# Surgical Intervention for Symptomatic Benign Prostatic Hyperplasia is Correlated With Expression of the AP-1 Transcription Factor Network

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**BACKGROUND.** Approximately one-third of patients fail medical treatment for benign prostatic hyperplasia and associated lower urinary tract symptoms (BPH/LUTS) requiring surgical intervention. Our purpose was to establish a molecular characterization for patients undergoing surgical intervention for LUTS to address therapeutic deficiencies.

**METHODS.** Clinical, molecular, and histopathological profiles were analyzed in 26 patients undergoing surgery for severe LUTS. Incidental transitional zone nodules were isolated from 37 patients with mild symptoms undergoing radical prostatectomy. Clinical parameters including age, prostate volume, medication, prostate specific antigen, symptom score, body mass index, and incidence of diabetes were collected. Multivariate logistic regression analysis with adjustments for potential confounding variables was used to examine associations between patient clinical characteristics and molecular targets identified through molecular profiling.

**RESULTS.** Compared to incidental BPH, progressive symptomatic BPH was associated with increased expression of the activating protein-1 transcription factor/chemokine network. As expected, inverse correlations were drawn between androgen receptor levels and age, as well as between 5α-reductase inhibitor (5ARI) treatment and tissue prostate specific antigen levels; however, a novel association was also drawn between 5ARI treatment and increased *c-FOS* expression.

**CONCLUSIONS.** This study provides molecular evidence that a network of pro-inflammatory activating protein-1 transcription factors and associated chemokines are highly enriched in symptomatic prostate disease, a profile that molecularly categorizes with many other chronic autoimmune diseases. Because 5ARI treatment was associated with increased *c-FOS* expression, future studies should explore whether increased activating protein-1 proteins are causal factors in the development of symptomatic prostate disease, inflammation or resistance to traditional hormonal therapy. *Prostate 74:669–679, 2014.* 

Abbreviations: AP-1, activating protein-1; AUASS, American Urological Association Symptom Score; BPH, benign prostatic hyperplasia; LUTS, lower urinary tract symptoms; BMI, body mass index; 5ARI, 5 alpha reductase inhibitor.

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## INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is a stromal and epithelial proliferation of the periurethral prostate [1]. Clinical manifestations of Lower Urinary Tract Symptoms (LUTS) secondary to BPH increases approximately 10% per decade of life after 50 years of age and include frequency, urgency, nocturia, hesitancy, and incomplete voiding [2]. According to the American Urological Association Symptom Score (AUASS) patient questionnaire, LUTS are graded as mild (0–7), moderate (8–20), or severe (21–35) [3]. EAU and AUA guidelines for the treatment of BPH suggest that medical intervention be discussed with patients when symptoms become moderate [4,5].

First line medical therapy for symptomatic BPH frequently involves treatment with $\alpha_1$-adrenergic blockers (α-blockers) to relax smooth muscle tone [6,7]. If α-blockers do not adequately reduce symptom severity, 5α-reductase inhibitors (5ARI) may be administered to inhibit dihydrotestosterone (DHT) production and androgen receptor (AR) signaling, decreasing prostatic volume [8]. 5ARI's may also be chosen as first line therapy in certain patients, particularly those with large prostates. According to several studies, approximately one-third of patients respond to these therapies individually, while approximately two-thirds of patients respond to combination therapy with both α-blockers and 5ARIs [9–12]. Nevertheless, a significant number of patients will become refractory to existing medical treatments, often then requiring surgical intervention. Due to these clinical deficiencies, a new therapeutic strategy that targets the underlying cause of BPH as well as associated LUTS is needed.

Several studies have drawn correlations among LUTS, inflammation and resistance to medical therapy, but a clear molecular etiology for the pathogenesis of BPH/LUTS has yet to be outlined, making it difficult to develop new approaches [13–15]. The purpose of this study was to establish a molecular signature associated with the progression of symptom severity to surgical intervention. We compared prostate tissues from a cohort of patients who underwent surgery for moderate to severe BPH/LUTS to a cohort of patients with, mildly symptomatic BPH incidental to radical prostatectomy for prostate cancer. Results from microarray analysis, qPCR, and histopathology revealed that prostate tissues from patients that progress to require surgery for BPH/LUTS have increased activating protein-1 (AP-1) transcription factor expression compared to BPH tissue from mildly symptomatic men. AP-1 proteins are a family of hetero- and homodimer transcription factors that belong to the JUN (c-JUN, JUNB, and JUND), FOS (c-FOS, FOSB, FRA-1, and FRA-2) and activating transcription factor (ATF2, ATF3, and B-ATF) families. These proteins are known to regulate innate immune and inflammatory responses and induce proliferation upon upstream activation by growth factors and stress signals [16–18] and may represent a novel molecular etiology for inflammation, proliferation, resistance to therapy and progression to surgery in BPH/LUTS.

## MATERIALS AND METHODS

### Patients

Prostate specimens used in this study were obtained from 26 patients undergoing holmium laser enucleation of the prostate (HoLEP) for symptomatic BPH (referred to as "Surgical BPH"). In addition, transitional zone nodules were isolated from 37 patients undergoing radical prostatectomy for small volume, low risk, clinically localized prostate cancer (referred to as "Incidental BPH") at Vanderbilt University Medical Center (Nashville, TN) from January 2012 to March 2013. Institutional Review Board approval was obtained for medical record review to collect retrospective clinical and pathological data. Study data were collected and managed using REDCap electronic data capture tools hosted at Vanderbilt University. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies (http://www.sciencedirect.com/science/article/pii/S1532046408001226).

### Tissue Processing and Pathology

After gross pathological examination, all prostate samples used for this study were stored at 4°C and processed within 24 hr. Processing of samples involved flash freezing in liquid nitrogen followed by storage at −80°C until use, as well formalin fixation for paraffin embedding. Samples were reviewed by pathologist (O.H.) to confirm histologic findings and exclude samples with any foci of cancer.

### Microarray, qPCR, Western Blot, and IHC

For microarray analysis, 50 mg flash-frozen tissue was ground by mortar and pestle in liquid nitrogen and RNA was extracted with Trizol (Ambion, Austin,

TX) from 10 Surgical BPH specimens and 10 Incidental BPH samples and stored at −80°C. Samples were hybridized to an Affymetrix Human Gene 2.1 ST 24-array plate and scanned using the Affymetrix Gene Titan AGCC v.3.2.4 followed by analysis on the Affymetrix Expression Console v.1.2 using a RMA normalization algorithm producing log base 2 results.

For qPCR, RNA was extracted as described above from 26 Surgical BPH and 37 Incidental BPH specimens. Subsequently, 500 ng RNA was reverse transcribed into cDNA using RT$^2$ First Strand Kit (SABiosciences, Valencia, CA). qPCR was performed using IQ SYBR Green Supermix (BioRad, Hercules, CA) at 58°C for 45 cycles and results were analyzed using BioRad CFX manager software. All results were calculated using ΔΔCt analysis and normalized to *GAPDH* expression. Primer sequences are listed in supplementary figure 2.

For Western blotting, approximately 50 mg of flash-frozen human prostate tissue was ground in liquid nitrogen using a mortar and pestle. Protein was extracted with 2% SDS buffer and 30 μg protein was run on pre-made 10% polyacrylamide gels (Life Technologies, Grand Island, NY). Primary antibodies were incubated in 5% BSA in TBST overnight at 4°C and included β-actin (1:10,000, Sigma, St. Louis, MO) and androgen receptor (1:500, Santa Cruz Biotechnology, Santa Cruz, CA). Phospho ERK1/2, phospho JNK1/2, phospho p38 as well as cyclin D1, phospho NFκB p65 (Ser 276) were purchased from Cell Signaling (Beverly, MA) and used at 1:1,000. Secondary antibodies in 5% milk in TBST were incubated for 45 min at room temperature.

Immunohistochemistry was performed as previously described [19]. Briefly, 5 μM sections were dewaxed, rehydrated, and endogenous peroxidases were blocked with hydrogen peroxide. Sections were then boiled in citrate and blocked in 5% serum for 1 hr. Primary antibodies were incubated overnight at 4°C and included the following: desmin (1:2,000, Sigma), c-JUN (1:500, Santa Cruz), and c-FOS (1:500, Santa Cruz).

### Statistical Analysis

For analysis of microarray data between Surgical BPH and Incidental BPH patients, we used *t*-test with Bonferonni and step-up multiple testing corrections to reduce the chance of a false-positive finding. Networks and canonical pathway maps were generated using INGENUITY software (https://apps.ingenuity.com). Wilcoxon signed rank and chi-square ($\chi^2$) tests were used to compare median values and categorical levels, respectively, between Surgical and Incidental BPH groups. We calculated mean analyte levels within each group after adjusting for age (continuous), BMI (continuous), use of a 5ARI (Y/N) or α-blocker (Y/N) or diagnosis of diabetes mellitus (Y/N) in a linear regression model. Distributions of AR approached a normal distribution, with low kurtosis and skewness, thus did not require transformation to meet statistical assumptions. Other analyte distributions were normalized through natural log transformation prior to analysis, and we report adjusted analyte levels after back transformation. Statistical analysis was performed with SAS (Cary, NC) and $P < 0.05$ was considered statistically significant.

## RESULTS

### Surgical BPH Patients Are Clinically Distinct From Incidental BPH Patients

Table I shows a comparison of the clinical characteristics of patients who underwent surgical intervention for symptomatic BPH ("Surgical BPH" cohort) versus patients who had transitional zone BPH nodules found incidentally on gross examination after radical prostatectomy for prostate cancer ("Incidental BPH"

**TABLE I. Clinical Characteristics of Incidental and Surgical BPH Cohorts**

| Characteristic | Incidental BPH | Surgical BPH | *P*-value |
|---|---|---|---|
| Age, year, median (n, IQR) | 61 (37, 56–65) | 67 (26, 62–71) | <0.002 |
| Prior systemic therapy | | | |
| Alpha blocker, yes (n, %) | 10 (37, 27) | 21 (25, 84) | <0.005 |
| 5α-reductase inhibitor, yes (n, %) | 6 (37, 16) | 17 (25, 65) | <0.005 |
| PSA level, ng/ml, median (n, IQR) | 4.8 (37, 4.3–6.4) | 6.5 (17, 3.9–9.9) | =0.488 |
| Prostate volume, cm$^3$, median (n, IQR) | 41 (30, 30–55.9) | 99 (24, 67.6–130) | <0.001 |
| AUASS, median (n, IQR) | 6 (37, 3–12) | 23 (24, 18–26.5) | <0.001 |
| BMI, median (n, IQR) | 28 (37, 56–65) | 29.5 (26, 3.4) | =0.118 |
| Diabetes mellitus, yes (n, %) | 5 (37, 14) | 7 (26, 27) | =0.182 |

n, number counted; IQR, interquartile range; PSA, prostate specific antigen; IPSS, International Prostate Symptom Score; BMI, body mass index.

cohort). The Surgical BPH cohort was significantly older ($P < 0.002$), displayed higher prostate volume ($P < 0.001$), and higher AUASS ($P < 0.001$), but were not statistically different as determined by circulating PSA levels ($P = 0.488$) or body mass index (BMI, $P = 0.118$). Surgical BPH patients were also more likely than Incidental BPH patients to be on individual medical therapy with α-blockers (31% vs. 17%) or 5ARIs (15% vs. 6%), or on combination medical therapy (50% vs. 11%).

### Surgical BPH Specimens Are Histologically Distinct From Incidental BPH Specimens

Embryonic urogenital mesenchyme instructs epithelial differentiation [20], and BPH has long been thought to result from a reawakening of these stromal-epithelial interactions [1]. Even in the absence of a full molecular profile, numerous stromal and epithelial factors have been implicated in the etiology of BPH/LUTS including hormones, chemokines, and growth factors, as well as downstream effects of systemic metabolic diseases [21–23]. As illustrated in Figure 1, a histopathological survey of our Incidental versus Surgical BPH specimens typically demonstrated a loss of smooth muscle differentiation (Fig. 1) suggesting our patient population and tissue were similar to those studied previously [15,24–27]. Confirmation of increased fibrosis and decreased smooth muscle differentiation was demonstrated by Masson's trichrome staining (Fig. 1C, D) and immunoreactivity for the

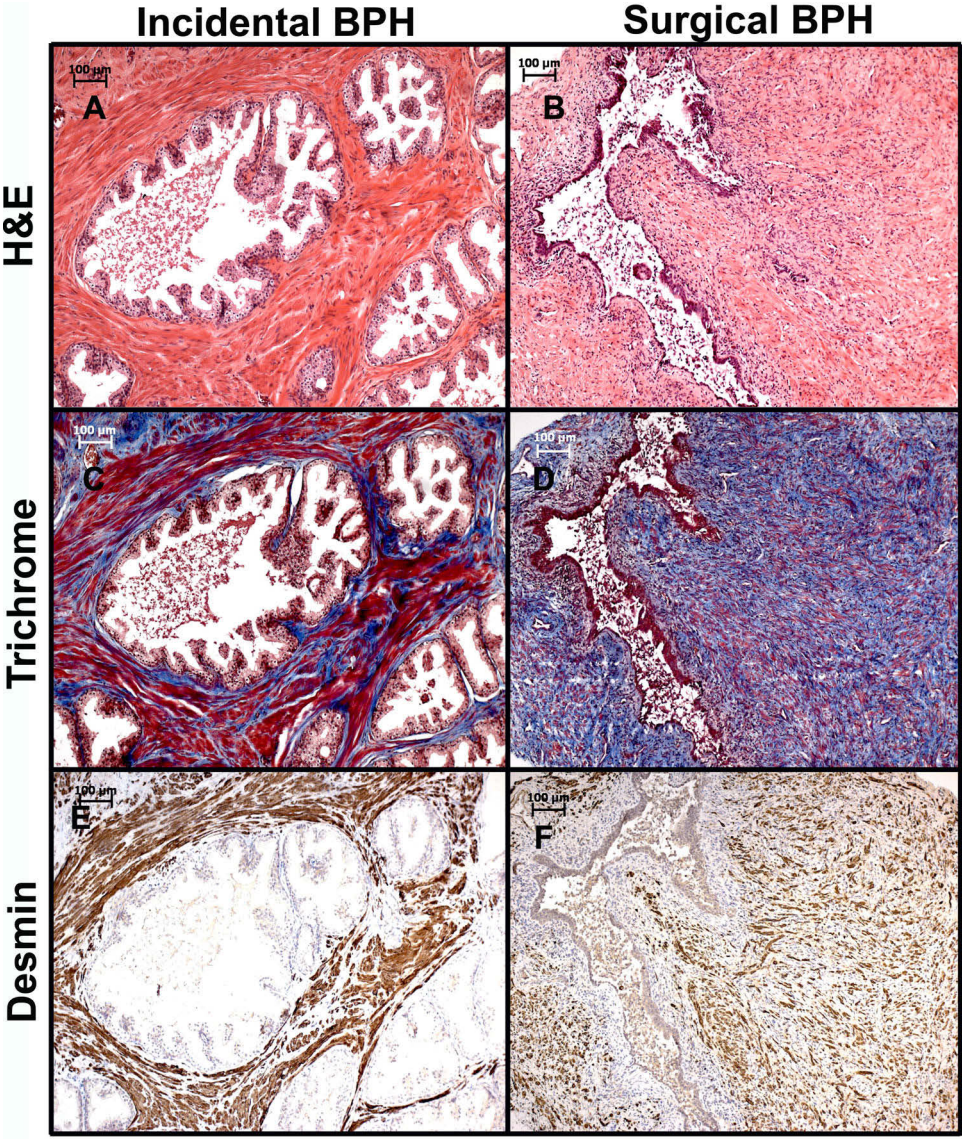


**Fig. 1.** Histological analysis of Surgical BPH specimens reveals reduced smooth muscle differentiation in Surgical versus Incidental BPH as shown by H&E (**A**, **B**), Masson's trichrome (**C**, **D**), and desmin immunoreactivity (**E**, **F**).

late-stage smooth muscle marker desmin (Fig. 1E, F). These data qualitatively confirm the quantitation of increased collagen content in symptomatic BPH performed previously [15]. The decreased desmin immunoreactivity was confirmed by microarray profiling of multiple samples as shown below.

### Surgical BPH Specimens Are Molecularly Distinct From Incidental BPH Specimens

To gain a molecular understanding of symptomatic BPH, we performed microarrays on 10 Surgical BPH and 10 Incidental BPH samples. After unsupervised hierarchical clustering of statistically significant genes, INGENUITY Systems Interactive Pathway Analysis (IPA) revealed alterations in a number of pathways as shown in Table II. Specific categories sorted by *P*-value and activation z-score (measures of whole pathway activation) show that Surgical BPH tissue is molecularly distinct from Incidental BPH in its inflammatory response, proliferation of stromal and epithelial cells, and angiogenesis. The top 30 statistically significant up- and down-regulated genes are listed in Supplementary figure 1.

As links between inflammation and fibrosis in BPH/LUTS are established [13,15], the decrease in smooth muscle differentiation markers provided confidence that the tissue and data set were reliable. However, we were particularly intrigued that a third of the top genes most upregulated in Surgical BPH specimens were members of the activating protein-1 (AP-1) family of transcription factors and their downstream chemokines (shown by nodal network analysis in Figure 2). These proteins are known to regulate proliferation upon upstream activation by growth factors and stress signals (reviewed in [28]). Because of the potential novelty for AP-1 factors as an etiological factor driving many characteristics in progressive BPH/LUTS, we decided to focus this study on the changes in a few of the AP-1 family members.

To confirm the increase in AP-1 protein expression and determine tissue specificity, we performed immunohistochemistry for c-JUN and c-FOS in Surgical and Incidental BPH samples. As shown in representative images in Figure 3, Incidental BPH tissues displayed mild expression of AP-1 factors, predominantly restricted to basal epithelium. However, in Surgical

**TABLE II. Top Functional Networks Altered in Surgical Versus Incidental BPH Tissues as Generated by Ingenuity Pathway Analysis Software**

| Associated network functions | IPA score |
|---|---|
| Cellular movement, cardiac hypertrophy, cardiovascular disease | 32 |
| Cancer, endocrine system disorders, gastrointestinal disease | 30 |
| Cancer, cell death and survival, cell morphology | 28 |
| Inflammatory response, connective tissue disorders, inflammatory disease | 27 |
| Cell death and survival, cellular movement, cellular growth and proliferation | 25 |

| Category | Functions annotation | *P*-value | Activation z-score | Molecules |
|---|---|---|---|---|
| Inflammatory response | Chemotaxis of monocytes | 6.30E-03 | 3.043 | ANXA1,CCL2,CCL3,CCL3L1/CCL3L3, CCL4,CCL8,IL8,PLAUR,SELE,SERPINE1 |
| Skeletal and muscular system development and function | Proliferation of smooth muscle cells | 2.10E-07 | 2.531 | CCL2,CCL3,EDN1,FHL1,FOS,HBEGF, HSPD1,IGF1,IL1A,IL6,IL8,mir-10,NR4A3, PLAUR,SERPINE1,THBS1,TNFAIP3, TRIB1,WISP2 |
| Cardiovascular system development and function | Angiogenesis | 1.77E-07 | 2.33 | ADM,ANGPT1,BMP2,BMPR1A,CAV1,CCL2, CH RNA7,COL4A3,CRYAB,CYR61,DCN, EDN1,FBLN 2,FLNA,FN1,HOXA1, HOXD10,IL1A,IL6,IL8,ITG Al,ITGB3, KCNMAl,KLF2,mir-10,NR4Al,PLAU R, PRKCA,PRKG1,PTGS2,PTX3,SELE, SERPINE1, SLIT2,SULF1,TGFB2,THBS1, TRPC1,TRPC4 |
| Cellular growth and proliferation | Proliferation of cancer cells | 1.02E-04 | 1.537 | BAMBL,CAV1,CYR61,DCN,DDIT3,EDN1, EGR1, FOS,ICMT,IGFl,IL6,ILK,mir-21, MYC,NKX3-l,N R4A2,PLAUR,PRKCA, RXRA,TFF3,TGFB2,THBS1 |

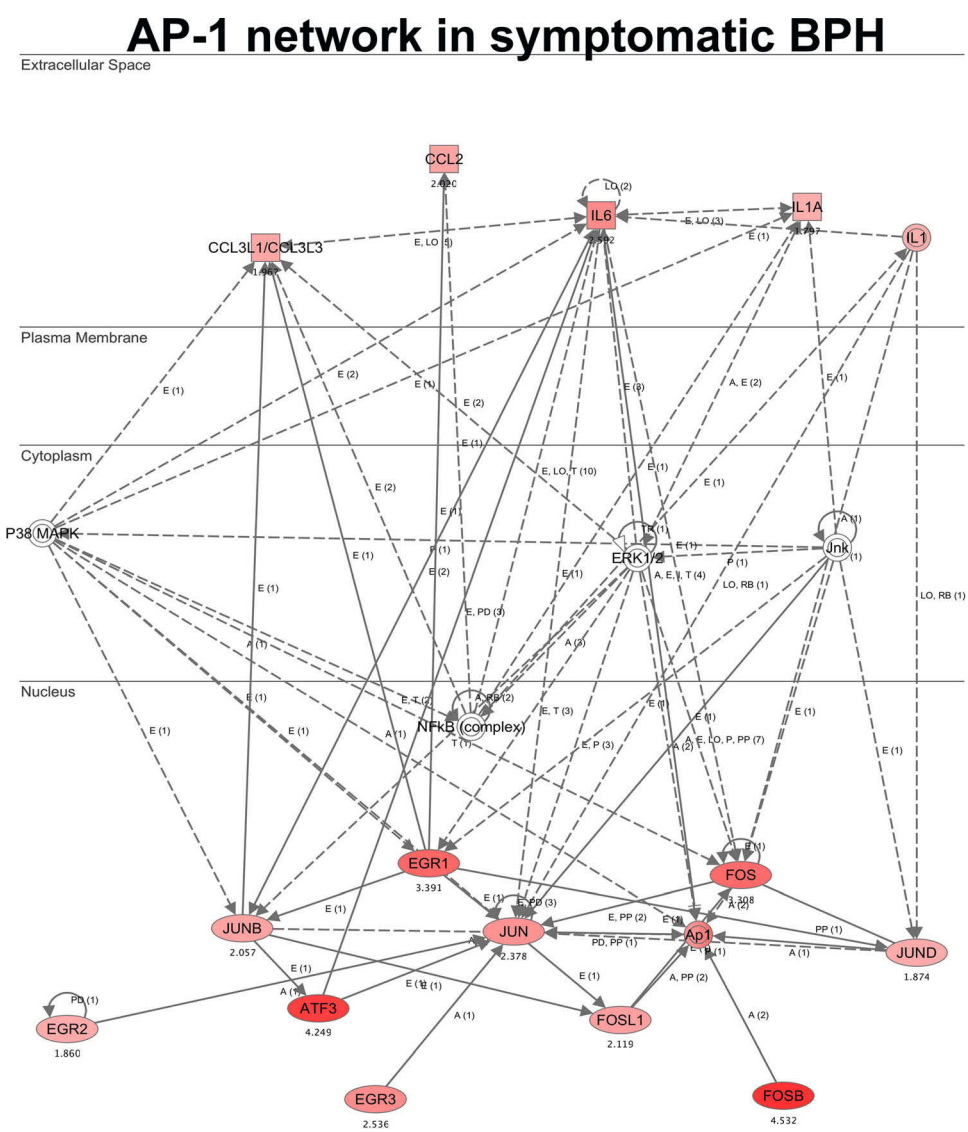


**Fig. 2.** Network analysis of *AP-1* transcription factors and chemokines upregulated in surgical BPH specimens. Intensity of red shading and numbers directly below indicate fold increase over Incidental BPH specimens.

BPH samples, c-JUN, and c-FOS were dramatically increased in basal epithelium as well as in stroma (arrows).

AP-1 transcription factors are post-translationally regulated by upstream activators such as NFκB, JNK, ERK, and p38 (shown by Network analysis in Fig. 2) [29]. Therefore, we also determined by Western blot whether any of these upstream signaling nodes were activated. As shown in Figure 3E, Surgical and Incidental BPH were similar in their activation of NFκB and p38, but Surgical BPH specimens displayed higher JNK and ERK activation as well as cyclin D1 expression, a known downstream mediator of AP-1-mediated proliferation [28].

### Statistical Correlations for AP-1 Transcription Factors and Chemokines by Group and Medical Treatment

To provide broader confirmation of the microarray data, we performed qPCR on select *AP-1* genes (*c-JUN* and *c-FOS*) and chemokines (*IL-6* and *IL-8*), as well as markers of prostate differentiation such as *p63*, *AR*, and *PSA* by group comparison of 26 Surgical BPH samples and 37 Incidental BPH samples (primer sequences listed in Supplementary figure 2). Analyte levels were adjusted for age, BMI, diabetes mellitus, and usage of α-blockers or 5ARIs. As shown in Table III, *AR* mRNA expression levels were similar

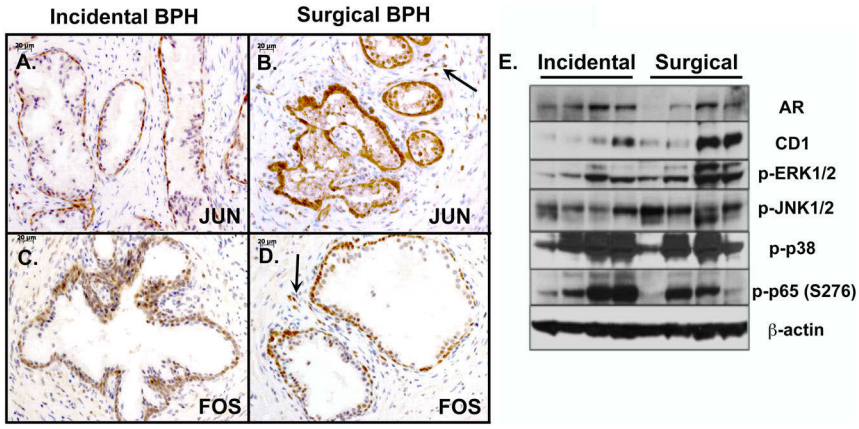


**Fig. 3.** Expression of AP-1 transcription factors in Surgical versus Incidental BPH. Representative IHC images demonstrate increased epithelial and stromal cell expression of c-JUN (**A** vs. **B**), c-FOS (**C** vs. **D**). Western blot analysis displays increased activation of JNK and ERK as well as increased cyclin DI expression in Surgical BPH specimens (**E**).

between the two groups ($P = 0.345$). While tissue *PSA* levels were similar between the two groups ($P = 0.621$), levels were expectedly lower in patients who were treated with 5ARIs ($P = 0.046$). *c-FOS* and *c-JUN* were significantly increased in the Surgical versus the Incidental group ($P < 0.001$), but intriguingly only *c-FOS* was associated with 5ARI treatment ($P = 0.040$). While *IL-6* ($P = 0.041$) was significantly higher in the Surgical BPH group, *IL-8* did not significantly differ between the groups and α-blocker use did not significantly effect any of the analytes analyzed. Since we had observed enhanced expression of *AP-1* factors in the basal epithelium of incidental BPH specimen (see Fig. 3), we also examined the basal cell marker *p63*, which did not show significant differences by any analysis.

## DISCUSSION

The estimated yearly costs related to medical and surgical intervention for BPH/LUTS in the US is nearly $4 billion [30]. Despite these statistics, a clear molecular etiology has not been established for BPH/LUTS. This is particularly relevant given the gap in therapeutic efficacy in approximately one-third of patients on combination therapy [11]. Given the high incidence and progression of LUTS [31] and the likely increase in the number of men afflicted due to an increase in lifespan and the prevalence of metabolic diseases [32,33], this represents a very large number of patients whose disease is not well controlled medically (approximately 34%) or who suffer adverse side effects leading to discontinued use (approximately 18% for combination therapy) [11]. In order to avoid urinary retention after progression to resistance to medical therapy, surgical intervention is used to alleviate symptoms. However, given the age profile of the patients, this is not always an ideal option. Understanding the pathobiology of BPH may permit us to offer better disease prevention and/or non-surgical therapy in these men who are often elderly and suffer from a wide range of co-morbid conditions.

Generating a molecular profile of symptomatic BPH has been particularly challenging in regards to the acquisition of both symptomatic tissue and "normal" controls for comparison. Limited by the practical concern that men without cancer or severe LUTS do not easily cede their prostates, we are relegated to comparing molecular profiles of severely symptomatic patient tissue to mildly symptomatic transitional zone nodules taken from prostate cancer patients, a surgical distinction that is routine practice in other studies of this disease [15,24–26]. Although it may be reasonable to assume the possibility of some sort of field effect due to the presence of peripheral zone cancer in our "Incidental" BPH tissues, this effect would presumably tend to reduce the comparative difference with symptomatic prostate tissue.

Using molecular and clinical profiling of symptomatic BPH patients, we demonstrate that expression of AP-1 transcription factors is associated with surgical intervention for BPH/LUTS. Insights into the functional roles of AP-1 factors in development and disease have shown aberrant regulation of AP-1 factors is associated with immune inflammatory diseases such as psoriasis, allergic asthma, chronic obstructive pulmonary disease, inflammatory bowel disease, type 2 diabetes, atherosclerosis, and rheumatoid arthritis [16,17]. In each of these diseases, chemotactic proteins and cytokines attract innate and adaptive immune cells, triggering an inflammatory cascade mediated in part by AP-1 transcription factor complexes.

**TABLE III. Statistical Analysis of Analyte Levels Compared by Group or Medical Treatments**

| I. Analyte | Group | | |
|---|---|---|---|
| | Incidental | Surgical | *P*-value |
| *AR* | 4.3 (3.4–5.1) | 4.9 (3.9–5.9) | 0.345 |
| *PSA* | 104.5 (73.7–200.3) | 82.5 (54.6–181.3) | 0.621 |
| *p63* | 4.5 (3.0–6.7) | 6.6 (4.1–11.0) | 0.219 |
| *c-FOS* | 7.0 (4.5–10.0) | 28.5 (18.2–44.7) | **<0.001** |
| *c-JUN* | 3.5 (2.5–4.9) | 9.5 (6.4–14.0) | **<0.001** |
| *IL-6* | 93.7 (43.4–204.4) | 330.3 (131.6–820.6) | **0.041** |
| *IL-8* | 379.9 (144.0–1002.2) | 395.4 (125.2–1248.9) | 0.956 |
| II. Analyte | 5ARI | | |
| | No | Yes | *P*-value |
| *AR* | 4.5 (3.7–5.3) | 4.6 (3.7–5.6) | 0.835 |
| *PSA* | 159.2 (97.5–262.4) | 75.9 (42.5–137.0) | **0.046** |
| *p63* | 5.8 (3.9–8.3) | 5.3 (3.7–8.3) | 0.792 |
| *c-FOS* | 10.4 (7.1–15.3) | 19.1 (12.1–30.3) | **0.040** |
| *c-JUN* | 5.9 (4.3–8.0) | 5.6 (3.9–8.2) | 0.850 |
| *IL-6* | 145.5 (70.1–301.9) | 212.7 (90.0–507.8) | 0.483 |
| *IL-8* | 555.6 (223.6–1394.1) | 270.4 (90.9–804.3) | 0.289 |
| III. Analyte | α-blocker | | |
| | No | Yes | *P*-value |
| *AR* | 4.5 (3.5–5.4) | 4.7 (3.9–5.4) | 0.698 |
| *PSA* | 121.5 (66.7–223.6) | 99.5 (62.2–159.2) | 0.580 |
| *p63* | 5.5 (3.5–8.8) | 5.5 (3.9–7.9) | 0.991 |
| *c-FOS* | 13.7 (8.6–22.2) | 14.4 (10.0–20.9) | 0.879 |
| *c-JUN* | 6.3 (4.3–9.2) | 5.3 (3.9–7.1) | 0.435 |
| *IL-6* | 177.7 (72.2–432.7) | 175.9 (87.4–350.7) | 0.483 |
| *IL-8* | 415.7 (135.6–1274.1) | 361.4 (151.4–871.3) | 0.843 |

I, mean analyte levels compared by group after adjusting for age (continuous), BMI (continuous), use of a 5ARI; Y/N, or alpha blocker (Y/N) or diagnosis of diabetes mellitus (Y/N) in a linear regression model; II and III, mean analyte levels compared by use of 5ARI (Y/N) or alpha blocker (Y/N).

Given the links between chemokines, inflammation, and fibrosis in BPH/LUTS [15,34] and the molecular profile indicating a role for AP-1 factors in BPH/LUTS progression presented here (see Fig. 3), we may be providing further evidence for describing benign prostatic disease as an autoimmune disease as others have suggested [24,35]. AP-1 factors are constitutively expressed in the basal cell layer of the epidermal epithelium and have been shown to be important regulators of skin inflammation. In fact, our symptomatic BPH molecular signature shares many of the same features as those in immune inflammatory diseases such as psoriasis (angiogenesis, inflammation, AP-1 expression), where investigators have suggested that a primary trigger of skin inflammation comes from dysregulation of AP-1 signaling in the epithelium [16].

Although prostate volume, inflammation, and fibrosis correlate with LUTS severity [13,15], it has been difficult to determine the molecular etiology underlying these findings [36–38]. The widespread and specific types of chronic and acute inflammation observed in BPH have prompted propositions that this is an autoimmune response [24,35], but the functional effects of specific types of inflammation on prostate hyperplasia and fibrosis are only beginning to be investigated [39]. Given that stromal-epithelial interactions govern prostatic homeostasis and differentiation, it will be imperative to determine experimentally whether dysregulation of AP-1 signaling in prostate epithelium is a causal factor in prostatic growth, the immune/inflammatory response, and fibrosis, or whether it is simply a stress response to existing tissue

damage or inflammation. Although the prostate volumes of Surgical BPH patients were extremely high and a laser-based surgical technique was used for Surgical BPH patients, it should be noted that the association between analytes and medication were assessed in all samples including the Incidental BPH patients, ruling out potential conflation with surgical technique or prostate volume. As has been experimentally demonstrated previously [40] and shown here by immunohistochemistry (Fig. 3), AP-1 factor expression in the stroma may also be the source of growth and inflammation. Furthermore, given the associations between LUTS and metabolic diseases [41–43], the effects of obesity and diabetes on AP-1 activity and inflammation should be examined.

There is a large body of work relating to opportunities for anti-AP-1 therapy in chronic immune/inflammatory diseases [44]. Glucocorticoids are used in the treatment of autoimmune diseases such as rheumatoid arthritis and act by repressing AP-1-mediated expression of several cytokines [45,46]. Our molecular network analysis of symptomatic BPH revealed that a number of genes represented in the AP-1 signaling network were upregulated including c-JUN, c-FOS, FOSB, cyclin D1, c-Myc, HB-EGF, MMP1, IL-6, and IL-8 (See Fig. 2, Supplementary figures 1 and 2). These data provide the beginnings of an overarching molecular context for the observed alterations in chemokines, growth factors and matrix-remodeling enzymes that have become part of our characterization of BPH/LUTS. Moreover, the data may indicate that, similar to other diseases such as atherosclerosis, anti-AP-1 therapy may provide therapeutic benefit in cases where α-blockers and 5ARIs prove insufficient to alleviate symptoms.

Although it was expected that PSA levels would decrease with 5ARI use (Table III), it was intriguing to note the coordinate increase in c-FOS. Although the functional effect of increased c-FOS on prostate is unclear, these data potentially suggest a link between AP-1 factors and therapeutic resistance to 5ARIs. Alternatively, these data could imply that 5ARI treatment induces an additional insult to further potentiate inflammation and proliferation through activation of AP-1 factors. It will be important to determine whether AP-1 factor expression and activation is a potential biomarker for BPH/LUTS progression or resistance to specific types of medical therapy such as 5ARIs as indicated here. Finally, the association between obesity and AP-1 factor expression in the prostate should be explored after a retrospective analysis of the Prostate Cancer Prevention Trial revealed that obese men were more likely to fail 5ARI therapy [47].

## CONCLUSIONS

As with many conditions the concept of BPH as a monolithic disease amenable to a single "one size fits all" therapeutic approach is starting to recede. However, the details of how specific comorbidities and their responses and reactions to medical options are presently unclear and need further exploration. Due to the potential for adverse events (approximately 20% of patients have to be taken off medication) and deficiencies of combination therapy (approximately a third of patients progress to surgery) [10,11], alternative therapies for BPH/LUTS such as phosphodiesterase inhibitors are being examined [48]. Recent data have directly linked the age- or obesity-related decreases in androgen levels with the development of inflammation [49]. In addition, obesity and diabetes are still associated with prostatic enlargement even after controlling for testosterone levels [50]. In such cases, targeting the androgen axis is not likely to provide therapeutic benefit; therefore, it will be important to utilize molecular profiles of severely symptomatic BPH, of the sort generated here as a basis to develop or repurpose therapies.

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