Letter to the Editor

The Effect of *PCDH15* Gene Variations on the Risk of Noise-induced Hearing Loss in a Chinese Population*

XU Xiang Rong[1,^], WANG Jing Jing[1,^], YANG Qiu Yue[1,2], JIAO Jie[3], HE Li Hua[1,#], YU Shan Fa[3,#], GU Gui Zhen[3], CHEN Guo Shun[4], ZHOU Wen Hui[3], WU Hui[3], LI Yan Hong[3], ZHANG Huan Ling[4], ZHANG Zeng Rui[3], and JIN Xian Ning[1]

Noise-induced hearing loss (NIHL) is a complex disease caused by interactions between environmental and genetic factors. This study investigated whether genetic variability in protocadherin related 15 (*PCDH15*) underlies an increased susceptibility to the development of NIHL in a Chinese population. The results showed that compared with the TT genotype of rs11004085, CT/CC genotypes were associated with an increased risk of NIHL [adjusted odds ratio (*OR*) = 2.64; 95% confidence interval (*CI*): 1.14-6.11, *P* = 0.024]. Additionally, significant interactions between the rs11004085 and rs978842 genetic variations and noise exposure were observed in the high-level exposure groups (*P* < 0.05). Furthermore, the risk haplotype TAGCC was observed when combined with higher levels of noise exposure (*P* < 0.05). Thus, our study confirms that genetic variations in *PCDH15* modify the susceptibility to NIHL development in humans.

Noise-induced hearing loss (NIHL) is a major occupational health risk in industrialized countries worldwide that affects people of all ages, sex, and races. About 22-30 million US workers are exposed to hazardous noise levels at work and an estimated US $242 million is spent annually on compensation for disability associated with hearing loss[1-2]. Data from the Centers for Disease Control and Prevention (China) showed that NIHL is the third most serious occupational disease in China[3]. NIHL not only affects workers' health, but also causes social isolation, impaired communication, and decreased productivity.

NIHL is a complex form of hearing loss induced by interactions between genetic and environmental factors. Noise is the most studied environmental factor associated with hearing loss; it is harmful over 85 dB and causes both mechanical and metabolic damage. However, not all workers develop NIHL after exposure to identical noise levels. Thus, genetic factors might also influence the susceptibility to NIHL.

PCDH15 is a member of the cadherin superfamily of calcium-dependent cell-cell adhesion molecules[4], which is localized in the inner ear hair cell stereocilia and retinal photoreceptors. Mutations in *PCDH15* have been associated with both non-syndromic (DFNB23) and syndromic hearing loss (Usher syndrome type1F, USH1F)[5-6]. In 2009, research in Swedish and Polish populations first reported that single nucleotide polymorphisms (SNPs) in *PCDH15* were associated with NIHL risk[7]. Even though the reported variation is relatively common in certain European populations, racial differences need to be considered. Zhang et al. were the first to conduct research on this topic in China, reporting that the rs11004085 genetic variation in *PCDH15* was associated with NIHL[8]. However, the findings in this study were restricted to men living in South China; thus, more studies in other independent samples are necessary to confirm these findings. Therefore, this study investigated whether susceptibility to NIHL was associated with *PCDH15* genetic variations in a northern Chinese population.

This study included 6,309 workers exposed to continuous and steady occupational noise in a steel factory in Henan province, China. A detailed description of the inclusion and exclusion criteria can

doi: 10.3967/bes2017.019

*This study was supported by the National Natural Science Foundation of China (81372940); and research funds from the National Science and Technology Infrastructure Program of the People's Republic of China (2014BAI12B03).

1. Department of Occupational and Environmental Health, School of Public Health, Peking University Health Science Center, Beijing 100191, China; 2. National Center of Occupational Safety and Health, Beijing 102308, China; 3. Henan Provincial Institute for Occupational Health, Zhengzhou 450052, Henan, China; 4. Wugang Institute for Occupational Health, Wugang 462500, Henan, China

be found elsewhere[9-10]. The case group was defined as those individuals with an average high-frequency (3, 4, and 6 kHz) binaural hearing level (HL) ≥ 40 dB. The individually matched control group was defined as binaural HL of any frequency (0.5, 1, 2, 3, 4, and 6 kHz) < 25 dB. Finally, 344 matched pairs of participants were recruited from the steel factory cohort from September to December in 2013 and September to November in 2014. The study was approved by the Ethics Committee of the Henan Institute of Occupational Medicine.

All the subjects answered a structured questionnaire and underwent physical examinations by trained physicians. Noise exposure levels were assessed during their working time, which was evaluated with equivalent continuous dB(A)-weighted sound pressure levels ($L_{Aeq,8h}$). Cumulative noise exposure (CNE) was calculated to determine the actual noise exposure for each subject[9-10].

A total of 12 candidate SNPs were selected based on the inclusion criteria[9-10], including rs10825112, rs10825113, rs1900443, rs12258253, rs2135720, rs11004085, rs11004142, rs996320, rs7081730, rs978842, rs11004439, and rs7922254. The genotypes were determined using the commercial SNPscan™ multiplex SNP genotyping kit (Genesky Biopharm Technology Co., Ltd, Shanghai, China).

Hardy-Weinberg equilibrium (HWE) was checked for each SNP in the control subjects using χ^2-tests. Paired samples *t*-tests were used to compare demographic information for continuous variables, while χ^2-tests were used for categorical variables. Adjusted *ORs* with 95% *CIs* were computed by conditional logistic regression analysis to test for associations between NIHL risk and the genotypes. Bonferroni correction was performed to control for multiple testing, which resulted in a corrected significance level of 0.004 (P = 0.05/12 = 0.004). Haploview was used to estimate the haplotypes and investigate the linkage disequilibrium (LD) between the SNPs. All statistical analyses were two-sided and performed using IBM SPSS Statistics for Windows, Version 20.0, with a significance level of 0.05.

The basic characteristics of the subjects are shown in Table S1 (www.besjournal.com). The average binaural HL of high frequency noise exposure in the case group was significantly higher than that of the control subjects (P = 0.024). The percentage of smokers was also higher in the case group (P < 0.001). The two groups appeared to be well matched by age, gender, body mass index (BMI), hypertension, drinking status, exposure time, the use of earplug, noise exposure level, and CNE (P > 0.05).

The distributions of the *PCDH15* genotypes and alleles in the case and control subjects are shown in Table 1. Of the 12 SNPs, only one significant association of genotype rs11004085 was observed between the two groups (P = 0.039). As shown in Table S2 (www.besjournal.com), for rs11004085, after adjusting for BMI, smoking, drinking, and CNE, the frequencies of the CT compared with the TT genotype in the case group was significantly higher than that in the control group (adjusted *OR* = 3.03; 95% *CI*: 1.26-7.33, P = 0.014). Compared with subjects carrying the TT genotype, subjects with CT/CC genotypes were at increased risk of NIHL (adjusted *OR* = 2.64; 95% *CI*: 1.14-6.11, P = 0.024). Thus, this SNP genotype (CT/CC on rs11004085) was identified as a risk factor associated with NIHL. These findings were in agreement with two previously published studies of Polish and Swedish populations and a southern Chinese population[7-8]. Mutations on rs11004085 might decrease calcium binding capacity, weaken its interaction with other genes such as *CDH23*, and affect its adhesion function, which increase the stereocilia bundle susceptibility to noise injury[4-6]. However, no significant differences between two groups in terms of the distribution of genotypes or alleles of the other 11 SNPs were found in our study, as shown in Table S3 (www.besjournal.com). Similarly, no significant differences were detected when Zhang and colleagues compared allele and genotype frequencies for the SNPs on rs12258253[8]. This SNP did not seem to play an important role in NIHL in the Chinese population, although more studies are needed to confirm these findings.

As noise is the most common cause of NIHL, stratified analysis by noise exposure level or CNE was conducted, the results of which are shown in Table 2. For rs11004085, compared with the TT genotype, CT/TT genotypes resulted in an increased risk of NIHL for noise exposure levels > 85 dB (A) (adjusted *OR* = 4.61; 95% *CI*: 1.25-17.04, P = 0.022); for CNE > 95 dB(A), the CT/TT genotypes were also at increased risk (P = 0.011), with adjusted *OR* of 5.26 and 95% *CI* of 1.47-18.79. For rs978842, no overall significant associations with NIHL were observed before stratification. Compared to those with TT genotypes, noise exposure levels > 85 dB(A) were associated with an increased risk for NIHL among

subjects carrying TC/CC genotypes (adjusted *OR* = 1.58; 95% *CI*: 1.03-2.42, *P* = 0.035). Therefore, this study identified significant SNP-environment interactions between two SNPs, rs11004085 and rs978842, and noise exposure, a finding concordant with that of previous research[8]. It is not surprising that NIHL is positively correlated with noise exposure, as workers are more susceptible to NIHL when exposed to greater noise exposure levels. Taken together, these data suggest that the interactions between *PCDH15* polymorphisms and noise exposure might play important roles in the incidence of NIHL.

In order to identify true associations that might be missed because of the incomplete information provided by the individual SNP, we primarily applied a haplotype-centric approach, taking into account different levels of noise exposure, to test for interactions. The pairwise LD between the 12 SNPs is shown in Table S4 (www.besjournal.com). From Table 3, no significant *P*-values were obtained before stratification (*P* > 0.05). When noise exposure level > 85 dB(A) were compared with haplotype TAGCT, the frequencies of haplotype TAGCC was significantly

Table 1. Distributions of *PCDH15* Alleles and Genotypes in the Case and Control Subjects

SNPs	Minor/major Allele (A1/A2)	Location	Minor Allele Frequency			*P* (H-W)[a]	A1A1/A1A2/A2A2		*P*[b]
			HapMap-CHB	Case	Control		Case	Control	
rs10825112	C/A	3'UTR	0.057	0.071	0.078	0.693	0/49/295	3/48/293	0.537
rs10825113	A/G	intron32	0.146	0.214	0.201	0.116	17/113/212	19/100/225	0.593
rs1900443	T/C	intron27	0.306	0.259	0.230	1.000	22/134/185	18/122/204	0.251
rs12258253	C/T	intron25	0.278	0.243	0.219	0.509	22/123/197	14/123/204	0.479
rs2135720	G/A	exon20	0.427	0.507	0.480	0.792	89/171/82	78/174/89	0.335
rs11004085	C/T	intron16	0.023	0.061	0.030	0.129	0/21/323	1/8/333	**0.039**
rs11004142	C/A	intron9	0.159	0.156	0.153	0.504	9/89/246	10/85/249	0.957
rs996320	A/G	intron9	0.159	0.177	0.154	0.328	14/94/236	11/84/247	0.364
rs7081730	T/C	intron8	0.232	0.195	0.170	0.085	18/98/228	15/87/242	0.312
rs978842	C/T	intron7	0.222	0.193	0.235	0.550	15/103/225	18/126/200	0.118

Note. [a]HWE test was performed using χ^2 test for each SNP among control subjects; [b]Adjusted for BMI, drinking, smoking, and CNE.

Table 2. Stratified Analysis of *PCDH15* by Noise Exposure Level or CNE

Variables	SNPs	Case		Control		*OR* (95% *CI*)[a]	*P*[a]
		Genetype	*N* (%)	Genetype	*N* (%)		
Noise exposure level [dB(A)]							
≤ 85	rs11004085	TT	151 (93.8)	TT	144 (96.6)	1.00	0.212
		CT/CC	10 (6.2)	CT/CC	5 (3.4)	2.03 (0.67-6.19)	
> 85		TT	172 (94.0)	TT	190 (98.4)	1.00	**0.022**
		CT/CC	11 (6.0)	CT/CC	3 (1.6)	4.61 (1.25-17.04)	
≤ 85	rs978842	TT	94 (58.4)	TT	94 (63.1)	1.00	0.457
		TC/CC	67 (41.6)	TC/CC	55 (36.9)	1.19 (0.75-1.91)	
> 85		TT	106 (57.9)	TT	131 (68.2)	1.00	**0.035**
		TC/CC	77 (42.1)	TC/CC	61 (31.8)	1.58 (1.03-2.42)	
CNE [dB(A)]							
≤ 95	rs11004085	TT	85 (92.4)	TT	85 (93.4)	1.00	0.523
		CT/CC	7 (66.6)	CT/CC	6 (6.6)	1.47 (0.45-4.73)	
> 95		TT	238 (94.4)	TT	250 (98.8)	1.00	**0.011**
		CT/CC	14 (5.6)	CT/CC	3 (1.2)	5.26 (1.47-18.79)	

Note. [a]Adjusted for BMI, drinking, smoking, and CNE.

Table 3. Assessment of the Associations between Haplotypes and NIHL

Haplotype[a,b]	Case (*n*, %)	Control (*n*, %)	Adjusted OR (95% *CI*)[c]				
			Total	Noise Exposure Level ≤ 85 dB(A)	Noise Exposure Level > 85 dB(A)	CNE ≤ 95 dB(A)	CNE > 95 dB(A)
TAGCT	479 (69.6)	516 (75.0)	1.00	1.00	1.00	1.00	1.00
TCATC	59 (8.6)	61 (8.9)	1.03 (0.70-1.51)	1.27 (0.72-2.24)	0.87 (0.51-1.47)	1.08 (0.53-2.18)	1.03 (0.65-1.63)
TAGCC	59 (8.6)	42 (6.1)	1.51 (1.00-2.30)	1.32 (0.72-2.41)	**1.84 (1.01-3.33)**[d]	1.13 (0.52-2.46)	**1.79 (1.08-2.95)**[d]
TCATT	23 (3.3)	25 (3.5)	0.94 (0.52-1.69)	1.48 (0.58-3.79)	0.68 (0.32-1.47)	3.55 (0.72-17.58)	0.72 (0.37-1.34)

Note. [a]Haplotype analysis was restricted to the SNPs that were in one block (D' > 0.4); [b]Haplotypes of *PCDH15* were deduced for the following SNPs: rs11004085, rs11004142, rs996320, rs7081730, and rs978842; [c]Adjusted for BMI, smoking and drinking; only haplotype with frequency >3% was shown in this table; [d]Bold signifies $P < 0.05$.

higher in the case group (adjusted *OR* = 1.84; 95% *CI* = 1.01-3.33, $P < 0.05$); for CNE > 95 dB(A), subjects with haplotype TAGCC were more susceptible to NIHL (adjusted *OR* = 1.79; 95% *CI* = 1.08-2.95, $P < 0.05$), indicating it to be a risk haplotype. Our finding was concordant with that of a previous study[8], suggesting that multiple genetic variations in *PCDH15* modify NIHL risk and that higher noise exposure might increase risk.

However, after applying Bonferroni correction, the associations were no longer statistically significant.

These findings are of value for the prevention of NIHL. People with the CT/CC rs11004085 genotype or the TAGCC *PCDH15* risk haplotype should take care to avoid high levels of noise exposure in their workplaces.

The limitations of this study should be acknowledged when interpreting the results. Firstly, although some workers might be exposed to community noise, such as sleeping with the television on and listening to music with headphones, these factors were too complicated to consider in the current study. Secondly, while this study identified TAGCT as a potential risk haplotype, the molecular mechanism are not clear and further functional studies are warranted.

In conclusion, our findings indicated that genetic variations in *PCDH15* may play an important role in genetic susceptibility to NIHL. The effect of gene-environment interactions and multiple loci on the development of NIHL were detected. However, after Bonferroni correction, these differences were not found to be significant. Further studies involving a larger number of individuals and independent populations are required to assess these findings.

Received: September 22, 2016;
Accepted: February 1, 2017

REFERENCES

1. National Institute for Occupational Safety and Health (NIOSH). Noise and hearing loss prevention. 2013. http://www.cdc.gov/niosh/topics/noise/. [2013-7-11]
2. Basner M, Babisch W, Davis A, et al. Auditory and onauditory effects of noise on health. Lancet, 2014; 383, 1325-32.
3. National Health and Family Planning Commission of the People,s Republic of China. Briefing on the prevention of occupational diseases in 2013. [EB/OL]. http://www.nhfpc.gov.cn/jkj/s5899t/201406/ed8ed220d0b74010bcb6dcd8e340f4fb.shtml. [2016-6-30]
4. Ahmed ZM, Riazuddin S, Bernstein SL, et al. Mutations of The protocadherin gene PCDH15 cause Usher syndrome type 1F. Am J Hum Genet, 2001; 69, 25-34.
5. Ahmed ZM, Riazuddin S, Ahmad J, et al. PCDH15 is Expressed in the neurosensory epithelium of the eye and ear and mutant alleles are Responsible for both USH1F and DFNB23. Hum Mol Genet, 2003; 12, 3215-23.
6. Zheng QY, Yan D, Ouyang XM, et al. Digenic inheritance of deafness caused by mutations in genes encoding cadherin 23 and protocadherin 15 in mice and humans. Hum Mol Genet, 2005; 14, 103-11.
7. Konings A, Van Laer L, Wiktorek-Smagur A, et al. Candidate gene association study for noise-induced hearing loss in two independent noise-exposed populations. Ann Hum Genet, 2009; 73, 215-24.
8. Zhang X, Liu Y, Zhang L, et al. Genetic variations in protocadherin15 and their interactions with noise exposure associated with noise-induced hearing loss in Chinese population. Environ Res, 2014; 135, 247-52.
9. Xu XR, Yang QY, Jiao J, et al. Genetic variation in POU4F3 and GRHL2 associated with noise-induced hearing loss in Chinese population: A case-control study. Int J Environ Res Public Health, 2016; 13, 561.
10. Yang QY, Xu XR, Jie J, et al. Genetic variation in EYA4 on the risk of noise-induced hearing loss in Chinese steelworks firm sample. Occup Environ Med, 2016; 73, 823-8.

Table S1. Basic Characteristics of Case and Control Subjects

Variables	Case (*n* = 344)	Control (*n* = 344)	*P*
Age (y), mean ± SD	40.7 ± 8.4	40.1 ± 8.4	0.998
Tenure (y), mean ± SD	19.1 ± 9.2	18.6 ± 9.0	0.747
HL[a], mean ± SD	51.1 ± 8.9	10.2 ± 8.6	**0.024**
BMI[b], mean ± SD	25.5 ± 3.4	25.3 ± 3.4	0.964
Gender, *n* (%)			
male	327 (95.1)	327 (95.1)	1.000
female	17 (4.9)	17 (4.9)	
Hypertension, *n* (%)			
yes	140 (40.7)	142 (41.3)	0.877
no	204 (59.3)	202 (58.7)	
Smoking, *n* (%)			
yes	217 (63.1)	172 (50.0)	**< 0.001**
no	127 (36.9)	172 (50.0)	
Drinking (Alcohol), *n* (%)			
yes	233 (71.6)	228 (69.0)	0.685
no	111 (28.4)	116 (31.0)	
Protector (Earplug), *n* (%)			
yes	140 (40.7)	148 (43.0)	0.536
no	204 (59.3)	196 (57.0)	
Noise exposure level, dB(A)	85.7 ± 3.9	85.7 ± 3.7	0.152
CNE[c], dB(A)	97.9 ± 4.6	97.9 ± 4.4	0.152

Note. [a]HL: hearing level in high frequency; [b]BMI (Body Mass Index) was calculated as body weight (kg)/height (m)^2; [c]CNE: cumulative noise exposure.

Table S2. Single SNP Analysis of Association of rs11004085 with the Risk of NIHL

Genotypes	Case		Control		*OR* (95% *CI*)[a]	*P*[a]
	N	%	*N*	%		
rs11004085						
TT	323	94.2	333	97.7	1.00	
CT	21	5.8	8	2.0	3.03 (1.26-7.33)	**0.014**
CC	0	0.0	1	0.3	-	0.978
CC/CT	21	5.8	8	2.3	2.64 (1.14-6.11)	**0.024**
T Allele	667	96.9	674	98.0	1.00	
C Allele	21	3.1	10	1.5	-	0.978

Note. [a]Adjusted for BMI, drinking, smoking and CNE.

Table S3. Associations of Candidate SNPs with the Risk of NIHL

SNPs	Genotypes	Case		Control		*OR* (95% *CI*)*	*P**
		N	%	*N*	%		
rs10825112	AA	295	85.8	293	85.2	1.00	
	CA	49	14.2	48	14.0	0.10 (0.63-1.56)	0.985
	CC	0	0.0	3	0.9	-	0.975
	CA/CC	49	14.2	51	14.8	0.93 (0.60-1.45)	0.741
	A allele	639	92.9	634	92.2	1.00	
	C allele	49	7.1	54	7.8	0.92 (0.59-1.43)	0.715
rs10825113	GG	212	61.6	225	65.4	1.00	
	GA	113	32.8	100	29.1	1.19 (0.85-1.67)	0.307
	AA	17	4.9	19	5.5	0.93 (0.48-1.83)	0.840
	GA/AA	130	37.8	119	34.6	1.15 (0.83-1.58)	0.398
	GG/GA	325	94.5	325	94.5	1.00	
	AA	17	4.9	19	5.5	0.88 (0.45-1.71)	0.706
	G allele	537	78.1	550	79.9	1.00	
	A allele	147	21.4	138	20.1	0.88 (0.45-1.71)	0.703
rs1900443	CC	185	53.8	204	59.3	1.00	
	TC	134	39.0	122	35.5	1.19 (0.86-1.63)	0.293
	TT	22	6.4	18	5.2	1.26 (0.66-2.40)	0.485
	TC/TT	156	45.3	140	40.7	1.19 (0.88-1.60)	0.268
	CC/TC	319	92.7	326	94.8	1.00	
	TT	22	6.4	18	5.2	1.19 (0.63-2.24)	0.599
	C allele	504	73.3	530	77.0	1.00	
	T allele	178	25.9	158	23.0	1.20 (0.89-1.61)	0.243
rs12258253	TT	197	57.3	204	59.3	1.00	
	TC	123	35.8	123	35.8	0.99 (0.72-1.37)	0.955
	CC	22	6.4	14	4.1	1.50 (0.76-2.99)	0.245
	TC/CC	145	42.2	137	39.8	1.06 (0.78-1.44)	0.710
	TT/TC	320	93.0	327	95.1	1.00	
	CC	22	6.4	14	4.1	1.51 (0.76-2.98)	0.238
	T allele	517	75.1	531	77.2	1.00	
	C allele	167	24.3	151	21.9	1.50 (0.76-2.96)	0.243
rs2135720	CC	82	23.8	89	25.9	1.00	
	TC	171	49.7	174	50.6	0.85 (0.59-1.22)	0.380
	TT	89	25.9	78	22.7	0.81 (0.52-1.27)	0.353

Continud

SNPs	Genotypes	Case		Control		*OR* (95% *CI*) *	*P* *
		N	%	*N*	%		
	TC/TT	260	75.6	252	73.3	0.84 (0.60-1.18)	0.319
	CC/TC	253	73.5	263	76.5	1.00	
	TT	89	25.9	78	22.7	0.90 (0.62-1.31)	0.592
	C allele	335	48.7	352	51.2	1.00	
	T allele	349	50.7	330	48.0	1.20 (0.85-1.70)	0.297
rs11004142	AA	246	71.5	249	72.4	1.00	
	CA	89	25.9	85	24.7	1.01 (0.70-1.45)	0.953
	CC	9	2.6	10	2.9	0.92 (0.34-2.44)	0.861
	CA/CC	98	28.5	95	27.6	1.00 (0.71-1.41)	1.000
	AA/CA	335	97.4	334	97.1	1.00	
	CC	9	2.6	10	2.9	0.92 (0.34-2.44)	0.859
	A allele	581	84.4	583	84.7	1.00	
	C allele	107	15.6	105	15.3	1.01 (0.71-1.42)	0.977
rs996320	GG	236	68.6	247	71.8	1.00	
	GA	94	27.3	84	24.4	1.11 (0.78-1.58)	0.558
	AA	14	4.1	11	3.2	1.41 (0.60-3.31)	0.426
	GA/AA	108	31.4	95	27.6	1.14 (0.82-1.60)	0.436
	GG/GA	330	95.9	331	96.2	1.00	
	AA	14	4.1	11	3.2	1.37 (0.59-3.20)	0.461
	G allele	566	82.3	578	84.0	1.00	
	A allele	122	17.7	106	15.4	1.41 (0.61-3.30)	0.423
rs7081730	CC	228	66.3	242	70.3	1.00	
	TC	98	28.5	87	25.3	1.14 (0.81-1.60)	0.467
	TT	18	5.2	15	4.4	1.37 (0.65-2.86)	0.406
	TC/TT	116	33.7	102	29.7	1.16 (0.84-1.61)	0.369
	CC/TC	326	94.8	329	95.6	1.00	
	TT	18	5.2	15	4.4	1.30 (0.63-2.69)	0.476
	C allele	554	80.5	571	83.0	1.00	
	T allele	134	19.5	117	17.0	1.15 (0.83-1.60)	0.391
rs978842	TT	225	65.4	200	58.1	1.00	
	TC	103	29.9	126	36.6	1.25 (0.90-1.72)	0.184
	CC	15	4.4	18	5.2	1.47 (0.70-3.09)	0.305
	TC/CC	118	34.3	144	41.9	1.29 (0.94-1.76)	0.113

Continud

SNPs	Genotypes	Case		Control		*OR* (95% *CI*)*	*P**
		N	%	*N*	%		
	TT/TC	328	95.3	326	94.8	1.00	
	CC	15	4.4	18	5.2	1.37 (0.66-2.83)	0.403
	T allele	553	80.4	526	76.5	1.00	
	C allele	133	19.3	162	23.5	1.34 (0.65-2.77)	0.427
rs11004439	AA	243	70.6	241	70.1	1.00	
	CA	89	25.9	98	28.5	0.94 (0.66-1.34)	0.736
	CC	12	3.5	5	1.5	2.43 (0.83-7.12)	0.105
	CA/CC	101	29.4	103	29.9	1.01 (0.72-1.43)	0.946
	AA/CA	332	96.5	339	98.5	1.00	
	CC	12	3.5	5	1.5	2.49 (0.86-7.23)	0.094
	A allele	575	83.6	580	84.3	1.00	
	C allele	113	16.4	108	15.7	1.01 (0.72-1.42)	0.953
rs7922254	AA	204	59.3	189	54.9	1.00	
	TA	117	34.0	139	40.4	0.90 (0.57-1.12)	0.187
	TT	23	6.7	16	4.7	1.41 (0.72-2.76)	0.323
	TA/TT	140	40.7	155	45.1	0.86 (0.63-1.19)	0.359
	AA/TA	321	93.3	328	95.3	1.00	
	TT	23	6.7	16	4.7	1.55 (0.81-3.00)	0.189
	A allele	525	76.3	517	75.1	1.00	
	T allele	163	23.7	171	24.9	0.86 (0.63-1.18)	0.353

Note. *Adjusted for BMI, smoking, drinking, and CNE.

Table S4. Linkage Disequilibrium test of PCDH15 Gene

SNPs	rs10825112	rs10825113	rs1900443	rs12258253	rs2135720	rs11004085	rs11004142	rs996320	rs7081730	rs978842	rs11004439	rs7922254
rs10825112	-	0.728	0.046	0.013	0.704	0.443	0.134	0.182	0.207	0	0	0
rs10825113	0.124	-	0.593	0.472	0.601	0.174	0.06	0.071	0.047	0.062	0	0
rs1900443	0.001	0.283	-	0.919	0.289	0.47	0.126	0.146	0.109	0.073	0	0
rs12258253	0	0.191	0.795	-	0.211	0.495	0.121	0.148	0.121	0.095	0	0
rs2135720	0.041	0.095	0.027	0.014	-	0.616	0.021	0.003	0.028	0.017	0	0
rs11004085	0.056	0.003	0.016	0.019	0.009	-	0.431	0.729	0.953	0.899	0	0
rs11004142	0.008	0.003	0.009	0.009	0	0.023	-	0.925	0.843	0.541	0.275	0
rs996320	0.013	0.004	0.013	0.015	0	0.062	0.784	-	0.923	0.644	0.094	0
rs7081730	0.015	0.002	0.008	0.011	0	0.094	0.58	0.758	-	0.689	0.235	0
rs978842	0	0.004	0.005	0.008	0	0.068	0.195	0.302	0.388	-	0.176	0.23
rs11004439	0	0	0	0	0	0	0.003	0	0	0.002	-	0.907
rs7922254	0	0	0	0	0	0	0	0	0	0.002	0.492	-

Note. The upper triangle was D' value and the lower triangle was r^2 value.