Published in final edited form as:

J Urol. 2010 August ; 184(2): 501–505. doi:10.1016/j.juro.2010.04.032.

Genetic Prostate Cancer Risk Assessment: Common Variants in 9 Genomic Regions are Associated with Cumulative Risk

Brian T. Helfand,

Northwestern University, Feinberg School of Medicine, Department of Urology, Chicago IL 60611

Angela J. Fought,

Northwestern University, Feinberg School of Medicine, Department of Urology, Chicago IL 60611

Stacy Loeb², Joshua J. Meeks,

Northwestern University, Feinberg School of Medicine, Department of Urology, Chicago IL 60611

Donghui Kan, and

Northwestern University, Feinberg School of Medicine, Department of Urology, Chicago IL 60611

William J. Catalona

Northwestern University, Feinberg School of Medicine, Department of Urology, Chicago IL 60611

²Johns Hopkins University, James Buchanan Brady Urological Institute, Baltimore, MD 21287

Abstract

INTRODUCTION—Five genetic variants along chromosomes 8q24 and 17q were previously shown to have a cumulative association with prostate cancer (CaP) risk. Our research group has previously demonstrated an association between these variants and clinicopathologic characteristics. More recently, 4 additional CaP susceptibility variants were identified on chromosomes 2p15, 10q11, 11q13 and Xp11. Our objectives were to examine a cumulative risk assessment incorporating all 9 genetic variants, and to determine the relationship of the new variants with clinicopathologic tumor features.

METHODS—The genotype for all 9 variants was determined in 687 men of European ancestry who underwent radical prostatectomy (2002-2008) and 777 healthy volunteer controls. We compared the frequencies of these variants between CaP cases and controls and examined a cumulative model. In addition, we determined the relationship between carrier status for the 4 new variants and clinical and pathology tumor features.

RESULTS—CaP cases had an increased frequency of all 9 risk variants compared to controls. A cumulative model that included the 9 SNPs provided greater CaP risk stratification than a model restricted to the original 5 SNPs described. Specifically, men with ≥ 6 variants had a >6 -fold increased risk of CaP. Although 2p15 and 11q13 carriers were more likely to have aggressive features, other clinical-pathology features were similar between carriers and non-carriers.

CONCLUSIONS—Genetic variants located in 9 regions have a cumulative association with CaP risk. The identification of an increasing number of SNPs may provide a greater understanding of their combined relationship with CaP risk and disease aggressiveness.

Introduction

Prostate cancer (CaP) is among the most heritable of human cancers, with an estimated heritable risk as high as ~50%¹. Additionally, the presence of a positive family history of CaP continues to be one of the strongest risk factors for developing the disease. Historically, men with a family history are considered likely to have inherited a greater genetic susceptibility for developing CaP compared to those without a positive family history. However, the underlying mechanism(s) of this genetic predisposition has remained largely unknown. Recent technologic advances using a combination of genome-wide association scans, linkage analyses and fine-mapping techniques have permitted researchers to identify genetic variants (single nucleotide polymorphisms) that are associated with an increased risk of CaP.

Genetic variants contained in chromosomal regions along 8q24 and 17q have reproducibly been associated with CaP risk²⁻⁸. Fine mapping and additional genome wide association studies (GWAS) have distinguished three distinct regions within 8q24 referred to as region 1 (128.54-128.62 Mb), region 2 (128.12-128.28 Mb) and region 3 (128.47-128.54 Mb)⁹. In addition, variants identified on chromosome 17q lie in 2 regions: one in the non-coding region at 17q24 and the other in the second intron of the TCF2/HNF1 β at 17q12⁶. Within the past year, a rapidly growing number of CaP susceptibility variants have been identified through GWAS, supporting the hypothesis of polygenic inheritance. For example, genetic variants contained within the EHBPI and MSMB genes located along chromosomal regions 2p15 and 10q11, respectively, were found to be associated with CaP¹⁰⁻¹². In addition, although not residing in gene- rich areas, variants located along Xp11¹¹ and 11q13^{13, 14} have also been associated with the risk of CaP. Although the aforementioned variants have all been related to CaP susceptibility, their interactions and mechanisms influencing CaP development are largely unknown.

Zheng et al. used multiple CaP variants in 5 chromosomal regions along 8q24 and 17q to study their possible interactions associated with CaP risk¹⁵. Taken together with family history, the 5 genetic variants had a cumulative association with CaP and were estimated to account for 46% of CaP cases in their Swedish population. Additionally, men with 5 of the 6 risk factors (i.e. 5 variants and a positive family history), had a >9- fold increased risk of CaP. Confirmation of these results has been reported in an external population, with an 11-fold increase in the relative risk of CaP¹⁶. While the results of these studies are impressive, it is important to note that few patients in the studied populations were carriers of all 5 genetic variants (~1%). Accordingly, it has been hypothesized that other genetic variants outside these 5 chromosomal regions may also contribute to CaP risk.

While some studies have reported that the aggressive forms CaP are influenced by the same genetic variants associated with susceptibility^{2, 4, 5, 17-20}, several others have reported conflicting results²¹⁻²⁴. Interestingly, the results of a recent study reported the presence of a genetic variant which was specific for aggressive disease²⁵. Therefore, although the newly identified variants located along 2p15, 10q11, 11q13 and Xp11 have been associated with the CaP susceptibility, their association with CaP aggressiveness remains to be determined. Therefore, the objective of the present study was to examine the cumulative association of these new variants with CaP risk, in addition to the original 5 SNPs, and also to examine their relationship with pathology tumor features.

Methods

We initially identified 1,614 men, of which 150 (9.3%) were excluded because of a lack of genetic data and/or incomplete clinical information. The current case-control study thus

included 1,464 men of European ancestry. Prostate cancer cases included 687 consecutive men who underwent radical prostatectomy at Northwestern Memorial Hospital between June 2002 and May 2008. Of these men, 90% were treated by a single surgeon (WJC), and the remainder by other urologists from the Northwestern University Specialized Program of Research Excellence (SPORE) research group. The study received IRB approval, and all participants provided informed consent prior to enrollment. The demographics, biopsy and pathology findings at the time of prostatectomy were prospectively recorded for all CaP patients. The comparison group included 777 healthy volunteer male controls of European ancestry that have previously been described elsewhere¹⁹.

We examined 9 CaP genetic variants, including 3 along 8q24 [rs1447295 (region 1), rs16901979 (region 2), and rs6983267 (region 3)] and 2 along 17q [rs4430796 (17q 12) and rs1859962 (17q24)]. In addition, we analyzed genetic variants at 2p15 (rs2710646), 10q11 (rs10993994), 11q13 (rs10896449), and Xp11 (rs5945572). Genotyping for all cases and controls was performed by deCODE Genetics, Inc. as previously described^{2, 5, 6, 11}. The quality control and genotyping accuracy of the genetic variants has previously been reported^{2, 5, 6, 11, 13}.

All genetic variants were found to be in Hardy-Weinberg equilibrium (data not shown). Differences in allele frequencies between cases and controls were tested for each SNP using a logistic regression model, and the odds ratios for CaP risk were estimated from the regression coefficients. For each of the 9 genetic variants, genotype information was compared using Akaike's information criteria to choose the best-fit genetic model (dominant or recessive), as previously described¹⁹. Based upon the best-fit genetic model, we analyzed the cumulative risk of CaP as previously described¹⁵. In addition, we adjusted the best-fit genetic models and cumulative models for age quartiles. Receiver operating characteristic (ROC) curves were constructed, with and without adjustment for age, and compared as a "ROCcontrast" statement in SAS 9.2 for the models including 5 genetic variants versus all 9 genetic variants. Finally, the Fisher's exact and Kruskal-Wallis tests were used to compare the clinical-pathology features between carriers (defined using the best-fit genetic model) and non-carriers of the 9 genetic variants. A p-value <0.05 was considered significant. All statistical analysis was performed using SAS 9.2 (Cary, NC).

Results

The clinical and pathology characteristics of the 687 CaP cases and 777 controls is shown in Table 1. Controls were significantly younger (mean age 58 years) and had lower PSA levels (mean 1.4 ng/ml) compared to CaP cases (mean age 69.8 years, mean PSA 6.0 ng/ml). The genotypes for the 9 CaP susceptibility variants along chromosomes 8q24, 17q, 2p15, 10q11, 11q13 and Xp11 are shown in Table 2 for cases and controls. All variants were present at a significantly greater frequency in CaP cases compared to controls, with the exception of 17q24 and 2p15 (p=0.18 and p=0.13, respectively).

We next determined the best-fit genetic model for each genetic variant (Table 3) and used this to examine the cumulative relationship between the original 5 SNPs with CaP risk in our population (p-trend <0.001; Table 4a). Overall, men with ≥ 4 variants had approximately a 3-fold risk of CaP. Table 4b shows the cumulative association between all 9 SNPs with CaP risk. Carriers of 2,3,4,5 or >6 CaP risk alleles had a progressively increasing odds of CaP, as compared with carriers of only one or none of the genetic variants (p-trend <0.0001). Also, carriers of at least 6 of the 9 genetic variants had a >6 fold increased risk of CaP (Table 4b), suggesting that the model using all 9 variants appeared to provide additional stratification regarding CaP risk. ROC curves were next used to compare the two models. The AUC for the model including all 9 variants was significantly greater than for the model with 5 genetic

variants (AUC 0.61 vs. 0.58; $p=0.007$). After adjustment for age, the 9 variant model also showed a marginally significant improvement in AUC compared to the 5-variant model (AUC 0.66 vs. 0.65; $p=0.05$).

Using the best-fit genetic models, we also compared the frequency of adverse clinical and pathology features between carriers and non-carriers of the 4 recently identified genetic variants. Since we have previously described an association between aggressive clinical-pathology features with the genetic variants located along 8q24 and 17q, our present analyses were limited to the genetic variants on 2p15, 10q11, 11q13 and Xp11. Age and PSA at diagnosis were similar between carriers and non-carriers of these genetic variants. Although there carriers of the 2p15 variant were more likely to have a prostatectomy Gleason grade ≥ 7 (55.2% carriers vs. 48.6% non-carriers, $p=0.18$), and carriers of the 11q13 variant tended to have a higher frequency of positive surgical margins compared with non-carriers (55.2% carriers vs. 48.6% non-carriers, $p=0.14$), neither comparison reached statistical significance

Discussion

This study examined the association of 9 genetic variants with CaP risk and pathology tumor features in a U.S. population of European ancestry. We found that carriers of an increasing number of genetic variants had a progressively greater CaP risk.

It has previously been demonstrated that there is a strong independent association of 5 genetic variants along 8q24 and 17q with CaP risk^{15, 16}. In prior studies, although each individual variant had a modest association with CaP risk, combinations of these variants had more robust cumulative relationship with CaP susceptibility (OR 3.8-3.9)¹⁵. Subsequent studies have identified additional chromosomal regions that are independently associated with increased CaP risk^{3, 7, 10, 11, 14, 20, 26-28}. Altogether, >50 SNPs have now been implicated in CaP susceptibility. While a recent study estimated the absolute CaP risk associated with many of these variants to be ~4.9 fold, it did not take into account best-fit genetic models, and thus may have over- or under-estimated their risk²⁹. In the present study, we used carrier status (previously defined using either dominant or recessive models^{15, 16}) to assess the cumulative risk associated with 9 genetic variants. We found that carriers of 6 or more variants had a >6-fold increased risk of CaP. ROC analysis demonstrated that this model offered improved, although modest, risk stratification compared to a base model including only the initial 5 variants along 8q24 and 17q¹⁵. However, the additional benefit of the 4 genetic variants should be confirmed in a larger sample. In addition, we could not assess the effect of family history since this information is not currently available for our control population. However, based on prior studies¹⁵, we would expect an improvement in the prediction of CaP risk by including family history in the analysis.

Our research group has previously demonstrated that specific genetic variants are over-represented in patients with adverse clinical features. Specifically, we reported that carriers of SNPs on 8q24 and 17q12 were more likely to have high-grade disease^{18,19}. Other groups have confirmed these findings between the genetic variants and aggressive features^{2, 4, 5, 17}, and recently a variant located along 17p12 was found to be over-represented in patients with advanced grade and stage disease²⁵. In the current study, we studied the relationship between the 4 additional variants with pathology tumor features. Although there were non-significant trends toward more high-grade disease and positive surgical margins in carriers of the 2p15 and 11q13 variants, respectively, we did not find a significant relationship between any of the other individual variants with aggressive tumor features. Possible explanations for the lack of robust findings include the relatively small surgical population

and the restriction of our analysis to a dominant or recessive best-fit model, as we have previously shown that the genetic model used can alter the apparent association with surgical outcomes¹⁹. Alternatively, despite their association with overall CaP risk, these 4 variants may not provide additional information about CaP aggressiveness. Future studies are warranted to re-examine these relationships in heterogeneous populations and to identify additional genetic variants which can help to distinguish aggressive disease.

Several limitations of our study deserve mention. Our study population was limited to a relatively small number of Caucasian men of European descent. Since prior studies have shown that allele frequencies differ among different ethnic groups^{3, 4, 30}, separate studies are needed to examine the cumulative effects of these variants in other populations. In addition, all patients with CaP in our study underwent radical prostatectomy, which may have introduced selection bias, as they may have fundamental differences from patients receiving other forms of treatment. Therefore, these variants should also be examined in patients with more advanced disease undergoing primary radiation and/or hormonal therapy.

Despite these limitations, our results suggest that genetic variants may enhance CaP risk assessment. In the future, genetic variants may be useful to help identify men at greatest risk for developing CaP, and the identification of additional variants associated with CaP aggressiveness could potentially enhance our ability to predict which patients have life-threatening disease.

Conclusion

In conclusion, 9 genetic variants were associated with a cumulative increase in CaP risk. Larger studies are warranted to evaluate the contribution of additional genetic variants to CaP risk stratification and to identify variants which can better distinguish aggressive disease.

Acknowledgments

Supported by the Urologic Research Foundation (URF), Northwestern University SPORE Grant P50 CA90386-05S2, Northwestern University Robert H. Lurie Comprehensive Cancer Center and deCODE Genetics.

References

1. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med*. 2000; 343:78. [PubMed: 10891514]
2. Amundadottir LT, Sulem P, Gudmundsson J, et al. A common variant associated with prostate cancer in European and African populations. *Nat Genet*. 2006; 38:652. [PubMed: 16682969]
3. Eeles RA, Kote-Jarai Z, Giles GG, et al. Multiple newly identified loci associated with prostate cancer susceptibility. *Nat Genet*. 2008; 40:316. [PubMed: 18264097]
4. Freedman ML, Haiman CA, Patterson N, et al. Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. *Proc Natl Acad Sci U S A*. 2006; 103:14068. [PubMed: 16945910]
5. Gudmundsson J, Sulem P, Manolescu A, et al. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet*. 2007; 39:631. [PubMed: 17401366]
6. Gudmundsson J, Sulem P, Steinthorsdottir V, et al. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet*. 2007; 39:977. [PubMed: 17603485]
7. Thomas G, Jacobs KB, Yeager M, et al. Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet*. 2008; 40:310. [PubMed: 18264096]

8. Yeager M, Orr N, Hayes RB, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet.* 2007; 39:645. [PubMed: 17401363]
9. Witte JS. Multiple prostate cancer risk variants on 8q24. *Nat Genet.* 2007; 39:579. [PubMed: 17460686]
10. Chang BL, Cramer SD, Wiklund F, et al. Fine mapping association study and functional analysis implicate a SNP in MSMB at 10q11 as a causal variant for prostate cancer risk. *Hum Mol Genet.* 2009; 18:1368. [PubMed: 19153072]
11. Gudmundsson J, Sulem P, Rafnar T, et al. Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. *Nat Genet.* 2008; 40:281. [PubMed: 18264098]
12. Lou H, Yeager M, Li H, et al. Fine mapping and functional analysis of a common variant in MSMB on chromosome 10q11.2 associated with prostate cancer susceptibility. *Proc Natl Acad Sci U S A.* 2009; 106:7933. [PubMed: 19383797]
13. Gudmundsson J, Sulem P, Gudbjartsson DF, et al. Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. *Nat Genet.* 2009; 41:1122. [PubMed: 19767754]
14. Zheng SL, Stevens VL, Wiklund F, et al. Two independent prostate cancer risk-associated Loci at 11q13. *Cancer Epidemiol Biomarkers Prev.* 2009; 18:1815. [PubMed: 19505914]
15. Zheng SL, Sun J, Wiklund F, et al. Cumulative association of five genetic variants with prostate cancer. *N Engl J Med.* 2008; 358:910. [PubMed: 18199855]
16. Sun J, Chang BL, Isaacs SD, et al. Cumulative effect of five genetic variants on prostate cancer risk in multiple study populations. *Prostate.* 2008; 68:1257. [PubMed: 18491292]
17. Cheng I, Plummer SJ, Jorgenson E, et al. 8q24 and prostate cancer: association with advanced disease and meta-analysis. *Eur J Hum Genet.* 2008; 16:496. [PubMed: 18231127]
18. Helfand BT, Loeb S, Cashy J, et al. Tumor characteristics of carriers and noncarriers of the deCODE 8q24 prostate cancer susceptibility alleles. *J Urol.* 2008; 179:2197. [PubMed: 18423739]
19. Helfand BT, Loeb S, Meeks JJ, et al. Pathological outcomes associated with the 17q prostate cancer risk variants. *J Urol.* 2009; 181:2502. [PubMed: 19371897]
20. Pal P, Xi H, Guha S, et al. Common variants in 8q24 are associated with risk for prostate cancer and tumor aggressiveness in men of European ancestry. *Prostate.* 2009
21. Kader AK, Sun J, Isaacs SD, et al. Individual and cumulative effect of prostate cancer risk-associated variants on clinicopathologic variables in 5,895 prostate cancer patients. *Prostate.* 2009; 69:1195. [PubMed: 19434657]
22. Salinas CA, Koopmeiners JS, Kwon EM, et al. Clinical utility of five genetic variants for predicting prostate cancer risk and mortality. *Prostate.* 2009; 69:363. [PubMed: 19058137]
23. Xu J, Isaacs SD, Sun J, et al. Association of prostate cancer risk variants with clinicopathologic characteristics of the disease. *Clin Cancer Res.* 2008; 14:5819. [PubMed: 18794092]
24. Wiklund FE, Adami HO, Zheng SL, et al. Established prostate cancer susceptibility variants are not associated with disease outcome. *Cancer Epidemiol Biomarkers Prev.* 2009; 18:1659. [PubMed: 19423541]
25. Xu J, Zheng SL, Isaacs SD, et al. Inherited genetic variant predisposes to aggressive but not indolent prostate cancer. *Proc Natl Acad Sci U S A.*
26. Duggan D, Zheng SL, Knowlton M, et al. Two genome-wide association studies of aggressive prostate cancer implicate putative prostate tumor suppressor gene DAB2IP. *J Natl Cancer Inst.* 2007; 99:1836. [PubMed: 18073375]
27. Hsu FC, Sun J, Wiklund F, et al. A novel prostate cancer susceptibility locus at 19q13. *Cancer Res.* 2009; 69:2720. [PubMed: 19318570]
28. Sun J, Zheng SL, Wiklund F, et al. Sequence variants at 22q13 are associated with prostate cancer risk. *Cancer Res.* 2009; 69:10. [PubMed: 19117981]
29. Xu J, Sun J, Kader AK, et al. Estimation of absolute risk for prostate cancer using genetic markers and family history. *Prostate.* 2009; 69:1565. [PubMed: 19562736]
30. Haiman CA, Patterson N, Freedman ML, et al. Multiple regions within 8q24 independently affect risk for prostate cancer. *Nat Genet.* 2007; 39:638. [PubMed: 17401364]

Table 1

Clinico-pathologic Characteristics Prostate Cancer Cases and Healthy Volunteer Controls

	Cases (n=687) %	Controls (n=777) %
European Ancestry	100	100
Age (years) in Quartiles *		
≤50	10.2	26.4
51-57	27.9	23.7
58-64	34.5	22.5
≥65	27.4	27.4
PSA (ng/ml) *		
0.0 - < 2.5	7.4	81.2
2.5 - < 4.0	14.6	3.9
4.0 - < 7.0	49.2	2.6
7.0 - < 10.0	15.0	0.8
≥10.0	9.5	0.6
Not available	4.4	10.9
Percent Clinical Stage (≤T1c)	71.8	-
Percent Biopsy Gleason Score		
≤6	68.5	-
≥7	31.5	-
Percent Organ Confined Disease (≤T2)	79.0	-
Percent Prostatectomy Gleason Score		
≤6	51.1	-
≥7	48.9	-
Percent Positive Surgical Margins	18.8	-
Percent Extracapsular Extension	18.5	-
Percent Seminal Vesicle Invasion	5.2	-
Percent Lymph Node Metastases	0.9	-

* Statistically significant difference ($p < 0.001$) in distribution among cases and controls tested using χ^2 test.

Table 2

Comparison of the Frequencies of the Genetic Variants (Single Nucleotide Polymorphisms) in Prostate Cancer Cases and Healthy Volunteer Controls

SNP	Chromosomal Location	Variant Allele	Percent of		OR (95% CI)
			Cases	Controls	
rs1447295	8q24 (region 1)	A	12.6	10.1	1.28 (1.02-1.61)
rs16901979	8q24 (region 2)	A	5.3	3.2	1.69 (1.17-2.44)
rs6983267	8q24 (region 3)	G	54.6	50.6	1.17 (1.02-1.36)
rs4430796	17q12	A	56.8	51.0	1.27 (1.10-1.47)
rs1859962	17q24	G	51.5	49.0	1.10 (0.96-1.28)
rs2710646	2p15	A	19.4	17.3	1.16 (0.96-1.40)
rs10993994	10q11	T	47.5	39.9	1.36 (1.17-1.58)
rs10896450	11q13	G	54.2	50.1	1.18 (1.02-1.37)
rs5945572	Xp11	A	40.5	35.3	1.25 (1.01-1.54)

Table 3

Best-Fitting Genetic Model (Dominant or Recessive) for the 9 Prostate Cancer Genetic Variants

SNP	Chromosomal Location	Best-fit Genetic Model	OR (95% CI)	OR (95% CI) Adjusted for Age
rs1447295	8q24 (region 1)	Dominant	1.30 (1.01-1.67)	1.32 (1.02-1.71)
rs16901979	8q24 (region 2)	Dominant	1.69 (1.15-2.47)	1.69 (1.14-2.49)
rs6983267	8q24 (region 3)	Dominant	1.43 (1.11-1.83)	1.39 (1.08-1.79)
rs4430796	17q12	Dominant	1.49 (1.56-1.93)	1.46 (1.13-1.90)
rs1859962	17q24	Recessive	1.22 (0.96-1.55)	1.17 (0.92-1.49)
rs2710646	2p15	Recessive	1.67 (0.93-2.98)	1.70 (0.94-3.08)
rs10993994	10q11	Recessive	1.63 (1.26-2.11)	1.64 (1.26-2.14)
rs10896450	11q13	Dominant	1.34 (1.05-1.72)	1.36 (1.06-1.74)
rs5945572	Xp11	Dominant	1.25 (1.01-1.54)	1.23 (0.99-1.53)

Table 4

Cumulative relationship between genetic variants with prostate cancer risk, including (a) the original five SNPs along 8q24 and 17q, and (b) addition of the four recently described variants on 2p15, 10q11, 11q13 and Xp11 (total 9 variants).

(a)				
Number of Carried Variants	Percent		OR (95% CI)*	OR (95% CI) Adjusted for Age*
	Cases n=687	Controls n=777		
0-1	17.3	29.2	1.00	1.00
2	47.5	44.4	1.80 (1.38-2.36)	1.74 (1.32-2.29)
3	28.7	23.0	2.10 (1.55-2.83)	2.00 (1.47-2.71)
4-5	6.6	3.4	3.30 (1.94-5.62)	3.19 (1.85-5.50)

(b)				
Number of Carried Variants	Percent		OR (95% CI)*	OR (95% CI) Adjusted for Age*
	Cases n=687	Controls n=777		
0-1	2.0	5.5	1.00	1.00
2	10.8	19.8	1.48 (0.76-2.87)	1.46 (0.74-2.86)
3	30.9	33.6	2.49 (1.33-4.86)	2.46 (1.29-4.66)
4	32.0	27.3	3.19 (1.69-6.00)	3.05 (1.60-5.79)
5	18.8	11.3	4.50 (2.32-8.72)	4.39 (2.24-8.61)
>6	5.5	2.5	6.14 (2.71-13.90)	5.75 (2.50-13.24)

* P-trend <0.001