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# Association of Fatty Acid Synthase Polymorphisms and Expression with Outcomes after Radical Prostatectomy

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## Abstract

Fatty acid synthase (FASN), selectively overexpressed in prostate cancer cells, has been described as linked to the aggressiveness of prostate cancer (PCa). Constitutional genetic variation of the FASN gene and the expression levels of FASN protein in cancer cells could thus be expected to predict outcome after radical prostatectomy (RP). This study evaluates the associations of malignant tissue status, neoadjuvant androgen deprivation treatment (NADT) and single nucleotide polymorphisms of FASN with FASN protein expression in prostate tissue. The study then examines the associations of FASN single nucleotide polymorphisms (SNPs) and gene expression with 3 measures of post-prostatectomy outcome.

Seven tagging FASN SNPs were genotyped in 659 European American men who underwent RP at Roswell Park Cancer Institute (RPCI) between 1993 and 2005. FASN protein expression was assessed using immunohistochemistry. The patients were followed for an average of 6.9 years (range: 0.1 to 20.6 years). Outcome was assessed using 3 endpoints: biochemical failure, treatment failure and development of distant metastatic PCa. Cox proportional hazards analyses were used to evaluate the associations of the tagging SNPs and FASN expression with these endpoints. Bivariate associations with outcomes were considered; the associations also were controlled for known aggressiveness indicators.

Overall, no SNPs were associated with any known aggressiveness indicators. FASN staining intensity was stronger in malignant than in benign tissue, and neoadjuvant androgen deprivation therapy (NADT) was associated with decreased FASN staining in both benign and malignant tissue. The relationships of FASN SNPs and staining intensity with outcome were less clear. One SNP, rs4246444, showed a weak association with outcome. FASN staining intensity also showed

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Author Contributions

Conceived and designed the experiments: JC and JRM. Performed the experiments: JC, RPO, JLM, DCM, BX, SG, GA and CDM. Analyzed the data: JC, RPO, JLM, KAK, BX, GA, SY, CDM and JRM. Wrote the paper: JC, RPO, JRM and JLM.

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a weak and seemingly contradictory relationship with outcome. Additional study with longer follow-up and populations that include more metastatic patients is warranted.

## INTRODUCTION

PCa remains one of the most frequently diagnosed cancers among men in the United States[1]. Since the advent of the PSA test, most PCa cases are diagnosed and treated early in their course, and only a small percentage of patients diagnosed develop aggressive, lethal forms of the disease[1]. Unfortunately, identifying patients whose PCa at biopsy is or will become aggressive remains difficult. The parameters currently used to identify patient risk (particularly PSA, but also clinical Gleason sum) are neither specific nor sensitive to aggressive disease[2–4]. This leads to over-diagnosis of prostate cancer among patients who never develop clinically significant disease, yet still misses the potential of some patients to later develop aggressive PCa[2–5]. New predictors of unfavorable outcome, especially metastasis, are needed[3,5].

FASN, a key enzyme in *de novo* lipogenesis, is highly expressed in PCa and high-grade prostatic intraepithelial neoplasia (PIN)[6–9]. Overexpression of FASN, which may lead to increased proliferation and impart growth and survival advantages to malignant cells[10], has been associated with the recurrence or relapse of a number of cancers[11–17]. At the molecular level, tumor-associated FASN could promote PCa growth in several ways: 1) generating lipids, such as phospholipids, for membrane production[18]; 2) providing the components for lipid rafts, which serve as membrane platforms for signal transduction[19]; or 3) modifying important signaling molecules at the post-translational level, by palmitoylation and myristoylation[20–22].

Several studies of families and twins have documented a higher risk of prostate cancer among family members of prostate cancer patients; that these associations are especially strong among monozygotic twins suggests that this risk stems from genetic effects.[23,24] Recent advances in genetics have allowed more directed studies of the genetics, and specifically SNPs-inherited variations within coding sequences, non-coding regions or in the intergenic regions of genes associated with indolent prostate cancer, as well as aggressive prostate cancer.[25–28] Most SNPs studied have shown a weak relationship with aggressive prostate cancer, though men with multiple risk alleles may have significantly higher risk of aggressive prostate cancer.[29,30] SNPs can be associated with expression of the gene and the activities of its protein products[31]. Polymorphisms of several genes which include 5α-reductase type 1 (SRD5A1), 5α-reductase type 2 (SRD5A2) and PSA, have been shown to be involved in PCa pathogenesis and progression[32,33]. A recently reported retrospective cohort study found germline FASN polymorphisms to be associated with more aggressive prostate cancer[34]. These studies suggest a genetic link for more aggressive prostate cancer. Genotyping for SNPs could provide a convenient biomarker for predicting poor PCa prognosis. If genetic variations in the FASN gene and its expression mark a subset of patients at risk for a poor outcome after RP, these patients may benefit from early therapeutic intervention.

This study investigated the association of FASN SNPs and its expression with treatment outcome among a cohort of 659 European American RP patients.

## METHODS

### Patients and Data Collection

After approval from the RPCI Institutional Review Board, detailed information was collected from the records of 659 European American patients who had undergone open or robotic RP at RPCI between 1/5/1993 and 12/29/2005. Annotating data included clinical stage and Gleason score, surgery date, pathological stage and Gleason score, margin and lymph node status, pre- and post-surgery serum PSA tests and prostate cancer-related treatments and dates before or after RP. Recurrence risk categories have been defined by the National Comprehensive Cancer network (NCCN)[35]; of the total of 659 patients, 317 (48%) were low recurrence risk, 261 (40%) were intermediate risk, 74 (11%) were high risk, and 7 (1%) were very high risk.

All patients were followed by patient, urologist, and primary care practitioner correspondence, in order to track biochemical recurrence, prostate cancer survival and overall survival.

### Single Nucleotide Polymorphisms, Sample preparation and Genotyping

Tissue cores (14g needle, 3–4 mm length) of benign epithelial glands were retrieved from FFPE blocks of the patients. Genomic DNA from the deparaffinized FFPE tissue cores was extracted using the QIAamp Blood and Tissue Kit and BioRobot Universal (Qiagen) per manufacturer instructions. DNA yield and concentration were determined using the PicoGreen dsDNA Quantitation Kit (Invitrogen) and spectrofluorimetric micro-plate reader (Molecular Devices).

SNP genotyping was performed using the MassARRAY Compact system (Sequenom) on a panel of 54 custom SNP assays designed using RealSNP and MassARRAY Assay Designer (Sequenom)[36,37]. PCR amplification of 20 ng DNA using SNP-specific primers (IDT) was followed by a base extension reaction, using the iPLEX Gold chemistry (Sequenom). The final base extension products were treated and spotted on a 384-pad SpectroCHIP (Sequenom) using a ChipSpotter LT Nanodispenser (Samsung). A MassARRAY Analyzer Compact MALDI-TOF MS (Sequenom) was used for data acquisition from the SpectroCHIP. The resultant genotypes were called using MassARRAY Typer Analyzer v4.0 (Sequenom). The successful genotyping call rate was ~95%.

The HapMap database (National Center for Biotechnology Information Build 35) was used to generate a list of SNPs that cover most of the genetic variation ($R^2 > 0.80$) within the genomic region between 5 kb 5’ of the beginning of the first known exon and 5 kb 3’ of the end of the last known exon with a minor allele frequency (MAF) ≥ 5%. The Tagger algorithm identified 7 tagging SNPs (rs1127678, rs6502051, rs8066956, rs4246444, rs12949488, rs1140616 and rs4485435) to represent the entire set of FASN SNPs. Two of these SNPs (rs6502051 and rs1140616) were found to be out of Hardy-Weinberg equilibrium (HWE p-values <0.001), and were dropped from further analysis.

### Tissue microarrays (TMA) construction, immunohistochemistry (IHC) staining and image analysis

Paraffin-embedded tissue was used for TMA construction. A tissue cylinder with a diameter of 0.6 mm was punched from a region of cancer and a distant region of benign tissue in each donor block and placed in a recipient paraffin block. Figure 1 shows (a) a typical tumor core and (b) a typical benign core. This procedure was repeated a total of three times for each patient to provide three replicate sets of malignant and benign tissue for each of the 659 patients.

Sections of each TMA set were stained with a FASN antibody (Lifespan BioSciences, Inc. Seattle, WA) per the manufacturer's protocol. TMA sections were scored semi-quantitatively based on the percentage of cells stained [quantity (Q)] and the staining intensity (I) to obtain the final score as the product of I × Q as previously described[38]. Staining intensity was evaluated as follows: unstained cells were scored as 0, lightly stained cells were scored as 1, moderately stained cells were scored as 2 and intensely stained cells were scored as 3. Each IHC core image was viewed by two independent pathologists, with consensus reached for each image. Each patient's 3 benign cores were averaged to yield a benign FASN staining intensity average. Likewise, each patient's 3 malignant cores were averaged to yield a malignant FASN staining intensity average.

### Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate the associations among genotypic polymorphisms and clinical and pathological characteristics. Student t-tests, as well as ANOVA, were used to evaluate the association between FASN staining and each clinical and pathological characteristic. If the prevalence of the least common homozygote category was 5% or less, that category was combined with the heterozygote category for analysis. Cox proportional hazards analyses were used to evaluate the associations of each tagging SNP and FASN staining intensity with each endpoint. Bivariate associations with outcomes were considered for each tagging SNP and FASN stain; the analyses were controlled for recognized risk factors: age at surgery, body mass index (BMI), diagnostic PSA, clinical and pathologic stage and Gleason score. Relative risk per unit change in minor allele frequency was estimated. Patients who had not reached biochemical failure, treatment failure or distant metastasis during the study period were censored at death, loss to follow-up or at last follow-up. For analyses linking FASN polymorphisms to staining, patients who had NADT prior to surgery were excluded due to the possible confounding effects of NADT.

## RESULTS

European American patients who underwent RP at RPCI from 1993 to 2005 were studied; their characteristics are summarized in Table 1. During a median follow-up of 6.9 years, 27% patients developed biochemical failure (either persistent disease or biochemical recurrence after RP), 33% developed clinical failure (either biochemical failure or treatment after RP) and 5% developed distant metastatic prostate cancer.

Genotyping success rates ranged from 90.1% for rs4246444 to 96.1% for rs4485435. Malignant cells generally expressed higher levels of FASN protein than their benign counterparts (Table 2). This difference between average benign and malignant FASN staining intensity is statistically significant among all patients, and persists when patients are stratified for NADT. NADT was associated with a statistically significant, decreased average FASN staining intensity in both benign and malignant cells. The decrease in staining intensity associated with NADT was most substantial - greater than 40% - in benign tissue; the difference, though still significant, was smaller in malignant tissue.

Increased average benign FASN staining intensity tended to be associated with increased risk of biochemical and of treatment failure, though only one hazard ratio was statistically significant (Table 3). Average benign FASN staining intensity was associated with essentially no alteration of the hazard ratio for distant metastatic cancer. Patients with average malignant FASN staining intensity between 2 and 3 had decreased risk of biochemical failure. This same group of patients showed an increased risk of distant metastatic disease, though this hazards ratio was not statistically significant. The association of average benign and malignant FASN staining with biochemical and treatment failure, and with distant metastatic disease, did not differ when stratified for BMI.

Clinical and pathological risk factors were distributed similarly among the different FASN genotype groups (Table 4). These results indicate that FASN polymorphisms and expression are independent of any well-known risk factors.

Individual FASN SNP and clinical outcomes were associated only weakly with biochemical failure, treatment failure and distant metastatic disease (Table 5). Both the unadjusted and adjusted hazard ratios for biochemical failure were increased among those with the variant A allele of rs4246444, and both were significant. All associations were examined with adjustment for known risk factors, including age at surgery, race, BMI, PSA, clinical and pathological stage and grade, margin status, NADT, date of surgery and lymph node status. The three outcomes did not differ for each SNP when stratified for BMI above vs. below the median. Among patients with BMI below the median, there was a significantly higher risk for treatment failure among those with the variant A allele of rs4246444, which persisted when adjusted for known risk factors.

No associations were found between FASN SNP and average malignant FASN staining intensity (Table 6). However, the minor variant T allele of SNP rs8066956 was associated with increased average benign FASN staining intensity (1.21 vs. 1.07, F=4.39, p=0.037). The minor variant A allele of SNP rs1127678 was very nearly significantly associated with increased average benign FASN staining intensity (1.21 vs. 1.07, F=3.61, p=0.058). No other SNPs were significantly associated with average benign FASN staining intensity.

## DISCUSSION

This report, based on a carefully followed and well annotated cohort of 659 European American RP patients from RPCI, confirmed that FASN protein expression is higher in malignant than in benign tissue. The analysis also showed that FASN protein expression

decreased among those treated by NADT. The results linking FASN SNPs and staining intensity with outcome are less clear; there is some association with FASN SNPs and outcome as defined by recurrence and malignancy, but this analysis shows FASN to be weakly associated with outcome. Only one FASN SNP (rs4246444) was associated with biochemical failure, which persisted after adjusting for known aggressiveness indicators. However, no significant relationship was observed between rs4246444 and treatment failure or distant metastatic disease, suggesting that the association between rs4246444 and outcome may not help in identifying patients with the most aggressive forms of the disease. In no SNP or FASN staining analysis was there a dose response, and adjustment for aggressiveness indicators frequently made substantial differences in hazards ratios and significance. This analysis of a well-documented data set provides no evidence that FASN SNPs and staining intensities are independent predictors of outcome.

The functions of these SNPs are not known, so the mechanisms by which these SNPs might affect the progression of PCa remain to be identified. SNP rs424644 is located in an intron of the FASN gene. A recently-revealed link between rs424644 and LDL peak particle diameter suggests that this genetic variant could indirectly affect the prognosis of PCa through modulation of circulatory LDL[44]. On the human genomic map, SNP rs1127678 is positioned upstream of the FASN gene, simultaneously falling into the 3' un-translated region (UTR) of Coiled-coil domain containing 57 (CCDC57), which may play a role in lipid metabolism[45]. This could enable genetic variation at rs1127678 to directly affect FASN gene expression and function, or to indirectly affect the FASN gene through regulation by CCDC57. Additional research to decipher how FASN SNPs are involved in PCa aggressiveness is warranted to clarify the function of these FASN SNPs and their interaction with the delicate networks of lipid metabolism.

A recent study found that genetic variation in the FASN gene was not associated with PCa risk[46]. The RPCI results revealed that FASN expression is greatly increased in malignant tissue and FASN expression is decreased by NADT, but any associations between the FASN gene and PCa risk remain unclear. These results suggest that these associations are weak at best.

Nguyen et al. found that the minor variant T allele of SNP rs4246444 was associated with decreased PCa-specific mortality[34]. Weak and seemingly contradictory associations between this genotype and PCa progression were found in patients who underwent RP at RPCI. Those with the variant T allele were at increased risk of biochemical failure (HR significant), but decreased risk of distant metastatic disease (HR not significant).

Differences between the Nguyen et al. results and those herein may be explained in part by variation in treatment modalities in the Physicians' Health Study (PHS) sample of Nguyen et.al.; over 10 percent of patients in Nguyen et al. had T3 or T4 disease, and few of these would have undergone surgery. Only 2 percent of the RPCI patients had T3 or T4 disease, and all RPCI patients underwent RP. Nguyen et al. found an association between FASN SNPs and BMI[34]; no such association was found among RPCI patients. Whether patients with higher BMI reported with higher grade disease in Nguyen et al. was not reported. Nguyen et al. observed significant interactions between BMI and the associations of genetic

polymorphisms with outcome. In the RPCI series, the weak associations of genetic polymorphisms and treatment outcome were equivalent among those with BMI above and below the median value. Similarly, the associations between FASN staining and outcome were similar among those above and below the median of BMI.

As in Nguyen et al.'s report, no associations between FASN SNPs and FASN protein expression in PCa tissue were found in the RPCI study. However, there were associations between two SNPs—rs1127678 (significant) and rs8066956 (nearly significant)—and staining intensity in benign tissue.

Some associations between FASN staining intensity in benign tissue and outcome were observed. Patients with low to moderate average benign FASN staining intensity were at increased risk for treatment failure. Patients with moderate to average malignant FASN staining intensity were at decreased risk of biochemical failure. However, elevated malignant staining intensity was associated with increased risk of metastatic disease. Because NADT affects FASN staining intensity, the patients with pre-surgery NADT were excluded from the staining analysis. These results show that FASN protein expression, in either benign or malignant tissue, is weakly associated with outcome, and suggests contradictory outcomes.

Androgen, an important element in the development of prostate cancer, could regulate the expression of FASN; several different mechanisms may be involved. Heemers, et.al. found that androgen stimulates FASN transcription by activation of sterol regulatory element-binding protein (SREBP) pathway.[47] Graner, et.al. found that androgen up-regulates the isopeptidase USP2a that can stabilize FASN protein and prevent its degradation.[48] Thus, FASN protein expression may reflect androgen deprivation. In our study, FASN staining intensity in benign and malignant epithelial tissue was decreased by 30% and 6%, respectively. However, the results could be complicated by TMA construction; a TMA may possibly under-represent PCa heterogeneity.

Because of the association between certain SNPs and ethnicity, combined with the lack of non-European Americans among the patients studied, and because the study was not powered to detect modification of associations by ethnicity, analyses were limited to only European Americans. Evaluation and validation of these findings in cohorts with diverse ethnicity is needed.

These results suggest that SNPs in genetic determinants of lipid metabolism and its expression in PCa cells are weak, but worth further study. Patients with different FASN SNPs and increased FASN staining intensity in malignant cells might be candidates for chemoprevention agents that specifically target lipogenic signaling pathways. Hamilton et al. recently reported that statin use was associated with reduced risk of biochemical recurrence after RP[49]. Prospective clinical prevention trials with anti-lipogenic therapies to test this hypothesis are warranted. The information gathered from this study may be useful for the design of clinical trials that specifically target tumor metabolic pathways.

In conclusion, this study confirmed that, although FASN protein concentration staining is much greater in malignant than in benign tissue, genetic polymorphisms in the FASN gene

and FASN protein expression in PCa cells were only weakly associated with outcome after RP.

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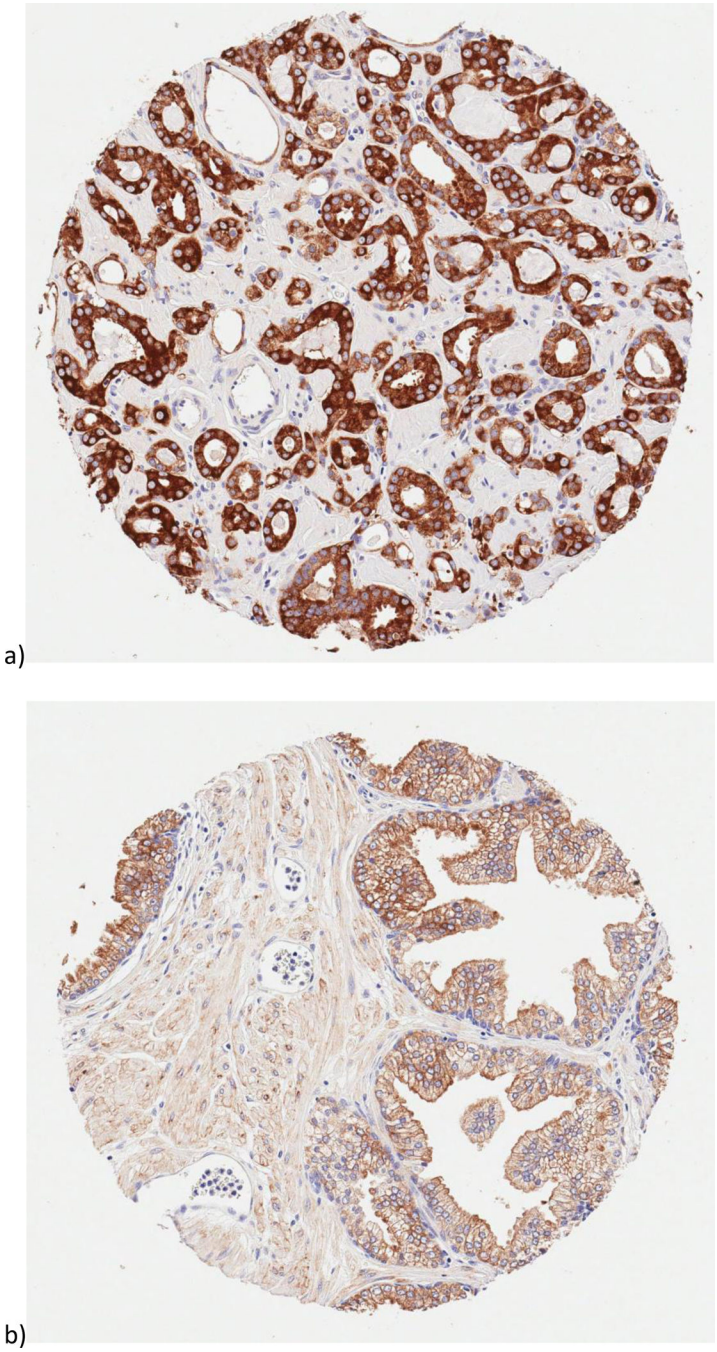


**Fig. 1.**
Images of FASN expression in a typical a) tumor core and b) benign core.

## Table 1

Characteristics of radical prostatectomy patients, European Americans only.

| | | Total N = 659 | |
|---|---|---|---|
| **Characteristic** | | **N** | **%** |
| Age at surgery, years | <45 | 8 | 1 |
| | 45 – <55 | 137 | 21 |
| | 55 – <60 | 166 | 25 |
| | 60 – <65 | 144 | 22 |
| | 65 – <75 | 204 | 31 |
| BMI, kg/m$^2$ | <25 | 121 | 18 |
| | 25 – <30 | 313 | 47 |
| | 30+ | 212 | 32 |
| | missing | 13 | 2 |
| Diagnostic PSA, ng/ml | <4 | 73 | 11 |
| | 4–10 | 437 | 66 |
| | 10–20 | 100 | 15 |
| | >20 | 49 | 7 |
| Clinical Gleason sum | <7 | 420 | 64 |
| | =7 (3+4) | 121 | 18 |
| | =7 (4+3) | 55 | 8 |
| | >7 | 42 | 6 |
| | missing | 21 | 3 |
| Clinical stage | T1a/T1c | 384 | 58 |
| | T2a/T2b/T2x | 210 | 32 |
| | T2c | 28 | 4 |
| | T3a/T3b/T4/T1b | 12 | 2 |
| | missing | 25 | 4 |
| Pathologic Gleason sum | <7 | 257 | 39 |
| | =7 (3+4) | 235 | 36 |
| | =7 (4+3) | 84 | 13 |
| | >7 | 83 | 13 |
| Pathologic stage | T2a/T2b/T2x | 76 | 12 |
| | T2c | 428 | 65 |
| | T3a | 100 | 15 |
| | T3b | 47 | 7 |
| | T4 | 8 | 1 |
| Positive surgical margin | | 164 | 25 |
| Lymph nodes at surgery | Not assessed | 118 | 18 |

| | | Total N = 659 | |
|---|---|---|---|
| **Characteristic** | | **N** | **%** |
| | Negative | 530 | 80 |
| | Positive | 11 | 2 |
| Neoadjuvant ADT | No | 540 | 82 |
| | Yes | 119 | 18 |
| Outcomes | Biochemical failure | 178 | 27 |
| | Treatment failure | 216 | 33 |
| | Distant metastatic prostate cancer | 35 | 5 |

## Table 2

Comparison of FASN staining, by tissue type and NADT treatment for European Americans.

| | | Average FASN Staining Intensity | | Benign vs. Malignant |
|---|---|---|---|---|
| **Patients** | **N** | **Benign** | **Malignant** | **β (P)** |
| All | 631 | 1.07 | 2.37 | 1.30 (p<0.001) |
| NADT | 116 | 0.78 | 2.22 | 1.44 (p<0.001) |
| Non-NADT | 515 | 1.13 | 2.40 | 1.27 (p<0.001) |
| NADT vs. non-NADT β (P) | | −0.34 (p<0.001) | −0.16 (p=0.027) | |

**Table 3**

Cox proportional hazards analyses: Association between FASN stain and outcomes for European Americans, excluding patients with NADT.

| | | Biochemical failure | | | | | | Treatment failure | | | | | | Distant Metastatic disease | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | unadjusted | | | adjusted[a] | | | unadjusted | | | adjusted[a] | | | unadjusted | | | adjusted[a] | | |
| **Average Staining Intensity** | **N** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** |
| **Benign cores** | | | | | | | | | | | | | | | | | | | |
| 0 | 70 | ref | | | ref | | | ref | | | ref | | | ref | | | ref | | |
| >0, ≤1 | 213 | 1.45 | 0.313 | 0.70–3.00 | 2.05 | 0.084 | 0.91–4.63 | 1.11 | 0.744 | 0.60–2.06 | 1.98 | 0.060 | 0.97–4.04 | 0.62 | 0.512 | 0.18–2.34 | 1.01 | 0.995 | 0.18–5.78 |
| >1, ≤2 | 190 | 1.17 | 0.677 | 0.55–2.49 | 2.14 | 0.073 | 0.93–4.93 | 1.08 | 0.804 | 0.58–2.03 | 2.38 | 0.018 | 1.16–4.88 | 0.64 | 0.475 | 0.19–2.19 | 1.33 | 0.736 | 0.25–7.11 |
| >2, ≤3 | 42 | 1.71 | 0.270 | 0.70–4.44 | 2.48 | 0.085 | 0.88–6.96 | 1.19 | 0.694 | 0.51–2.78 | 1.88 | 0.196 | 0.72–4.89 | -- | -- | -- | -- | -- | -- |
| continuous variable | | 1.13 | 0.401 | 0.85–1.50 | 1.3 | 0.080 | 0.97–1.75 | 1.11 | 0.432 | 0.86–1.42 | 1.26 | 0.083 | 0.97–1.63 | 0.73 | 0.342 | 0.38–1.40 | 1.03 | 0.945 | 0.44–2.41 |
| **Malignant cores** | | | | | | | | | | | | | | | | | | | |
| ≥0, <2 | 85 | ref | | | ref | | | ref | | | ref | | | ref | | | ref | | |
| ≥2, <3 | 211 | 0.58 | 0.092 | 0.31–1.09 | 0.48 | 0.035 | 0.24–0.95 | 0.64 | 0.103 | 0.37–1.10 | 0.71 | 0.268 | 0.39–1.30 | 1.54 | 0.594 | 0.32–7.45 | 1.54 | 0.709 | 0.16–14.95 |
| 3 | 197 | 0.96 | 0.897 | 0.54–1.73 | 0.86 | 0.648 | 0.46–1.62 | 0.95 | 0.845 | 0.57–1.59 | 1.05 | 0.876 | 0.59–1.85 | 1.91 | 0.404 | 0.42–8.78 | 3.14 | 0.341 | 0.30–33.05 |
| continuous variable | | 1.19 | 0.331 | 0.84–1.68 | 1.12 | 0.555 | 0.77–1.62 | 1.16 | 0.317 | 0.87–1.56 | 1.20 | 0.273 | 0.87–1.66 | 1.65 | 0.245 | 0.71–3.83 | 3.37 | 0.121 | 0.73–15.62 |

[a]Estimated with control for age at surgery, BMI, PSA, clinical stage, clinical grade, pathologic stage, pathologic grade, margin status, surgery date, and lymph node status

**Table 4**

Clinical/pathologic characteristics by genotype for each SNP for European Americans.

| | | Diagnostic PSA | | | | Clinical Gleason | | | | Clinical Stage | | | | | Pathologic Gleason | | | | Pathologic Stage | | | | | Surgical margin | | | BMI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SNP** | **N** | **mean** | **sd** | **β** | **p** | **mean** | **sd** | **β** | **p** | **%T1** | **%T2** | **%T3–4** | **F** | **p** | **mean** | **sd** | **β** | **p** | **%T2** | **%T3** | **%T4** | **F** | **p** | **% positive** | **β** | **p** | **mean** | **sd** | **β** | **p** |
| **rs1127678** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| GG | 367 | 8.95 | 8.56 | ref | | 6.21 | 0.94 | ref | | 61 | 38 | 1 | 0.29 | 0.589 | 6.71 | 0.95 | ref | | 76 | 23 | 2 | 0.44 | 0.509 | 25 | ref | | 28.78 | 4.78 | ref | |
| GA+AA | 230 | 7.92 | 5.79 | −1.03 | 0.109 | 6.24 | 0.89 | 0.03 | 0.712 | 59 | 40 | 1 | | | 6.77 | 0.88 | 0.06 | 0.421 | 77 | 22 | 1 | | | 27 | 0.03 | 0.492 | 28.72 | 4.21 | −0.05 | 0.893 |
| **rs8066956** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| GG | 357 | 8.41 | 8.34 | ref | | 6.18 | 0.93 | ref | | 62 | 37 | 1 | 0.31 | 0.581 | 6.7 | 0.88 | ref | | 74 | 24 | 2 | 1.85 | 0.175 | 25 | ref | | 28.6 | 4.54 | ref | |
| GT+TT | 255 | 8.64 | 6.58 | 0.23 | 0.714 | 6.29 | 0.91 | 0.11 | 0.136 | 61 | 37 | 2 | | | 6.8 | 1.00 | 0.10 | 0.192 | 79 | 21 | 1 | | | 26 | 0.01 | 0.793 | 28.8 | 4.52 | 0.22 | 0.555 |
| **rs4246444** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CC | 304 | 8.50 | 7.23 | ref | | 6.24 | 0.93 | ref | | 61 | 38 | 1 | 0.04 | 0.844 | 6.74 | 0.92 | ref | | 78 | 21 | 1 | 0 | 0.992 | 23 | ref | | 28.7 | 4.78 | ref | |
| CA+AA | 274 | 8.64 | 8.23 | 0.14 | 0.824 | 6.19 | 0.91 | −0.05 | 0.491 | 63 | 35 | 2 | | | 6.72 | 0.95 | −0.02 | 0.783 | 77 | 22 | 1 | | | 27 | 0.04 | 0.327 | 28.9 | 4.39 | 0.19 | 0.625 |
| **rs12949488** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| GG | 355 | 8.46 | 6.98 | ref | | 6.24 | 0.94 | ref | | 62 | 37 | 1 | 0.01 | 0.906 | 6.76 | 0.970 | ref | | 77 | 22 | 1 | 0.66 | 0.416 | 28 | ref | | 28.5 | 4.31 | ref | |
| GT+TT | 243 | 8.58 | 8.52 | 0.12 | 0.851 | 6.2 | 0.88 | −0.04 | 0.620 | 62 | 36 | 1 | | | 6.72 | 0.849 | −0.04 | 0.633 | 75 | 23 | 2 | | | 23 | −0.05 | 0.188 | 29.0 | 4.94 | 0.5 | 0.188 |
| **rs4485435** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| GG | 415 | 8.43 | 6.97 | ref | | 6.2 | 0.92 | ref | | 61 | 38 | 1 | 0.04 | 0.849 | 6.7 | 0.95 | ref | | 76 | 23 | 1 | 0.08 | 0.782 | 26 | ref | | 28.7 | 4.39 | ref | |
| GC+CC | 199 | 8.80 | 8.98 | 0.37 | 0.577 | 6.2 | 0.91 | 0.02 | 0.797 | 62 | 36 | 2 | | | 6.8 | 0.89 | 0.12 | 0.130 | 77 | 21 | 2 | | | 26 | 0 | 0.929 | 28.9 | 4.78 | 0.21 | 0.590 |

## Table 5

Cox proportional hazards analyses: Association between FASN SNPs and outcome for European Americans.

| | | Biochemical Failure | | | | | | Treatment Failure | | | | | | Distant Metastatic disease | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | unadjusted | | | adjusted[a] | | | unadjusted | | | adjusted[a] | | | unadjusted | | | adjusted[a] | | |
| **SNP** | **N** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** | **HR** | **p** | **95% CI** |
| **rs1127678** | | | | | | | | | | | | | | | | | | | |
| GG | 374 | | ref | | ref | | | ref | | | ref | | | ref | | | ref | | |
| GA+AA | 231 | 0.91 | 0.644 | 0.62–1.34 | 1.02 | 0.916 | 0.68–1.53 | 1.09 | 0.594 | 0.79–1.52 | 1.13 | 0.502 | 0.80–1.59 | 0.50 | 0.102 | 0.21–1.15 | 0.43 | 0.108 | 0.16–1.20 |
| per allele | | 0.88 | 0.497 | 0.63–1.25 | 0.96 | 0.842 | 0.68–1.38 | 1.00 | 0.990 | 0.75–1.34 | 1.01 | 0.945 | 0.75–1.37 | 0.49 | 0.091 | 0.22–1.12 | 0.43 | 0.104 | 0.16–1.19 |
| **rs8066956** | | | | | | | | | | | | | | | | | | | |
| GG | 362 | | ref | | ref | | | ref | | | ref | | | ref | | | ref | | |
| GT+TT | 258 | 0.96 | 0.847 | 0.66–1.40 | 1.11 | 0.636 | 0.73–1.68 | 0.87 | 0.412 | 0.63–1.21 | 0.93 | 0.712 | 0.64–1.35 | 1.16 | 0.665 | 0.60–2.25 | 1.14 | 0.758 | 0.50–2.56 |
| per allele | | 0.99 | 0.933 | 0.70–1.39 | 1.04 | 0.813 | 0.73–1.50 | 0.91 | 0.543 | 0.67–1.23 | 0.96 | 0.802 | 0.67–1.34 | 1.32 | 0.379 | 0.71–2.43 | 1.34 | 0.425 | 0.65–2.75 |
| **rs4246444** | | | | | | | | | | | | | | | | | | | |
| CC | 308 | | ref | | ref | | | ref | | | ref | | | ref | | | ref | | |
| CA+AA | 278 | 1.38 | 0.1 | 0.94–2.01 | 1.39 | 0.107 | 0.93–2.07 | 1.21 | 0.253 | 0.87–1.68 | 1.26 | 0.197 | 0.89–1.78 | 0.92 | 0.81 | 0.46–1.82 | 0.64 | 0.342 | 0.26–1.60 |
| per allele | | 1.40 | 0.035 | 1.02–1.93 | 1.41 | 0.046 | 1.01–1.96 | 1.22 | 0.151 | 0.93–1.61 | 1.29 | 0.091 | 0.96–1.73 | 1.10 | 0.749 | 0.61–1.99 | 1.00 | 0.998 | 0.46–2.18 |
| **rs12949488** | | | | | | | | | | | | | | | | | | | |
| GG | 360 | | ref | | ref | | | ref | | | ref | | | ref | | | ref | | |
| GT+TT | 247 | 0.96 | 0.824 | 0.66–1.39 | 1.06 | 0.773 | 0.71–1.59 | 0.98 | 0.925 | 0.71–1.36 | 1.10 | 0.589 | 0.77–1.58 | 0.80 | 0.549 | 0.39–1.64 | 1.59 | 0.337 | 0.62–4.08 |
| per allele | | 1.04 | 0.815 | 0.75–1.44 | 1.06 | 0.734 | 0.75–1.49 | 1.05 | 0.758 | 0.79–1.39 | 1.11 | 0.501 | 0.82–1.50 | 0.83 | 0.570 | 0.44–1.58 | 1.70 | 0.221 | 0.73–3.98 |
| **rs4485435** | | | | | | | | | | | | | | | | | | | |
| GG | 420 | | ref | | ref | | | ref | | | ref | | | ref | | | ref | | |
| GC+CC | 203 | 1.07 | 0.716 | 0.73–1.59 | 1.20 | 0.397 | 0.79–1.82 | 0.92 | 0.641 | 0.65–1.30 | 0.91 | 0.612 | 0.63–1.32 | 0.69 | 0.336 | 0.32–1.48 | 0.89 | 0.823 | 0.33–2.40 |
| per allele | | 1.08 | 0.648 | 0.77–1.54 | 1.16 | 0.435 | 0.80–1.69 | 0.93 | 0.641 | 0.68–1.27 | 0.90 | 0.558 | 0.64–1.27 | 0.67 | 0.287 | 0.32–1.40 | 0.87 | 0.770 | 0.33–2.25 |

[a]Estimated with control for age at surgery, BMI, PSA, clinical stage, clinical grade, pathologic stage, pathologic grade, margin status, NADT, surgery date, and lymph node status

**Table 6**

FASN stain by genotype for each SNP for European Americans, excluding NADT patients.

| | | Average benign FASN staining intensity | | | | | Average malignant FASN staining intensity | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **SNP** | **N** | **mean** | **sd** | **F** | **p** | **N** | **mean** | **sd** | **F** | **p** |
| **rs1127678** | | | | | | | | | | |
| GG | 292 | 1.07 | 0.75 | 3.61 | 0.058 | 276 | 2.38 | 0.75 | 0.71 | 0.400 |
| GA+AA | 185 | 1.21 | 0.79 | | | 180 | 2.43 | 0.63 | | |
| **rs8066956** | | | | | | | | | | |
| GG | 286 | 1.07 | 0.76 | 4.39 | 0.037 | 272 | 2.37 | 0.72 | 1.48 | 0.224 |
| GT+TT | 201 | 1.21 | 0.76 | | | 196 | 2.45 | 0.64 | | |
| **rs4246444** | | | | | | | | | | |
| CC | 237 | 1.16 | 0.81 | 0.36 | 0.550 | 238 | 2.4 | 0.70 | 0.17 | 0.680 |
| CA+AA | 223 | 1.12 | 0.73 | | | 205 | 2.38 | 0.71 | | |
| **rs12949488** | | | | | | | | | | |
| GG | 280 | 1.12 | 0.79 | 0.11 | 0.736 | 270 | 2.37 | 0.72 | 0.87 | 0.351 |
| GT+TT | 195 | 1.14 | 0.76 | | | 187 | 2.43 | 0.69 | | |
| **rs4485435** | | | | | | | | | | |
| GG | 327 | 1.14 | 0.77 | 0.05 | 0.827 | 318 | 2.38 | 0.71 | 1.31 | 0.254 |
| GC+CC | 164 | 1.13 | 0.77 | | | 153 | 2.46 | 0.68 | | |