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The *Monoamine Oxidase A* Gene Promoter Repeat and Prostate Cancer Risk

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Abstract

Background—Amine catabolism by Monoamine Oxidase A (MAOA) contributes to oxidative stress, which plays a role in prostate cancer (PCa) development and progression. An upstream variable-number tandem repeat (uVNTR) in the *MAOA* promoter influences gene expression and activity, and may thereby affect PCa susceptibility.

Methods—Caucasian (n=2,572) men from two population-based case-control studies of PCa were genotyped for the *MAOA*-VNTR. Logistic regression was used to assess PCa risk in relation to genotype.

Results—Common alleles of the *MAOA*-VNTR were not associated with the relative risk of PCa, nor did the relationship differ by clinical features of the disease. The rare 5-copy variant (frequency: 0.5% in cases; 1.8% in controls), however, was associated with a reduced PCa risk (odds ratio, OR=0.30, 95% CI 0.13–0.71).

Conclusions—A rare polymorphism of the *MAOA* promoter previously shown to confer low expression was associated with a reduced risk of developing prostate cancer. This novel finding awaits confirmation in other study populations.

Keywords

MAOA; polymorphism; prostate cancer

INTRODUCTION

Monoamine oxidase A (MAOA) is a mitochondrial membrane enzyme that catalyzes the oxidative deamination of serotonin, melatonin, catecholamines and other biogenic amines. This activity generates aminoaldehyde and hydrogen peroxide (1), reaction byproducts that increase oxidative stress and associated cellular responses. Though primarily studied in the context of a wide array of antisocial spectrum disorders (2), MAOA may also contribute to cancer, as the deaminated products of MAOA activity have been shown to promote cellular proliferation and inhibit apoptosis (3–5). Studies of gene expression determined that *MAOA*

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transcripts are elevated in malignant prostate epithelia relative to normal adjacent epithelia, and MAOA protein levels are associated with higher Gleason grades (6). MAOA activity has been shown to promote a more aggressive, de-differentiated PCa phenotype through undetermined mechanisms (7). Thus, since *MAOA* expression is associated with PCa and tumor severity, it follows that genetic variation in *MAOA* may be associated with PCa risk.

MAOA is located on chromosome Xp11.3. An upstream simple sequence repeat in the *MAOA* promoter region (*MAOA-uVNTR*) alters *MAOA* expression 2- to 10-fold depending on the number of repeats (8). One full repeat (ACCGGCACCGGCACCGTACCCGCACCGT) is 30 nucleotides long and contains smaller repetitive sequence blocks. The combined allele frequency of the high-expression 3.5-copy and 4-copy promoter repeats is approximately 65% among Caucasians and 39% among African-Americans; most of the remaining men carry 3- or 5-copy low-expression repeats (8). The 3.5-copy and 5-copy alleles are rare. To our knowledge, no studies examining the relationship between *MAOA* polymorphisms and risk of PCa have been published to date.

The observation of *MAOA* dysregulation in PCa led to the hypothesis that genetic polymorphisms affecting *MAOA* expression and activity might influence risk of developing PCa and confer a phenotype of more clinically aggressive disease. The purpose of this study was to examine the association between the *MAOA* promoter repeat polymorphism and the risk of developing PCa in the context of a population-based study.

MATERIALS AND METHODS

Study Population

Subjects were derived from two population-based case-control studies examining risk factors for PCa among Caucasian and African American residents of King County, Washington (9, 10). Briefly, eligible cases were men with histologically confirmed prostate adenocarcinoma identified via the Seattle-Puget Sound Survival, Epidemiology and End Results (SEER) cancer registry. Controls with no history of a physician's diagnosis of PCa were recruited by random-digit dialing (11) and frequency-matched to cases by 5-year age group. In the first study (study I), 917 men aged 40–64 who were diagnosed between 1993 and 1996 were eligible. Of these, 631 (84%) were interviewed and donated blood for DNA isolation. Of 941 potential controls, 565 (80%) of those interviewed provided a blood sample. Between 2002 and 2005 a second study of similar methodology (study II) ascertained 1,327 incident PCa cases aged 35–74; 1,001 (75%) agreed to be interviewed, and 827 (83%) also provided blood. Of 1,507 potential controls, 942 (63%) were interviewed and 787 (84%) also provided blood. For the analyses presented here, only Caucasians with DNA available were included (1,309 cases and 1,266 controls). The Institutional Review Board of the Fred Hutchinson Cancer Research Center has reviewed and approved the design of this study. Written informed consent was obtained from all participants.

Data collection was completed for exposures and events that occurred prior to reference date, which was the date of diagnosis for cases, and a randomly assigned date for controls based on the distribution of cases' diagnosis dates. The PSA level at diagnosis, Gleason score at diagnosis, stage of cancer at diagnosis, and primary treatment were ascertained for cases from SEER records. A case was characterized as having more aggressive PCa if the Gleason score at diagnosis was ≥ 7 (4+3), or the cancer was regional or distant stage, or if serum PSA was ≥ 20 ng/mL.

Genotyping

Genomic DNA was purified from peripheral blood lymphocytes using phenol-chloroform extraction preceded by proteinase K treatment (12). The subset of interviewed men who provided DNA did not differ significantly from the overall group with regard to demographic characteristics; interviewed cases did not differ from cases who declined interview with regard to clinical characteristics (9, 10).

The *MAOA* promoter variant repeat size was determined for all cases and controls for whom purified genomic DNA was available. PCR primers bracketing the repeat region, which varies between 224 and 344 nucleotides (nt) in length, were used to generate PCR products that are readily distinguished by length on a capillary sequencer: forward primer is 5'-TGCTCCAGAAACATGAGCAC-3', and the reverse primer is 5'-GTAGGAGGTGTCGTCCAAGC-3'. Primer specificity was confirmed by resequencing 20 samples and by comparing PCR products to those generated using previously published primers (8).

Similar numbers of case and control DNAs were included in each genotyping batch, and laboratory personnel were blinded as to case-control status. In addition, blanks and blind duplicates (5% of DNAs) were included and evaluated for agreement as part of quality control.

Statistical analysis

Unconditional logistic regression was used to calculate odds ratios (ORs) as estimates of the relative risk (13), and 95% confidence intervals for the association between PCa and individual *MAOA* promoter alleles. Polytomous regression was used to calculate ORs and 95% confidence intervals (14) for *MAOA* risk alleles, stratifying cases by clinicopathological features of tumor severity. Associations of these genotypes with dichotomized Gleason score (2–6 or 7=3+4 vs. 7=4+3 or 8–10) at diagnosis, tumor stage at diagnosis (local vs. regional or distant) and a composite score of PCa aggressiveness at diagnosis were evaluated. The composite measure of aggressiveness was: 1) less aggressive (local stage and Gleason score ≤ 7 (3+4) and PSA < 20 ng/mL); or 2) more aggressive (regional or distant stage, Gleason score ≥ 7 (4+3) or PSA ≥ 20 ng/mL). Potentially confounding covariates were examined according to genotype among controls using the χ^2 test, and were included in the model when a significant association ($p < 0.05$) and meaningful impact on the main effect of genotype (a change in magnitude of the OR of 10% or more) were detected.

RESULTS

Cases were significantly more likely than controls to report a family history of PCa (21.6% vs. 11.2%) and were more likely to have received a PSA test within the five years prior to the reference date (72.6% vs. 48.7%). Cases and controls were otherwise similar on selected characteristics: BMI, smoking status, alcohol consumption and exercise frequency (Table 1). The majority of cases had local stage PCa (78.2%) and/or a Gleason score of 6 or less (57%) at the time of diagnosis.

All men had between two and five copies of the 30-nucleotide repeat sequence in the *MAOA* promoter (Table 2). The two most common alleles (3 and 4 repeats) jointly accounted for approximately 90.7% of the controls' genotypes. Although family history of PCa and PCa screening history differed between cases and controls, neither variable confounded the association with *MAOA* genotype. Previous studies have determined that promoter repeats of 3.5 or 4 copies confer substantially greater *MAOA* transcriptional activity (high-expression repeats) than repeat lengths of 3 or 5 copies (low-expression

repeats). In this study the relative risk of PCa did not differ between the common high-expression 4-repeat promoter and the low-expression 3-repeat promoter (OR = 1.06, 95% CI 0.89–1.25). However, the relative risk was significantly lower in carriers of the rare 5-copy low-expression allele than the more common 3-copy low-expression allele (OR=0.30, 95% CI 0.13–0.71), though there were only 7 case and 23 control carriers of the 5-copy allele. Polytomous regression revealed that risk estimates did not differ between the high-expression 4-copy allele and low-expression 3-copy allele when cases were partitioned by Gleason scores, stage at time of diagnosis or disease aggressiveness (Table 3).

DISCUSSION

A large number of studies have linked variation in the *MAOA* gene with behavioral traits that include smoking, alcohol dependence, aggression, and depression, most thought to involve differential regulation of the MAOA substrate neurotransmitters that include serotonin and norepinephrine (2). In the context of neoplasia, these neurotransmitters have been implicated in tumor growth through activation of MAP kinase, PI3K/Akt, and other signal transduction programs (17). Further, the major byproducts of amine catabolism that include reactive oxygen species such as hydrogen peroxide (1), have clear roles in tumorigenesis through genotoxic mutagenesis and the promotion of invasive phenotypes.

This population-based case-control study examined whether alleles of the *MAOA* promoter shown to confer differential expression were associated with PCa risk among Caucasian men. Study subjects were interviewed regarding recognized risk factors for PCa and screening history, and these factors were considered in the analysis. None of the common alleles of the *MAOA* promoter were found to predispose men to developing PCa, nor were they related to clinicopathological features of more aggressive disease. Carriers of the rare 5-copy allele (0.5% of cases; 1.8% of controls), however, had a significantly lower risk of a PCa relative to 3-copy allele carriers. Although this estimate is based on small numbers, it is possible that this allele is biologically relevant for mediating PCa risk and warrants further study.

While the regulation of MAOA expression is known to be influenced by a simple sequence repeat in the *MAOA* promoter region located ~1,000 nucleotides upstream of the transcriptional start site (tss) (8), there are other mechanisms that have the potential to regulate MAOA levels or the activity of the MAOA enzyme. For example, a recent report identified a second small VNTR ~1,500 nucleotides upstream of the MAOA tss, that also appears to influence MAOA transcription (18). It has not been determined if this regulatory component is functional in benign or neoplastic prostate epithelium. Further, MAOA has been shown to be regulated by epigenetic mechanisms including methylation of promoter sequences (19). Variation in the inherent or inducible activity of other modulators of biogenic amine levels such as catechol-O-methyltransferase (COMT) may also influence the functional end-point of MAOA variation through the redundant synthesis or catabolism of precursors and products (1). Thus, future studies may benefit from analysis of additional genetic variants that influence MAOA activity.

The observation that a low MAOA activity allele is associated with reduced PCa risk is intriguing and warrants further evaluation in larger studies. Given the potential influence of MAOA on concentrations of reactive oxygen species within cells, studies designed to evaluate the influence of MAOA variation on PCa risk and outcomes may be enlightening.

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Table 1
Selected characteristics of population-based Caucasian prostate cancer (PCa) cases and controls, King County, WA

	Characteristic	Cases (%)	Controls (%)	OR ¹	95% CI
Age (years)	35–49	102 (7.8)	107 (8.5)		
	50–54	189 (14.4)	178 (14.1)		
	55–59	325 (24.8)	343 (27.1)		
	60–64	395 (30.2)	334 (26.4)		
	65–69	153 (11.7)	160 (12.6)		
Family history of PCa	70–74	145 (11.1)	144 (11.4)		
	No, 1st degree	1026 (78.4)	1124 (88.8)	1.00	Ref.
	Yes, 1st degree	283 (21.6)	142 (11.2)	2.17	1.77, 2.66
BMI (kg/m ²)	< 25.0	429 (32.8)	389 (30.7)	1.00	Ref.
	25.0 – 29.9	638 (48.7)	618 (48.8)	0.94	0.78, 1.12
	30+	242 (18.5)	259 (20.5)	0.85	0.68, 1.06
Smoking status	Nonsmoker	523 (40.0)	541 (42.7)	1.00	Ref.
	Former smoker	631 (48.2)	561 (44.3)	1.16	0.98, 1.36
	Current smoker	155 (11.8)	164 (13.0)	0.98	0.76, 1.26
Alcohol consumption ²	<= 1/mo for 6mo+	230 (17.6)	267 (21.1)	1.00	Ref.
	> 1/mo for 6mo+	1079 (82.4)	999 (78.9)	1.25	1.03, 1.53
Exercise ³	None	271 (20.7)	258 (20.4)	1.00	Ref.
	1× per week	249 (19.0)	279 (22.0)	0.84	0.66, 1.07
	2–3× per week	439 (33.5)	400 (31.6)	1.04	0.84, 1.30
PCa screening ⁴	4–5× per week	283 (21.6)	256 (20.2)	1.05	0.83, 1.33
	6+ per week	67 (5.1)	73 (5.8)	0.86	0.59, 1.25
	Never	135 (10.3)	167 (13.2)	1.00	Ref.
PSA (ng/mL) ⁵	DRE only	224 (17.1)	483 (38.2)	0.56	0.43, 0.75
	PSA	950 (72.6)	616 (48.7)	2.01	1.55, 2.60
	<4	178 (13.6)	1175 (92.8)		
	4–9.9	722 (55.2)	74 (5.8)		
	10–19.9	189 (14.4)	15 (1.2)		
	20+	118 (9.0)	2 (0.2)		

	Characteristic	Cases (%)	Controls (%)	OR ¹	95% CI
Gleason score	Unknown / Missing	102 (7.8)	0 (0)		
	2-4	67 (5.1)			
	5-6	680 (51.9)			
	7=3+4	356 (27.2)			
	7=4+3	76 (5.8)			
	8-10	126 (9.6)			
Stage	Unknown / Missing	4 (0.3)			
	Local	1023 (78.2)			
	Regional	254 (19.4)			
	Distant	32 (2.4)			
Aggressiveness ⁶	Less	874 (66.8)			
	More	435 (33.2)			
Primary treatment ⁷	RP	770 (58.8)			
	RAD and RAD+ADT	359 (27.4)			
	ADT	61 (4.7)			
	Other (only)	4 (0.3)			
	Expectant management	115 (8.8)			

¹ Models adjusted for 5-year age group

² Lifetime exposure

³ Within the year prior to reference date

⁴ Within the 5 year period prior to reference date

⁵ At time of diagnosis for cases, and at time of interview for controls

⁶ Aggressive cases had Gleason score 7(4+3) or PSA ≥ 20 ng/mL or regional / distant stage at diagnosis

⁷ RP (radical prostatectomy); RAD (radiation therapy); ADT (androgen deprivation therapy)

Table 2Odds ratios (OR) for prostate cancer associated with *MAOA* promoter repeats

<i>MAOA</i> promoter repeats	Cases (%)	Controls (%)	OR ^I	95% CI
2	0 (0.0)	1 (0.1)	-	-
3	412 (31.5)	408 (32.2)	1.00	Ref.
3.5	39 (3.0)	42 (3.3)	0.91	0.57, 1.44
4	792 (60.5)	740 (58.5)	1.06	0.89, 1.25
5	7 (0.5)	23 (1.8)	0.30	0.13, 0.71
Unknown	59 (4.5)	52 (4.1)		

^IORs adjusted for 5-year age group

Table 3
Odds ratios (OR) for prostate cancer associated with *MAOA* promoter repeats in cases stratified by clinical features

By Gleason Score		Controls (n=1,214) ²			Cases, Gleason score 7=3+4 (n=1,052) ²			Cases, Gleason score 7=4+3 (n=194) ²		
		No. (%)	No. (%)	OR ¹	95% CI	No. (%)	OR ¹	95% CI	No. (%)	OR ¹
<i>MAOA</i> promoter repeats	2	1 (0.1)	0 (0.0)	-	-	0 (0.0)	-	-	0 (0.0)	-
	3	408 (33.6)	337 (32.0)	1	Ref.	73 (37.6)	1	Ref.	73 (37.6)	1
	3.5	42 (3.5)	34 (3.2)	0.99	0.60, 1.62	5 (2.6)	0.72	0.27, 1.89	5 (2.6)	0.72
	4	740 (61.0)	674 (64.1)	1.07	0.89, 1.29	116 (59.8)	0.90	0.65, 1.24	116 (59.8)	0.90
	5	23 (1.9)	7 (0.7)	0.37	0.15, 0.90	0 (0.0)	-	-	0 (0.0)	-
By Stage		Controls (n=1,214) ²			Cases, Localized stage (n=974) ³			Cases, Regional/Distant stage (n=276) ³		
<i>MAOA</i> promoter repeats	2	1 (0.1)	0 (0.0)	-	-	0 (0.0)	-	-	0 (0.0)	-
	3	408 (33.6)	331 (34.0)	1	Ref.	81 (29.3)	1	Ref.	81 (29.3)	1
	3.5	42 (3.5)	26 (2.7)	0.79	0.46, 1.34	13 (4.7)	1.45	0.73, 2.85	13 (4.7)	1.45
	4	740 (61.0)	612 (62.8)	1	0.83, 1.20	180 (65.2)	1.18	0.88, 1.58	180 (65.2)	1.18
	5	23 (1.9)	5 (0.5)	0.26	0.10, 0.71	2 (0.7)	0.43	0.10, 1.90	2 (0.7)	0.43
By Aggressiveness		Controls (n=1,214) ²			Cases, Less aggressive (n=831) ³			Cases, More aggressive (n=419) ³		
<i>MAOA</i> promoter repeats	2	1 (0.1)	0 (0.0)	-	-	0 (0.0)	-	-	0 (0.0)	-
	3	408 (33.6)	274 (33.0)	1	Ref.	138 (32.9)	1	Ref.	138 (32.9)	1
	3.5	42 (3.5)	24 (2.9)	0.87	0.50, 1.50	15 (3.6)	1.04	0.55, 1.96	15 (3.6)	1.04
	4	740 (61.0)	528 (63.5)	1.03	0.85, 1.26	264 (63.0)	1.04	0.81, 1.32	264 (63.0)	1.04
	5	23 (1.9)	5 (0.6)	0.32	0.12, 0.87	2 (0.5)	0.25	0.06, 1.08	2 (0.5)	0.25

¹ ORs were obtained from polytomous regression models utilizing controls as the comparison group and were adjusted for 5-year age group and PCa screening history in the 5-year period prior to reference date

² 52 controls and 59 cases missing *MAOA* genotype and 4 cases missing Gleason score were excluded from analysis

³ 52 controls and 59 cases missing *MAOA* genotype were excluded from analysis