activity, maximum androgen-binding capacity, the apparent
equilibrium dissociation constant (Kd), the rate constant of dissociation
(k), and thermostability of various A–R complexes or of the free AR
were determined on monolayer cultures as described previously (29).
The same methods were used for COS-1 cells except that they were
washed and lysed in 0.5 N NaOH directly in the culture dishes. The
androgens used were: [17α-methyl-3H]mibolerone (MB; 7α, 17α-di-
methyl-19-nortestosterone; 80 Ci/mmol); [1,2,4,5,6,7-3H]5α-dihy-
drotestosterone (DHT; 120 Ci/mmol); and [17β-methyl-3H]meth-
yltestosterone (MT; 17β-hydroxyl-17α-methyl-4,9,11-estriene-3-one; 87
Ci/mmol). The synthetic androgens are nonmetabolizable.

PCR amplification and direct DNA sequencing of AR genomic exons.
These were modified (30) from methods described in reference 12.

Construction of cDNA expression vectors with R839H or R839C.

The germline sequence alterations identified in the DNA of representa-
tive affected subjects were reproduced using a modification of Higuchi’s
(31) site-directed PCR mutagenesis method. Four separate primary PCR
reactions were performed using 2 μg of the human (h) AR cDNA expression vector pSVhAR.BHEX as template and 5 pmol each of the
following primers (mutated bases are underlined and bold faced):

1a. inside, AEL-A (5'-CAAGGAATCGATCATATCATGCG-3')
   outside, SVEcO (5'-GAATTGCCATGAGGGTTGAAAT-AAG-3')

2a. inside, AEL-B (5'-GATGCAATGATGATGCGAG-3')
   outside, Primer B (5'-TCACACATGAGAGCTGTA3')

3a. inside, 335002-A (5'-CAAGGAATCGATGATGCGAG-3')
   outside, Primer P3 (5'-GTCGCTATCGAAGGTTGGA-3')

4a. inside, 335002-B (5'-GCAATGAGAGCAATGATC-3')
   outside, Primer B (see 2b above).

The primary fragments were amplified for 25 cycles by Pfu DNA
polymerase (Stratagene, La Jolla, CA) with the following parameters:
denaturation at 95°C for 1 min, annealing at 55°C for 75 s, and extension
at 75°C for 90 s. The fragments from the four primary PCR reactions
were gel-purified and resuspended in 20 μl H2O, and 1–2 μl of the
latter was used for the secondary PCR in the following combination

3. pSVhAR.BHEX is a version of pSVhAR (32) that we have made
more useful by making three of its restriction sites unique and by intro-
ducing a fourth site at codons 676–677 in the AR gene. The PCR
primers are underlined. Arg-839, the cysteine (C) to thymine (T)
transition leading to Cys-839, and the guanine (G) to adenine (A)
transition leading to His-839 are in bold. The Pvu recognition
sequence that is altered by each mutation is bracketed.
reactions: for A.E.L. (R839H) fragments from reactions 1 and 2; for 335002 (R839C) fragments from reactions 3 and 4. The secondary PCR reactions contained 0.5 pmol of each of the respective internal primers and 50 pmol of each of the outside primers: for 335002, P3' and Primer B; for A.E.L., SVEco and Primer B. Except for the greater number of cycles (35 cycles), the cycle parameters were the same as the primary PCR reactions. The fragments from the four secondary PCR reactions were gel-purified. R839C (335002) fragments were double-digested with restriction enzymes EcoRI and BamHI, and R839H (A.E.L.) fragments were digested with EcoRI. The digested fragments were ligated into pSVhAR.BHEX3 or pSVhAR (R839H), each deleted for the corresponding restriction fragments. XL1 Blue competent bacteria were transformed with the ligation products, and the colonies were screened by PvuI restriction enzyme digestion (both mutations abolish a PvuI site in the hAR cDNA). The appropriate clones were grown in 500 ml of liquid cultures, and the plasmid vectors were purified by QIAGEN columns (QIAGEN Inc., Chatsworth, CA). The inserted fragments were sequenced by the dideoxy method to confirm exclusivity of the site-directed nucleotide substitutions.

Assessment of transactivational efficiency of mutant ARs with R839H or R839C. The construction of the mouse mammary tumor virus/human growth hormone (MMTV.hGH) reporter plasmid and its cotransfection with the pCMV.β-gal vector to assess transfection efficiency has been reported (12, 13). To achieve consistent relative transactivational activities among replicate experiments, the prior transfection protocol (13) was modified as follows: we electroporated confluent COS-1 cells at 350 V/960 μF (~20 ms) in the presence of 1 μg normal or mutant pSVhAR and 20 μg pMMTV.GH, and 6 h later we exposed the cells to various concentrations of milborene for an additional 72 h.

Western analysis of the AR in transfected COS-1 cells. Confluent monolayers (~1 million cells) in 30-mm multiwell petri dishes were washed twice with 20 mM Tris/HCl (pH 7.4) containing 0.15 M NaCl, and the scraped cells from duplicate dishes were combined in 1 ml of the same buffer. After microfuging, the cells were resuspended in 160 μl of 0.25 M Tris/HCl (pH 7.8) containing 3.5% (vol/vol) aprotinin (Sigma Immunochemicals, St. Louis, MO), lysed by three freeze-thaw cycles, and microfuged (12,000 g, 5 min, 4°C). 50 μl of the supernatant was used for protein assay (33), and 100 μg of supernatant protein from each sample was subjected to SDS-PAGE and Western transfer by electroblotting according to standard methods (34). The nitrocellulose filters were blocked by immersion in TBS (20 mM Tris/HCl [pH 7.4] and 500 mM NaCl) with 0.5% Tween 20 for 1 h at room temperature and incubated overnight at 4°C with a monoclonal antibody (F39.4.1) to peptide (561) corresponding to amino acids 301–320 in the NH2-terminal portion of the hAR (35). The antibody was diluted 1:10,000 in TBS with 0.05% Tween 20. After three washes in TBS with 0.5% Tween 20, the filter was incubated with a 1:5,000 dilution of horseradish peroxidase goat anti-mouse IgG for 1 h at room temperature. After five more washes in TBS with 0.5% Tween 20, the blot was developed using the ECL chemiluminescence detection system (Amersham Corp., Arlington Heights, IL). Using the LKB Ultrascan XL densitometer and the Pharmacia Gel Scan XL program (Pharmacia LKB Biotechnology Inc., Piscataway, NJ), we validated the chemiluminescence method by demonstrating linearity with 10–300 fmol of androgen-binding activity in the standard Western blotting procedure and by exposing film to the Lumilin reaction for various times (30 s–20 min) to define a window during which relative band intensity remained constant densitometrically.

Results

Identification and restriction-site analysis of the mutations. By PCR amplification and direct sequencing of exons 1–8, we found the guanine to adenine transition responsible for Arg839His first in the GSF of subject 4308 and then in those of the half-sisters A.E.L./K.E.L. (Fig. 2). In the same way, we later found the cysteine to thymine transition responsible for Arg839Cys in the brothers 335002/333203 (Fig. 2). The ablation of a PvuI site by either substitution allowed us to probe maternal heterozygosity for the half-sisters A.E.L./K.E.L. (Fig. 3, top) and for the brothers 335002/333203 (Fig. 3, bottom). The families of 4308 and A.E.L./K.E.L. are historically unrelated. They also differ in the highly polymorphic length (36) of the poly-glutamine tract in exon 1 of the AR gene: 4308 has
19 repeats, A.E.L./K.E.L. have 18, their mother has ones of 18 and 20; and 335002 has 20.

Androgen-binding activity in GSF. We first determined various androgen-binding parameters of the mutant AR in the GSF of the half-sisters A.E.L./K.E.L. and the brothers 335002/333203. Fig. 4 shows that 335002’s AR has modestly increased \( K_d \) values for MB, MT, and DHT that are comparable with those published previously (28) for 4308 (0.57, 2.2, and 1.0 nM, respectively; normal < 0.3 nM) and to those of A.E.L./K.E.L. (0.35, 1.0, and 0.56 nM, respectively).

The MB- and MT- R complexes preformed in the GSF of 333203 or A.E.L. at 37°C were much more thermolabile than normal when the cells were postincubated at 41°C with 100 \( \mu \)M cycloheximide in the presence of 3 nM of the appropriate \( ^{[3}H \)androgen (Fig. 5). Furthermore, the Arg839His MB-receptor complexes in A.E.L. were more thermolabile than the Arg839Cys ones in 333203. However, when the GSF of subject 333203 or A.E.L. were incubated at 37°C in serum-free medium overnight and then exposed to 100 \( \mu \)M cycloheximide at 41°C, they lost their androgen-binding activity during the next 2 h at the same rate as control GSF (\( t = 1.5 \) h; data not shown). These disparate responses of A.E.L.’s GSF to thermal stress closely resembled those in 4308’s GSF under slightly different conditions (28).

Table I shows the rates at which various \( ^{[3}H \)A-R complexes dissociated in GSF chased by a great excess of the corresponding radioisotopic androgen at specific temperatures. A.E.L.’s DHT- R complexes dissociated moderately faster than normal not only at 37°C, as suggested by those of 4308, but also at 40 and 42°C. In contrast, 335002/333203 had normal DHT k values (‘‘off-rates’’) at 37 and 42°C. Likewise, these brothers had a normal mean MT k value at 37°C, whereas the corresponding value for A.E.L. and 4308 was suspiciously high. Notably, A.E.L., 4308, and the brothers 333203/335002 all had normal MB k values at 37°C; yet, all three tested had clearly increased MB k values at 40 and/or 42°C. We have reported such patterns of ligand-specific, temperature-restricted departures from normal k values in the GSF of other subjects with partial androgen resistance (37).

Androgen-binding and transactivational activity of trans-
fected ARs. To prove that Arg839His or Cys are pathogenic substitutions, we created each mutation in an otherwise normal hAR cDNA expression vector and transiently transfected it into COS-1 cells. Table II shows that the cells with the Arg839His AR, but not those with the Arg839Cys AR, had higher 37°C k values for all three androgens than those with the normal AR. These results conserved the pattern of 37°C k values in normal and either type of mutant GSF.

Likewise, the MB–Arg839His receptor complexes produced in COS-1 cells at 37°C were more thermostable than their Arg839Cys counterparts when shifted to 42.5°C (Fig. 6 A). In contrast, as previously shown for GSF, when the transfected COS-1 cells were first exposed to a temperature of 42.5°C in the presence of 100 µM cycloheximide and then measured for androgen-binding activity at various intervals, there was no appreciable difference between the thermostability of the normal receptor and either of the two mutant ARs (Fig. 6 B).

Fig. 7 A shows that in COS-1 cells treated with MB 6–80 h after cotransfection the Arg839His AR had a clear tendency to be less active in promoting transcription of the GH gene than the Arg839Cys AR and that both of them were less active than the normal hAR.

We also assessed stability of the normal and either type of mutant AR in COS-1 cells by measuring their androgen-binding activities during various intervals after transfection. Fig. 8 shows that the normal binding activity was stable, or relatively so, during prolonged (46–120 h) incubation at all MB concentrations tested, whereas both types of mutant activities were clearly unstable at the same MB concentrations. Again, the Arg839His mutant was inferior to the Arg839Cys. The approximate 50% difference between the 2-h binding activities at the beginning and the end of the prolonged exposure to MB reflects the transience of the transfections. In this respect, all three transfections behaved very similarly.

In another transfection experiment using only 3 nM MB (Fig. 9), the normal and mutant androgen-binding activities were subjected to immunoblot analysis. As above, the 118–120-h binding activities were 40–50% of those in their 46–48-h counterparts, and their respective blot intensities were appropriate. As before (Fig. 8), prolonged (46–120 h) incubation with MB caused cells with Arg839His to lose more (> 75%) of their starting binding activity than cells with Arg839Cys (50%). Under the same conditions, the cells with the wild-type hAR essentially maintained their initial androgen-binding activity. After prolonged MB incubation, the immunoreactivities of the wild-type hAR and both mutant forms increased relative to their respective androgen-binding activities. This implies that fractions of their respective pools of ARs were in a

Table I. Rate Constants of Dissociation (k: 10⁻¹¹ min⁻¹) of Control and Mutant A–R Complexes in GSF Monolayers at Various Temperatures

<table>
<thead>
<tr>
<th>Ligand</th>
<th>DHT 37°C</th>
<th>DHT 40°C</th>
<th>DHT 42°C</th>
<th>MT 32°C</th>
<th>MT 37°C</th>
<th>MT 40°C</th>
<th>MT 42°C</th>
<th>MB 37°C</th>
<th>MB 40°C</th>
<th>MB 42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4308</td>
<td>9±1</td>
<td>10±1</td>
<td>11±1</td>
<td>30±2</td>
<td>43±2</td>
<td>43±2</td>
<td>43±2</td>
<td>43±2</td>
<td>43±2</td>
<td>43±2</td>
</tr>
<tr>
<td>A.E.L.</td>
<td>10±1</td>
<td>15±1</td>
<td>28±1</td>
<td>12±2</td>
<td>3±1</td>
<td>3±1</td>
<td>3±1</td>
<td>3±1</td>
<td>3±1</td>
<td>3±1</td>
</tr>
<tr>
<td>335002</td>
<td>6±2</td>
<td>6±2</td>
<td>6±2</td>
<td>6±2</td>
<td>6±2</td>
<td>6±2</td>
<td>6±2</td>
<td>6±2</td>
<td>6±2</td>
<td>6±2</td>
</tr>
<tr>
<td>Control</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
<td>±1 (30)</td>
</tr>
</tbody>
</table>

The monolayers were incubated in serum-free medium at 37°C with 3 nM [³H]androgen alone or together with 0.6 µM radioinert androgen. Specific androgen-binding activity was measured at 1 h, then the cells were washed and chased with the same medium containing only radioinert androgen, and the rate of decline of the activity was measured at intervals for 2 h. * 4308 and A.E.L. (unrelated) share Arg839His. † From Table 1 in reference 22. ‡ 335002/333203, brothers, share Arg839Cys.

Table II. Ligand-specific k of the Normal and Mutant hARs in GSF and COS-1 Cells at 37°C

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Normal GSF</th>
<th>Normal COS-1</th>
<th>Arg839Cys GSF</th>
<th>Arg839Cys COS-1</th>
<th>Arg839His GSF</th>
<th>Arg839His COS-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>3.0±0.1</td>
<td>3.1±0.10</td>
<td>3.0±0.00</td>
<td>3.2±0.30</td>
<td>4.3±0.33</td>
<td>4.9±0.35</td>
</tr>
<tr>
<td>DHT</td>
<td>6.3±0.25</td>
<td>7.1±0.35</td>
<td>5.3±0.75</td>
<td>6.9±0.65</td>
<td>9.3±0.48</td>
<td>9.2±0.70</td>
</tr>
<tr>
<td>MT</td>
<td>12.0±0.5</td>
<td>12.3±0.30</td>
<td>14.3±2.25</td>
<td>10.9±0.70</td>
<td>20.7±4.70</td>
<td>15.6±0.20</td>
</tr>
</tbody>
</table>

* The GSF values have been combined for affected members of the same family or unrelated subjects with the same mutation. † The values are the mean±SE.
non-androgen-binding state; it is evident that these fractions were greater for the mutants.

In Fig. 7 B, for each of three experiments, we replotted GH production (shown in aggregate in Fig. 7 A) against relative MB-binding activity during prolonged (6–78 h) MB incubation. In two experiments, the normal and mutant receptors were indistinguishable; in the third, the differences were slight.

Discussion

In our initial report (28) on the partial androgen insensitivity syndrome in 4308, we noted that her GSF AR phenotype was distinctive; for instance, her DHT–receptor complexes had questionably increased 37°C $k$ values, yet the 42°C $k$ values for her MB–receptor complexes were clearly high. Many years later, we realized that 4308 and the unrelated half-sisters, A.E.L./K.E.L., shared not only the Arg839His AR but also a very similar GSF AR phenotype and predominantly female external genitalia. The broad phenotypic similarity in the two

![Diagram](image.png)

**Figure 6.** (A) MB–receptor complexes were formed in COS-1 cells incubated with 2.4 nM MB at 37°C for 2 h (46–48 h after transfection; 0 time). The cells were then postincubated at 42.5°C under the conditions defined in the legend to Fig. 5, and residual MB–receptor complexes were measured at intervals up to 6 h. ○, Two samples of normal hAR cDNA expression plasmid transfected separately. (B) 48 h after transfection, COS-1 cells at 37°C were incubated at 42.5°C in the presence of 100 μM cycloheximide. Thereafter, at intervals up to 6 h, the decline of mibolerone-binding activity was measured by incubating with 3 nM MB for 1 h at 37°C.

![Diagram](image.png)

**Figure 7.** (A) COS-1 cells were transfected with normal hAR expression plasmid, or with a plasmid containing either mutant AR, and also with both the MMTV.GH and CMV.B-gal plasmids. 6 h after transfection, different concentrations of MB were added to the medium, and 72 h later samples of medium and cells were taken to measure GH and B-gal activity, respectively. The latter activity was used to assess transfection efficiency, and the GH values were normalized for variation in that efficiency. *P \(< 0.05\) versus normal; **P \(< 0.05\) versus normal or R839C. (B) The GH data above are replotted against specific MB-binding activities at a given concentration of MB in the 6–78-h intervals after transfection.
families with Arg839His AR appreciated in value both by similarity and by contrast with the Arg839Cys AR in the brothers (335002/333203) with partial androgen insensitivity (AI). For example, in GSF both mutant ARs had variably elevated but overlapping $K_d$ values for all three androgens, and their respective MB $k$ values were normal at 37°C but elevated at 40–42°C. By contrast, the GSF with Arg839His had slightly to moderately elevated DHT $k$ values at 37–42°C, while the corresponding values for those with Arg839Cys were normal.

The fact that the GSF with Arg839His had abnormal DHT $k$ values while those with Arg839Cys did not corresponded with the greater undermasculinization of the subjects with Arg839His than those with Arg839Cys. This was borne out by the relative transregulatory dysfunction of each mutant receptor within transfected COS-1 cells: the Arg839His AR was less active than the Arg839Cys AR when GH production was plotted against MB concentration. Importantly, however, the difference disappeared when GH production was plotted against specific MB-binding activity. This suggested that the mutant MB–receptor complexes had normal intrinsic transactivational competence, but that they were unstable during prolonged exposure to MB. An immunoblot analysis revealed that some of the mutant AR instability reflected conversion of the mutant AR to a non–androgen-binding state. Such a state may be a part of normal intracellular AR recycling (38), but the mutant ARs may be abnormally disposed to occupy it. Despite these observations, it is important to appreciate that there is strong theoretical and experimental (39) reason to believe that the transregulatory activity of normal or mutant (40) steroid receptors is influenced by cell type and by the promoter context of a particular target gene. Hence, the disparity we observed among the normal and mutant ARs using the MMTV.hGH reporter gene in COS-1 cells might well change in degree and/or quality, if we were to use a reporter gene with a different androgen-responsive promoter context and a transfectant cell type with a different set of cofactors that affect the transregulatory activity (or stability) of the normal and mutant ARs. The ideal candidates would be physiologic target cells rendered AR-free and reporter genes whose products are essential for male sexual differentiation.

Figure 8. COS-1 cells were transfected with normal hAR expression plasmid or with plasmid containing either mutant AR. Specific MB-binding activity at various concentrations of MB was measured for 2 h, at 46 and 118 h after transfection, and for 74 h, from 46 to 120 h after transfection.

Figure 9. Androgen-binding activity (top, not corrected for transfection efficiency) and immunoreactivity (middle and bottom) were measured concurrently after the addition of 3 nM MB for various intervals to replicate wells of COS-1 cells that had been cotransfected with pMMTV.GH and pCMV.β-gal alone (Mock) or additionally with pSV.hAR.BHEX (Normal) or one of its two mutant versions, R839H or R839C.
(Fig. 1) imply that the hAR must have Arg or Lys at position 839 to function normally. Our data also suggest that His-839 tends to disrupt the AR more than Cys-839 does: the former is likely to cause predominantly female external genitalia and a female sex-of-rearing; the latter is likely to cause predominantly male external genitalia and a male sex-of-rearing. The two unrelated individuals with Arg839Cys reported by others (25) also had partial Al with a male phenotype (Reifenstein syndrome) and, as we have found, in the GSF of the one tested, MB-receptor complexes had normal 37°C k values. Others have found the Arg839His mutation in four unrelated individuals. All four had partial Al: two with a female phenotype (25, 27), one with a male phenotype (25), and one ambigous (41). Importantly, as we found, in the GSF from the one subject with a female phenotype tested, the AR had an increased k value for MB at 37°C (25). Remarkably, the GSF of the one with a male phenotype had almost unmeasurable androgen-binding activity, typical of subjects with complete Al (25). This is a striking indication that “background” factors can mitigate the typical clinical expression of an AR mutation.

The fact that neither the His nor the Cys substitution of Arg-839 disrupts the AR sufficiently to cause complete AI is provocative, in light of information available on the same set of substitutions at two other Arg codons in the AR’s androgen-binding domain. For instance, Arg773Cys disrupts the AR phenotype more than Arg773His (12), yet both cause complete AI exclusively (12, 24, 26), possibly because Arg-773* is within an ultrasanerved region of the androgen-binding domain. Contrarily, Arg854Cys has caused complete AI in three out of three subjects (25, 26, 42), while Arg854His has caused partial AI in three out of four subjects (24, 25, 43), yet Arg-854* is at the NH2-terminal limit of an 11-amino acid stretch that is absolutely identical among the members of the AR subfamily. The facts that Arg-839 is not within a highly conserved region of the androgen-binding domain and that neither Cys-839 nor His-839 causes complete AI indicate that Arg-839 is less important than Arg-773 or Arg-854 to the AR’s androgen-binding properties. It is also noteworthy that Cys-839 appears to be less disruptive than His-839, whereas Cys-773 and Cys-854 appear to be more disruptive than His-773 and His-854, respectively. Perhaps this is because Cys-839 does not provoke a harmful disulphide bridge, whereas Cys-773 and Cys-854 do. This hypothesis is strengthened by the ligand- and temperature-restricted character of the moderately abnormal nenequilibrium rate constants of dissociation that we have observed in GSF carrying either of the present mutations. In the GSF of most subjects with complete AI due to missense mutations in their androgen-binding domains, these rate constants of dissociation are more abnormal absolutely and seldom ligand- or temperature-restricted (14).

Arg839Cys and Arg839His represent G:C → A:T transitions. This reflects the tendency for cytosines at CpG dinucleotides to become thymines by methylation at carbon 5 and spontaneous deamination at carbon 4. Indeed, 9 out of 28 point mutations that we have identified in the hAR gene fall into this category (14).

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References


