ANTICANCER RESEARCH *40*: 5503-5508 (2020)
doi:10.21873/anticanres.14562

Association of Caspase-8 Genotypes With the Risk for Nasopharyngeal Carcinoma in Taiwan

LIANG-CHUN SHIH[1,2,3*], CHIA-WEN TSAI[1,3*], WEN-SHIN CHANG[1,3*], TE-CHUN SHEN[3], YUN-CHI WANG[1,3], JIA-SING YANG[3,4], MENG-LIANG LIN[5], ZHI-HONG WANG[6] and DA-TIAN BAU[1,3,7]

[1]*Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, R.O.C.;*
[2]*Department of Otorhinolaryngology, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[3]*Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[4]*Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[5]*Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan, R.O.C.;*
[6]*Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan, R.O.C.;*
[7]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: Accumulating evidence shows that caspase-8 (Cas-8) rs3834129 genotypes determine susceptibility to various cancers, but their association with nasopharyngeal carcinoma (NPC) has not been examined. We aimed at investigating the association of Cas-8 rs3834129 with NPC risk. Materials and Methods: Cas-8 rs3834129 genotypes and their associations with NPC risk were investigated among 176 NPC patients and 352 non-cancer subjects by the PCR-RFLP method. Additionally, the interaction of Cas-8 rs3834129 genotypes with smoking was examined. Results: The II, ID and DD frequencies were 56.8, 36.9 and 6.3% among NPC patients and 54.8, 38.1 and 7.1% among control subjects (p_{trend}=0.8830). Allelic frequency distribution analysis also indicated that the D allele is not a risk factor for NPC (p=0.6183). There was no interaction between Cas-8 rs3834129 and smoking and NPC risk (p=0.8305). Conclusion: Cas-8 rs3834129 genotypes play a minor role in the risk for NPC.*

Nasopharyngeal carcinoma (NPC), or nasopharynx cancer, is a rapidly growing squamous cell carcinoma in nasopharynx which attacks both children and adults (1). Epidemiological studies have shown Epstein–Barr virus (EBV) infection to be a major risk factor for NPC and metastasis (2, 3). In addition, cigarette smoking and alcohol consumption have been also shown to serve as behavioral determiners of NPC risk (4, 5). In NPC-prevalent areas, every individual is exposed to similar environment factors, however, only a subpopulation may develop NPC, suggesting that genetic differences such as single-nucleotide polymorphisms (SNPs) may also contribute to NPC. The molecular mechanisms of NPC induced by these genetic factors are largely remaining unrevealed. The understanding of these genomic factors and how they contribute to NPC's etiology may help in providing novel biomarkers for early detection and prediction of NPC susceptibility and prognosis outcomes. For instance, mounting evidence indicates that the variant genotypes of genes involved in the production of inflammatory cytokines (6, 7), extracellular matrix metabolism (8), cell cycle regulation (9), and DNA repair capacity (10) could determine individual susceptibility to NPC.

Apoptosis is an essential mechanism, mediated by intrinsic and/or extrinsic pathways, triggered by cellular stress, DNA damage and immune surveillance, to alter cell morphology, and control the death rate in a stable population (11, 12). In the literature, mounting evidence indicates that the loss of homeostasis of the apoptotic pathway is associated with the development of cancer (13), while few FDA-approved anticancer agents are small molecules designed to inhibit anti-apoptotic BCL-2 family members (12).

Caspase-8 (*Cas-8*), one of the most important components of the caspase family, plays a critical role in the extrinsic apoptosis signaling (14, 15). Among the polymorphic sites

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau and Zhi-Hong Wang, Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422053366 (Ext. 5805), e-mail: datian@mail.cmuh.org.tw; artbau2@gmail.com (D-T B)

Key Words: Case–control study, *caspase-8*, genotype, nasopharyngeal carcinoma, polymorphism, Taiwan.

at *Cas-8*, rs3834129 (-652, 6N insertion/deletion), a six-nucleotide insertion (I)/deletion (D) variant, has been functionally identified to down-regulate the levels of *Cas-8* mRNA (16). There are at least 353 SNPs in *Cas-8* reported in the NCBI dbSNP database. *Cas-8* SNPs, such as D302H (rs1045485), IVS12-19G/A (rs3769818), and the promoter rs3834129 that will be the focus of the current study, have been reported to be potential genomic markers for the prediction of risk for several types of cancer such as neuroblastoma (17), breast cancer (18-20), lung cancer (21), digestive tract cancer (22, 23), prostate cancer (24), and bladder cancer (25). However, the role of *Cas-8* polymorphisms in NPC is not known.

Based on the above, in the current study, we aimed at examining and evaluating the association of *Cas-8* rs3834129 genotypes with NPC risk, as well as revealing the joint effects of *Cas-8* rs3834129 genotypes with smoking behaviors on NPC risk in a representative Taiwanese population.

Materials and Methods

Study subjects. During the period of 2003 to 2009, 176 NPC patients were recruited in the department of general surgery outpatient clinics at the China Medical University Hospital. Clinically, the histological status and type of each NPC patient were identified and classified according to 1991 WHO classification system. For each case of NPC, two age-, gender- and behavior-matched cancer-free healthy subjects were manually selected among subjects of the regular health examination pool and included into the final comparison and evaluation. Thus, 352 healthy controls and 176 NPC patients were subjected to *Cas-8* genotyping and final statistical analysis. All the protocols in the study were approved by the institutional review board of the China Medical University Hospital (DMR101-IRB1-306).

DNA preparation and storage. Genomic DNA was isolated from the peripheral blood leukocytes using the QIAamp Blood Mini Kit (Qiagen, Valencia, CA, USA), stored for long term at −80˚C, diluted, and aliquoted for genotyping as a working stock at −20˚C, as we have previously described (26, 27).

Cas-8 rs3834129 genotyping methodology. In brief, the polymerase chain reaction (PCR) cycling conditions were set as: 1) start with one cycle at 94˚C for 5 min; 2) 35 cycles at 94˚C for 30 s, 59˚C for 30 s and 72˚C for 30 s, 3) a final extension step at 72˚C for 10 min. The self-designed sequences for *Cas-8* rs3834129 genotyping primers were 5'-ACTCTGCATGCCAGGAGCTA-3' and 5'-CTGGGGA AGCCTCACTGTAT-3'. After PCR amplification by a PCR Thermocycler (Bio-RAD, Hercules, CA, USA), the PCR products were digested with *Pvu* II (New England Biolabs, Beverly, MA, USA) and the products of the digestion were separated by 3% agarose gel electrophoresis. All the genotypic procedures were independently and blindly conducted by at least two well-experienced researchers. To confirm the PCR-RFLP results, 5% of the samples were randomly chosen and sent for direct sequencing. The results of direct sequencing and PCR-RFLP methodologies were 100% consistent to each other.

Statistical analysis. First, to make sure that the control subjects are representative of the Taiwan population, the deviations of the genotypic frequencies of *Cas-8* rs3834129 of the control group from those expected under the Hardy–Weinberg equilibrium were assessed using the goodness-of-fit test. Second, the Pearson's chi-square test was used for examining the distributions of *Cas-8* rs3834129 genotypes. Third, Student's *t*-test was used to examine the distribution of ages among the case and control groups. Last, the association between NPC risk and *Cas-8* genotypes was estimated by calculating the odds ratios (ORs) and related 95% confidence intervals (CIs) in the logistic regression analysis, adjusted or unadjusted for potential confounders for NPC risk. Any *p*-value less than 0.05 was considered to be statistically significant.

Results

Comparisons of basic characteristics between the NPC and the control groups. The recorded information on age, gender, and personal behavioral habits of the investigated 176 NPC patients and the 352 non-cancer control subjects are summarized in Table I. As we matched the patients with controls by these indexes, no significant differences for age and gender between the case and control groups was observed ($p>0.05$). Also, no significant differences were observed between the case and control groups regarding personal behavioral habits, including cigarette smoking, alcohol drinking, and areca chewing consumption (all $p>0.05$) (Table I). The histological status for each NPC patient was identified and classified by experienced surgeons. There were 5 (2.8%) keratinizing squamous cell carcinoma (WHO type I) and 171 (97.2) non-keratinizing carcinoma (WHO type II) cases. Those type II NPC patients were further divided into 28 (15.9%) non-keratinizing differentiated carcinoma (WHO type IIa) and 143 (81.3%) non-keratinizing undifferentiated carcinoma (WHO type IIb) cases (Table I).

Association of Cas-8 promoter rs3834129 genotypes and NPC risk among Taiwanese. The distributions of *Cas-8* rs3834129 genotypes among healthy controls and patients with NPC are presented in Table II. The distribution of *Cas-8* rs3834129 genotypes in the control group fitted well with the Hardy-Weinberg Equilibrium ($p_{HEW}=0.7921$). The genotypic percentages of *Cas-8* rs3834129 were not differentially distributed among the NPC cases and the controls (p for trend=0.8830) (Table II). In detail, neither the heterozygous ID nor the homozygous DD genotype was associated with decreased NPC risk compared with the wild-type II genotype at *Cas-8* rs3834129 (adjusted OR=0.93 and 0.86, 95%CI=0.68-1.31 and 0.56-1.83, $p=0.7353$ and 0.6686, respectively). In addition, the results of the dominant model of analysis showed that there was no significant association between ID+DD genotypes and NPC risk compared with II genotype at *Cas-8* rs3834129 (adjusted OR=0.89, 95%CI=0.66-1.47, $p=0.6647$).

In order to validate the results in Table II, we performed the analysis of allelic frequency distributions for *Cas-8*

Table I. *Demographic characteristics of the 352 healthy controls and 176 NPC patients.*

Characteristics	Controls (n=352)			Cases (n=176)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			48.7 (10.8)			49.3 (9.4)	0.7138
Gender							1.0000
Male	256	72.7%		128	72.7%		
Female	96	27.3%		48	27.3%		
Personal behaviors							
Cigarette smoker	150	42.6%		73	41.4%		0.8519
Alcohol drinker	124	35.2%		72	40.9%		0.2150
Areca chewer	115	32.7%		54	30.7%		0.6926
Classification							
KSCC (WHO type I)				5	2.8%		
NKC (WHO type II)				171	97.2%		
NKDC (WHO type IIa)				28	15.9%		
NKUC (WHO type IIb)				143	81.3%		

SD: Standard deviation; KSCC: keratinizing squamous cell carcinoma; NKC: non-keratinizing carcinoma; NKDC: non-keratinizing differentiated carcinoma; NKUC: non-keratinizing undifferentiated carcinoma; [a]Based on Chi-square test or Student's *t*-test.

Table II. *Distributions of Cas-8 rs3834129 genotypic frequencies among the NPC patients and healthy controls.*

Genotype	Cases, n (%)	Controls, n (%)	Adjusted OR (95%CI)[a]	*p*-Value[b]
II	100 (56.8)	193 (54.8)	1.00 (Reference)	
ID	65 (36.9)	134 (38.1)	0.93 (0.68-1.31)	0.7353
DD	11 (6.3)	25 (7.1)	0.86 (0.56-1.83)	0.6686
ID+DD	76 (43.2)	159 (45.2)	0.89 (0.66-1.47)	0.6647
p_{trend}				0.8830
p_{HWE}				0.7921

I: Insertion; D: deletion; OR: Odds ratio; CI: confidence interval; HWE: Hardy-Weinberg Equilibrium; [a]Data adjusted for confounding factors: age, gender, smoking, alcohol and betel quid consumption; [b]Based on chi-square test without Yates' correction.

rs3834129 and the results are shown in Table III. Supporting the finding that *Cas-8* rs3834129 genotypes are not associated with NPC risk, the variant allele D was also non-significantly associated with decreased NPC risk (adjusted OR=0.91, 95%CI=0.73-1.35, p=0.6183) (Table III).

Interaction between Cas-8 rs3834129 genotypes and behavioral factors on NPC risk. From the epidemiological viewpoint, cigarette smoking, alcohol drinking and betel quid consumption may contribute to NPC in Taiwan. Therefore, we were interested in evaluating the interaction of *Cas-8* rs3834129 genotypes with these personal behaviors among Taiwanese. First, among non-smokers, people with ID and DD genotypes at *Cas-8* rs3834129 were at 1.01- and 0.83-fold odds ratio for NPC risk compared to those with the II genotype (95%CI=0.62-1.66 and 0.30-2.29, p=0.9681 and 0.7245) (Table IV, left panel). After adjusting for confounding factors including age, gender, alcohol drinking and betel quid chewing status, the statistical results still stayed at non-significant levels for ID and DD genotypes (Table IV, left panel). Meanwhile, similar non-significant associations were found among smokers (Table IV, right panel). The similar findings found among the subgroups of non-alcohol drinkers, alcohol drinkers, non-betel quid chewers, and betel quid chewers, showed that sub-groups of people with ID and DD genotypes at *Cas-8* rs3834129 were not significantly different regarding the risk of having NPC compared with those with the II genotype (data not shown).

Discussion

Apoptosis plays critical roles in a wide variety of physiological and pathological processes during fetal development, adult maturation, and aging, while defects in

Table III. *Allelic frequencies for Cas-8 rs3834129 polymorphisms among the NPC patients and healthy controls.*

Allele	Cases, n (%) (n=352)	Controls, n (%) (n=704)	Adjusted OR (95%CI)[a]	*p*-Value[b]
I	265 (75.3)	520 (73.9)	1.00 (Reference)	
D	87 (24.7)	184 (26.1)	0.91 (0.73-1.35)	0.6183

I: Insertion; D: deletion; OR: Odds ratio; CI: confidence interval. [a]Data adjusted for confounding factors: age, gender, smoking, alcohol and betel quid consumption. [b]Based on chi-square test without Yates' correction.

Table IV. *Odds ratios for association of Cas-8 rs3834129 genotype with NPC after stratification by personal smoking status.*

Genotype	Non-smokers, n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value[c]	Smokers, n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value[c]
	Controls	Cases				Controls	Cases			
II	109	56	1.00 (ref)	1.00 (ref)		84	44	1.00 (ref)	1.00 (ref)	
ID	79	41	1.01 (0.62-1.66)	0.96 (0.73-1.56)	0.9681	55	24	0.83 (0.46-1.52)	0.88 (0.53-1.47)	0.5521
DD	14	6	0.83 (0.30-2.29)	0.87 (0.45-1.86)	0.7245	11	5	0.87 (0.28-2.66)	0.86 (0.46-2.13)	0.8036
Total	202	103				150	73			
p_{trend}					0.9335					0.8305

I: Insertion; D: deletion; CI: confidence interval; aOR: adjusted odds ratio; [a]Multivariate logistic regression analysis; [b]multivariate logistic regression analysis after adjusting for age, gender and alcohol drinking status; [c]Chi-square without Yates' correction.

the homeostatic regulation of apoptosis may contribute to human diseases, such as several types of cancer and autoimmune lymphoproliferative syndrome (ALPS) (28, 29). However, the knowledge of how genomic variants of caspases are involved in NPC etiology is still lacking. The *Cas-8* gene encodes cysteine-aspartic acid protease 8, which plays a central role in the execution of programmed cell death (30). As originally identified, Cas-8 initiates apoptotic processes after being activated by various apoptotic stimuli, such as Fas and FADD, the Fas-interacting protein (31, 32). The activated Cas-8 may work alone or cooperate with other initiators, such as caspase-10, to activate the downstream executor caspases, such as caspase-3, to complete the apoptotic process (33). Among the SNPs on *Cas-8*, rs3834129 is the most commonly examined (34). In 2007, Sun and his colleagues firstly examined the contribution of *Cas-8* rs3834129 polymorphism to the risk of many types of cancer (35). They obtained valuable pilot results indicating that the D allele at *Cas-8* rs3834129 is associated with a decreased risk for some types of cancer including lung, colorectal, esophageal, breast, cervical and gastric cancer (35). After that, the genotypes of *Cas-8* rs3834129 were found to be associated with the risk for melanoma (36), breast cancer (20), kidney cancer (37), and worse prognosis of neuroblastoma (17). Furthermore, the D allele at *Cas-8* rs3834129 has been found to destroy the affinity of stimulatory protein 1 binding element for its promoter region, which results in a significantly decreased *Cas-8* transcription and eventually a reduced apoptosis of T lymphocytes (35). However, the contribution of the *Cas-8* rs3834129 genotypes to NPC risk had not been examined.

In the current study, we firstly revealed that the ID or DD genotypes at *Cas-8* rs3834129 were not significantly associated with NPC risk in a representative Taiwanese population containing 176 NPC patients and 352 control subjects (Tables II and III). In addition, after adjusting for the confounding factors, there was still no association between the *Cas-8* rs3834129 genotypes with NPC risk. Furthermore, we analyzed the interaction of *Cas-8* rs3834129 genotypes with several risk behaviors among Taiwanese including smoking, alcohol drinking, and betel quid chewing. The results indicated that there was no interaction among *Cas-8* rs3834129 genotypes and cigarette smoking, alcohol drinking, or betel quid consumption on determining personal susceptibility for NPC (Table IV). We also examined the correlations between genotypes at *Cas-8* rs3834129 and clinicopathological features of NPC patients. Gender, age and the pathological status of patients did not affect the influence of *Cas-8* rs3834129 genotypes on NPC risk (data not shown). The finding in NPC was consistent with our previous study investigating the association of *Cas-8* rs3834129 genotypes with oral cancer, another head and neck cancer (38). These highlight findings are also consistent with a previous report investigating the contribution of *Cas-8* rs3834129 genotypes to oral cancer susceptibility in a South Indian population. Noticeably, Du and his colleagues

have conducted a meta-analysis to investigate the association between the *Cas-8* rs3834129 polymorphisms and the risk for digestive tract cancers (39). They found an evident association between *Cas-8* rs3834129 polymorphisms and reduced digestive cancer risk, especially for Asians (22). Future studies investigating the association of *Cas-8* rs3834129 polymorphism and the risk of NPC in larger and different populations are urgently required to confirm our findings. These results may contribute to revealing the influence of genomic factors among different ethnicities.

In conclusion, the study provided evidence showing that *Cas-8* rs3834129 genotypes were not associated with altered risk for NPC in a representative Taiwanese population. In addition, in the stratified analyses, no interaction between *Cas-8* rs3834129 and personal behaviors such as cigarette smoking, alcohol drinking, and betel quid consumption, on NPC risk determination was found.

Conflicts of Interest

The Authors declare no conflicts of interest in regard to this study.

Authors' Contributions

Research design: Shih LC, Tsai CW and Chang WS; patient and questionnaire summaries: Shih LC and Shen TC; experimental work: Wang YC and Yang JS; statistical analysis: Wang ZH and Lin ML; article writing: Wang ZH and Bau DT; review and revision: Chang WS, Tsai CW and Bau DT.

Acknowledgements

The Authors appreciate the TissueBank of China Medical University Hospital for their excellent technical assistance and all the subjects, doctors, nurses and colleagues. The perfect technological expertise and efforts from Kai-Cheng Chan, Yu-Ting Chin, Tai-Lin Huang, Yu-Hsin Lin, Yi-Ru Huang and Yu-Chen Hsiau are also appreciated. This study was supported mainly by the China Medical University and Hospital (DMR-109-143) to Dr. Bau.

References

1 Lin CL, Lo WF, Lee TH, Ren Y, Hwang SL, Cheng YF, Chen CL, Chang YS, Lee SP, Rickinson AB and Tam PK: Immunization with Epstein-Barr Virus (EBV) peptide-pulsed dendritic cells induces functional CD8+ T-cell immunity and may lead to tumor regression in patients with EBV-positive nasopharyngeal carcinoma. Cancer Res *62(23)*: 6952-6958, 2002. PMID: 12460912.

2 O'Neil JD, Owen TJ, Wood VHJ, Date KL, Valentine R, Chukwuma MB, Arrand JR, Dawson CW and Young LS: Epstein-Barr virus-encoded EBNA1 modulates the AP-1 transcription factor pathway in nasopharyngeal carcinoma cells and enhances angiogenesis *in vitro*. J Gen Virol *89(Pt 11)*: 2833-2842, 2008. PMID: 18931081. DOI: 10.1099/vir.0.2008/003392-0

3 Yoshizaki T: Promotion of metastasis in nasopharyngeal carcinoma by Epstein-Barr virus latent membrane protein-1. Histol Histopathol *17(3)*: 845-850, 2002. PMID: 12168795. DOI: 10.14670/HH-17.845

4 Cheng YJ, Hildesheim A, Hsu MM, Chen IH, Brinton LA, Levine PH, Chen CJ and Yang CS: Cigarette smoking, alcohol consumption and risk of nasopharyngeal carcinoma in Taiwan. Cancer Causes Control *10(3)*: 201-207, 1999. PMID: 10454065. DOI: 10.1023/a:1008893109257

5 Long M, Fu Z, Li P and Nie Z: Cigarette smoking and the risk of nasopharyngeal carcinoma: a meta-analysis of epidemiological studies. BMJ Open *7(10)*: e016582, 2017. PMID: 28982817. DOI: 10.1136/bmjopen-2017-016582

6 Huang CY, Chang WS, Tsai CW, Hsia TC, Shen TC, Bau DT and Shui HA: Interleukin-18 promoter genotype is associated with the risk of nasopharyngeal carcinoma in Taiwan. Cancer Manag Res *10*: 5199-5207, 2018. PMID: 30464617. DOI: 10.2147/CMAR.S179367

7 Tsai CW, Tsai MH, Shih LC, Chang WS, Lin CC and Bau DT: Association of interleukin-10 (IL10) promoter genotypes with nasopharyngeal carcinoma risk in Taiwan. Anticancer Res *33(8)*: 3391-3396, 2013. PMID: 23898109.

8 Tsai CW, Chang WS, Gong CL, Shih LC, Chen LY, Lin EY, Li HT, Yen ST, Wu CN and Bau DT: Contribution of matrix metallopeptidase-1 genotypes, smoking, alcohol drinking and areca chewing to nasopharyngeal carcinoma susceptibility. Anticancer Res *36(7)*: 3335-3340, 2016. PMID: 27354591.

9 Shih LC, Tsai CW, Tsai MH, Tsou YA, Chang WS, Li FJ, Lee MH and Bau DT: Association of cyclin D1 genotypes with nasopharyngeal carcinoma risk. Anticancer Res *32(3)*: 1093-1098, 2012. PMID: 22399638.

10 Huang CY, Tsai CW, Hsu CM, Shih LC, Chang WS, Shui HA and Bau DT: The role of XRCC6/Ku70 in nasopharyngeal carcinoma. Int J Oral Maxillofac Surg *44(12)*: 1480-1485, 2015. PMID: 26149939. DOI: 10.1016/j.ijom.2015.06.008

11 Chen M and Wang J: Initiator caspases in apoptosis signaling pathways. Apoptosis *7(4)*: 313-319, 2002. PMID: 12101390. DOI: 10.1023/a:1016167228059

12 Carneiro BA and El-Deiry WS: Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol *17(7)*: 395-417, 2020. PMID: 32203277. DOI: 10.1038/s41571-020-0341-y

13 Wang LH, Ting SC, Chen CH, Tsai CC, Lung O, Liu TC, Lee CW, Wang YY, Tsai CL and Lin YC: Polymorphisms in the apoptosis-associated genes FAS and FASL and risk of oral cancer and malignant potential of oral premalignant lesions in a Taiwanese population. J Oral Pathol Med *39(2)*: 155-161, 2010. PMID: 20359312. DOI: 10.1111/j.1600-0714.2009.00873.x

14 Kuwana T, Smith JJ, Muzio M, Dixit V, Newmeyer DD and Kornbluth S: Apoptosis induction by caspase-8 is amplified through the mitochondrial release of cytochrome c. J Biol Chem *273(26)*: 16589-16594, 1998. PMID: 9632731. DOI: 10.1074/jbc.273.26.16589

15 Yu J, Zhang L, Hwang PM, Kinzler KW and Vogelstein B: PUMA induces the rapid apoptosis of colorectal cancer cells. Mol Cell *7(3)*: 673-682, 2001. PMID: 11463391. DOI: 10.1016/s1097-2765(01)00213-1

16 Hashemi M, Eskandari-Nasab E, Fazaeli A, Rezaei H, Mashhadi MA, Arbabi F and Taheri M: Bi-directional PCR allele-specific amplification (bi-PASA) for detection of caspase-8 -652 6N ins/del promoter polymorphism (rs3834129) in breast cancer.

Gene *505(1)*: 176-179, 2012. PMID: 22659694. DOI: 10.1016/j.gene.2012.05.043

17 Rihani A, De Wilde B, Zeka F, Laureys G, Francotte N, Tonini GP, Coco S, Versteeg R, Noguera R, Schulte JH, Eggert A, Stallings RL, Speleman F, Vandesompele J and Van Maerken T: CASP8 SNP D302H (rs1045485) is associated with worse survival in MYCN-amplified neuroblastoma patients. PLoS One *9(12)*: e114696, 2014. PMID: 25502557. DOI: 10.1371/journal.pone.0114696

18 Kuhlmann JD, Bankfalvi A, Schmid KW, Callies R, Kimmig R, Wimberger P, Siffert W and Bachmann HS: Prognostic relevance of caspase 8 -652 6N InsDel and Asp302His polymorphisms for breast cancer. BMC Cancer *16*: 618, 2016. PMID: 27507139. DOI: 10.1186/s12885-016-2662-x

19 Park HL, Ziogas A, Chang J, Desai B, Bessonova L, Garner C, Lee E, Neuhausen SL, Wang SS, Ma H, Clague J, Reynolds P, Lacey JV, Jr., Bernstein L and Anton-Culver H: Novel polymorphisms in caspase-8 are associated with breast cancer risk in the California Teachers Study. BMC Cancer *16*: 14, 2016. PMID: 26758508. DOI: 10.1186/s12885-015-2036-9

20 Zhang Y, Li W, Hong Y, Wu G, He K and Liu D: A systematic analysis of the association studies between CASP8 D302H polymorphisms and breast cancer risk. J Genet *96(2)*: 283-289, 2017. PMID: 28674227. DOI: 10.1007/s12041-017-0774-y

21 Son JW, Kang HK, Chae MH, Choi JE, Park JM, Lee WK, Kim CH, Kim DS, Kam S, Kang YM and Park JY: Polymorphisms in the caspase-8 gene and the risk of lung cancer. Cancer Genet Cytogenet *169(2)*: 121-127, 2006. PMID: 16938569. DOI: 10.1016/j.cancergencyto.2006.04.001

22 Du H, Song GX, Fang MZ, Shu YQ, Zhao X and Zhu LJ: A meta-analysis of caspase-8 -652 6N del polymorphism and digestive tract cancer risk. J Biomed Res *33(3)*: 173-180, 2019. PMID: 30057371. DOI: 10.7555/JBR.32.20160030

23 Wu Z, Li Y, Li S, Zhu L, Li G, Yu Z, Zhao X, Ge J, Cui B, Dong X, Tian S, Hu F and Zhao Y: Association between main Caspase gene polymorphisms and the susceptibility and prognosis of colorectal cancer. Med Oncol *30(3)*: 565, 2013. PMID: 23715747. DOI: 10.1007/s12032-013-0565-0

24 Zhang CD, Li HT, Liu K, Lin ZD, Peng QL, Qin X, He M, Wu H, Mo ZN and Yang XL: Impact of caspase-8 (CASP8) -652 6N del and D302H polymorphisms on prostate cancer in different ethnic groups. Asian Pac J Cancer Prev *15(18)*: 7713-7718, 2014. PMID: 25292051. DOI: 10.7314/apjcp.2014.15.18.7713

25 Srivastava K, Srivastava A and Mittal B: Caspase-8 polymorphisms and risk of gallbladder cancer in a northern Indian population. Mol Carcinog *49(7)*: 684-692, 2010. PMID: 20564345. DOI: 10.1002/mc.20641

26 Shih LC, Chang WS, Lee HT, Wang YC, Wang ZH, Chao CY, Yu CC, Lin HY, Shen TC, Kuo CC, Tsai CW and Bau DT: Interaction of interleukin-16 genotypes with betel quid chewing behavior on oral cancer in Taiwan. In Vivo *34(4)*: 1759-1764, 2020. PMID: 32606144. DOI: 10.21873/invivo.11969

27 Li CH, Shih LC, Hsu CL, Lee HT, Wang YC, Chang WS, Tsai CW, Li CY and Bau DT: The contribution of interleukin-12A genotypes to oral cancer risk in Taiwanese. Anticancer Res *40(7)*: 3707-3712, 2020. PMID: 32620609. DOI: 10.21873/anticanres.14359

28 Jackson CE and Puck JM: Autoimmune lymphoproliferative syndrome, a disorder of apoptosis. Curr Opin Pediatr *11(6)*: 521-527, 1999. PMID: 10590910. DOI: 10.1097/00008480-199912000-00009

29 Reed JC: Mechanisms of apoptosis. Am J Pathol *157*: 1415-1430, 2000. PMID: 11073801. DOI: 10.1016/S0002-9440(10)64779-7

30 Kruidering M and Evan GI: Caspase-8 in apoptosis: the beginning of "the end"? IUBMB Life *50(2)*: 85-90, 2000. PMID: 11185963. DOI: 10.1080/713803693

31 Ghavami S, Hashemi M, Ande SR, Yeganeh B, Xiao W, Eshraghi M, Bus CJ, Kadkhoda K, Wiechec E, Halayko AJ and Los M: Apoptosis and cancer: mutations within caspase genes. J Med Genet *46(8)*: 497-510, 2009. PMID: 19505876. DOI: 10.1136/jmg.2009.066944

32 Li J and Yuan J: Caspases in apoptosis and beyond. Oncogene *27(48)*: 6194-6206, 2008. PMID: 18931687. DOI: 10.1038/onc.2008.297

33 Ashkenazi A: Targeting the extrinsic apoptotic pathway in cancer: lessons learned and future directions. J Clin Invest *125(2)*: 487-489, 2015. PMID: 25642709. DOI: 10.1172/JCI80420

34 Zhang F, Yang Y, Guo C and Wang Y: CASP8 -652 6N del polymorphism and cancer risk: a meta-analysis of 30 case-control studies in 50,112 subjects. Mutagenesis *27(5)*: 559-566, 2012. PMID: 22513478. DOI: 10.1093/mutage/ges019

35 Sun T, Gao Y, Tan W, Ma S, Shi Y, Yao J, Guo Y, Yang M, Zhang X, Zhang Q, Zeng C and Lin D: A six-nucleotide insertion-deletion polymorphism in the CASP8 promoter is associated with susceptibility to multiple cancers. Nat Genet *39(5)*: 605-613, 2007. PMID: 17450141. DOI: 10.1038/ng2030

36 Li C, Zhao H, Hu Z, Liu Z, Wang LE, Gershenwald JE, Prieto VG, Lee JE, Duvic M, Grimm EA and Wei Q: Genetic variants and haplotypes of the caspase-8 and caspase-10 genes contribute to susceptibility to cutaneous melanoma. Hum Mutat *29(12)*: 1443-1451, 2008. PMID: 18563783. DOI: 10.1002/humu.20803

37 de Martino M, Haitel A, Schatzl G, Klingler HC and Klatte T: The CASP8 -652 6N insertion/deletion promoter polymorphism is associated with renal cell carcinoma risk and metastasis. J Urol *190(2)*: 717-722, 2013. PMID: 23313206. DOI: 10.1016/j.juro.2013.01.008

38 Shih LC, Tsai CW, Sun KT, Hsu HM, Shen TC, Tsai YT, Chang WS, Lin ML, Wang YC, Gong CL and Bau DT: Association of caspase-8 genotypes with oral cancer risk in Taiwan. In Vivo *33(4)*: 1151-1156, 2019. PMID: 31280204. DOI: 10.21873/invivo.11585

39 Arunkumar G, Murugan AK, Nagarajan M, Ajay C, Rajaraman R and Munirajan AK: Absence of the frequently reported PIK3CA, CASP8, and NOTCH1 mutations in South Indian oral cancers. Oral Dis *23(5)*: 669-673, 2017. PMID: 28181739. DOI: 10.1111/odi.12655

Received July 27, 2020
Revised August 6, 2020
Accepted August 7, 2020