Association of *Megalin* Genetic Polymorphisms with Prostate Cancer Risk and Prognosis

Sarah K. Holt,[1] Danielle M. Karyadi,[4] Erika M. Kwon,[4] Janet L. Stanford,[1,3] Peter S. Nelson,[2] and Elaine A. Ostrander[4]

Abstract

Purpose: Megalin, an endocytic receptor expressed by prostate epithelial cells, can internalize biologically active androgens bound to sex hormone binding globulin. Genetic variation within *megalin* could potentially influence levels of steroid hormone uptake.

Experimental Design: Forty haplotype-tagging single-nucleotide polymorphisms (htSNP) were analyzed in a population-based, case-control study of 553 Caucasian men who were diagnosed with prostate cancer between the ages of 40 and 64 years from the Seattle-Puget Sound region and 534 control men. Prostate cancer risk was estimated using adjusted unconditional logistic regression for both individual SNPs and haplotypes. Risks of disease recurrence/progression and prostate-specific cancer mortality were estimated using Cox proportional hazards regression.

Results: We found no strong evidence of altered risk of developing prostate cancer for any of the htSNPs when they were assessed individually or in haplotypes. However, three htSNPs were significantly associated with both disease recurrence/progression and mortality. Risk of recurrence/progression alone was also associated with five additional htSNPs, and six other htSNPS showed evidence of modification by primary androgen deprivation therapy. Two additional htSNPs were significantly associated with altered risk of death from prostate cancer.

Conclusions: Preliminary results suggest that common genetic variation within the *megalin* gene could alter both risk of recurrence/progression and prostate-specific cancer mortality. In addition, androgen deprivation therapy effectiveness may be modified by the activity of this gene. To our knowledge, this is the first study that has examined polymorphisms within the *megalin* gene for associations with prostate cancer risk and outcomes.

Androgens are believed to contribute to both the etiology and progression of prostate cancer (1). Previously, it was thought that the only way the androgen hormone entered the cell and became biologically active was after disassociation from its plasma carrier protein, sex hormone binding globulin, and subsequent internalization of the lipophilic compound via free diffusion (2). A recent study by Hammes and colleagues (3) showed that megalin, a member of the LDL receptor gene family located on chromosome 2q24-q31, may play a role in the internalization of androgen into the prostatic cell. These researchers showed that megalin, a multiligand endocytic receptor, carries a specific binding site for androgen still bound to sex hormone binding globulin and serves as an active uptake mechanism of the entire steroid hormone complex. The role of megalin in the uptake of androgen in the mature healthy human prostate cell is not known, but our preliminary immunohistochemical assay studies of *megalin* gene expression have found increased levels of the protein in malignant relative to benign prostate cells.[5]

Polymorphisms within the *megalin* gene [low density lipoprotein–related protein 2 (*LRP2*)] could potentially alter the activity of the encoded protein to either promote or inhibit active transport of androgen into the prostate cell, resulting in a phenotype that has altered levels of androgen in the prostate cell. Whether this phenotype would have greater effect on normal versus malignant prostate cells is difficult to predict. If' megalin does in fact play a significant role in the internalization of androgen in normal mature prostate cells, then genetic variation could potentially affect the risk of developing prostate cancer. It is also plausible that prostate cancer cells may benefit from increased internalization of androgens via the active transport mechanism of megalin (4). Indeed, our immunohistochemical assay evidence does support a role of megalin in tumor progression. This lends credence to the hypothesis that a megalin phenotype that alters the uptake of androgen could

Authors' Affiliations: Divisions of [1]Public Health Sciences and [2]Human Biology, Fred Hutchinson Cancer Research Center; [3]Department of Epidemiology, University of Washington, Seattle, Washington; and [4]National Human Genome Institute, Cancer Genetics Branch, NIH, Bethesda, Maryland

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Requests for reprints: Elaine A. Ostrander, Building 50, Room 5351, 50 South Drive, Bethesda, MD 20892. Phone: 301-594-5284; Fax: 301-480-0472; E-mail: eostrand@mail.nih.gov.

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[5] P.S. Nelson and E. Mostaghel, unpublished data.

modify the progression from a localized androgen-dependent state into the androgen-independent and metastatic forms of prostate cancer. This association could be observed in either the risks of clinically diagnosed aggressive forms of prostate cancer or by disease outcomes. In addition, changes in protein function mediated by genetic variants could theoretically modify the reaction of prostate cells to androgen deprivation therapy (ADT). The goal of this study was to measure common genetic variation within *megalin* and to explore potential associations with disease risk, recurrence/progression, prostate-specific mortality, and possible interactions with primary ADT in a population-based, case-control study.

Materials and Methods

Study population. Study subjects were enrolled in a population-based prostate cancer case-control study that has previously been described (5). Prostate cancer cases were identified from the metropolitan Seattle-Puget Sound population-based tumor registry that is operated as part of the National Cancer Institute Surveillance, Epidemiology, and End Results program. The Surveillance, Epidemiology, and End Results registry also provided information on tumor characteristics, primary therapy, and vital status. Underlying cause of death was confirmed from death certificates. Eligible individuals were Caucasian or African American men, ages 40 to 64 y, who were newly diagnosed with histologically confirmed prostate cancer between January 1, 1993 and December 31, 1996. Of the 916 men eligible for the study, 752 (82.1%) completed structured in-person interviews. We obtained peripheral blood leukocyte samples yielding sufficient DNA for genotyping from 585 (77.8%) of the interviewed cases. Analysis was further limited to Caucasian men, bringing the total number of cases to 553 (94.5%).

Controls were identified through random digit telephone dialing using a clustering factor of five residences per sampling unit. Controls were frequency matched to cases by age (same 5-year group). Eligible controls were Caucasian or African American men, ages 40 to 64 y at the reference date, with no history of prostate cancer. Complete household census information was obtained for 94% of the 21,116 residential telephone numbers contacted. Of the 941 men who were identified and agreed to participate, 703 (74.7%) were interviewed, making the overall level of participation 70.2% (94% × 74.7%). From these potential controls, our analysis was limited to the 549 (78.1%) from whom we had sufficient DNA for genotyping. Analysis was further restricted to Caucasian men, bringing the total number of controls to 534 (97%).

Cases and controls completed detailed in-person interviews covering demographic, medical, lifestyle, and family cancer history conducted by trained male interviewers. Blood specimens were processed and DNA isolated using standard protocols (6). There were no differences between the nongenotyped and genotyped cases and controls with respect to age, race, family history of prostate cancer, or clinical characteristics of the patients. In January 2004, a self-administered follow-up questionnaire collecting information on quality of life, secondary therapies, follow-up prostate-specific antigen (PSA) results, and prostate cancer recurrence/progression was sent to the cases. Of the 630 cases who were alive and previously consented to future contact, the survey was completed by 520 (82.4%). Of these respondents, 405 (77.9%) had DNA and were Caucasian. There were no differences between follow-up survey respondents versus nonrespondents with respect to tumor characteristics or primary therapy. This study was approved by the Fred Hutchinson Cancer Research Center Institutional Review Board and genotyping was approved by the Internal Review Board of the National Human Genome Research Institute.

Single-nucleotide polymorphism selection and genotyping. Single-nucleotide polymorphisms (SNP) that captured the genetic variability in the gene were selected using publicly available data from the HapMap consortium[6] from a 248-kb region that comprised the *LRP2* locus and 13 kb of the 5′ end/promoter (from 169,804,367 to 170,048,683 in National Center for Biotechnology Information Build 35). There were 219 SNPs with minor allele frequencies >5% in the Caucasian HapMap data set. Haploview software version 3.11 was used to generate pairwise linkage disequilibrium estimates and define haplotype blocks using criteria outlined first by Gabriel and colleagues and then by the Solid Spline approach (7, 8). The user-defined linkage disequilibrium criteria for defining the blocks were fraction of informative pairwise comparisons $\geq$0.95 and $D' > 0.8$. This excludes SNPs with a minor allele frequency <5% by default. A total of 40 haplotype tagging SNPs (htSNP) were then selected to capture the variation within each block and then regions in between blocks (9). We defined common haplotypes as those with a frequency of >5%. The set of optimal htSNPs included three nonsynonymous SNPs that were forced into the htSNP selection.

The Applied Biosystems SNPlex Genotyping System was used to genotype SNPs in individual DNA samples. Proprietary GeneMapper software was used for calling alleles.[7] Discrimination of the specific SNP allele is carried out with the Applied Biosystems 3730*xl* DNA Analyzer and is based on the presence of a unique sequence assigned to the original allele-specific oligonucleotide.

Quality control included genotyping of 76 blind duplicate samples, which revealed 99% agreement on genotyping calls across all SNPs assayed. In addition, each batch of DNA aliquots genotyped incorporated similar numbers of case and control samples, and laboratory personnel were blinded to the case-control status of samples. The overall call rate was 99.0%. Subjects had to have at least 75%, or 30 of the 40 SNPs, successfully genotyped to be included in the study. Of the 56 case specimens included who did not have completely successful genotyping, 34 had only one SNP with unknown genotype, 12 had two SNPs with unknown genotype, and 10 had three to nine SNPs with unknown genotypes. Of the 30 controls who did not have complete genotyping, 16 had only one SNP with unknown genotype, 6 had two SNPs with unknown genotype, and 8 had three to nine SNPs with unknown genotypes.

Statistical analysis. SNP genotype frequencies were examined for Hardy-Weinberg equilibrium using the χ^2 statistics. Data were analyzed using unconditional logistic regression to calculate odds ratios as estimates of the relative risk of prostate cancer associated with any given individual htSNP. For each htSNP, we classified carriers of the variant or less common allele as the exposure group and then used both dominant and codominant models, excepting the polymorphic loci for which no individuals were homozygous for the variant genotype. We included the design variable of age in all regression models. We also assessed possible confounding effects of first-degree family history of prostate cancer, PSA testing history (none versus any PSA test done in the previous 5 y), history of benign prostatic hyperplasia (none, diagnosis $\leq$2 y before reference date, diagnosis >2 y before reference date), and body mass index (<25, 25-29, $\geq$30 kg/m^2). Because adjustment for none of these variables appreciably altered risk estimates, we did not include them as covariates. Trend tests were used to assess gene dosage and global *P* values were estimated to assess overall gene effect. Associations with individual htSNPs according to Gleason score [2-6 or 7 (3 + 4) versus 7 (4 + 3) or 8-10], tumor stage (local versus regional/distant), and a composite prostate cancer aggressiveness score were examined using polytomous regression. Classification parameters of the composite "nonaggressive" phenotype included diagnosis at local stage, and Gleason score of 2 to 6 or 7 (3 + 4), and serum PSA <20 ng/mL, whereas the "aggressive" phenotype included diagnosis at regional/distant stage, or Gleason score 7 (4 + 3) or 8 to 10, or serum PSA $\geq$20 ng/mL.

[6] http://www.hapmap.org/

[7] http://www.appliedbiosystems.com/

Table 1. Selected characteristics of Caucasian prostate cancer cases and controls

	Cases (n = 553), %	Controls (n = 534), %	Adjusted OR* (95% CI)
Age group (y)			
40-49	33 (6.0)	47 (8.8)	
50-54	122 (22.1)	108 (20.2)	
55-59	187 (33.8)	202 (37.8)	
60-64	211 (38.2)	177 (33.1)	
First-degree relative with prostate cancer			
No	444 (80.3)	477 (89.3)	1.0 (reference)
Yes	109 (19.7)	57 (10.7)	2.1 (1.5-2.9)
Body mass index (kg/m^2)			
<25	200 (36.2)	178 (33.3)	1.0 (reference)
25-29	267 (48.3)	264 (49.4)	0.9 (0.7-1.2)
30+	86 (15.6)	92 (17.2)	0.9 (0.6-1.2)
History of benign prostatic hyperplasia †			
None reported	367 (66.4)	437 (82.0)	1.0 (reference)
Diagnosis ≤2 y before reference date	63 (11.4)	23 (4.3)	2.5 (1.5-4.3)
Diagnosis >2 y before reference date	123 (22.2)	73 (13.7)	1.3 (1.0-2.0)
History of PSA and or DRE screening ‡			
None	37 (6.7)	72 (13.5)	1.0 (reference)
DRE only	113 (20.4)	278 (52.1)	0.8 (0.5-1.2)
PSA with or without DRE	403 (72.9)	184 (34.4)	4.2 (2.7-6.5)
Stage of disease at diagnosis			
Local	399 (72.2)		
Regional/distant	149 (26.9)		
Unknown	5 (0.9)		
Gleason score			
Low/moderate [2-6, 7 (3 + 4)]	480 (86.8)		
High [7 (4 + 3), 8-10]	72 (13)		
Unknown	1 (0.2)		
Composite aggressiveness score§			
Low	351 (63.5)		
High	202 (36.5)		
Primary androgen deprivation therapy‖			
No	459 (17.0)		
Yes	94 (83.0)		

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; DRE, digital rectal examination.
*Adjusted for age.
†Benign prostatic hyperplasia diagnosed at specific time periods before reference date. History of benign prostatic hyperplasia also adjusted for history of PSA tests.
‡Screening history limited to tests done in the 5 y before reference date.
§Composite aggressiveness: Low, diagnosed at local stage, and a Gleason score of 2 to 6 or 7 (3 + 4), and serum PSA <20 ng/mL; High, diagnosed at regional/distant stage, or Gleason score 7 (4 + 3) or 8 to 10, or serum PSA ≥20 ng/mL.
‖Androgen deprivation shots, pills, and/or orchiectomy within 12 mo of initial diagnosis date.

Because the htSNPs were chosen to represent haplotype variation, it is necessary to include both individual SNP and haplotype analyses for a complete assessment of genetic variation with disease risk. Two haplotype analyses were carried out. In the first, haplotype risk was assessed within each block using criteria defined by Gabriel and colleagues (7). Using data from control subjects, Haploview software version 3.2 was used to estimate pairwise linkage disequilibrium between the htSNPs and define haplotype blocks. In the second analysis, haplotype risk was assessed for a haplotype containing only htSNPs, which showed an individual significant association with prostate cancer risk. Risk was assessed using Haplostat software (version 1.2.1)[8] for R (version 2.1.0),[9] which uses the expectation-maximization algorithm to estimate haplotype frequencies and an iterative two-step expectation-maximization model to estimate the association between individual haplotypes and risk assuming an additive model (10). Overall differences in risk of prostate cancer across haplotypes were assessed using global score test adjusted for age (11). Only haplotypes with estimated frequencies of >5% were included.

To examine associations between individual htSNPs with prostate cancer recurrence/progression and prostate cancer mortality, we used Cox proportional hazards regression models adjusting for age, Gleason score, stage at diagnosis, and diagnostic PSA level. For estimating risk in the recurrence/progression model, the time-dependent variable was defined as time from diagnosis to the first self-reported evidence of development of metastasis. This variable was calculated using the data from the self-administered follow-up questionnaire as previously described (12). The censoring date for cases without a metastasis event was the date that their follow-up questionnaire was returned. Cases who had an initial diagnosis of metastatic disease were excluded from the analysis (n = 5) as were cases who were alive and did not fill out a follow-up questionnaire (n = 83). To account for cases diagnosed with localized or regional disease who died of metastatic prostate cancer before the follow-up survey was administered, the proportional hazards model was weighted. The weights were the inverse of the probability of having a metastasis date for men with a metastatic event and 1.0 for the men who were censored (13). For calculation of prostate-specific mortality, the time-dependent variable was defined as the time from

[8] http://ndc.mayo.edu/mayo/research/schaid_lab/
[9] http://CRAN.R-project.org/doc/FAQ/R-FAQ.html

Table 2. Genotype distribution and odds ratios (95% confidence intervals) for the association between *megalin* htSNPs and prostate cancer risk

Block*	htSNP	Genotype	Controls† (*n* = 534), %	Cases† (*n* = 553), %	Adjusted OR‡ (95% CI)
A	rs6433107	GG	303 (57.2)	305 (55.5)	1.0
		GT	192 (36.2)	211 (38.4)	1.1 (0.8-1.4)
		TT	35 (6.6)	34 (6.2)	1.0 (0.6-1.6)
		GT + TT			1.1 (0.8-1.3)
A	rs1003456	AA	340 (64.6)	345 (64.6)	1.0
		AT	166 (31.6)	166 (31.1)	1.0 (0.7-1.3)
		TT	20 (3.8)	23 (4.3)	1.1 (0.6-2.1)
		AT + TT			1.0 (0.8-1.3)
None	rs990626	TT	310 (58.2)	309 (56.6)	1.0
		CT	187 (35.1)	201 (36.8)	1.1 (0.8-1.4)
		CC	36 (6.8)	36 (6.6)	1.0 (0.6-1.7)
		CT + CC			1.1 (0.8-1.3)
B	rs4667592	GG	148 (27.8)	139 (25.2)	1.0
		AG	261 (49.1)	274 (49.7)	1.1 (0.8-1.5)
		AA	123 (23.1)	138 (25)	1.2 (0.9-1.7)
		AG + AA			1.2 (0.9-1.5)
B	rs3944004	TT	313 (58.8)	327 (59.5)	1.0
		GT	179 (33.6)	205 (37.3)	1.1 (0.8-1.4)§
		GG	40 (7.5)	18 (3.3)	**0.4 (0.2-0.8)**§
		GT + GG			1.0 (0.8-1.2)§
B	rs2075252‖	GG	311 (58.7)	316 (58.1)	1.0
		AG	187 (35.3)	196 (36)	1.0 (0.8-1.3)
		AA	32 (6)	32 (5.9)	1.0 (0.6-1.7)
		AG + AA			1.0 (0.8-1.3)
B	rs2239602	AA	426 (79.9)	442 (80.7)	1.0
		AG	100 (18.8)	103 (18.8)	1.0 (0.7-1.3)
		GG	7 (1.3)	3 (0.5)	0.4 (0.1-1.6)
		AG + GG			1.0 (0.7-1.3)
None	rs7588584	CC	264 (49.5)	264 (48.2)	1.0
		CT	217 (40.7)	236 (43.1)	1.1 (0.8-1.4)
		TT	52 (9.8)	48 (8.8)	0.9 (0.6-1.4)
		CT + TT			1.1 (0.8-1.3)
None	rs7592152	CC	228 (42.8)	207 (37.9)	1.0
		AC	240 (45)	268 (49.1)	1.3 (1.0-1.6)
		AA	65 (12.2)	71 (13)	1.2 (0.8-1.8)
		AC + AA			1.3 (1.0-1.6)
None	rs10490131	TT	368 (69.2)	388 (70.7)	1.0
		CT	143 (26.9)	146 (26.6)	1.0 (0.7-1.3)
		CC	21 (3.9)	15 (2.7)	0.7 (0.3-1.3)
		CT + CC			0.9 (0.7-1.2)
C	rs4668123‖	CC	283 (53.2)	283 (51.6)	1.0
		CT	214 (40.2)	224 (40.9)	1.1 (0.8-1.4)
		TT	35 (6.6)	41 (7.5)	1.1 (0.7-1.9)
		CT + TT			1.1 (0.8-1.4)
C	rs2239600	GG	264 (49.7)	270 (49.5)	1.0
		AG	226 (42.6)	232 (42.6)	1.0 (0.8-1.3)
		AA	41 (7.7)	43 (7.9)	1.0 (0.6-1.6)
		AG + AA			1.0 (0.8-1.3)
C	rs2268373	GG	279 (52.3)	300 (54.7)	1.0
		CG	215 (40.3)	223 (40.7)	1.0 (0.7-1.2)
		CC	39 (7.3)	25 (4.6)	**0.6 (0.3-1.0)**
		CG + CC			0.9 (0.7-1.1)
None	rs2300446	AA	178 (33.5)	162 (29.7)	1.0
		AG	247 (46.5)	284 (52)	1.3 (1.0-1.7)
		GG	106 (20)	100 (18.3)	1.0 (0.7-1.5)
		AG + GG			1.2 (0.9-1.6)
D	rs2302693	GG	138 (25.9)	131 (23.7)	1.0
		AG	256 (48)	302 (54.7)	1.3 (0.9-1.7)
		AA	139 (26.1)	119 (21.6)	0.9 (0.6-1.3)
		AG + AA			1.1 (0.9-1.5)
D	rs1362996	AA	432 (81.4)	433 (78.7)	1.0
		AG	94 (17.7)	112 (20.4)	1.2 (0.9-1.6)
		GG	5 (0.9)	5 (0.9)	1.0 (0.3-3.4)
		AG + GG			1.2 (0.9-1.6)

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Table 2. Genotype distribution and odds ratios (95% confidence intervals) for the association between *megalin* htSNPs and prostate cancer risk (Cont'd)

Block*	htSNP	Genotype	Controls† (*n* = 534), %	Cases† (*n* = 553), %	Adjusted OR‡ (95% CI)
D	rs2239598	TT	247 (46.5)	216 (39.6)	1.0
		CT	218 (41.1)	268 (49.2)	**1.4 (1.1-1.8)**§
		CC	66 (12.4)	61 (11.2)	1.0 (0.7-1.5)§
		CT + CC			1.3 (1.0-1.7)§
D	rs2239595	CC	385 (72.4)	398 (72.9)	1.0
		CT	131 (24.6)	140 (25.6)	1.0 (0.8-1.4)
		TT	16 (3)	8 (1.5)	0.5 (0.2-1.1)
		CT + TT			1.0 (0.7-1.3)
D	rs830958	TT	411 (77.5)	446 (81.2)	1.0
		GT	111 (20.9)	95 (17.3)	0.8 (0.6-1.1)
		GG	8 (1.5)	8 (1.5)	0.9 (0.3-2.5)
		GT + GG			0.8 (0.6-1.1)
E	rs830994	AA	236 (44.3)	229 (41.7)	1.0
		AG	235 (44.1)	256 (46.6)	1.1 (0.9-1.4)
		GG	62 (11.6)	64 (11.7)	1.1 (0.7-1.6)
		AG + GG			1.1 (0.9-1.4)
E	rs830995	GG	274 (51.6)	267 (48.7)	1.0
		AG	210 (39.5)	240 (43.8)	1.2 (0.9-1.5)
		AA	47 (8.9)	41 (7.5)	0.9 (0.6-1.4)
		AG + AA			1.1 (0.9-1.4)
E	rs831003	CC	328 (61.7)	309 (56.1)	1.0
		CG	171 (32.1)	218 (39.6)	**1.3 (1.0-1.7)**§
		GG	33 (6.2)	24 (4.4)	0.8 (0.4-1.3)§
		CG + GG			**1.3 (1.0-1.6)**§
None	rs831006	CC	187 (35.3)	185 (34.1)	1.0
		CG	252 (47.6)	266 (49.1)	1.1 (0.8-1.4)
		GG	90 (17)	91 (16.8)	1.0 (0.7-1.5)
		CG + GG			1.0 (0.8-1.3)
None	rs831012	TT	242 (45.5)	229 (41.9)	1.0
		CT	220 (41.4)	250 (45.8)	1.2 (0.9-1.5)
		CC	70 (13.2)	67 (12.3)	1.0 (0.7-1.5)
		CT + CC			1.2 (0.9-1.5)
F	rs831016	GG	194 (36.3)	190 (34.7)	1.0
		CG	249 (46.6)	258 (47.1)	1.1 (0.8-1.4)
		CC	91 (17)	100 (18.2)	1.2 (0.8-1.6)
		CG + CC			1.1 (0.9-1.4)
F	rs16856748	GG	421 (79.4)	441 (81.7)	1.0
		AG	99 (18.7)	93 (17.2)	0.9 (0.6-1.2)
		AA	10 (1.9)	6 (1.1)	0.6 (0.2-1.7)
		AG + AA			0.8 (0.6-1.2)
None	rs3770613	TT	162 (30.7)	166 (30.2)	1.0
		CT	263 (49.8)	263 (47.8)	1.0 (0.8-1.3)
		CC	103 (19.5)	121 (22)	1.2 (0.8-1.7)
		CT + CC			1.0 (0.8-1.4)
G	rs16856759	AA	294 (55.3)	294 (54)	1.0
		AG	206 (38.7)	210 (38.6)	1.0 (0.8-1.3)
		GG	32 (6)	40 (7.4)	1.2 (0.8-2.0)
		AG + GG			1.0 (0.8-1.3)
G	rs2892802	CC	388 (72.7)	407 (74)	1.0
		AC	138 (25.8)	130 (23.6)	0.9 (0.7-1.2)
		AA	8 (1.5)	13 (2.4)	1.5 (0.6-3.8)
		AC + AA			0.9 (0.7-1.2)
G	rs700550	TT	185 (34.8)	187 (34)	1.0
		CT	245 (46.1)	262 (47.6)	1.1 (0.8-1.4)
		CC	101 (19)	101 (18.4)	1.0 (0.7-1.4)
		CT + CC			1.0 (0.8-1.3)
G	rs830971	GG	336 (63.2)	359 (66.2)	1.0
		CG	173 (32.5)	164 (30.3)	0.9 (0.7-1.2)
		CC	23 (4.3)	19 (3.5)	0.8 (0.4-1.5)
		CG + CC			0.9 (0.7-1.1)
H	rs2673169	CC	270 (50.7)	289 (52.9)	1.0
		CG	230 (43.2)	218 (39.9)	0.9 (0.7-1.1)
		GG	33 (6.2)	39 (7.1)	1.1 (0.7-1.8)
		CG + GG			0.9 (0.7-1.2)

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Table 2. Genotype distribution and odds ratios (95% confidence intervals) for the association between *megalin* htSNPs and prostate cancer risk (Cont'd)

Block*	htSNP	Genotype	Controls† (*n* = 534), %	Cases† (*n* = 553), %	Adjusted OR‡ (95% CI)
H	rs6716834‖	TT	254 (47.8)	269 (48.8)	1.0
		CT	240 (45.2)	238 (43.2)	0.9 (0.7-1.2)
		CC	37 (7)	44 (8)	1.1 (0.7-1.8)
		CT + CC			1.0 (0.8-1.2)
H	rs831023	GG	435 (81.8)	466 (84.9)	1.0
		GT	94 (17.7)	81 (14.8)	0.8 (0.6-1.1)
		TT	3 (0.6)	2 (0.4)	0.7 (0.1-4.2)
		GT + TT			0.8 (0.6-1.1)
H	rs831030	TT	214 (40.4)	223 (40.9)	1.0
		CT	254 (47.9)	254 (46.6)	1.0 (0.7-1.2)
		CC	62 (11.7)	68 (12.5)	1.1 (0.7-1.6)
		CT + CC			1.0 (0.8-1.3)
None	rs6730118	AA	339 (63.8)	336 (61.3)	1.0
		AG	174 (32.8)	186 (33.9)	1.1 (0.8-1.4)
		GG	18 (3.4)	26 (4.7)	1.5 (0.8-2.7)
		AG + GG			1.1 (0.9-1.4)
None	rs1990842	AA	236 (44.6)	247 (45.3)	1.0
		AC	228 (43.1)	238 (43.7)	1.0 (0.8-1.3)
		CC	65 (12.3)	60 (11)	0.9 (0.6-1.3)
		AC + CC			1.0 (0.8-1.2)
None	rs3845732	CC	353 (66.5)	360 (66.2)	1.0
		CT	163 (30.7)	167 (30.7)	1.0 (0.8-1.3)
		TT	15 (2.8)	17 (3.1)	1.1 (0.6-2.3)
		CT + TT			1.0 (0.8-1.3)
None	rs3755166	GG	175 (33.8)	169 (31.8)	1.0
		AG	248 (47.9)	249 (46.8)	1.0 (0.8-1.4)
		AA	95 (18.3)	114 (21.4)	1.2 (0.9-1.7)
		AG + AA			1.1 (0.8-1.4)
None	rs700552	GG	219 (41.2)	218 (39.6)	1.0
		CG	241 (45.3)	260 (47.3)	1.1 (0.8-1.4)
		CC	72 (13.5)	72 (13.1)	1.0 (0.7-1.5)
		CG + CC			1.1 (0.8-1.4)

*Blocks of htSNPs are defined by Gabriel et al. (7). SNPs are in contiguous order.
†Variable numbers of cases and controls reflect instances of failed genotyping.
‡Adjusted for age. Odd ratios with a $P \leq 0.05$ are in boldface.
§Global $P < 0.05$. $P_{trend} > 0.05$.
‖These are nonsynonymous SNPs.

diagnosis to death or censoring for men who remained alive. Living cases were censored on the date of the most recent match with the cancer registry, December 1st, 2006. Cases that died from other causes were censored at the time of death.

We also assessed the possibility of an interaction between genotype and ADT with both disease recurrence/progression and survival. Using both data from the Surveillance, Epidemiology, and End Results registry and the self-report questionnaire, we defined cases who received androgen deprivation shots, pills, and/or orchiectomy within 12 mo of initial diagnosis as having received primary ADT. Due to small numbers of cases receiving primary ADT overall (n = 94), we limited both our mortality and recurrence/progression proportional hazards models to risk estimates assuming a dominant genetic model, combining heterozygotes and homozygous variants as the comparison group. Models with and without interaction terms were compared using the likelihood ratio test.

In an effort to account for multiple comparisons, the false discovery rate was used to calculate corrected critical P value to evaluate the robustness of all risk estimates (14). After consideration of SNP genotypes individually, all significant SNPs were included in a stepwise selection model using Akaike's Information Criterion to select the most parsimonious model (15). SNPs that remained significant ($P \leq 0.05$) after adjustment for each other were included in the final model (16). Both forward and backward selection models were compared, with equivalent results. All analyses were done using the STATA statistical package (version 9.2, STATA Corp.).

Results

Cases and controls were similar in age (mean: cases, 57 years; controls, 57 years). Cases were more likely to report a family history of prostate cancer, history of benign prostatic hyperplasia, and history of PSA tests (Table 1). The majority of prostate cancers were local stage tumors with low/moderate Gleason scores. All SNPs included in this study were statistically consistent ($P > 0.05$) with the Hardy-Weinberg equilibrium.

There was no strong evidence of altered risk of developing prostate cancer for any of the htSNPs evaluated (Table 2). Two SNPs (rs3944004 and rs2268373) showed slightly lower relative risks of prostate cancer for the homozygous variant carriers versus homozygous wild-type carriers. Two SNPs (rs2239598 and rs831003) showed slightly increased relative risks for the heterozygote carriers as compared with homozygous wild-type carriers. However, the P for trend for all four of these SNPs was >0.05, and after adjustment for multiple comparisons none of these associations remained significant.

When we assessed risk estimates by measures of tumor aggressiveness (Gleason score, stage, and composite score), we found no differences in risk estimates for any of the polymorphisms examined.

Within the 40 htSNPs included in this study, we defined 8 haplotype blocks (A-H; see Table 2) ranging from two to five htSNPs. Thirteen htSNPs did not fall into any haplotype block structure. We found no evidence of altered relative risks of prostate cancer for any of the haplotypes in any of the blocks. The four SNPs that showed slightly altered risks at the individual level (rs3944004, rs2268373, rs2239595, and rs831003) displayed no evidence of altered risks for prostate cancer when a haplotype containing only these four htSNPs was considered.

There were 86 cases with physician-diagnosed recurrence/progression, with an average of 8.8 years of follow-up (range, 0.2-11.9 years) after diagnosis. Of these 86 cases, 67 (78%) were diagnosed at low/moderate Gleason score, 55 (64%) were diagnosed at a localized stage, and 14 (16%) were treated with primary ADT. Eight htSNPs showed altered risks of disease recurrence/progression overall (Table 3). When these SNPs were adjusted for multiple comparisons, only three SNPs (rs830994, rs830995, and rs831003), all within the same haplotype block "E," retained significance. We then considered all eight SNPs and then only the three block "E" SNPs using stepwise regression and found that only rs830994 remained significant at $P = 0.001$, independent of all other SNPs in the model. An additional six htSNPs, although not showing altered risks for all cases combined, did show some evidence of different risks of recurrence/progression by ADT (Table 3).

There were 33 cases who died of prostate cancer in the average 10.4 years of follow-up (range, 1.3-13.4 years). Of these 33 cases, 12 (36%) were diagnosed at low/moderate Gleason score [2-6 or 7 (3 + 4)], 6 (18%) were diagnosed at a localized

Table 3. Association of *megalin* htSNPs genotypes with prostate cancer recurrence/progression by primary ADT

htSNP	Genotype	All cases* (*n* = 405)		Received primary ADT† (*n* = 51)		No primary ADT (*n* = 354)		*P*‡
		No. events§ (*n* = 86)	HR (95% CI)‖	No. events§ (*n* = 14)	HR (95% CI)‖	No. events§ (*n* = 72)	HR (95% CI)‖	
rs6433107	GG	39	1.0 (reference)	6	1.0 (reference)	33	1.0 (reference)	
	GT + TT	46	**1.7 (1.1-2.7)**	8	**4.0 (1.1-14.3)**	38	1.4 (0.9-2.4)	NS
rs1003456	AA	47	1.0 (reference)	9	1.0 (reference)	38	1.0 (reference)	
	AT + TT	37	**1.6 (1.0-2.6)**	4	1.1 (0.3-4.1)	33	**1.7 (1.0-2.9)**	NS
rs990626	TT	42	1.0 (reference)	8	1.0 (reference)	34	1.0 (reference)	
	CT + CC	44	**1.8 (1.1-2.8)**	6	2.1 (0.6-6.7)	38	**1.7 (1.0-2.8)**	NS
rs3944004	TT	47	1.0 (reference)	7	1.0 (reference)	40	1.0 (reference)	
	GT + GG	39	1.3 (0.8-2.1)	7	**3.6 (1.1-12.1)**	32	1.1 (0.7-1.8)	0.05
rs2239602	AA	71	1.0 (reference)	10	1.0 (reference)	61	1.0 (reference)	
	AG + GG	15	0.8 (0.4-1.4)	4	1.6 (0.3-8.6)	11	0.6 (0.3-1.1)	0.02
rs7592152	CC	40	1.0 (reference)	7	1.0 (reference)	33	1.0 (reference)	
	AC + AA	45	**0.5 (0.3-0.9)**	7	0.6 (0.2-2.3)	38	**0.6 (0.3-0.9)**	NS
rs830958	TT	62	1.0 (reference)	11	1.0 (reference)	51	1.0 (reference)	
	GT + GG	24	**1.9 (1.2-3.1)**	3	0.9 (0.2-3.8)	21	**2.0 (1.2-3.4)**	NS
rs830994¶	AA	42	1.0 (reference)	8	1.0 (reference)	34	1.0 (reference)	
	AG + GG	44	**0.4 (0.3-0.7)**** ††	6	**0.3 (0.1-1.0)**	38	**0.5 (0.3-0.9)** ††	NS
rs830995¶	GG	48	1.0 (reference)	8	1.0 (reference)	40	1.0 (reference)	
	AG + AA	38	**0.5 (0.3-0.7)****	6	0.5 (0.2-1.8)	32	**0.5 (0.3-0.8)**	NS
rs831003¶	CC	57	1.0 (reference)	9	1.0 (reference)	48	1.0 (reference)	
	CG + GG	29	**0.5 (0.3-0.7)****	5	0.5 (0.1-1.7)	24	**0.5 (0.3-0.9)** ††	NS
rs3770613	TT	26	1.0 (reference)	3	**1.0 (reference)**	23	1.0 (reference)	
	CT + CC	60	1.4 (0.9-2.3)	11	**6.4 (1.0-43.3)** ††	49	1.1 (0.7-1.9)	0.05
rs2673169	CC	46	1.0 (reference)	10	1.0 (reference)	36	1.0 (reference)	
	CG + GG	39	1.2 (0.7-1.9)	4	0.3 (0.1-1.3)	35	1.6 (0.9-2.6)	0.001
rs6716834‡‡	TT	43	1.0 (reference)	9	1.0 (reference)	34	1.0 (reference)	
	CT + CC	42	1.1 (0.7-1.8)	5	0.3 (0.1-1.2)	37	1.4 (0.9-2.4)	0.005
rs831023	GG	68	1.0 (reference)	10	1.0 (reference)	58	**1.0 (reference)**	
	GT + TT	17	1.5 (0.8-2.7)	3	0.6 (0.1-3.4)	14	**2.3 (1.2-4.3)** ††	0.01

Abbreviation: NS, not significant.
*Includes only subjects who completed the self-administered follow-up questionnaire.
†Category includes cases who received androgen deprivation shots, pills, and/or orchiectomy within 12 mo of initial diagnosis date.
‡*P* value based on the likelihood ratio test comparing models with and without an interaction term.
§Category includes a self-reported physician's diagnosis of prostate cancer recurrence/progression, a positive bone scan, biopsy, or magnetic resonance imaging showing cancer after primary treatment, or a PSA value >0.2 ng/mL after radical prostatectomy.
‖Cox proportional hazards models are adjusted for age, Gleason score, tumor stage at diagnosis, and PSA screening history. Hazard ratios with significance at $P \leq 0.05$ are in boldface.
¶These htSNPs are also associated with altered risks of prostate-specific cancer mortality by genotype.
**These associations remain significant after adjustment for multiple comparisons.
††These associations remain significant in a stepwise regression model.
‡‡This is a nonsynonymous SNP.

Table 4. Association of *megalin* htSNPs genotypes with prostate cancer-specific mortality by primary ADT

htSNP	Genotype	All cases (n = 553)		Received primary ADT* (n = 94)		No primary ADT (n = 459)	
		No. deaths (n = 33)	HR (95% CI) †	No. deaths (n = 26)	HR (95% CI) †	No. deaths (n = 7)	HR (95% CI) †
rs2300446	AA	14	1.0 (reference)	11	1.0 (reference)	3	1.0 (reference)
	AG + GG	19	**0.3 (0.2-0.7)** ‡	15	0.4 (0.2-1.1)	4	0.3 (0.1-1.6)
rs2239598	TT	18	1.0 (reference)	14	1.0 (reference)	4	1.0 (reference)
	CT + CC	15	**0.5 (0.2-1.0)**	12	0.6 (0.2-1.5)	3	0.3 (0.1-1.6)
rs830994§	AA	19	1.0 (reference)	17	1.0 (reference)	2	1.0 (reference)
	AG + GG	14	**0.3 (0.2-0.8)** ‡	9	**0.4 (0.1-0.9)** ‡	5	1.2 (0.2-6.8)
rs830995§	GG	21	1.0 (reference)	18	1.0 (reference)	3	1.0 (reference)
	AG + AA	12	**0.3 (0.2-0.8)**	8	0.4 (0.2-1.1)	4	0.9 (0.2-4.3)
rs831003§	CC	22	1.0 (reference)	19	1.0 (reference)	3	1.0 (reference)
	CG + GG	11	**0.4 (0.2-1.0)**	7	**0.3 (0.1-0.9)**	4	2.4 (0.5-12.3)

*Category includes cases who received androgen deprivation shots, pills, and/or orchiectomy within 12 mo of initial diagnosis date.
†Cox proportional hazards models are adjusted for age, Gleason score, tumor stage at diagnosis, and PSA screening history. Hazard ratios with significance at $P \leq 0.05$ are in boldface.
‡These associations remain significant in a stepwise regression model.
§These htSNPs are also associated with altered risks of disease recurrence/progression by genotype.

stage, and 26 (79%) were treated with primary ADT. Five htSNPs were significantly associated with altered risks of death from prostate cancer, with no evidence of interaction with ADT (Table 4). None of these five SNPs remained significant after adjustment for multiple comparisons. Using stepwise regression, we observed independence for only two SNPs: rs2300446 at $P = 0.02$ and rs830094 at $P = 0.03$. The htSNP rs830994 was found to have genotypes that had altered risks of both recurrence/progression and mortality independent of all other SNPs under the uncorrected multiple comparison models.

Discussion

Because of the apparent etiologic role for androgens in the development of prostate cancer, several candidate gene studies have focused on genetic polymorphisms found in the cascade of enzymes involved in the androgen metabolic pathway. This is one of the first epidemiologic studies to examine *megalin*, a gene that functions to actively transport androgen into the prostatic cell. Results from this study do not support a relationship between *megalin* polymorphisms and the risk of developing prostate cancer. The megalin-deficient mouse model described by Hammes and colleagues showed the importance of megalin in the developmental stage by the impaired descent of testes in males, a phenotype similar to animals treated with androgen receptor antagonists. However, the importance of megalin as an active uptake mechanism in the mature prostatic cell is unknown, and under normal physiologic conditions, the primary means of androgen internalization is likely to be via free diffusion across the cell membrane. Thus, under most conditions, megalin may not substantially influence the development of prostate cancer.

Whereas megalin may not play an important role for androgen internalization in normal prostate tissue, it may serve as a means for the malignant cell to increase androgen uptake. Using immunohistochemical assays, we have determined that neoplastic prostate epithelium expresses greater levels of megalin protein than adjacent benign epithelium.[10] We hypothesize that polymorphisms in the *megalin* gene that result in altered transport activity may influence the outcome of the disease. Our preliminary results suggest that genetic variants within the *megalin* gene may alter both risk of prostate cancer-specific death and disease recurrence/progression.

ADT takes advantage of the dependence of the prostate cell on androgen to temporarily arrest further malignant growth. This approach is not curative, and with time the cells will become resistant to ADT. Although this phase is termed ''androgen independence,'' it has been shown that the androgen receptor retains activity as shown by the continued expression of androgen-regulated genes such as PSA. If megalin provides an important secondary means of steroid uptake to circumvent the efficiency of ADT and has a true altered activity phenotype, outcomes could be modified by influences from genotypic variation within *megalin* and the physiologic pressures of ADT. Although we were limited by power, our results do suggest a potential interaction at least with disease recurrence/progression for six of the *megalin* htSNPs tested.

This was a population-based study with detailed information allowing for analysis of potential interaction and confounders. Although this study had enough power to detect modest relative risks for prostate cancer at the individual SNP level, we were limited by sample size in analyses of outcomes and in subset analyses. In addition, our Caucasian-only population may make our findings less generalizable. It is possible that some control men may have had undiagnosed prostatic cancer, leading to misclassification of disease and attenuation of the risk estimates toward the null. However, >86% of the controls in this study reported that they had previously had a digital rectal examination and/or PSA test for the detection of prostate cancer. In addition, we measured PSA in the serum in a random sample of 400 controls within our study and found only 39 (9.8%) men with a PSA $\geq$4.0 ng/mL and only 6 (1.5%) of these men with a PSA $\geq$10.0 ng/mL. Because of the large size of the

[10] P.S. Nelson and E. Mostaghel, unpublished data.

megalin gene and the lack of any previously published findings on functional polymorphisms, we elected to use the haplotype tagging approach. This was a cost-effective means to identify susceptibility alleles across the entire gene region.

In conclusion, although this study does not support a role between *megalin* polymorphisms and the risk of developing prostate cancer, several polymorphisms seem to play a role in the prostate cancer outcomes following diagnosis. Both the htSNPs identified in this study and SNPs in linkage disequilibrium with these htSNPS need to be examined to determine if, and how, they affect megalin protein function. A true test for the robustness of our findings will be replication in a larger data set. In addition, a larger study may be able to expand on the initial findings here, suggesting a role for *megalin* polymorphisms and outcomes associated with use of ADT.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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