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Introduction

Benign prostatic hyperplasia (BPH) is a disease associated with aging, with >210 million cases worldwide; nearly all men will encounter some clinical lower urinary tract symptoms (LUTS) in their lifetime. However, African American (AA) men have a higher incidence of BPH with increased incidence of non-surgical treatment failure, larger prostates at time of surgery, and surgery occurring at a younger age. The Flint Men's Health Study was the first report of LUTS in AA men. The study found AA men score similarly to Caucasian American (CA) men on the AUASS; however, AA men were more likely to report significant bother from their symptoms and were significantly less likely to be diagnosed with an enlarged prostate. Out of 127 patients reporting significant bother, only 11 (3%) reported having been medically managed for their symptoms. This study examines the changes in estrogen receptors alpha and beta (ER α , ER β) and steroid metabolism genes due to race, age, location, and disease.

Hypothesis

We hypothesize that steroid metabolism enzymes associated with estrogens are differentially expressed between AA and CA men. With BPH, the expression of these proteins are further dysregulated, leading to differing treatment responses and contributes to the racial disparity.

Materials and Methods

- Surgical specimens from 69 men were obtained from the biorepository at UT Southwestern and the University of Pittsburgh
- 23 were normal transition zone control samples, 49 were BPH samples (Both normal adjacent and BPH tissue was obtained from 3 patients)
- Steroid hormone-related protein expression was identified using multispectral quantitative multiplex IHC on one FFPE tissue section
- Proteins of interest: ER α , ER β , CYP7B1, AKR1C1, and COX-2
- Tissue segmentation, as well as fluorophore unmixing and quantification was accomplished through InForm[®] software
- Cell and tissue segmentation was used to examine protein localization through system training to differentiate stroma from epithelium
- Staining intensity of each fluorophore as a measure of expression was quantified by the optical density of each respective chromogen per unit area in pixels
- All analysis in present study examined differences in epithelial expression of targets of interest
- Comparisons between normal prostate and BPH, and CA and AA groups were analyzed using a two-tailed students t-test for proportions.
- Statistical analysis was performed using GraphPad Prism.

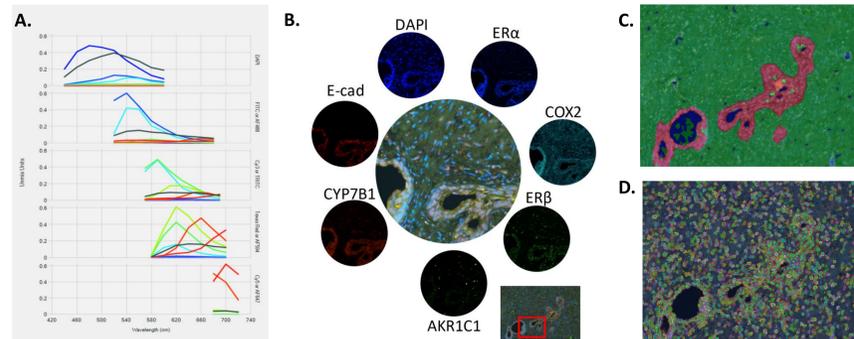


Figure 1. Image processing of multispectral, multiplex IHC. A. Spectral library of Opal stains account for autofluorescence inherent to tissues. B. Spectral unmixing using the spectral library allows for quantitation of each protein. Area represented in red inset. C. Machine learning algorithms using E-cadherin as a marker to segment epithelium (pink) from stroma (Green). D. Using the DAPI nuclear stain, cell segmentation identifies nucleus, cytoplasm, and membrane.

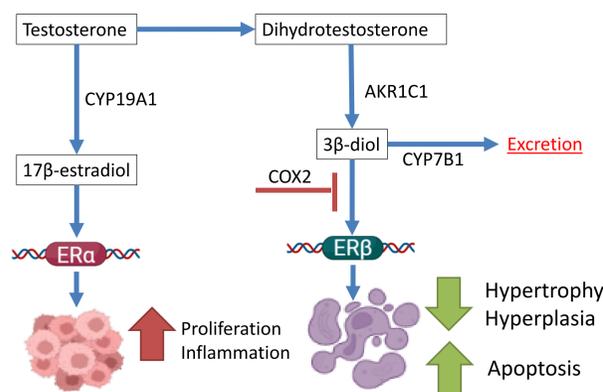


Figure 2. Androgen metabolism generates ER α and ER β ligands in the prostate. Androgen metabolism in the prostate has the capacity to produce estrogenic steroids that stimulate either ER α or ER β , depending on the predominate pathway. ER α stimulation leads to pro-proliferative changes in prostate, while ER β stimulation is implicated in anti-proliferative processes. COX2, a product of the arachidonic acid pathway, inhibits the action of ER β . CYP7B1 metabolizes 3 β -diol, leading to a decreased stimulation of ER β . Imbalances in this estrogenic pathway have been implicated in the pathogenesis of benign prostatic hyperplasia.

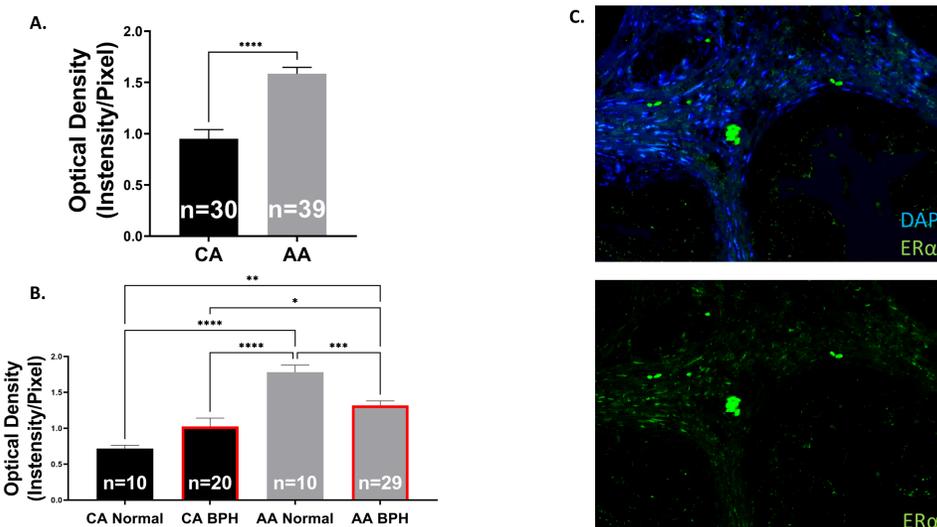


Figure 3. Multiplex fluorescent IHC shows altered expression of estrogen pathway genes between CA and AA prostates. A. ER α expression in prostate epithelial cells stratified by race. B. ER α expression in prostate epithelial cells stratified based on race and disease state C. Representative single-channel image of prostate (Blue-DAPI; Green- ER α)
 *, p < 0.05; **, p < 0.01; ***, p < 0.005; ****, p < 0.0001

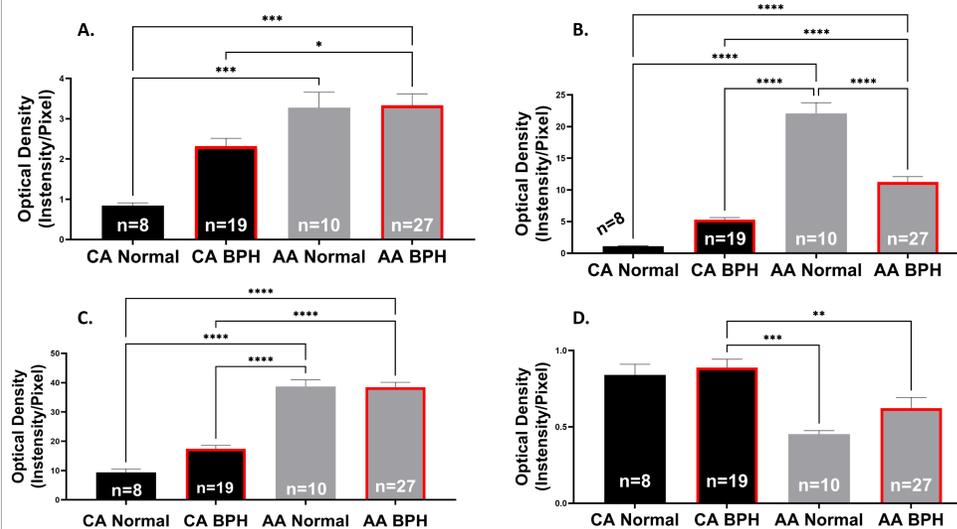


Figure 4. Multiplex fluorescent IHC shows altered expression of estrogen pathway genes in the epithelium in CA and AA prostates. A. ER β expression. B. AKR1C1 expression C. CYP7B1 expression. D. COX2 expression
 *, p < 0.05; **, p < 0.01; ***, p < 0.005; ****, p < 0.0001

Results

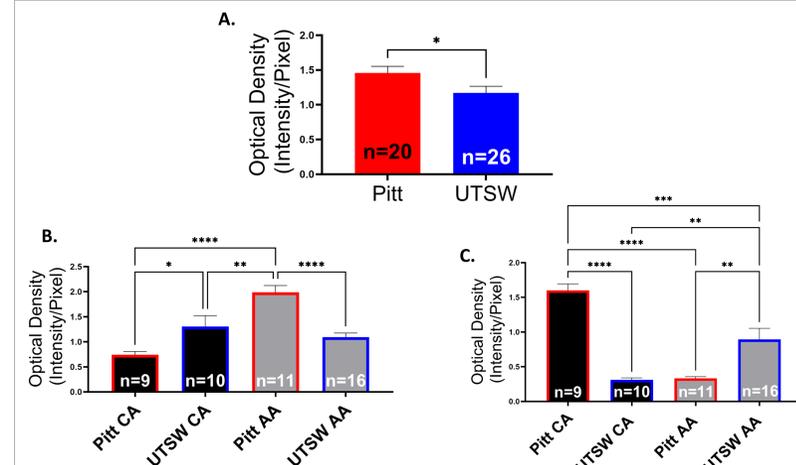


Figure 5. Alterations in ER α expression in patient samples when stratified by race. A. Difference in ER α expression seen between locations where tissue samples were obtained. B. Stratification of ER α expression based on race and location C. Stratification of COX2 expression based on race and location.
 *, p < 0.05; **, p < 0.01; ***, p < 0.005; ****, p < 0.0001

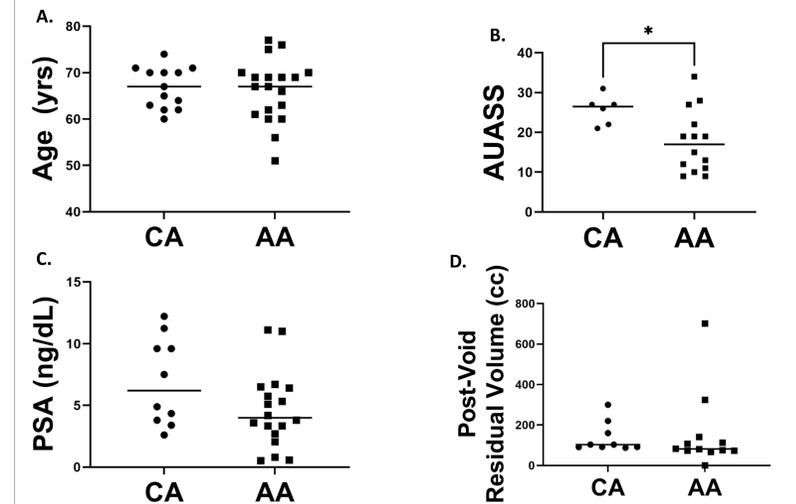


Figure 6. Comparison of clinical characteristics between race. A. Age B. AUASS C. PSA D. Post-Void Residual Volume; *, p < 0.05

Conclusions

- There is a racial difference in steroid metabolism enzymes affecting the expression of ER α and ER β
- Dysfunction in this metabolic pathway of estrogen may play a role in the regulation of ER α and ER β , complicating the treatment strategies targeting the estrogen pathway
- There is an upregulation of ER α and downregulation of COX2 in the Pittsburgh cohort that is not seen in the Texas cohort, suggesting that environmental exposures may be playing a role in susceptibility for estrogen dysregulation.

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