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The *XPD* Asp³¹²Asn and Lys⁷⁵¹Gln polymorphisms, corresponding haplotype, and pancreatic cancer risk

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Summary

We evaluated the association between the *XPD* exon 10 Asp³¹²Asn and exon 23 Lys⁷⁵¹Gln polymorphisms and the risk of pancreatic cancer in a hospital-based study of 344 patients and 386 controls frequency matched by age, gender, and race. Stratified analyses showed ever smokers carrying the Asn³¹²Asn genotype had a significantly reduced risk when compared with those carrying the ³¹²Asp allele (OR= 0.46, 95% confidence interval 0.24–0.88) (*P* for interaction = 0.03). The ³¹²Asp-⁷⁵¹Gln was identified as the putative at risk haplotype. Our study shows that the *XPD* gene polymorphism could be a genetic risk modifier for smoking-related pancreatic cancer.

Keywords

XPD; Single nucleotide polymorphism; Smoking; Pancreatic cancer

Abbreviations

CI: confidence interval; HWE, Hardy-Weinberg equilibrium; NER, nucleotide excision repair; OR, odds ratio; SNP, single nucleotide polymorphism; *XPD*, xeroderma pigmentosum-D

1. Introduction

From 1998 to 2002, pancreatic cancer ranked fourth in cancer-related mortality among both genders and all races in the United States. The mortality rate for pancreatic cancer is almost equal to its incidence rate (10.5/100,000 versus 11.0/100,000) [1]. Although the etiology of sporadic pancreatic cancer is still largely unknown, smoking is the only established environmental risk factor, with a relative risk of 2- to 4-fold [2]. However, pancreatic cancer develops in only a small percentage of smokers, suggesting that host susceptibility factors play a role in this disease.

The genetic profiles of pancreatic cancer indicate that genomic instability mediated by DNA repair deficiency may be an important event in pancreatic carcinogenesis. DNA repair machinery plays a critical role in protecting cells from environmental hazards such as cigarette smoke. One of the DNA repair systems, nucleotide excision repair (NER), involves a multienzyme complex that is responsible for repairing a wide variety of structurally unrelated

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DNA lesions, including bulky adducts, cross-links [3], oxidative DNA damage, thymidine dimers [4], and alkylating damage [5]. Xeroderma pigmentosum-D (XPD) is one of the important proteins involved in the NER pathway. XPD, together with xeroderma pigmentosum-B (XPB), works as an adenosine triphosphate-dependent 5'-3' helicase and is a subunit of the basal transcription factor IIIH, which is required for separation of the double helix during both global genomic repair and transcription-coupled repair [6]. Both XPD and XPB DNA helicases are components of the p53-mediated apoptosis pathway [7]. Deficient NER caused by XPD mutations is known to be associated with xeroderma pigmentosum, trichothiodystrophy, and Cockayne's syndrome [8]. Although these conditions represent extreme defects in NER, variations in the repair processes in the general population are highly plausible, and such variations may be partially attributable to variations of the *XPD* gene. The *XPD* gene spans ~54.3 kb at chromosome 19q13.3 and comprises 23 exons. Two common nonsynonymous single nucleotide polymorphisms (SNPs) in the coding region of the *XPD* gene have been identified: a G to A substitution at nucleotide position 23591 causing an exon 10 codon 312 Asp (D) to Asn (N) amino acid exchange, and an A to C substitution at nucleotide position 35931 causing an exon 23 codon 751 Lys (K) to Gln (Q) substitution [9].

To our knowledge, there have been no documented studies of the effect of *XPD* gene polymorphisms on pancreatic cancer risk. In the hospital-based study described herein, we tested the hypothesis that a subtle difference in DNA repair capacity conferred by the Asn³¹²Asp and Lys⁷⁵¹Gln polymorphisms predisposes an individual to pancreatic cancer. Because XPD is involved in the repair of DNA damage caused by carcinogens in cigarette smoke and because smoking is a well-established risk factor for pancreatic cancer, our secondary hypothesis is that the effects of these polymorphisms are more prominent in individuals with known exposure to cigarette smoke.

2. Materials and methods

2.1. Study subjects and data collection

This case-control study was conducted at The University of Texas M. D. Anderson Cancer Center from January 2000 to July 2005. The study design, patient recruitment, and data-collection methods were described previously [10]. In brief, the patients were individuals with pathologically confirmed primary pancreatic ductal adenocarcinoma (International Classification of Diseases for Oncology code C25.3, World Health Organization, 2000). Controls were patient companions who were not genetically related to their respective patients who were diagnosed with any cancer except for pancreatic cancer. Patients and controls had no prior cancer history (except for non-melanoma cancer of the skin) and they were frequency matched by age (± 5 years), race, and gender. Potential patients were identified in the Gastrointestinal Center at M. D. Anderson Cancer Center, and controls were recruited from various clinics at M. D. Anderson. Controls were selected by using a brief screening questionnaire designed to obtain information about demographics, cancer history, state of residence, and willingness to participate in a research project. The participation rate was 78% (consented/approached) among both the patients and controls. Written informed consent was obtained from each participant for an in-person interview and blood donation. The research protocol was approved by the M. D. Anderson Institutional Review Board.

Among those who consented to take part, the success rate for blood-sample collection and the in-person interview was 85% and 100%, respectively, in the patients and 100% and 95%, respectively, in the controls. There were 385 eligible patients and 427 eligible controls for this study. Because of the known ethnic variation in pancreatic cancer risk and genotype distribution, the current analysis was focused on non-Hispanic white individuals only (344 patients and 386 controls).

Trained interviewers conducted direct interview with patients and controls to collect information on demographics, smoking exposure, medical history (pancreatitis and type II diabetes), and family history of cancer with the use of a structured questionnaire. The definitions of the smoking-exposure parameters were described previously [10]. Briefly, subjects were classified as ever smokers or never smokers according to whether they had ever smoked more than 100 cigarettes in their lifetime.

2.2. DNA isolation and genotyping

Peripheral blood mononuclear cells were separated from freshly drawn blood by using Ficoll-Hypaque density gradient centrifugation (Amersham Pharmacia Biotech, Piscataway, NJ) and stored at -80°C . DNA was extracted using a FlexiGene DNA kit (Qiagen, Valencia, CA). The genotype of exon 10 Asp³¹²Asn was determined using a PCR-based allele-specific genotyping assay with the Masscode™ technology (BioServe Biotechnologies, Ltd., Laurel, MD). The genotype of the exon 23 Lys⁷⁵¹Gln variants was determined by using the PCR-restriction fragment length polymorphism method (RFLP) described by Matullo et al. [11]. For quality control of PCR-RFLP experiment, a blank control without a DNA template was included in each experiment to monitor any contamination. Two personnel who were blinded to the case-control status read the results independently. Ten percent of the samples were selected randomly for repeat analysis revealing 99.2% concordance. Discordant data were resolved with further genotyping. For the PCR-based allele-specific genotyping assay, positive allele controls were run in every experiment, and the random 10% sample repeats were imbedded for quality control. Final calls of genotype were made in the most conservative manner possible, and all ambiguous calls were coded as missing in the data analysis. The no-call rate was 3.7% and 1.4% for the Asp³¹²Asn polymorphism and Lys⁷⁵¹Gln polymorphism, respectively.

2.3. Statistical analysis

All statistical analyses were performed by using the Stata software program (version 9.0; StataCorp, College Station, TX). All of the tests were two sided, and statistical significance was defined as $P \leq 0.05$. Pearson's χ^2 test was used to test the difference in the distribution of categorical variables. Hardy-Weinberg equilibrium (HWE) of the *XPB* genotypes was tested by performing a goodness-of-fit χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated as a measure of association between each polymorphism and pancreatic cancer risk by using an unconditional logistic regression model. No significant confounding factors were identified in the association analyses. The matching factors age (continuous) and gender were included in the multivariate logistic regression models. Both the dominant and recessive inheritance modes of *XPB* polymorphisms were evaluated. In the dominant mode, the *XPB* Asp³¹²Asp or Lys⁷⁵¹Lys was the reference group; in the recessive mode, the homozygous variant Asn³¹²Asn or Gln⁷⁵¹Gln was compared with the combined wild-type and heterozygous variant. The gene-environment interaction effect was examined by performing stratified analyses of the variables of interest, including age, gender, smoking status (never *versus* ever), history of pancreatitis and type II diabetes, and family history of cancer (yes *versus* no). The cross-product term was generated with the use of the logistic regression model correspondingly. The significance of the interaction term was tested by using a likelihood ratio test, with the full model containing the interaction term, the main effect of the genotype, and the exposure variable and reduced model lacking the interaction term. Linkage disequilibrium of the two polymorphic loci was tested by using the SNPalyze software program (DYNACOM Co., Ltd., Mobara, Japan). Haplotypes were reconstructed from the genotype data by using the PHASE program (version 2) [12]. Pearson's χ^2 test was used to test the difference in the distribution of the haplotypes between the patients and controls. Finally, overall ORs and 95% CIs were calculated.

3. Results

Selected variables and risk factors for the 344 patients and 386 controls are listed in Table 1. There were no significant differences between the patients and controls in terms of distribution by age and gender. A similar percentage of patients and controls had a family history of cancer among their first-degree relatives ($P < 0.001$). A significantly higher percentage of patients than controls reported having a history of type II diabetes ($P = 0.01$) and pancreatitis ($P < 0.001$). Ever smokers accounted for 59.6% of the patients and 50.3% of the controls ($P = 0.03$). There was also a higher proportion of former smokers and current smokers among the patients than among the controls ($P = 0.03$). Individuals who smoked 22 pack-years or more were more likely to have pancreatic cancer than never smokers were ($P = 0.001$).

Table 2 presents the difference in genotype distribution between the patients and controls. The frequency of the ³¹²Asn and ⁷⁵¹Gln allele in the controls was 0.36 and 0.35, respectively. With respect to controls, the genotype distribution of the Asp³¹²Asn polymorphism was in HWE ($P = 0.93$). However, the genotype distribution of the Lys⁷⁵¹Gln polymorphism departed from HWE ($P = 0.007$). There was not a significant association between the two genotypes and the risk of pancreatic cancer. The stratified analyses by gender, history of pancreatitis or type II diabetes, and family history of cancer also showed no association (data not shown). Furthermore, there was not a significant combined effect of the two polymorphisms on the risk of pancreatic cancer (data not shown).

We evaluated the gene-smoking interaction in modification of the risk of pancreatic cancer by performing stratified analyses (Table 3). The Asn³¹²Asn genotype had a protective effect against pancreatic cancer in ever smokers but not in never smokers ($P = 0.03$ for the likelihood ratio test with the logistic regression model). Among ever smokers, those carrying the Asn³¹²Asn genotype had an OR of 0.42 (95% CI 0.21–0.83; $P = 0.01$) when compared with those carrying the Asp³¹²Asp genotype, and an OR of 0.46 (95% CI 0.24–0.88; $P = 0.02$) when compared with those carrying at least one copy of the ³¹²Asp allele. We observed the interaction effect of the Asp³¹²Asn polymorphism and smoking in both current and former smokers (data not shown). There was not a significant interactive effect of the Lys⁷⁵¹Gln polymorphism and smoking (Table 3).

The *XPD* ³¹²Asp and ⁷⁵¹Lys alleles were in linkage disequilibrium ($P < 0.001$; $D' = 0.64$ and $D' = 0.68$ for controls and patients, respectively). Most of the study participants carrying the Asp³¹²Asp genotype also carried the Lys⁷⁵¹Lys genotype (212/287, 74%). The concordance between the two polymorphisms was 71% in the controls and 72% in the patients, which is similar to that reported previously [13]. Four haplotypes were putatively inferred for all participants, including ³¹²Asp-⁷⁵¹Lys (57.3%), ³¹²Asn-⁷⁵¹Gln (28.8%), ³¹²Asp-⁷⁵¹Gln (6.9%), and ³¹²Asn-⁷⁵¹Lys (7.1%) (Table 4). According to our priori hypothesis and the SNP-based analyses, we considered the individuals with the ³¹²Asn-⁷⁵¹Lys haplotype to be the reference group for OR estimations. Patients tended to be more likely to have the ³¹²Asp-⁷⁵¹Gln haplotype than did controls among all study participants with an OR of 1.60 (95% CI 0.89–2.90; $P = 0.09$) and among ever smokers in particular with OR of 3.01 (95% CI 1.32–6.93; $P = 0.004$). When compared with the ³¹²Asn-⁷⁵¹Lys haplotype, the order of magnitude in the increase in risk of pancreatic cancer was ³¹²Asp-⁷⁵¹Gln > ³¹²Asp-⁷⁵¹Lys > ³¹²Asn-⁷⁵¹Gln in all study subjects and in ever smokers. The frequency of the haplotypes in the patients was significantly different from that in the controls among ever smokers ($P = 0.02$).

4. Discussion

In this study, we did not find a significant association between the two *XPB* polymorphisms and the risk of pancreatic cancer in general. However, we did find that the Asn³¹²Asn polymorphism was inversely associated with the risk of pancreatic cancer in ever smokers. The exploratory haplotype analyses revealed that the ³¹²Asp-⁷⁵¹Gln haplotype was associated with an increased risk of pancreatic cancer in people with exposure to smoking. It infers that inefficient NER conferred by these naturally occurring, low-penetrance but common SNPs may exert moderate effect on the risk of smoking-related pancreatic cancer in the non-Hispanic white population.

The Asp³¹²Asn polymorphism is considered to be more functionally relevant than the Lys⁷⁵¹Gln polymorphism. The residue 312 is evolutionarily highly conserved and is in the seven-motif helicase domain of the RecQ family of DNA helicases [14]. The Asn variant has the acidic moiety of the aspartic acid removed [15], a change that may alter the folding of the resulting protein and hence the downstream sites of protein interaction and function [16]. Although the residue 751 in the carboxyl terminus of the *XPB* protein is not conserved, the SNP at amino acid 751 may be important in terms of *XPB* activity [15], because it is located in the interactive domain, i.e., its helicase activator, p44, is inside the basal transcription factor IIH complex [9,17]. More than a dozen studies have investigated the functional significance of these two polymorphisms. The results of selected independent studies (with sample sizes greater than 100) sorted by the types of phenotypic biomarkers are summarized in Table 5 [11,18–24]. Irrespective of the effect end points or experimental systems used, half (3/6) of the studies on the Asp³¹²Asn polymorphism and two thirds (6/9) of the studies on the Lys⁷⁵¹Gln polymorphism inferred that the ³¹²Asn and ⁷⁵¹Gln alleles were related to deficient DNA repair capacity. Hence, they have been considered to be deleterious alleles in cancer development. However, results from epidemiological studies have been less consistent than those of phenotypic studies have [15,25]. Our findings on the protective role of the Asp³¹²Asn polymorphism in ever smokers are in line with observation showing that the Asn allele is related to an enhanced apoptotic response [26]. We found that the protective effect of ³¹²Asn was more prominent among light smokers (<22 pack-years), but we had insufficient power to detect whether the effect of Asp³¹²Asn polymorphism differed by the number of pack-years smoked. Recently, Duell et al. [27] reported that the Asn³¹²Asn and Gln⁷⁵¹Gln homozygote were significantly inversely associated with the risk of pancreatic cancer in a population-based study. Our findings support the protective effect of the Asn³¹² allele on the risk of smoking-related pancreatic cancer. However, we did not find a significant association between the Lys⁷⁵¹Gln polymorphism and pancreatic cancer risk. This discrepancy must be explained in the future. It may imply a weak effect of these polymorphisms on pancreatic cancer risk overall.

As reported with other study populations [14,22,28], the *XPB* ³¹²Asp and ⁷⁵¹Lys alleles have been found to be in linkage disequilibrium. The four haplotypes inferred in our study participants are reminiscent of those in two other studies conducted in Polish [14] and German [29] populations. We further identified the Asp³¹²-Gln⁷⁵¹ haplotype, which accounted for 6.9% of the haplotypes in our study population, as the putative “at-risk” haplotype. Similarly, in studies of basal cell carcinoma and breast cancer, the frequency of the ³¹²Asp-⁷⁵¹Gln haplotype was shown to be higher in patients than in controls [29,30]; furthermore, it is the most potent risk-conferring haplotype in breast cancer development. In supporting the gene-smoking interaction in the SNP-based analyses in the present study, the ³¹²Asp-⁷⁵¹Gln haplotype was associated with triple the risk of pancreatic cancer when compared with the ³¹²Asn-⁷⁵¹Lys haplotype in ever smokers. This indicates that the functional significance of the *XPB* polymorphisms was manifested only when there was excessive exposure to carcinogens. However, there may be an inferred error in constructing a haplotype based on these two SNPs.

Other SNPs of *XPD* genes should be included to identify subsets of representative SNPs in assessment of the risk of pancreatic cancer.

Few molecular epidemiological studies of pancreatic cancer have been conducted thus far. The present investigation represents one of the largest studies with an ethnically homogeneous study population. The strength of this study is the low rate of misclassification of outcome, as all of the cases were pathologically confirmed. There likelihood of chance findings cannot be excluded. Therefore, the results regarding the protective effect of Asn³¹²Asn in ever smokers must be validated. Other inherent limitations of hospital-based case-control studies also apply, such as potential selection bias associated with the voluntary recruitment of patients and controls. Nevertheless, the genotype frequencies [25] and smoking prevalence [31] among controls were comparable with those in other American population-based studies. Moreover, the patients and controls were geographically homogeneous, because 52% of the patients and 57% of the controls were from Texas. Therefore, we believed that our data were unlikely to be significantly affected by selection bias.

In summary, despite its limitations, our study sheds light on how the DNA repair genotypes contribute to altered risk of pancreatic adenocarcinoma. This preliminary study shows that the *XPD* Asp³¹²Asn and Lys⁷⁵¹Gln polymorphisms had no overall effect on the risk of pancreatic cancer in our non-Hispanic white population. However, the variant allele of the Asp³¹²Asn polymorphism may have had a protective effect on smoking-related pancreatic cancer. The imbalance in allelic combination between the Asp³¹²Asn and Lys⁷⁵¹Gln polymorphisms may contribute to the development of pancreatic cancer. While the functional interpretation remains elusive, additional larger epidemiological studies are needed to validate our findings and explore the association between the *XPD* tagging SNP and risk of pancreatic cancer.

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Table 1

Selected variables in patients and controls

Variables	Number (%)		<i>P</i> value ^a
	Patients (<i>n</i> =344)	Controls (<i>n</i> =386)	
Age at recruitment (matching factor)			
<50 years	40 (11.6)	58 (15.0)	
50–59 years	91 (26.5)	105 (27.2)	
60–69 years	115 (33.4)	133 (34.5)	
≥70 years	98 (28.5)	90 (23.3)	0.320
Gender (matching factor)			
Men	182 (52.9)	190 (49.2)	
Women	162 (47.1)	196 (50.8)	0.270
Family history of cancer (first-degree relatives)			
No	107 (31.1)	135 (35.0)	
Yes	237 (68.9)	251 (65.0)	<0.001
History of pancreatitis			
No	319 (92.7)	382 (99.0)	
Yes	25 (7.3)	4 (1.0)	<0.001
History of type II diabetes			
No	277 (80.5)	359 (93.0)	
Yes	67 (19.5)	26 (6.7)	0.010
Smoking status			
Never	139 (40.4)	192 (49.7)	
Ever	205 (59.6)	194 (50.3)	0.030
Former	140 (40.7)	139 (36.0)	
Current	65 (18.9)	55 (14.2)	0.030
Smoking history ^b			
Never	130 (38.8)	192 (50.9)	
<22 pack-years	85 (25.4)	97 (25.7)	
≥22 pack-years	120 (35.8)	97 (25.7)	0.001

^a *P* value for Pearson's χ^2 test^b Categorized by the 50th percentile value of the controls who smoked. Pack-year data were missing for nine patients.

Table 2*XPD* Asp³¹²Asn and Lys⁷⁵¹Gln polymorphisms and pancreatic cancer risk

<i>XPD</i> genotype	Number (%) ^a		OR (95% CI) ^b
	Patients (n=344) (%)	Controls (n=386) (%)	
Exon 10 Asp ³¹² Asn			
Asp/Asp	140 (41.3)	147 (40.4)	1.00
Asp/Asn	163 (48.1)	169 (46.4)	1.01 (0.74–1.39)
Asn/Asn	36 (10.6)	48 (13.2)	0.79 (0.48–1.28)
Asp/Asn + Asn/Asn	199 (58.7)	217 (59.6)	0.97 (0.72–1.31)
Asp/Asp + Asp/Asn	303 (89.4)	316 (86.8)	1.00
Asn/Asn	36 (10.6)	48 (13.2)	0.78 (0.49–1.24)
Asn allele frequency	0.34	0.36	--
Exon 23 Lys ⁷⁵¹ Gln			
Lys/Lys	124 (36.7)	147 (38.5)	1.00
Lys/Gln	184 (54.4)	203 (53.1)	1.09 (0.80–1.50)
Gln/Gln	30 (8.9)	32 (8.4)	1.11 (0.64–1.94)
Lys/Gln + Gln/Gln	214 (63.3)	235 (61.5)	1.10 (0.81–1.49)
Lys/Lys + Lys/Gln	308 (91.1)	350 (91.6)	1.00
Gln/Gln	30 (8.9)	32 (8.4)	1.06 (0.63–1.78)
Gln allele frequency	0.36	0.35	--

^aNumbers do not add up to total because of missing data.^bAdjusted for age and gender.

Table 3
XPD genotype and pancreatic cancer risk stratified by smoking status

<i>XPD</i> genotypes	Never smokers			Ever smokers		
	Patients (<i>n</i> =139) %	Controls (<i>n</i> =192) %	OR (95% CI) ^a	Patients (<i>n</i> =205) %	Controls (<i>n</i> =194) %	OR (95% CI) ^a
Exon 10 Asp ³¹² Asn						
Asp/Asp	33.6	42.2	1.00	46.5	38.6	1.00
Asp/Asn	51.8	47.2	1.48 (0.90 – 2.42)	45.6	45.6	0.83 (0.54 – 1.27)
Asn/Asn	14.6	10.6	1.80 (0.86 – 3.76)	7.9	15.6	0.42 (0.21 – 0.83)
Asp/	85.4	89.4	1.00	92.1	84.2	1.00
Asp + Asp/Asn						
Asn/Asn	14.6	10.6	1.44 (0.73 – 2.85)	7.9	15.8	0.46 (0.24 – 0.88)
Exon 23 Lys ⁷⁵¹ Gln						
Lys/Lys	35.0	40.0	1.00	37.8	37.0	1.00
Lys/Gln	54.8	50.5	1.29 (0.80 – 2.09)	54.2	55.7	0.95 (0.62 – 1.45)
Gln/Gln	10.2	9.5	1.24 (0.56 – 2.77)	8.0	7.3	1.08 (0.49 – 2.37)
Lys/Lys + Lys/ Gln	89.8	90.5	1.00	92.0	92.7	1.00
Gln/Gln	10.2	9.5	1.07 (0.51 – 2.21)	8.0	7.3	1.11 (0.53 – 2.34)

^a Adjusted for age and gender.

Table 4
XP3D haplotype frequencies in patients and controls stratified by smoking status

Groups	Haplotype 1 ^{3f2} Asn- ⁷⁵¹ Lys	Haplotype 2 ^{3f2} Asn- ⁷⁵¹ Gln	Haplotype 3 ^{3f2} Asp- ⁷⁵¹ Lys	Haplotype 4 ^{3f2} Asp- ⁷⁵¹ Gln	<i>P</i> value
All subjects					
Patients n (%) 688	41 (6.0)	198 (28.8)	397 (57.7)	52 (7.6)	0.39
Controls n (%) 772	62 (8.0)	222 (28.2)	439 (56.9)	49 (6.3)	
OR (95%CI)	1.00	1.35 (0.85–2.15)	1.37 (0.88–2.13)	1.60 (0.89–2.90)	
Never smokers					
Patients n (%) 278	21 (7.6)	83 (29.9)	152 (54.7)	13 (4.7)	0.61
Controls n (%) 384	28 (7.3)	107 (27.9)	222 (57.8)	27 (7.0)	
OR (95%CI)	1.00	1.03 (0.52–2.06)	0.91 (0.48–1.76)	0.64 (0.24–1.67)	
Ever smokers					
Patients n (%) 410	20 (4.9)	106 (25.9)	245 (59.8)	39 (9.5)	0.02
Controls n (%) 388	34 (8.8)	115 (29.6)	217 (55.9)	22 (5.7)	
OR (95%CI)	1.00	1.57 (0.82–3.06)	1.92 (1.04–3.62)	3.01 (1.32–6.93)	