# Different Levels of 5α-Reductase Type I and II, Aromatase, and Androgen Receptor in Hair Follicles of Women and Men with Androgenetic Alopecia

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**In this study, 12 women and 12 men, ages 18–33 y, with androgenetic alopecia were selected for biopsies from frontal and occipital scalp sites. The androgen receptor, type I and II 5α-reductase, cytochrome P-450-aromatase enzyme were measured and analyzed in hair follicles from these scalp biopsies. Findings revealed that both women and men have higher levels of receptors and 5α-reductase type I and II in frontal hair follices than in occipital follicles, whereas higher levels of aromatase were found in their occipital follicles. There are marked quantitative differences in levels of androgen receptors and the three enzymes, which we find to be primarily in the outer root sheath of the hair follicles in the two genders. Androgen receptor content in female frontal hair follicles was approximately 40% lower than in male frontal hair follicle. Cytochrome P-450-aromatase content in women's frontal hair follicles was six times greater than in frontal hair follicles in men. Frontal hair follicles in women had 3 and 3.5 times less 5α-reductase type I and II, respectively, than frontal hair follicles in men. These differences in levels of androgen receptor and steroid-converting enzymes may account for the different clinical presentations of androgenetic alopecia in women and men.** *J Invest Dermatol 109:296–300, 1997*

The extent of hair loss in women with androgenetic alopecia (AGA) is usually much less than in men. It has been assumed that the hormonal basis for AGA in women is the same as in men, even though no studies have confirmed this. 5α-Dihydrotestosterone (DHT) is thought to be responsible for the gradual miniaturization of genetically marked hair follicles by shortening the duration of the anagen growth phase and reducing the cellular hair matrix volume (Messenger, 1993).

Previous work in young balding men has shown a substantial increase in the production of DHT in frontal anagen hair follicles compared to frontal hair follicles in nonbalding men with AGA (Schweikert and Wilson, 1974). Previous reports on the metabolism of androgens and estrogens by target tissue organs have shown that the metabolizing enzymes are critical for determining the cellular effects of steroids. Our studies describe the androgen receptor and three important androgen-converting enzymes, 5α-reductase (5α-R) types I and II and cytochrome P-450 aromatase (AROM), in scalp hair follicles of women and men with AGA.

## MATERIALS AND METHODS

**Chemicals** [1,2-$^3$H(N)]Testosterone (60 Ci/mmol), [1,2-$^3$H]androst-4-ene-3,17-dione (55 Ci/mmol), radioactive and nonradioactive [17a-methyl-$^3$H]methyltrienolone (R-1881, 86 Ci/mmol), and scintillation supplies were purchased from New England Nuclear (Boston, MA). The purity of steroids was verified by thin-layer chromatography. Nonradioactive steroids, coenzymes, silica gel H, Hanks balanced salt solution buffered with 25 mM N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) at pH 7.34, and other chemical reagents were from Sigma (St. Louis, MO).

**Patients** Twelve women (ages 14–33 y, mean = 23 y) with AGA were clinically evaluated and chosen for the study at the University of California (San Francisco, CA). The duration of hair loss was between 1 and 12 y (mean = 4), and all patients had either a Ludwig I or II grade hair loss. The history and physical exam revealed 11 with regular menstrual cycles, 1 with irregular menstrual cycles with polycystic ovaries, none of the women had hirsutism, and one patient had mild papular acne. All laboratory tests (free testosterone, dehydroepiandrosterone-sulfate, thyroid studies, iron, total iron binding capacity, and prolactin) were within normal limits. After obtaining a signed informed consent, we took two 4-mm punch biopsies, one from the thinning frontal temporal scalp and one from the unaffected occipital scalp.

Twelve men (18–30 y, mean = 26 y) with AGA were selected for the study. The hair loss was between 3 and 7 y in duration (mean = 4), Hamilton II–III grade. No patients had acne. These male patients were undergoing hair transplants and consented to donate one 4-mm punch biopsy specimen from the occipital scalp and all 3.5-mm punch frontal scalp biopsies, which are normally discarded after hair transplant surgery.

**Isolation of Hair Follicles and Tissue Processing** All scalp specimens were processed as previously described (Sawaya, 1992). Anagen hair follicles were microdissected, and approximately three-seven hair follicles were obtained from frontal specimens and 15–22 hair follicles were from occipital specimens. Microdissected hair follicles were stored at −70°C until used for assays.

Frontal and occipital hair follicles from each patient were subjected to sequential ultracentrifugation as previously described (Sawaya *et al*, 1988) to obtain nuclear (800 × *g*), mitochondrial (10,000 × *g*), microsomal (105,000 × *g*) pellets, and a cytosol fraction (105,000 × *g*). Nuclear and microsomal pellets were washed once by resuspension in buffer, recentri-

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Abbreviations: AROM, cytochrome P-450 aromatase; 5α-R, 5α-reductase; AGA, androgenetic alopecia.

fuged, and used for the enzyme studies. Nuclear and cytosol portions were used for androgen receptor analysis.

**Androgen Receptor Analysis** Cytosol (105,000 × *g*) and nuclear fractions (800 × *g*) prepared by sequential ultracentrifugation of the hair follicle homogenates were assessed for androgen receptor binding activity by the dextran-coated charcoal assay and steroid exchange assays (Sawaya, 1992). After correction for nonspecific binding, the data assessing receptor binding was plotted according to Scatchard (1949) to obtain $K_d$ (dissociation constant) and $B_{max}$ (maximum binding capacity) values.

**5α-R Type I and II Enzyme Analysis** Each incubation tube contained 50 μg of nuclear and microsomal protein, as determined by the method of Lowry (1951), along with 1 μM ($10^6$ dpm) 1,2-[$^3$H]testosterone, as previously described (Itami *et al*, 1990) with modifications made, because two forms of this enzyme may be found with type I predominating in skin (Itami *et al*, 1990, 1991). Type I and II differ in regard to their biochemical characteristics; therefore, pH, pyridine cofactor and time course studies were performed to distinguish these isoenzymes.

**pH, Pyridine Cofactors, and Time Course Studies** To assess the activity of the two 5α-R enzymes, different pH ranges, pyridine nucleotides, and incubations times were used to assess testosterone conversion to DHT, as described previously (Hsia *et al*, 1983; Sawaya *et al*, 1984; Itami *et al* 1990), with modifications made as specified.

All incubations were terminated by the addition of 1 ml of cyclohexane/ethanol (1:1, vol/vol) and extracted, and steroid products were analyzed as previously described (Sawaya *et al*, 1988). Activity of 5α-R type I and II isoenzymes was expressed as the sum of all 5α-reduced products including DHT, androstanediol, and androstanedione.

**AROM Analysis** AROM analysis consisted of assessing the enzyme activity from the microsomal pellets, as previously described (Schweikert *et al*, 1975; Berkovitz *et al*, 1984; Zimniski *et al*, 1987), by following the release of tritiated water from tritiated testosterone and androstenedione. Progesterone was added (5 μM) to all incubations to prevent loss of substrate via the 5α-R pathway. Incubations were carried out containing 100 nM [$^3$H]testosterone and/or androstenedione including 5 μM nonradioactive progesterone at 37°C for 3–6 h of incubation. Production of tritiated water was verified by measuring radioactivity in the reaction mixture after charcoal extraction of the steroids, and the production of estradiol and estrone was confirmed by high pressure liquid chromatography.

**Northern Blot Analysis** Northern blots were performed by the method of Fourney *et al* (1988). Briefly, RNAs were extracted from the tissues by the single-step guanidine-phenol-chloroform method (Chomczynski *et al*, 1987). The integrity of the extracted RNA was monitored by electrophoresis on a 1% agarose gel (Sambrook *et al*, 1989). RNA obtained was denatured at 65°C for 15 min in 45% formamide, 5.4% formaldehyde, and morpholinopropanesulfonic acid (Sigma, St. Louis, MO) in a 1× buffer, electrophoresed through a 1% agarose gel containing 1.9% formaldehyde, and transferred to nitrocellulose filters (Millipore, Bedford, MA). A 0.20- to 9.5-kilobase (kb) RNA ladder (GIBCO-BRL, Gaithersburg, MD) and 18S–28S ribosomal RNA were used as size markers.

The filters were pre-hybridized and hybridized at 44°C with a $^{32}$P-labeled cDNA probe or at 55°C with an anti-sense cRNA probe. The blots were washed in 0.1× sodium dodecyl sulfate at 52°C (cDNA probes) or 60°C (cRNA probe). Autoradiography was performed as previously described (Sambrook *et al*, 1989). Densitometric measurements of each specific mRNA were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to consider the amount of RNA loaded per lane. Relative comparisons of mRNA levels between lanes were quantified by densitometric scanning of the autoradiographs (Ultrogel Scan, LKB). On each blot, the sample presenting the highest intensity after normalization was arbitrarily set at 100. The other samples of the same blot were expressed as percentages of this maximum intensity.

**Probes** The androgen receptor cDNA was from rat and kindly provided by Dr. S. Liao (University of Chicago, Chicago, IL). The type I and II human steroid 5α-R cDNAs were donated from Dr. David Russell (University of Texas, Dallas, TX). The human P450 AROM cDNA was donated by Dr. E. R. Simpson (University of Texas, Dallas, TX).

[$^{32}$P]cRNA probes were synthesized in the presence of [$^{32}$P]CTP (New England Nuclear, Boston, MA). An anti-sense cRNA probe 464 bases long corresponding to the androgen receptor was generated by using an *Eco*RI-linearized pGEM vector and T7 RNA polymerase (Promega, Madison, WI). Anti-sense cRNA probes, respectively 807 and 595 bases long were also generated for type I and II 5α-R, using pBluescript vectors linearized with *Ava*I (type I) and *Pst*I (type II) as templates and $T_3$ RNA polymerase (Pharmacia, Piscataway, NJ). The 2.5 fragment of human AROM (*Eco*RI digestion of the pBR322 vector) was used. This cDNA insert was labeled by random priming (Boehringer Mannheim, Germany).

**Table I. Androgen Receptor Levels in Women and Men**[a]

| | Androgen Receptor | |
|---|---|---|
| | Nuclear (fmol per mg DNA) | Cytosol (fmol per mg protein) |
| Women | | |
| Frontal HF | 177 ± 24 | 48 ± 11 |
| Occipital HF | 132 ± 20 | 29 ± 10 |
| Men | | |
| Frontal HF | 302 ± 24 | 67 ± 12 |
| Occipital HF | 226 ± 20 | 38 ± 12 |

[a] Incubations were performed in triplicate with mean ± SD shown for the maximum binding capacity expressed as fmol per mg of DNA and fmol per mg protein, respectively, in the nuclear (800 × *g*) and cytosol (105,000 × *g*) subcellular fractions, for isolated hair follicles (HF) from frontal and occipital scalp.

**Immunohistochemical Detection of Androgen Receptors and 5α-R Type I and II** The immunohistochemical demonstration of androgen receptors and 5α-R type I and II was performed with the standard avidin-biotin-peroxidase complex system (Taylor, 1978), with methodology similar to that previously reported for AROM (Sawaya and Penneys, 1991). Polyclonal rabbit anti-androgen receptor antibody (Santa Cruz, Santa Cruz, CA) was used at a dilution of 1:100. For 5α-R type I and II, polyclonal rabbit antibodies were raised against synthetic peptides corresponding to the amino acids 210–240 for type I and amino acids 220–250 for type II as described by Luu-The *et al* (1994). Specificity for each enzyme was assessed by western blot analysis showing specific bands for each type I and II isoenzyme forms.

**Statistical Analysis** Assessing AROM for initial velocity was determined in triplicate from least squares linear regression analysis of tritiated water production *versus* time. 5α-R and AROM enzyme activities were measured in duplicate and are expressed as pmoles of product formed per 0.5 mg of protein.

The maximal specific activity and apparent $K_m$ for the enzymes 5α-R I and II and AROM was determined and the Lineweaver-Burk plot and linear least squares regression analysis were performed with a Hewlett-Packard HP41C computer with Statpac. Data (mean ± SD) were analyzed and Student's t tests and analysis of variance were used to compare differences between the groups concerning all descriptive data.

## RESULTS

**Androgen Receptor** The action of androgens are mediated by the androgen receptor, which modulates the transcription of androgen-responsive genes thought to play a major role in AGA (Sawaya, 1989, 1992). Therefore, total receptor levels were measured and found to be 30% greater in the frontal hair follicles than occipital follicles of both women and men **(Table I)**. The total receptor content in females was approximately 40% less than the amount found in males, for both frontal and occipital regions, indicating that women have less receptors in their hair follicles than men with AGA.

***In Vitro* Analysis of 5α-R Type I and II** Isoenzymes may be difficult to distinguish under general *in vitro* incubation conditions using physiologic pH with general reduced cofactors, i.e., pH 7.4 and NADH (Sawaya *et al*, 1984; Itami *et al*, 1990).

Optimum pH and cofactor requirements are described in **Fig 1** and **Table II**, respectively, to distinguish type I from type II *in vitro* enzyme activity. A bimodal pattern of 5α-R activity from frontal follicles showed large peaks of 5α-R activity at pH range of both 8.0 and 6.0, respectively, indicating greater enzyme activity for both type I and II 5α-R isoenzymes. Occipital follicles revealed the type I 5α-R activity at pH range 8.0, with very low detectable levels of type II found at pH 6. The use of the NADPH-generating system gave 30–40% greater reduction of testosterone to DHT in comparison to NADPH alone.

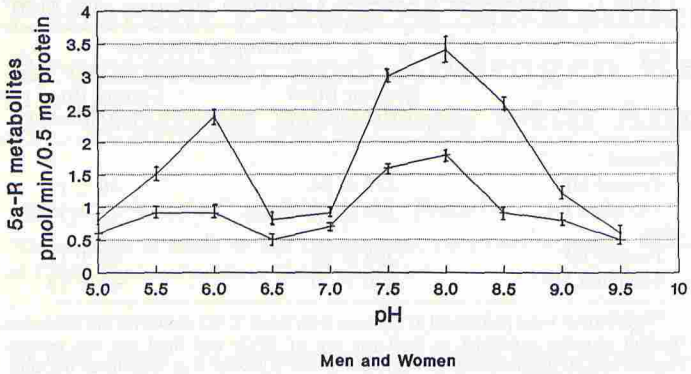


**Figure 1. pH optimum for 5α-R in men and women.** Nuclear (800 × *g*) and microsomal (105,000 × *g*) fractions of 50 μg of hair follicle protein were incubated for 30 min at 37°C in the presence of an NADPH-generating system **(Table I)**. The top curve is from frontal hair follicles of three men and three women; the bottom line is from occipital hair follicles of same patients. *Error bars,* SD (n = 3).

Establishing the optimum assay conditions resulted in finding differences in the isoenzymes in scalp hair follicles, as shown in **Table III**, in which type I is four to five times higher than type II in both women and men. Both isoenzyme forms were significantly higher ($p < 0.001$) in frontal than occipital follicles for women and men. Women had approximately 3 to 3.5 times less type I and II 5α-R, respectively, than hair follicles of men.

When comparing frontal to occipital hair follicles within women, results indicate approximately 40% more type I and type II isoenzymes in frontal than occipital follicles ($p < 0.001$). Frontal follicles in men had approximately 60% higher levels of both isoenzymes than occipital follicles ($p < 0.001$). The results indicate clear differences between as well as within women and men with regard to frontal *versus* occipital hair follicles.

**AROM** AROM, a microsomal enzyme (Schweikert *et al*, 1975; Berkovitz *et al*, 1984; Zimniski *et al*, 1987; Hickey *et al*, 1989), which converts testosterone and 4-androstenedione to estradiol and estrone, respectively, was found to be much higher in hair follicles of women than men **(Table IV)**. Frontal follicles in women had approximately six times more AROM than male frontal follicles ($p < 0.001$), and female occipital hairs had approximately four times greater AROM levels than hairs from male occipital follicles. In women, frontal follicles had approximately half the AROM of occipital follicles, suggesting that AROM may be an important enzyme limiting the level of androgens in hair follicles of women by converting androgens to estrogens.

**Northern Blot Analysis** Northern blots were performed to verify findings from the biochemical analysis, especially because 5α-R type I and II isoenzymes may overlap under *in vitro* conditions, i.e., when pH and cofactors are not adequately maintained through the incubations.

**Table II. Pyridine Cofactor Requirements by 5α-R[a]**

| Cofactor | 5α-Reduced Products Formed (pmol per 0.5 mg protein) | |
|---|---|---|
| | Type I 5α-R | Type II 5α-R |
| None | 0.33 ± 0.16 | 0.45 ± 0.19 |
| NADPH | 1.55 ± 0.30 | 2.44 ± 0.35 |
| NADPH-generating system | 2.42 ± 0.42 | 3.47 ± 0.38 |

[a] Incubations contained 50 μg of protein and were performed in triplicate, with 1 mM NADPH and an "NADPH-generating system" consisting of 5 μM glucose 6-phosphate, 0.2 μM NADP, and 0.2 unit of glucose-6-phosphate dehydrogenase. The data are the mean ± SD for the specific activity expressed after 30 min of incubation for isolated hair follicles from frontal scalp.

**Table III. Type I and II 5α-R Levels in Hair Follicles from Women and Men[a]**

| | 5α-R | |
|---|---|---|
| | Type I (pH 8.0) | Type II (pH 6.0) |
| Women | | |
| Frontal HF | 22.0 ± 2.8 | 4.6 ± 2.0 |
| Occipital HF | 13.0 ± 1.8 | 2.8 ± 1.0 |
| Men | | |
| Frontal HF | 63.3 ± 2.2 | 17.2 ± 1.4 |
| Occipital HF | 29.4 ± 2.2 | 6.9 ± 1.4 |

[a] Incubations were performed with a NADPH-generating system as described in **Table I**. Incubations were performed in triplicate with the mean ± SD shown for the specific activity expressed as (pmol per 30 min per 0.5 mg protein), for the isolated hair follicles (HF) from frontal and occipital scalp.

Hair follicle mRNA was extracted from all samples for northern blot analysis to verify the biochemical measurements performed for the androgen receptor, 5α-R enzymes, and AROM. **Figure 2*a*** shows results for the 12 women and **Fig 2*b*** shows results for men. Frontal and occipital hair follicle mRNA was normalized for GAPDH. The observed transcripts are described in the literature and indicate that the signals measured are specific (Sambrook *et al*, 1989). **Table V** describes the densitometric scanning results, in which higher levels of mRNA were found for receptor and 5α-R types I and II in frontal follicles from both women and men compared to occipital follicles. In contrast, mRNA probes for AROM show higher levels in occipital compared to frontal follicles from both men and women. mRNA levels densitometrically scanned are described in **Table V**, which gives a relative numerical percent value for mRNA probed that can be correlated with the cellular protein levels of androgen receptors and enzyme levels expressed found by the biochemical assays performed.

**Immunohistochemical Analysis** **Figure 3** shows androgen receptor expression in the outer root sheath and dermal papilla of hair follicles. This specimen was obtained from male frontal scalp, and similar distribution was found for women.

**Figure 4** shows 5α-R type I and II expression in the outer root sheath of hair follicles, with less intense expression in dermal papilla. Overall, androgen receptors and 5α-R types I and II were most apparent in the outer root sheaths of anagen hair follicles for both women and men, with less intense expression in dermal papilla, indicating an important role for outer root sheath in androgen regulation.

## DISCUSSION

The androgen receptor is the key factor that mediates androgen action by its molecular effects on transcription and translation of cellular proteins. Our studies indicate androgen receptor levels were 1.5 times greater in frontal than in occipital hairs in men.

**Table IV. AROM Levels in Hair Follicles from Women and Men[a]**

| Follicles | Aroamtase (pmol per min per 0.5 mg protein) |
|---|---|
| Women | |
| Frontal HF | 18 ± 3.4 |
| Occipital HF | 32 ± 4.0 |
| Men | |
| Frontal HF | 3 ± 2.4 |
| Occipital HF | 9 ± 2.8 |

[a] Incubations were performed in triplicate with the mean ± SD shown for the specific activity expressed as (pmol per min per 0.5 mg protein), for isolated hair follicles (HF) from occipital and frontal scalp.

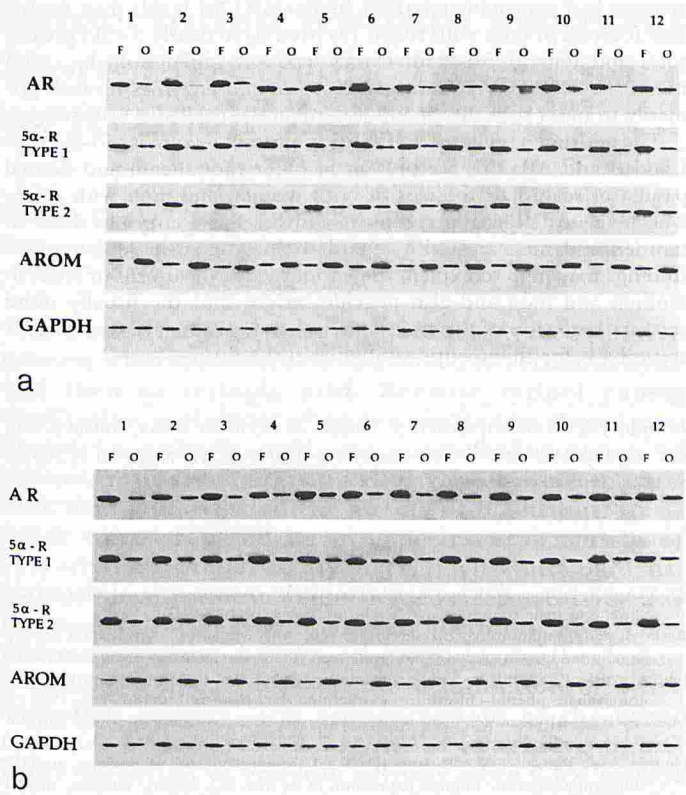


**Figure 2. Northern blot analysis of human hair follicle RNA of women and men.** Autoradiograph showing the transcripts obtained after hybridization of the complete series with anti-sense cRNA probes corresponding, respectively, to androgen receptor and type I and II 5α-R (50 μg of total RNA per lane). Specific transcripts are 9.4 kb for androgen receptor, 2.4 kb for 5α-R type I, 1.0 kb for 5α-R type II, and 2.5 kb for AROM. The filters were reprobed with a GAPDH probe and the data were normalized to the respective levels and showing mRNA levels between women (a) and men (b) for frontal and occipital hair follicles. There were no variations in transcript length recorded whatever the probe or sample used. Exposure time for film development was 10 d. Experiments were performed twice as stated in *Materials and Methods.*

Interestingly, when we compare men to women, we find 1.5 times more receptors in frontal than in occipital follicles.

Other studies have emphasized the importance of type I 5α-R found in skin (Itami at al, 1990, 1991) *versus* type II 5α-R in prostate and testes (Andersson *et al*, 1991; George *et al*, 1991). The

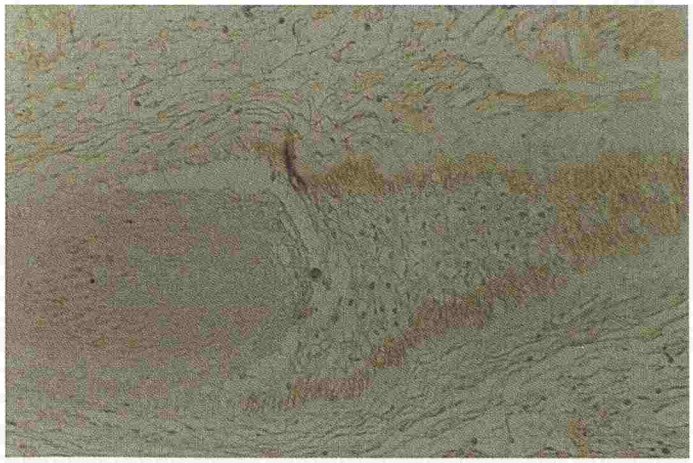

**Figure 3. In this frontal hair follicle, androgen receptor is detected in external root sheath, with less intense expression in dermal papilla.**

current study indicates that both forms of 5α-R may be present in scalp hair follicles. In AGA, the levels of these two isoenzymes may differ in frontal and occipital scalp hair follicles.

Previous work (Thigpen *et al*, 1993) using polyclonal antibodies to 5α-R isoenzymes indicated that type I 5α-R expression had no qualitative difference between adult balding and nonbalding scalp and that type II predominated in fetal genital skin, male accessory glands, and prostate, with no detection in any region of balding adult scalp. It was recently shown in whole skin sections that 5α-R type I is expressed strongly in sebaceous glands and hair follicles, whereas 5α-R type II is expressed in the dermal papilla and interfollicular dermal spindle cells (Russell *et al*, 1996). These studies examined the intensity of 5α-R staining in histologic sections by using polyclonal antiserum obtained by synthesizing amino acid segments of each 5α-R isoenzyme molecule. The studies revealed almost a 3-fold increase in 5α-R levels in frontal region AGA compared to "normal" occipital hair follicles. These findings are similar to ours if we consider occipital hair follicles in our studies as "normal" when we compare women to men with AGA. In this study we also examined the androgen receptor immunohistochemically, which revealed staining localized to outer root sheath and dermal papilla of hair follicles. Studies previously done (Sawaya and Penneys, 1991) for AROM found it to be located in external root sheath, dermal papilla of hair follicles, and sebaceous glands in scalp.

Previous studies (Itami *et al*, 1990, 1991) reported low levels of

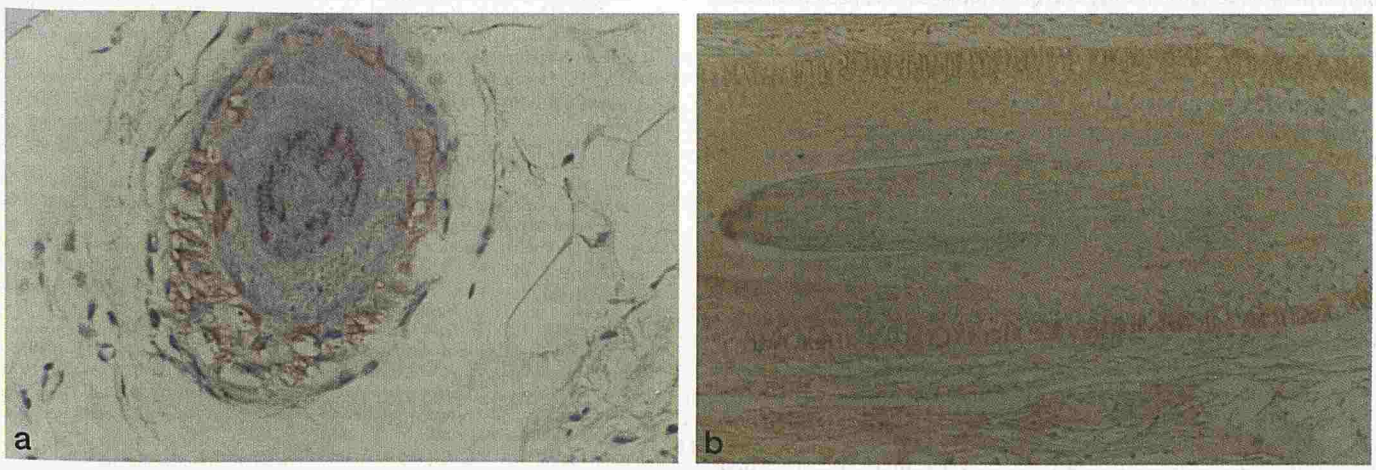


**Figure 4. 5α-R types I *(a)* and II *(b)* reveal frontal hair follicle with expression in outer root sheath with less expression in dermal papilla.** Expression for type I was more intense in outer root sheath than type II.

**Table V. Northern Blot Densitometric Scanning**[a]

| Source | AR[b] | 5α-R Type I | 5α-R Type II | AROM |
|---|---|---|---|---|
| Women | | | | |
| Frontal | 55.1 ± 9.2 | 46.2 ± 6.7 | 22.1 ± 4.8 | 43.5 ± 2.5 |
| Occipital | 28.3 ± 6.7 | 39.4 ± 6.1 | 11.4 ± 2.5 | 54.9 ± 6.0 |
| Men | | | | |
| Frontal | 91.1 ± 3.9 | 74.7 ± 5.8 | 42.4 ± 2.7 | 11.0 ± 1.0 |
| Occipital | 58.2 ± 12.4 | 53.4 ± 4.2 | 29.8 ± 3.8 | 27.0 ± 1.8 |

[a] The examples are expressed as the mean ± SD of sample for percentage values obtained by densitometric scanning after normalization of the intensity settings.
[b] AR, androgen receptor.

type II 5α-R in occipital follicles, perhaps due to methodology used, because there can be instability of NADPH when used alone at low pH levels. In previous animal studies of steroid enzymology (Hsia *et al*, 1983; Sawaya *et al*, 1984), we found that use of a "cofactor-generating system" such as the NADPH-generating system used in these experiments can be very beneficial, resulting in greater 5α-R enzyme activity for both forms of the enzyme, especially type II 5α-R (pH optimum of 6.0 form). It may be that use of such a cofactor-generating system shifts the equilibrium of the reaction in favor of DHT formation, because NADP is being removed with continuous generation of NADPH. In any case, use of cofactor-generating systems may better assess the total level of enzyme in biologic systems to quantitate their *in vivo* potential.

Accordingly, in this study both forms of 5α-R were present in frontal hair follicles of women and men with AGA, and lower but measureable levels were found in occipital, "androgen-resistant" hair follicles. In women, frontal hair follicles had 40% more of both enzyme forms than occipital follicles ($p < 0.001$). Men had 60% more of both enzyme forms in their frontal follicles compared to their occipital hairs ($p < 0.001$). Most importantly, men had nearly to 3.5 times greater 5α-R type I and II, respectively, in frontal hair follicles than women.

Our findings showed that the two isoenzymes have different biochemical characteristics. Type I 5α-R has a pH optimum near 8.0 and a $K_m$ approximately 24 μM, with less cofactor specificity, and is located primarily in the microsomal subcellular fraction. Type II 5α-R has a pH optimum at 6.0 and $K_m$ approximately 0.33 μM and is found in both the nuclear and microsomal fractions.

Finding differences in AROM, which converts androgens to estrogens, revealed levels of AROM significantly different in women *versus* men ($p < 0.001$). Women with AGA had nearly two times higher AROM in occipital compared to their frontal hair follicles, and 6 to 3.5 times higher levels in frontal and occipital sites, respectively, compared to men with AGA.

The level of AROM may be of key importance in limiting the formation of 5α-reduced substrates such as DHT and androstanediol, which have high affinity for the androgen receptor to initiate androgen cellular events. Hence, even though the role of estrogens in human hair growth has not been clearly delineated, it may be that AROM may serve to lower or regulate the level of androgens in the hair follicle (Sawaya and Penneys, 1991).

The low AROM levels may have an effect on the equilibrium of 5α-reduced products being formed. The greater levels of 5α-reduced products may stimulate up-regulation of receptor synthesis, which may have positive or negative effects on transcription and RNA translational processing of new proteins related to hair growth.

Several important conclusions can be drawn from this study. (i) Androgen receptor levels are nearly 1.5 times greater in frontal follicles of women and men than their occipital hair follicles; frontal follicles of women have approximately 40% fewer androgen receptors than frontal follicles of men. (ii) two forms of 5α-R, types I and II, may be present in hair follicles of women and men with AGA, even though type I is thought to be the primary form in skin (Itami *et al*, 1990). (iii) Women with AGA have approximately 40% more total 5α-R in frontal compared to occipital follicles but less than 50% of the levels found in men with AGA. (iv) Frontal follicles of women had approximately 80% higher AROM levels than frontal hair follicles of men with AGA. (v) Men have nearly 3-fold greater total and type II 5α-R in frontal *versus* occipital follicles, with minimal AROM. (vi) Androgen receptor and enzymes localize primarily to outer root sheath and less in dermal papilla in hair follicle.

The androgen receptor and three important enzymes, 5α-R types I and II and AROM, are present in outer root sheath and dermal papilla of scalp hair follicles of both women and men with AGA. The levels of androgen receptors and the three enzymes differ in women and men and with regard to specific scalp regions. Our findings may help to explain the various clinical patterns of AGA in women and men and also provide insight into the usually more severe expression of this trait in men than in women as well as offer some basis for therapeutic strategies in the future.

*In memory of my brother, Herbert S. Sawaya, M.D., whose humor, brilliance, love, and inspiration will always be a part of me. This work was supported in part by National Institutes of Health Grant R29 AR41924.*

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