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Differential Gene Expression in Prostate Tissue According to Ejaculation Frequency

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Abstract

In a prospective study of 31 925 men with 18 yr of follow-up, higher ejaculation frequency (EF) throughout adulthood was associated with lower rates of prostate cancer. To further explore this association, we evaluated whole transcriptome gene expression in the prostate tissue from study participants who developed prostate cancer between 1992 and 2004 (n = 157 tumor tissue, n = 85 adjacent normal). We tested for trends in gene expression according to the level of EF as self-reported in 1992 for ages 20–29 yr, 40–49 yr, and the year prior to the questionnaire, 1991. There were no associations between EF and gene expression in areas of tumor after accounting for multiple testing. In contrast, in the adjacent normal tissue, 409 genes and six pathways were differentially expressed at a false discovery rate $\leq$0.2 across categories of EF in 1991. These results suggest that ejaculation affects the expression of genes in the normal prostate tissue. The identified genes and pathways provide potential biological links between EF and prostate tumorigenesis.

Patient summary: To explore previous findings that men who ejaculate more frequently have lower risk of prostate cancer, we evaluated molecular alterations in the prostate tissue according to each man's frequency of ejaculation prior to diagnosis. We identified biological processes that could link ejaculation frequency and prostate cancer.

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1. Brief report

We recently reported results from a prospective study among 31 925 men in the Health Professionals Follow-Up Study (HPFS) demonstrating an association between higher ejaculation frequency (EF) during adulthood and lower prostate cancer incidence between 1992 and 2010 [1]. The association persisted when considering EF at different ages and controlling for potential confounding. The reason for the inverse association is unclear; however, one proposed causal explanation is the prostate stagnation hypothesis: carcinogenic substances accumulate in the prostate, and thus, longer intervals between ejaculations provide greater opportunity for tumorigenesis [2,3]. We hypothesized that

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if this were true, gene expression patterns in the prostate tissue may vary according to EF.

We, therefore, analyzed the expression of 20 254 genes in the prostate tumor (and adjacent normal) tissue of 157 (85) men with prostate cancer from the HPFS. Because these sample sizes are small, these analyses are exploratory; however, we believe they demonstrate that ejaculation is associated with molecular alterations in the prostate and support that the association we observed between EF and prostate cancer risk could operate through such molecular alterations.

Information on our study population is presented in Supplementary Table 1. In 1992, men self-reported EF in six frequency categories for the age ranges of 20–29 yr, 40–49 yr, and previous year (1991). A subset subsequently developed prostate cancer between 1992 and 2004 and had tissue available from radical prostatectomy or transurethral resection of the prostate (Gene Expression Omnibus accession number GSE79021) [4]. Individuals were selected for whole-transcriptome gene expression profiling based on the outcome. For this study, we included 33 lethal patients who died of cancer or developed distant metastases and 124 nonlethal patients who survived at least 8 yr after diagnosis and experienced no distant metastases during follow-up.

For each of 20 254 genes, we regressed expression level on EF, controlling for age, and year of diagnosis. Each EF variable was collapsed from six to three categories to maintain reasonable sample sizes in each category (details in Supplementary Material). By testing the coefficient associated with EF, we assessed increasing or decreasing trend in the gene expression levels across EF categories. We repeated this for EF in each time period, separately in tumor and normal tissue (when available). We first examined the distributions of p values of these tests, which can provide a macroscopic view of whether there are any relationships between gene expression and EF (Supplementary Material). We observed no notable relationship between EF and gene expression in the prostate tumor tissue. In the normal prostate tissue, however, while there was no signal when considering EF at ages 20–29 yr and only a slight signal at ages 40–49 yr, we found an enrichment of significant associations between gene expression and EF in 1991. Controlling for multiple testing using the false discovery rate (FDR), there were 409 genes significant at FDR $\leq$0.20 (Supplementary Table 2). Boxplots displaying expression patterns in the top 15 most significant genes are displayed in Fig. 1.

Next, focusing on EF in 1991, we tested for pathway-level associations to understand which biological processes demonstrated coordinated differences according to EF. We performed gene set enrichment analysis using the trend tests above [5,6]. The top 10 pathways are in Table 1. The most statistically significant pathway was ubiquitin-mediated proteolysis (FDR = 0.10), a pathway involved in cell cycle regulation. Interestingly, the citrate cycle pathway appears in this list, albeit with FDR = 0.30; this pathway may be particularly relevant here because increased ejaculation causes increased citrate production and export by prostate cells [7,8]. No pathways approached significance in the tumor tissue.

Our study has strengths and limitations to consider. This dataset is unique in its access to information on EF status in combination with gene expression in the tumor and adjacent normal tissue. We used a validated expression profiling platform for archival tissue specimens [9]. However, our sample size was small. Participants were aged 45–73 yr in 1992, and the time from 1992 to diagnosis (and tissue collection) ranges from 0 to 12 yr. The wide range in time between EF report and diagnosis likely attenuated the associations. We accounted for this heterogeneity by including age and year of diagnosis in our models; however, our sample size is too small to deeply dissect differences by age, time, or other factors that could confound or modify the association between EF and gene expression.

We hypothesize that associations identified here in the adjacent normal tissue could reflect associations that exist between ejaculation and gene expression in cancer-free patients. If EF impacts gene expression in the prostate tissue, then perhaps one of these pathways could influence tissue susceptibility to tumorigenesis and help explain the mechanism whereby EF affects prostate risk. However, gene expression data is from the time of diagnosis or treatment, not the time when EF was reported. Moreover, adjacent normal tissue in prostate cancer patients is known to exhibit subhistological molecular alterations that may differ from the tissue in men who are cancer-free [10–12]. Thus, we cannot be certain that the adjacent normal tissue is a good proxy for either normal or precancerous tissue.

It is interesting that the signal we see is exclusively in the adjacent normal tissue, but perhaps not surprising. In other work, we have seen large differences in tumor gene expression associated with clinical characteristics and found, for example, increasing variability in gene expression patterns among individuals with higher grade disease [13]. A small signal, such as what we see here with EF, could be obscured in the tumor tissue because a multitude of processes are dysregulated. Our results are also consistent with an early role for EF in prostate tumor development.

Finally, we emphasize that the study is observational; thus, we cannot rule out common causes of higher EF and gene expression such as overall health and other lifestyle factors. A follow-up study could look at gene expression changes before and after ejaculation.

Keeping these limitations in mind, this work makes two major contributions. First, our findings are consistent with EF leading to changes in gene expression in the prostate and could help substantiate the prostate stagnation hypothesis or another biological rationale for the relationship between EF and prostate cancer incidence. Second, we provide a list of genes and pathways to investigate further, which could potentially elucidate biological mechanisms underlying the observed, robust association between EF and risk of prostate cancer.

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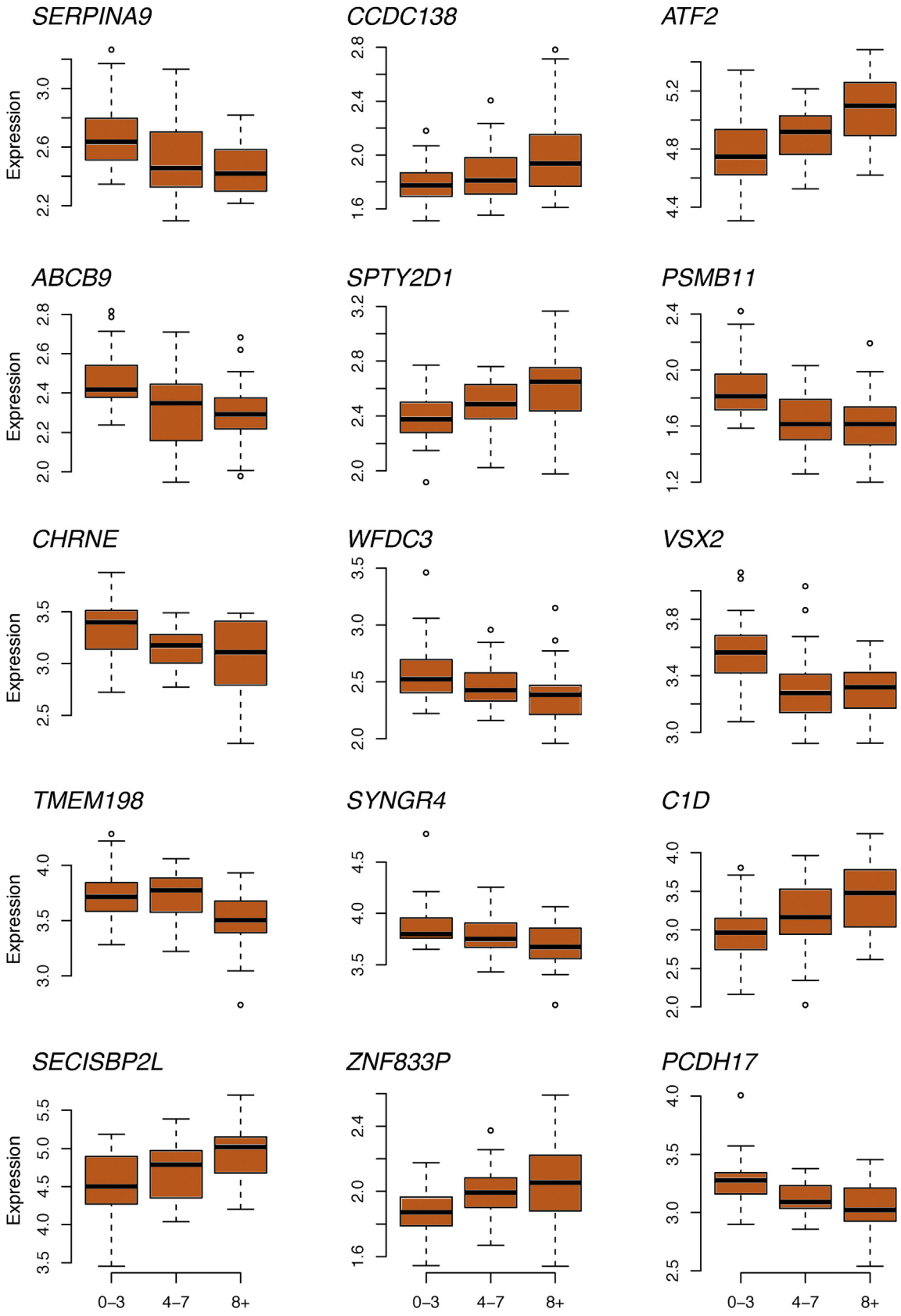

Fig. 1 – For the top 15 individual genes whose expression in the normal prostate tissue was associated with previous year ejaculation frequency, we present boxplots displaying the relationship between the gene expression levels and categories of ejaculation (0–3, 4–7, and 8+ ejaculations per month during the year prior to the questionnaire, as reported in 1992).

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Table 1 – The top 10 most statistically significant pathways from a Gene Set Enrichment Analysis (GSEA) performed in the normal prostate tissue for ejaculation frequency (EF) reported in the year before the questionnaire, 1991

Pathway	Size	Nominal *p* value	FDR *q* value
Ubiquitin-mediated proteolysis	132	0.001	0.10
Basal transcription factors	35	0.005	0.16
RNA degradation	57	0.006	0.16
Aminoacyl-tRNA biosynthesis	41	0.015	0.19
Spliceosome	124	0.003	0.19
Neuroactive ligand-receptor interaction	272	0.001	0.20
Oocyte meiosis	111	0.003	0.21
Citrate cycle (TCA cycle)	30	0.025	0.30
Valine, leucine, and isoleucine degradation	44	0.049	0.30
Adherens junction	73	0.038	0.31

FDR = false discovery rate; TCA cycle = tricarboxylic acid cycle.
Gene associations were measured by the t-statistics assessing the trend in the gene levels across categories of EF, controlling for age at and year of diagnosis. Pathway relevance was measured by the GSEA enrichment score. The *p* values were calculated by sample permutation; 10 000 permutations were used. All pathways are from the Kyoto Encyclopedia of Genes and Genomes and accessed through the molecular signatures database (MSigDB) version 6.0.

Author contributions: Jennifer Renee Rider had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Rider, Sinnott.
Acquisition of data: Rider, Mucci, Giovannucci.
Analysis and interpretation of data: Rider, Sinnott, Brumberg, Mucci, Giovannucci.
Drafting of the manuscript: Rider, Sinnott.
Critical revision of the manuscript for important intellectual content: Rider, Sinnott, Wilson, Ebot, Giovannucci.
Statistical analysis: Sinnott, Brumberg.
Obtaining funding: Mucci, Giovannucci.
Administrative, technical, or material support: None.
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2018.05.006.

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