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Corpora amylacea in prostatectomy tissue and associations with molecular, histological and lifestyle factors

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Abstract

Background: Corpora amylacea are amyloid bodies commonly found adjacent to damaged prostatic epithelium. Little is known about their formation or function. The current study sought to characterize corpora amylacea in prostate tissue and to describe their relationship with clinical, histological, molecular and lifestyle factors, especially with chronic inflammation which is associated with aggressive disease.

Methods: We studied a cohort of 355 men with prostate cancer and tissue specimens from the Health Professionals Follow-up Study. Pathologists examined H&E slides and undertook a standardized review for histologic data and inflammation. Trained observers counted corpora amylacea within the benign and predominately tumor areas. Immunohistochemistry biomarkers were available from tissue microarrays. We used multivariable logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) to assess associations of chronic inflammation, clinical, histological, molecular and lifestyle factors with the presence of corpora amylacea.

Results: Corpora amylacea were present in benign tissue area for 298 men (84%). Specimens with moderate to severe chronic inflammation were more likely to have corpora amylacea in

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Author Contributions:

ND, ST, RU carried out literature searches and drafted the manuscript. ND and ST led the data collection of corpora amylacea after being trained by RF. MF led the histological standardization of the slides. LM and JR developed the study design. ND led the analytical design. ND and EG led the data analyses. All authors were involved in interpreting the data, writing the paper, and had final approval of the submitted manuscript.

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Supporting Information: Supplementary information is available at Modern Pathology's website

benign regions (OR=5.4 95% CI 1.9, 15.6). Moreover, corpora amylacea were more common in men with higher body mass index (OR=1.13 95% CI 1.01, 1.26). In contrast, Gleason grade (OR=0.4 95% CI 0.2, 0.8), proliferation index (OR=0.6 95% CI 0.3, 1.2) and presence of the *TMPRSS2:ERG* fusion (OR=0.4 95% CI 0.2, 0.8) were inversely associated with corpora amylacea presence. TURP specimens were less likely to have corpora amylacea than prostatectomy specimens (OR=0.12 95% CI 0.03, 0.47). Age, PSA, stage, biomarkers of angiogenesis and PTEN, and vasectomy were not significantly associated with corpora amylacea.

Conclusion: Corpora amylacea were common among men with prostate cancer and were associated with pro-inflammatory factors, some markers of less aggressive disease, and lack of the *TMPRSS2:ERG* fusion.

Keywords

corpora amylacea; prostate; epidemiology; inflammation; *TMPRSS2:ERG*

Introduction

Prostate cancer is the most common cancer diagnosed, excluding non-melanoma skin cancer, and the second leading cause of cancer-death among men in the United States.¹ Chronic inflammation appears to be a key feature in prostate carcinogenesis,^{2,3} in both its development of high-grade tumors and progression to lethal disease.^{4,5} Contributing factors to prostatic inflammation are thought to include dietary factors, infections, hormonal variations, and prostatic cell injury, although specific risk factors have yet to be identified.³ Corpora amylacea are amyloid bodies commonly found within the lumen of cellular acini adjacent to damaged prostate epithelium.^{3,6} They are hypothesized to be reservoirs of acute inflammatory proteins indicative of past exposure to infections that may also contribute to further physical trauma and inflammation to the surrounding prostate tissue.^{6,7} Similar bodies have been identified in the brain, lung, and uterus^{8–10}, and have been implicated in health outcomes such as neurodegenerative diseases.⁸

Few epidemiologic studies have been conducted on corpora amylacea in the prostate.^{3,11–13} As such, the prevalence and significance of corpora amylacea in men with prostate cancer are unclear, particularly in terms of their relationship with histological, clinical and lifestyle factors. The goals of this study were to characterize corpora amylacea in benign prostate tissue adjacent to the tumor area and within the predominately tumor area among a cohort of men with prostate cancer, and to provide a comprehensive assessment on the relationship of corpora amylacea with clinical, lifestyle, histological and molecular factors, particularly chronic inflammation in the prostate.

Materials and Methods

Study Population

The Health Professionals Follow-up Study is an ongoing prospective cohort initiated in 1986 with 51,529 U.S. male health care professionals age 40 to 75 at baseline. Participants are sent questionnaires biennially to obtain self-reported information on health outcomes, anthropometrics, and various lifestyle factors.¹⁴ In this study, we included men who were

diagnosed with prostate cancer between 1986 and 2012, and for whom we had formalin-fixed, paraffin-embedded archival prostate tissue specimens (n=1043) from radical prostatectomy (95.5%) or transurethral resection of the prostate (TURP) procedures (4.5%). The study was approved by the human subjects committee of the Harvard T.H. Chan School of Public Health, Boston, MA (USA). Details of the prostate tumor cohort are available elsewhere.¹⁵ Within the tissue cohort, 890 specimens were reviewed for chronic inflammation and 395 were reviewed for corpora amylacea; these subgroups were similar to the full tumor tissue cohort in terms of diagnosis year, age at diagnosis, PSA, and pathological Gleason score. Our study sample was restricted to men whose prostate tissue was reviewed for chronic inflammation and corpora amylacea (n=355).

Evaluation of histology data: Gleason grade, inflammation and corpora amylacea

Study pathologists reviewed all available hematoxylin and eosin (H&E) slides of archival radical prostatectomy and TURP specimens to provide a standardized histological review of Gleason grade, presence of high-grade prostatic intraepithelial neoplasia (PIN), chronic and acute inflammation, and atrophy. We characterized chronic and acute inflammation, according to cells of the inflammatory infiltrate. Chronic inflammation was semi-quantitatively graded based on the area covered by inflammatory cells and classified as mild (<10% coverage), moderate (10-20% coverage), or severe (>20% coverage). Acute inflammation was categorized as present or absent.

Within each H&E slide, areas of PIN, post-atrophic hyperplasia (PAH), and areas of >95% tumor glands were noted and manually encircled on the slides by the study pathologists. Among 395 participants, all available H&E slides (n=1,575 slides) were reviewed for corpora amylacea by trained investigators, who were not pathologists, using the 10x objective of a microscope (Labomed CXL, Labo America, Inc. USA). The number of slides viewed per participant ranged from 1 to 37 slides, with a median of 3 slides per participant (IQR: 2-4) though the majority of the participants (81.77%) had 4 slides reviewed. The trained investigators counted the number of corpora amylacea for up to fifty-10x fields in the benign area. For example, a random field was selected to begin manually counting corpora amylacea and then we moved to an adjacent field to assess presence of corpora amylacea, and repeated for up to 50 fields in the benign tissue area. Similarly, within the encircled areas of predominately tumor glands, the trained investigators counted the number of corpora amylacea for up to fifty-10x fields; however, it was not distinguished whether the corpora amylacea within the predominately tumor area were found within an admixed benign gland or the tumor gland. The median of the total number of fields reviewed per participant in the benign area was 100 fields (IQR: 50-156) and the median total number of fields reviewed per participant in the tumor tissue was 17 fields (IQR: 8-31). We calculated the density of corpora amylacea for each participant across slides by summing the number of corpora amylacea and dividing it by the total area viewed, which was derived as the total number of fields observed multiplied by the area of one-10x field. The general size of the corpora amylacea was subjectively characterized in to categories of small, medium, and large or various sizes regardless of tissue area. Presence of corpora amylacea is the primary outcome of interest and we have presented outcomes of high-density and low-density of corpora amylacea and size of corpora amylacea in supplementary tables.

Evaluation of Other Covariates:

i. Immunohistochemical biomarkers—A range of tumor biomarker studies have been conducted within the Health Professionals Follow-up Study on tumor tissue microarrays (TMA) that were constructed by sampling 3 to 6 cores (0.6 mm) of tumor tissue.^{16–18} Briefly, the *TMPRSS2:ERG* gene fusion is a common molecular subtype of prostate cancer that was characterized using immunohistochemistry for protein overexpression of v-ets erythroblastosis virus E26 oncogene homolog (ERG),¹⁵ a method that was previously shown to have high concordance with other assays.¹⁹ A genitourinary pathologist reviewed the TMA slides and samples were considered positive if they had at least one core of ERG staining within prostate epithelial cells.²⁰ Similarly, immunohistochemistry of the tumor suppressor PTEN was performed on TMAs using a rabbit antihuman PTEN antibody (Clone D4.3 XP; Cell Signaling Technologies, Danvers, MA). A tissue core was considered to have PTEN protein loss based on the staining intensity of cytoplasmic and nuclear staining. The PTEN staining intensity was classified as entirely lost or markedly decreased across more than 10% of tumor cells compared to the surrounding benign glands and/or stroma, which provided an internal positive control for PTEN protein expression.²¹

To understand the relationship between corpora amylacea with biomarkers of disease aggressiveness, we also considered tumor biomarkers of proliferation and angiogenesis. Tumor proliferation was assessed by immunohistochemical staining with the polyclonal anti ki67 antibody diluted 1:2,000 after citrate-based antigen retrieval (Vector Labs). The ki67 proliferative index was scored using quantitative image analysis (Ariol SL-50 Applied Imaging).²² To characterize morphologic measures of angiogenesis, serial sections of prostatectomy blocks were evaluated for endothelial cell marker CD34, by immunohistochemical staining of primary mouse monoclonal antibody anti-CD34 (QBEN10; Biogenex, San Ramon, CA).²³ Semi-automated image analysis under pathology supervision was undertaken to derive vessel diameter and irregularity of the vessel shape, which were associated with lethal prostate cancer in this cohort; however, microvessel density was not associated with lethal prostate cancer and hence was not evaluated in this study.²³

ii. Clinical and lifestyle characteristics—We obtained clinical information including age at diagnosis, PSA at diagnosis, pathological tumor stage for radical prostatectomy specimens, and clinical tumor stage for TURP specimens through review of medical records, pathology reports, and physician- and participant-reported questionnaires. Information on weight and height to derive body mass index (BMI, kg/m²) and vasectomy status were self-reported on biennial mailed questionnaires. BMI at diagnosis was derived from the questionnaire immediately preceding a prostate cancer diagnosis. We identified men who had a vasectomy before their prostate cancer diagnosis by deriving the date and age at vasectomy from self-reported vasectomy information on biennial questionnaires between 1986 and 2000, which was associated with higher risk of lethal prostate cancer in this cohort.²⁴

Statistical Analyses

The main outcomes of interest were presence of corpora amylacea within benign area (n=353) and within predominately tumor area (n=340). The main exposure of interest was chronic inflammation status in prostate tissue. To assess whether chronic inflammation was associated with presence of corpora amylacea, we performed multivariable logistic regression models to estimate odds ratios (OR) and 95% confidence intervals (CI), separately for presence of corpora amylacea in benign and within predominately tumor tissue area. All analyses were conducted in SAS version 9.3 (Cary, NC). For all analyses, we report two-sided p-values.

Additionally, in this cross-sectional analysis we assessed other potential predictors of corpora amylacea, such as age, BMI, source of tissue, number of fields reviewed, PSA, acute inflammation, Gleason grade, stage, *TMPRSS2:ERG* fusion, ki67, angiogenesis markers and vasectomy status to better describe corpora amylacea and its potential drivers or consequences. We used the missing indicator method for those with missing PSA at diagnosis (n=38) and missing acute inflammation data (n=7), and used simple single imputation to assign values for those with missing *TMPRSS2:ERG* fusion status (n=3), vasectomy (n=1), and BMI at diagnosis (n=1).²⁵ The missingness of PSA was primarily among cases diagnosed before 1994, which suggests that most individuals missing PSA were diagnosed in the pre-PSA screening era. In multivariable logistic regression models, we considered the following clinical and histological features: Gleason grade (high grade 4+3 and 8-10 versus low grade 2-6 and 3+4), age at diagnosis (years, continuous), PSA at diagnosis (< 4 ng/mL, >4 ng/mL, and missing PSA at diagnosis), source of tissue specimen (radical prostatectomy, TURP, missing), TNM stage (T2, T3, T4/N1/M1), *TMPRSS2:ERG* fusion status (fusion-positive versus fusion-negative) and the following lifestyle factors: BMI at diagnosis (kg/m², continuous), and vasectomy status at diagnosis (ever versus never). We further considered the proliferative index and angiogenesis biomarkers in the multivariable models using categories based on the median value cut-offs of the ki67 marker of cellular proliferation (high expression, low expression, and missing), vessel size (large diameter >24.8 µm, small diameter ≤24.8 µm, and missing) or vessel irregularity (most roundly shaped vessels, most irregularly shaped vessels, and missing). Those missing proliferative index and angiogenesis markers were similar to the larger tissue cohort in terms of important prostate cancer factors, such as age, PSA, and Gleason grade, but were more likely to have been diagnosed more recently. The parsimonious model contained the following predictors: chronic inflammation, BMI, age, Gleason grade, source of tissue, *TMPRSS2:ERG* fusion status, ki67 proliferation index, PTEN status, and number of fields reviewed. The fully adjusted model additionally included PSA at diagnosis, TNM stage, angiogenesis markers and vasectomy status and are presented in supplementary tables. Due to the high concordance between chronic inflammation and acute inflammation based on a Chi-square test of categorical variables, we separately considered a model to enumerate the association between acute inflammation and presence of corpora amylacea adjusted for BMI, age, Gleason grade, source of tissue, *TMPRSS2:ERG* fusion status, ki67, PTEN status, and number of fields reviewed.

In supplementary analyses, we used multinomial logistic regression to test whether chronic inflammation was similarly associated with density and size of corpora amylacea in benign tissue after multivariable adjustment. We categorized corpora amylacea density as absent, present with low-density, or present with high-density based on the median value of corpora amylacea density. Furthermore, we categorized size of corpora amylacea as absent, present with generally small size, present with generally medium size, and present with generally large or various sizes.

Results

We found that 298 men (84%) had corpora amylacea in benign prostate tissue and 180 men (53%) had corpora amylacea within the predominately tumor area. Just over half of the men (51%) had corpora amylacea in both benign and predominately tumor areas, while corpora amylacea were absent in benign or tumor tissue for 14%. Clinical and histological characteristics of this cohort are displayed in Table 1 according to presence of corpora amylacea in the benign area. The corpora amylacea were predominately medium-sized. Images of the corpora amylacea in benign tissue and within the predominately-tumor area are presented in Supplementary Figure 1.

Presence of chronic inflammation was strongly associated with corpora amylacea in benign tissue in the parsimonious (Table 2) and fully adjusted models (Supplementary Table 1). Compared to those without chronic inflammation, the multivariable odds ratios for presence of corpora amylacea were 3.3 for mild chronic inflammation (95% CI 1.3, 8.5) and 5.4 for moderate/severe chronic inflammation (95% CI 1.9, 15.6) (p-trend=0.007, Table 2); findings were similar after accounting for additional covariates (Supplementary Table 1). These strong and positive associations with moderate to severe chronic inflammation were similar across levels of corpora amylacea density (Supplementary Table 2) and size categories (Supplementary Table 3), albeit there were few with large- or various-sized corpora amylacea.

BMI was also positively associated with presence of corpora amylacea in benign tissue, with 1.13 times higher odds of having corpora amylacea for every one kg/m² increase in BMI at diagnosis (95% CI 1.01, 1.26; p-value=0.03, Table 2). In the multivariate model, presence of acute inflammation was associated with corpora amylacea compared to those without acute inflammation (OR= 2.31 95% CI 0.94, 5.65; p-value=0.07). Due to the strong concordance between chronic inflammation and acute inflammation categories (Chi-square p-value <0.0001), after further adjusting for chronic inflammation the association between acute inflammation and corpora amylacea was attenuated and, as expected for collinear variables, had larger standard errors (OR=1.97 95% CI 0.67, 5.83; p-value=0.22). The associations between chronic inflammation status and corpora amylacea remained strong after adjustment for acute inflammation (mild chronic inflammation versus none OR=2.63 95% CI 0.96, 7.24; moderate to severe chronic inflammation vs none OR=2.95 95% CI 0.87, 9.97; p-value for trend=0.14).

Patients with corpora amylacea in benign tissue had tumors that were characterized by less aggressive disease based on lower Gleason grade and lower ki67 proliferative index in the

parsimonious (Table 2) and fully adjusted models (Supplementary Table 1). The presence of corpora amylacea was less common for those with higher Gleason scores compared to those with lower Gleason scores (OR=0.4 95% CI 0.2, 0.8, p-value=0.01) and for those with higher proliferative index (OR=0.6 95% CI 0.3, 1.2, p-value=0.11). In addition, tumors with the *TMPRSS2:ERG* fusion were significantly less likely to have corpora amylacea in the benign tissue compared to those with fusion negative tumors (OR=0.4, 95% CI 0.2, 0.8; p-value =0.01, Table 2). In contrast, there was a suggestion that PTEN loss was positively associated with presence of corpora amylacea (OR=2.0 95% CI 0.8, 5.2, p-value=0.16, Table 2) but this was not statistically significant.

Those with TURP specimens were much less likely to have corpora amylacea in benign tissue than those with radical prostatectomy tissue (OR=0.12 95% CI 0.03, 0.47; p-value=0.002, Table 2). There was a suggestion that older age at diagnosis (OR=1.04 95% CI 0.99, 1.10; p-value=0.13) was positively associated with corpora amylacea (Table 2). Corpora amylacea in benign tissue was not statistically significantly associated with disease TNM stage, high PSA at diagnosis, vasectomy, or angiogenesis markers of vessel shape (Supplementary Table 1) or vessel diameter (data not shown for vessel diameter). Of note, the association between TNM stage and corpora amylacea was in line with previous findings seen with Gleason and proliferative index where those with T3 stage disease were less likely to have corpora amylacea than those with less aggressive T2 disease (OR=0.5 95% CI 0.3, 1.1; p-value=0.11; Supplementary Table 1); similar associations were observed when comparing those with T3 and T4 stage disease to those with T2 stage disease (OR=0.5 95% CI 0.3, 1.2; p-value=0.12).

Overall, associations between clinical, histological, biomarkers, and lifestyle factors with corpora amylacea found within predominately tumor areas (Supplementary Table 4) were not statistically significant, except for vasectomy status. Corpora amylacea in predominately tumor-tissue areas were observed to be suggestively associated with moderate to severe chronic inflammation (OR=2.2 95% CI 0.9, 5.2) and *TMPRSS2:ERG* fusion status (OR=0.7 95% CI 0.4, 1.1). Lastly, those who had a vasectomy were less likely to have corpora amylacea in the tumor area than those who did not have a vasectomy (OR=0.6 95% CI 0.3, 1.0). Presence of corpora amylacea within predominately tumor tissue was not associated with Gleason grade, TNM stage, type of tissue specimen, PSA at diagnosis, age at diagnosis, ki67 proliferation, angiogenesis markers, PTEN status, or BMI at diagnosis.

Discussion

In this prostate cancer patho-epidemiology study, we found that corpora amylacea within the benign-area and within predominately tumor area was a more common histological feature among men with prostate cancer than previous reports^{11–13}. Furthermore, presence of corpora amylacea in benign tissue was strongly associated with pro-inflammatory factors (i.e. moderate to severe chronic inflammation and higher BMI), as well as some markers of less aggressive prostate cancer (i.e. lower Gleason and lower proliferative index), and with tumors that lack the *TMPRSS2:ERG* fusion (Table 3). The associations were similar when we considered the density and size of corpora amylacea, highlighting that their presence alone is informative.

In the current study of prostatectomy and TURP tissue specimens, we found that a large majority of prostate cancer patients have corpora amylacea in adjacent normal tissue. We also observed that corpora amylacea were commonly found within the predominately tumor area; however, we did not distinguish whether the corpora amylacea were within a tumor gland or a benign gland admixed in the tumor area, which may overestimate the prevalence of corpora amylacea in the predominately tumor area. In this study, the small TURP specimens were much less likely to have corpora amylacea compared to the whole-section radical prostatectomy tissue specimens. While this finding may be due to sampling variation, it is noteworthy that the TURP tissue tends to derive from the transitional zone of the prostate, whereas we quantified corpora amylacea predominantly in the peripheral zone of the prostatectomy specimens, which may suggest that corpora amylacea are more commonly found in the peripheral zone of the prostate.

Pro-inflammatory factors, especially chronic inflammation in the prostate, were associated with corpora amylacea. Corpora amylacea in the prostate are mainly composed of proteins involved in acute inflammatory and immune processes including lactoferrin, calprotectin, neutrophil-derived proteins (myeloperoxidase, α -defensins), β -microglobulin, and pro-inflammatory calcium-binding S100 proteins.^{7,26} Though the current cross-sectional study cannot distinguish the temporality of acute or chronic inflammation and corpora amylacea formation, the molecular structure of corpora amylacea suggest that their development is dependent on acute inflammatory proteins, immune proteins, and metal ions like Zn^{2+} and Ca^{2+} .^{7,9,27} Interestingly, the strong association between chronic inflammation and corpora amylacea persisted after adjustment for acute inflammation among this group of men with prostate cancer. While it appears that the formation of corpora amylacea is dependent on existing inflammation, some hypothesize that corpora amylacea may also induce additional physical trauma to the surrounding prostatic cells perpetuating the inflammatory response (i.e. positive feedback).³ If corpora amylacea caused additional inflammatory trauma to the surrounding cells, one would expect stronger associations with chronic inflammation among those with higher density of corpora amylacea or larger-size corpora amylacea and not as strong associations with chronic inflammation among those with less dense or smaller-sized corpora amylacea. Our exploration of corpora amylacea density and size were consistent with the main finding that moderate to severe chronic inflammation was strongly associated with presence of corpora amylacea compared to those without chronic inflammation; however, we observed that the associations with chronic inflammation were not substantially different across high versus low density or across sizes of corpora amylacea. The findings of corpora amylacea density and size show that chronic inflammation was similarly associated with presence of corpora amylacea regardless of the density or size, which would suggest that corpora amylacea were not causing additional damage and inflammation to the surrounding tissue; however, the findings on density and size are not conclusive given the small sample sizes for those within levels of density and size. Furthermore, BMI was a modest, independent predictor of presence of corpora amylacea in benign prostate tissue, even after adjusting for histological chronic inflammation. Obesity is marked by a chronic inflammatory condition whereby adipocytes secrete cytokines and experience phagocytosis.^{28,29} The associations with chronic inflammation and BMI highlight that corpora amylacea

are related to pro-inflammatory exposures and that there are potentially modifiable factors contributing to the inflammatory environment of the prostate.

These observations that presence of corpora amylacea was common and associated with less aggressive disease markers, and with tumors that do not harbor the *TMPRSS2:ERG* fusion are hypothesis generating. While this cross-sectional study cannot tease apart temporality, these findings suggest that corpora amylacea formation may be a normal response to early cancers and the corpora amylacea may act to consolidate inflammatory debris to prevent more aggressive or mutated tumors; or perhaps the *TMPRSS2:ERG* fusion and aggressive tumors work to prevent the formation of corpora amylacea. Limited literature suggests that *TMPRSS2:ERG* fusion-positive tumors are characterized by presence of inflammatory markers^{30–34} and that inflammation was associated with worse disease.^{4,5} *TMPRSS2:ERG* fusion-positive tumors had higher IL-6 expression than fusion-negative tumors³³ and the pro-tumorigenic effects of the NF-κB pathway were activated only in fusion-positive cancer patients.³² Furthermore, inflammation-related exposures such as body mass index^{35–37}, aspirin, and lycopene intake^{38,39} were associated with *TMPRSS2:ERG* fusion positive tumors and not fusion negative tumors. In this study, we found that *TMPRSS2:ERG* fusion-positive tumors were less likely to have corpora amylacea, suggesting that the fusion positive tumors could be evading a normal process that may protect cells from inflammation. However, the cross-sectional design of this study restricts us drawing conclusions about the temporal relationship of corpora amylacea and tumor formation. While the debate continues on whether corpora amylacea are protective or degenerative, research on corpora amylacea in the brain also points to their role in defending cells from further damage.⁸

This study has several limitations and strengths to consider when interpreting the study findings. The cross-sectional design of this study prohibits understanding the temporal development of prostatic corpora amylacea and their complex temporal relationship with the cellular environment. For example, corpora amylacea are composed of acute inflammatory proteins⁷ suggesting that their development depends on acute inflammation; however, in this cross-sectional study we could not fully distinguish the associations between acute inflammation and corpora amylacea independent of chronic inflammation as most men with prostate cancer who had acute inflammation also had chronic inflammation. Similarly, in a cross-sectional study one cannot distinguish whether the predictors influence development of corpora amylacea or if corpora amylacea may influence certain histological and tumor biomarkers. We did not measure the proximity of inflammatory infiltrates to the corpora amylacea, which would provide more compelling evidence for the relationship between inflammation and corpora amylacea; however, we still observed strong associations between chronic inflammation and corpora amylacea presence in benign tissue. The corpora amylacea found in predominately tumor area were not fully characterized as being within a tumor gland or being within a benign gland admixed in the tumor area, which can lead to misclassification and overestimation of corpora amylacea in the tumor area. Furthermore, while this is the first study to incorporate the size of corpora amylacea, the supplementary results on corpora amylacea size should be interpreted with caution because of small sample sizes and corpora amylacea size was not objectively measured that may lead to misclassification of size. In spite of these limitations, this was the first patho-epidemiologic study to assess corpora amylacea in prostatectomy and TURP tissue specimens and to

incorporate a wealth of data to gain insight in to their formation and function. We used an array of biomarkers, clinical, histological, and lifestyle factors to characterize their cross-sectional associations with presence, density, and size of corpora amylacea. To our knowledge, this is the largest and most comprehensive epidemiologic study of prostatic corpora amylacea and provides novel insights on the biology of corpora amylacea.

In conclusion, the presence of corpora amylacea in prostatectomy tissue was common among men with prostate cancer and was strongly associated with chronic inflammation and obesity. Additionally, having corpora amylacea in the benign prostate tissue was associated with markers of less aggressive disease and lacking the *TMPRSS2:ERG* fusion in tumors. Further research is needed to establish the temporality in corpora amylacea formation and to understand the complex nature of inflammatory responses and the immune system in relation to molecular subtypes of prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Clinical and histological characteristics of the Health Professionals Follow-up Study prostate cancer cohort according to presence of corpora amylacea in the benign tissue area (n=353)

	Present (n=298)	Absent (n=55)
Number of fields reviewed, median (IQR)	100 (67-171)	76 (40-120)
Median density of corpora amylacea (SD)	0.50 (0.79)	-
Size of corpora amylacea, n (%)		
- Small	71 (24.0)	-
- Medium	194 (65.5)	-
- Large or Various	31 (10.5)	-
<i><u>Clinical and Histological features</u></i>		
Chronic inflammation status, n (%)		
- None	20 (6.7)	12 (21.8)
- Mild	154 (51.7)	30 (54.6)
- Moderate to Severe	124 (41.6)	13 (23.6)
Acute inflammation present, n (%)	84 (28.2)	7 (12.7)
Pathological Gleason grade, n (%)		
- 6	56 (18.8)	5 (9.1)
- 3+4	120 (40.3)	18 (32.7)
- 4+3	62 (20.8)	18 (32.7)
- 8-10	60 (20.1)	14 (25.5)
Age at diagnosis, years (SD)	65.6 (6.0)	65.5 (5.8)
PSA at diagnosis, n (%) [*]		
- 4 ng/mL	46 (17.4)	8 (15.7)
- >4 ng/mL	218 (82.6)	43 (84.3)
Radical prostatectomy tissue specimen, n (%)	289 (97.0)	50 (90.9)
TNM stage, n (%)		
- T2 N0/Nx M0/Mx	224 (75.2)	32 (58.2)
- T3 N0/Nx M0/Mx	67 (22.5)	21 (38.2)
- T4/N1/M1	7 (2.4)	2 (3.6)
<i><u>Biomarkers</u></i> [^]		
<i>TMPRSS2:ERG</i> fusion-positive, n (%)	136 (45.6)	35 (63.6)
Proliferation index categories, n (%) [^]		
- Low ki67 expression	102 (54.0)	17 (37.8)
- High ki67 expression	87 (46.0)	28 (62.2)
Vessel Shape categories, n (%) [^]		
- Irregular vessels	88 (48.1)	22 (59.5)
- Round vessels	95 (51.9)	15 (40.5)
Vessel Diameter categories, n (%) [^]		
- Thin vessels (< 24.8 µm)	89 (48.6)	22 (59.5)
- Large vessels (> 24.8 µm)	94 (51.4)	15 (40.5)

	Present (n=298)	Absent (n=55)
PTEN status, n (%) [^]		
- PTEN intact	171 (75.0)	34 (81.0)
- Any PTEN loss	57 (25.0)	8 (19.1)
<i>Lifestyle factors</i>		
Pre-diagnostic BMI, kg/m ² (SD)	26.3 (4.0)	24.7 (2.6)
Had a vasectomy before diagnosis, n (%)	88 (29.5)	15 (27.3)

Values are means (SD) or sample sizes (percentages). Values of polytomous variables may not sum to 100% due to rounding.

Abbreviations: PSA: prostate specific antigen

* The sample size does not sum to the total sample size due to missingness of PSA. The percentages presented here are only among those with non-missing data.

[^] The proliferation index, angiogenesis and PTEN biomarkers were only measured on a subset of participants so the sample sizes do not sum to the total sample size with information on corpora amylacea in the benign-tissue area. The percentages presented here are only among those with biomarker data.

Table 2.

Mutually adjusted odds ratios and 95% confidence intervals (CI) of histological and patient characteristics in relation to presence of corpora amylacea in benign tissue area (n=353)

Presence of corpora amylacea in benign tissue		
	Odds Ratio (95% CI)	p-value
<u>Clinical & Histological predictors</u>		
Chronic inflammation status		p-trend=0.007
<i>None</i>	REF	
<i>Mild</i>	3.33 (1.30, 8.50)	0.01
<i>Moderate to Severe</i>	5.40 (1.87, 15.6)	0.002
Gleason grade		
<i>6, 3+4</i>	REF	
<i>4+3 or higher</i>	0.42 (0.22, 0.83)	0.01
Age at diagnosis, per year	1.04 (0.99, 1.10)	0.13
Source of tissue specimen		
<i>Radical Prostatectomy</i>	REF	
<i>TURP</i>	0.12 (0.03, 0.47)	0.002
<u>Biomarker predictors</u>		
<i>TMPRSS2:ERG</i>		
<i>Fusion negative</i>	REF	
<i>Fusion positive</i>	0.42 (0.21, 0.84)	0.01
Ki67 Proliferation Index		
<i>Low</i>	REF	
<i>High</i>	0.55 (0.27, 1.15)	0.11
<i>Missing</i>	1.61 (0.64, 4.04)	0.31
PTEN status		
<i>PTEN intact</i>	REF	
<i>Any PTEN loss</i>	2.00 (0.77, 5.22)	0.16
<i>Missing</i>	1.01 (0.44, 2.29)	0.98
<u>Lifestyle predictors</u>		
Pre-diagnostic BMI, kg/m ²	1.13 (1.01, 1.26)	0.03
Number of fields reviewed, per 10 fields	1.04 (0.99, 1.08)	0.09

Abbreviations: PSA: prostate specific antigen; TURP: transurethral resection of the prostate

Table 3.

Summary of associations between clinical, histological, and lifestyle factors with corpora amylacea.*

	Presence of corpora amylacea in benign tissue
<i>Inflammatory features</i>	
Chronic Inflammation	↑
BMI at diagnosis	↑
<i>Tissue Source</i>	
TURP tissue	↓
<i>Markers of Disease Aggressiveness</i>	
Gleason Grade	↓
Cellular proliferation	↓
Angiogenesis markers	-
Pathological TNM Stage	-
Vasectomy before diagnosis	-
Age at diagnosis	-
PSA at diagnosis	-
<i>Molecular Subtypes</i>	
PTEN loss	-
<i>TMPRSS2:ERG</i> fusion positive	↓

Abbreviations: TURP: transurethral resection of the prostate; PSA: prostate specific antigen

* An upward arrow (↑) indicates a positive association with the predictor and corpora amylacea. A downward arrow (↓) indicates an inverse association with the predictor and corpora amylacea. The (–) sign indicates no association between the predictor and corpora amylacea presence.