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The Contribution of DNA Ligase 4 Genetic Variations to Taiwanese Lung Cancer

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Abstract. *Background/Aim: Impaired non-homologous end-joining DNA repair capacity may have a significant role in maintaining genome integrity and triggering carcinogenesis. However, the specific impact of DNA ligase 4 (Lig4) genotypes remains unclear. This study aimed to assess the contribution of Lig4 genotypes to the risk of developing lung cancer. Materials and Methods: Polymerase chain reaction-restriction fragment length polymorphism analysis was used to examine the genotypes of Lig4 rs1805388, and their association with lung cancer risk was evaluated in a case-control study consisting of 358 lung cancer cases and 716 age- and sex-matched cancer-free control subjects. Results: The distribution of CC, CT, and TT genotypes for Lig4 rs1805388 among the cases was 45.0%, 41.6%, and 13.4%, respectively, compared to 58.0%, 36.3%, and 5.7% among the controls (p for trend=1.98×10^{-6}). Allelic analysis indicated that individuals carrying the T-allele for Lig4 rs1805388 had a 1.66-fold higher risk of developing lung cancer compared to those carrying the wild-type C-allele [95% confidence interval (CI)=1.36-2.02, p=4.04×10^{-7}]. Moreover, a significant interaction was observed between the Lig4 rs1805388 genotype and smoking status (p=1.32×10^{-7}). Conclusion: These findings suggest that the CT and TT variant genotypes of Lig4 rs1805388, combined with cigarette smoking, may contribute to a higher risk of developing lung cancer.*

Lung cancer is a prevalent cancer type worldwide, accounting for over 11% of all neoplasms and causing 18% of cancer-related deaths (1). In 2018, there were an estimated 2.1 million new cases and 1.8 million deaths related to lung cancer globally (2). The increasing number of cases and high mortality rates have prompted the search for reliable markers to predict lung cancer risk, prognosis, and drug treatment response. Multiple factors, including behavioral, environmental, and genetic factors, contribute to initiation and development of lung cancer. Among these factors, cigarette smoking is the most significant risk factor for lung cancer (3). However, the fact that 10% to 25% of lung cancer patients worldwide are non-smokers indicates that genetic influences also play a crucial role in personal lung cancer etiology (4). In Taiwan, lung cancer is ranked third in incidence and first in mortality among different types of cancer (5). Although several biomarkers have been examined for early lung cancer detection in recent years,

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there is still an urgent need to find clinically practical markers for early detection of lung cancer (6-10).

The human genome is frequently damaged by both endogenous and exogenous mutagens, and DNA repair systems are essential in preventing irreversible mutations that can cause carcinogenesis (11, 12). In the case of non-small cell lung cancer, patients over the age of 50 and those with adenocarcinoma exhibit significantly higher genomic instability compared to those with squamous-cell carcinoma. Additionally, genomic instability is negatively correlated with tumor grade (12). Double-strand breaks (DSBs) are a severe type of DNA damage resulting from both exogenous and endogenous factors such as ionizing radiation, free radicals, replication errors, and telomere dysfunction (13, 14). DSBs should be promptly repaired, as failure to do so can result in irreversible consequences.

The *Lig4* gene, located on the 13q33 chromosome, encodes a nuclear protein that is homologous to other ligases in the N-terminal region but not in the C-terminal region (15, 16). The importance of the Lig4 protein in maintaining genomic stability is underscored by the fact that mutations in this gene are associated with a rare autosomal syndrome (OMIM 606593), characterized by microcephaly, immunodeficiency, spontaneous genomic instability, and a higher susceptibility to various human diseases, including cancer (17-20). Cells from LIG4-deficient patients display increased radio-sensitivity and are defective in non-homologous end joining (NHEJ) repair capacity (18, 20, 21). In mice, knockout of *Lig4* resulted in late embryonic lethality with massive neuronal apoptosis and lymphocyte development arrest due to the lack of V(D)J recombination (22-24). Taken together, these findings emphasize the crucial role of Lig4 in maintaining normal DNA repair capacity and cellular viability.

The first report of an association between *Lig4* rs1805388 CT and TT genotypes and increased lung cancer risk among 152 non-small cell lung cancer patients in Taiwan was published in 2009 (25). Subsequently, in 2012, it was reported that these genotypes were associated with severe radiation pneumonitis in 195 non-small cell lung cancer patients in the USA (26). In 2015, Xu and his colleagues attempted to validate whether *Lig4* rs1805388 could serve as a practical marker for severe radiation pneumonitis in an Asian population of 160 non-small cell lung cancer patients but in vain (27). Although the association was not statistically significant, their findings, along with those of the previous studies, suggest that *Lig4* rs1805388 genotypes may be critical in determining phenotypes and may be of clinical significance in lung cancer therapy. However, the latter two reports (Yin's and Xu's) do not provide evidence for *Lig4* rs1805388 genotypes as predictive diagnostic markers. Moreover, the investigated populations in all three studies were not large enough and require further validation. Therefore, in this study, we aim to conduct a hospital-based case-control study in a representative population (control:case=716:358), validate the contribution of *Lig4* rs1805388, examine the interaction of *Lig4* genotypes with smoking status on lung cancer risk.

Materials and Methods

Lung cancer case and age- and sex-matched control population. A total of 358 patients with histologically confirmed lung cancer were recruited from the China Medical University Hospital, as described previously (28, 29). Exclusion criteria included a history of malignancy or other pulmonary diseases such as chronic obstructive pulmonary disease (COPD), pneumothorax, and asthma. Two healthy volunteers were selected as controls for each lung cancer patient, matched for age (within 5 years), sex, and smoking behavior to minimize the influence of smoking. Controls were selected from the Health Examination Cohort database of the China Medical University Hospital, which contained over 15,000 individuals. Exclusion criteria for controls included a history of malignancy or metastasized cancer, and any genetic or familial diseases. Both cases and controls were Taiwanese, and Table I summarizes several population characteristics.

Genotyping conditions for Lig4 rs1805388. Genomic DNA was extracted from the peripheral blood leukocytes of each participant within 24 h of blood collection using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC), as previously described (30). The extracted DNA was quantified, stored long-term at -80˚C, and aliquoted for genotyping as a working stock at -20˚C. The genotyping methodology for *Lig4* rs1805388 was designed by the Terry Fox Cancer Research Lab. The forward and reverse primer sequences were 5'-TCTGTATTCGTTCTAAAGTT-3' and 5'-TGCTTTACTAGTTAAA CGAG-3', respectively. Polymerase chain reaction (PCR) cycling conditions were as follows: one cycle at 94˚C for 5 min, 35 cycles of 94˚C for 30 s, 52˚C for 30 s, and 72˚C for 30 s and a final extension step at 72˚C for 10 min. After PCR amplification, the products were digested with the *HpyCH4* III restriction endonuclease and separated via 3% agarose gel electrophoresis for 25 min. The *Lig4* rs1805388 genotypes were identified as wild-type C/C with a 121-bp product, heterozygous variant C/T with 121-, 65-, and 56-bp products, and homozygous variant T/T with 65- and 56-bp products. Genotyping was repeated independently by two researchers in a blind manner, and all genotyping results were 100% successful and concordant.

Statistical analysis. The distribution of ages between the case and control groups was compared using the Student's *t*-test. The goodness-of-fit chi-square test was utilized to assess the Hardy-Weinberg equilibrium among the different *Lig4* rs1805388 genotypes in 716 non-cancer controls. Pearson's Chi-square methodology was used to compare the distributions of *Lig4* rs1805388 genotypes among subgroups, as well as to perform stratification analysis for the interaction between *Lig4* rs1805388 genotypes and smoking status. Logistic regression analysis was used to estimate the odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) to determine the associations between *Lig4* rs1805388 genotypes and lung cancer risk. A *p*-value less than 0.05 was considered significant in any comparison.

Results

Table I presents the frequency distributions of age, sex, and smoking status for 358 lung cancer patients and their 716 matched non-cancer healthy controls. Additionally, the

Table I. *Distribution of demographic data of 358 lung cancer patients and 716 matched non-cancer. controls.*

Characteristics	Controls (n=716)			Patients (n=358)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.8 (6.8)			64.0 (6.9)	0.5871
Sex							
Male	488	68.1%		254	70.9%		
Female	228	31.9%		104	29.1%		0.3642
Smoking status							
Ever smokers	563	78.6%		293	81.8%		
Non-smokers	153	21.4%		65	18.2%		0.2282
Histology							
Adenocarcinoma				218	60.9%		
SCC				106	29.6%		
Other				34	9.5%		

[a]Based on Chi-square test with Yates' correction. SCC, Squamous cell carcinoma; SD, standard deviation.

Table II. *Distribution of Lig4 rs1805388 genotypes among the 358 lung cancer and non-cancer 716 controls.*

Genotypes	Controls, N	%	Patients, N	%	OR (95% CI)	*p*-Value
Lig4 rs1805388						
CC	415	58.0	161	45.0	1.00 (Reference)	
CT	260	36.3	149	41.6	1.48 (1.13-1.94)	**0.0059**
TT	41	5.7	48	13.4	3.02 (1.91-4.76)	**0.0001**
P_{trend}						**1.98×10^{-6}**
P_{HWE}						0.9737
Carrier comparison						
CC+CT	675	94.3	310	86.6	1.00 (Reference)	
TT	41	5.7	48	13.4	2.55 (1.65-3.95)	**0.0001**
CC	415	58.0	161	45.0	1.00 (Reference)	
CT+TT	301	42.0	197	55.0	1.69 (1.31-2.18)	**0.0001**

N: Number; OR: odds ratio; CI: confidence interval; P_{HWE}: whether the frequencies in controls are consistent with Hardy-Weinberg Equilibrium. Statistically identified as significant *p*-values based on Chi-square test with Yates' correction are shown in bold.

Table III. *Distribution of Lig4 rs1805388 allelic frequencies among the 358 lung cancer and the non-cancer 716 controls.*

Allele	Controls, N	%	Patients, N	%	OR (95% CI)	*p*-Value[a]
Lig4 rs1805388						
C	1090	76.1%	471	65.8%	1.00 (Reference)	
T	342	23.9%	245	34.2%	1.66 (1.36-2.02)	**4.04×10^{-7}**

N: Number; OR: odds ratio; CI: Confidence interval; [a]Based on Chi-square with Yate's correction test; significant *p*-values are bolded.

histology of lung cancer patients is shown. Frequency matching was performed on age, sex, and smoking status to recruit the healthy controls, and the data demonstrated no significant differences in the distribution of age, sex, or smoking behavior between the two groups (all $p>0.05$). It is important to note that the high percentage of smokers (78.6%) in the control group is due to the frequency matching strategy and may not represent the entire Taiwanese population. Adenocarcinoma was the most common histological type, accounting for 60.9% (218/358) of lung cancer cases, followed by squamous cell carcinoma (29.6%, 106/358) and other types (9.5%, 34/358).

Table II displays the genotypic distributions of *Lig4* rs1805388 among the 716 non-cancer controls and the 358

Table IV. *Distribution of Lig4 rs1805388 genotypes among 358 lung cancer and 716 controls after stratification by smoking status.*

Genotype	Non-smokers, N		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value	Smokers, N		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value
	Controls	Cases				Controls	Cases			
CC	78	32	1.00 (ref)	1.00 (ref)		337	129	1.00 (ref)	1.00 (ref)	
CT	62	27	1.09 (0.44-2.21)	1.06 (0.58-1.96)	0.9718	198	122	1.58 (1.12-2.26)	1.61 (1.19-2.18)	**0.0026**
TT	13	6	1.18 (0.32-2.96)	1.13 (0.39-3.22)	0.8261	28	42	3.79 (2.21-5.84)	3.92 (2.33-6.59)	**0.0001**
Total	153	65				563	293			
p_{trend}					0.9669					**1.32×10^{-7}**

N: Number; OR: odds ratio; CI: Confidence interval; [a]Based on Chi-square with Yate's correction test; [b]Based on Chi-square with Yate's correction test after adjustment of age and sex; significant *p*-values are bolded.

lung cancer patients. The genotypic frequencies of *Lig4* rs1805388 among control subjects were found to be in Hardy-Weinberg equilibrium (p=0.9737, Table II). Furthermore, the genotypic frequencies of *Lig4* rs1805388 were observed to be differently distributed between the lung cancer and healthy control groups (p for trend=1.98E-6, Table II). Specifically, both the heterozygous CT and homozygous variant TT genotypes of *Lig4* rs1805388 were associated with increased lung cancer risks (OR=1.48 and 3.02, 95%CI=1.13-1.94 and 1.91-4.76, p=0.0059 and 0.0001, respectively; Table II). The recessive model showed a significant 2.55-fold increase in lung cancer risk for carriers of the *Lig4* rs1805388 TT genotype compared to those with CC+CT genotypes (95%CI=1.65-3.95, p=0.0001, Table II). In the dominant model, there was a significant 1.69-fold increase in lung cancer risk for carriers of the CT+TT genotypes of *Lig4* rs1805388 compared to those carrying CC genotypes (95%CI=1.31-2.18, p=0.0001, Table II).

To validate the findings in Table II, an analysis of allelic frequency distribution for *Lig4* rs1805388 was performed, and the results are presented in Table III. The results further support the notion that the *Lig4* rs1805388 genotype is associated with lung cancer risk, as the proportion of the risk T allele was found to be 34.2% among the lung cancer cases, which is significantly higher than the 23.9% observed among the cancer-free controls (OR=1.66, 95%CI=1.36-2.02, $p=4.04\times10^{-7}$).

We further performed a stratification analysis to investigate the interaction between the inherited *Lig4* rs1805388 genotype and personal cigarette smoking habits, as cigarette smoking is a well-known risk factor for lung cancer. Interestingly, no significant interaction was found among non-smokers (p>0.05) (Table IV). However, among smokers, those with *Lig4* rs1805388 CT and TT genotypes had 1.61- and 3.92-fold higher odds of having lung cancer, respectively (95%CI=1.19-2.18 and 2.33-6.59, p=0.0026 and 0.0001, respectively). The statistical significance persisted at a similar level after adjusting for age, sex, and alcohol drinking status (OR=1.58 and 3.79, 95%CI=1.12-2.26 and 2.21-5.84, respectively, Table IV).

Discussion

The DNA repair system is a crucial mechanism that evolved to maintain genomic stability and prevent cells from mutating into tumors. The primary pathways of the DNA repair system include base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), and double-strand break repair (DSBR). Inter-individual variations in DNA repair capacity may contribute to differences in susceptibility to various types of cancer (31). Among these pathways, Lig4 plays a pivotal role in the joining of DSBs through the NHEJ pathway. Existing literature has demonstrated that LIG4 interacts with XRCC4, which is critical to stabilize LIG4 protein in cells (32, 33). Cells deficient in XRCC4, such as XR-1 cells, exhibit reduced levels of LIG4 (34). Although the interaction between XRCC4 and DNA Lig4 remains unresolved, subtle alterations in DSB repair genes may contribute to the development and progression of all types of cancer, including lung cancer.

In recent years, several studies have reported associations between SNPs in genes involved in the DSB repair pathway and lung cancer, including Nijmegen breakage syndrome 1 (*NBS1*), X-ray repair cross-complementing group 3 (*XRCC3*), and ataxia telangiectasia mutated (*ATM*) (35-37). However, studies on the association between NHEJ pathway gene SNPs and lung cancer risk are limited and not well characterized. Interestingly, studies have shown that genotypes of *XRCC4*, *XRCC6*, and *XRCC7* are associated with lung cancer risk (38-40).

In this study, we investigated the genotypes of *Lig4* rs1805388 in Taiwan and assessed the reliability of our sampling and genotyping data. We compared the percentages of *Lig4* rs1805388 genotypic distribution in our cancer-free control group with those on the National Center for Biotechnology Information (NCBI) website for East Asian populations. Towards this aim, the percentages of *Lig4* rs1805388 genotypic distribution among the cancer-free controls were compared to those on National Center for

Biotechnology Information (NCBI) websites (41). According to the 1,170 and 3,128 subjects of East Asians genotyped and analyzed, the minor allelic frequencies at *Lig4* rs1805388 are 0.2205 and 0.2430, respectively (updated on 2023/05/07). In our data, the variant T allele has the frequency of 0.2390 and can represent the Taiwan population which located in East Asia. We then examined the association between the *Lig4* rs1805388 genotypes and lung cancer risk in a genetically homogeneous population in Taiwan consisting of 716 healthy subjects and 358 lung cancer cases. Our findings revealed that the CT and TT genotypes of *Lig4* rs1805388 were associated with an increased risk of lung cancer (Table II and Table III), and this genetic factor had a joint effect with cigarette smoking on determining lung cancer susceptibility (Table IV). Cigarette smoke contains compounds that induce DNA DSBs and promote lung carcinogenesis (42, 43). *Lig4* rs1805388 genotypes may influence protein function, resulting in differential capacity to repair DSB damage in lung cells. It has been reported in the literature that the T allele at *Lig4* rs1805388 is associated with reduced adenylation and ligation activities of the enzyme (44), which may result in a lower capacity for DSB removal and a higher risk of lung cancer. Further investigations are required to validate the genotype-phenotype correlation.

In the scientific literature, other *Lig4* polymorphisms have been investigated for their potential associations with various human diseases. For example, *Lig4* rs1805386 genotypes were initially linked to ovarian cancer risk (45), However, subsequent replication studies failed to confirm this association (46). Additionally, the genotypes of *Lig4* rs1805386 were not associated with either breast cancer risk (46, 47), or lung cancer risk (48), except for a borderline significant association with a decreased risk of breast cancer reported by Kuschel and colleagues (p=0.04) (49). Another *Lig4* polymorphic site, rs2232641, was not associated with breast cancer risk (50), but was linked to a reduced risk of developing lung cancer in Japanese individuals (p=0.03) (51).

There are several limitations of this study that should be acknowledged. First, the lack of recorded follow-up data restricted the analysis of the correlation between *Lig4* rs1805388 genotypes and prognosis indices, such as survival time. Second, the use of tumor and non-tumor samples restricted the investigation of differential expression of Lig4 mRNA and protein levels in lung cancer patients. Third, the relatively small sample size, particularly for subgroup analyses such as those shown in Table IV (especially the 6 TT carriers in non-smoker case group), may have caused in some sampling bias and reduced statistical power for further annotations. The findings of subgroup stratification should be further validated with larger samples in the future.

In summary, the present study demonstrated a correlation between the T allele at *Lig4* rs1805388 and increased lung cancer susceptibility, particularly in the context of smoking. Future research should investigate the impact of *Lig4* genotypes on mRNA and protein expression levels, as well as NHEJ capacity, to better understand the underlying mechanisms driving this association.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Research design: Chang WS, Bau DT and Hsia TC; Patient and questionnaire summary: Lee YH, Wang SC, Li CH, Chen LH, Shen TC, Liu YF, and Hsia TC; Experiment data clearing and checking: Lee YH, Wang YC and Chang WS; Statistical analysis: Chen LH, Wang SC and Chen LH; Literature review: Lee YH, Wang YC, and Tsai CW; Manuscript writing: Lee YH, Tsai CW, Hsia TC, Chang WS and Bau DT; Review and revision: Bau DT, Chang WS and Hsia TC.

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References

1 Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3): 209-249, 2021. DOI: 10.3322/caac.21660

2 Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6): 394-424, 2018. DOI: 10.3322/caac.21492

3 Malhotra J, Malvezzi M, Negri E, La Vecchia C, Boffetta P: Risk factors for lung cancer worldwide. Eur Respir J 48(3): 889-902, 2016. DOI: 10.1183/13993003.00359-2016

4 Rivera G, Wakelee H: Lung cancer in never smokers. Adv Exp Med Biol. 893: 43-57, 2016. DOI: 10.1007/978-3-319-24223-1_3

5 Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence: Cancer Registration Annual Report. Available at: https://www.hpa.gov.tw/Pages/List.aspx?nodeid=269 [Last accessed on June 19, 2023]

6 Wu M, Wang Y, Shen T, Chang W, Li H, Liao C, Gong C, Wang Z, Tsai C, Hsia T, Bau D: Significant association of interleukin-16 genetic variations to Taiwanese lung cancer. In Vivo 34(3): 1117-1123, 2020. DOI: 10.21873/invivo.11883

7 Bau D, Chen G, Wang S, Shen T, Tsai C, Chang W, Li H, Wu C, Chao C, Hsia T: The association of matrix metalloproteinas-2 promoter polymorphisms with lung cancer susceptibility in Taiwan. Chin J Physiol 62(5): 210, 2019. DOI: 10.4103/CJP.CJP_43_19

8 Chen G, Wang S, Huang W, Chang W, Tsai C, Li H, Shen T, Hsia T, Bau D: The association of *MMP-11* promoter polymorphisms with susceptibility to lung cancer in Taiwan. Anticancer Res 39(10): 5375-5380, 2019. DOI: 10.21873/anticanres.13731

9 Shen TC, Chang WS, Hsia TC, Li HT, Chen WC, Tsai CW, Bau DT: Contribution of programmed cell death 6 genetic variations, gender, and smoking status to lung cancer. Onco Targets Ther 12: 6237-6244, 2019. DOI: 10.2147/OTT.S205544

10 Wu M, Wang Y, Li H, Chen W, Liao C, Shih T, Chang W, Tsai C, Hsia T, Bau D: The contribution of interleukin-12 genetic variations to Taiwanese lung cancer. Anticancer Res 38(11): 6321-6327, 2018. DOI: 10.21873/anticanres.12989

11 Markovic J, Stojsic J, Zunic S, Ruzdijic S, Tanic N: Genomic instability in patients with non-small cell lung cancer assessed by the arbitrarily primed polymerase chain reaction. Cancer Invest 26(3): 262-268, 2008. DOI: 10.1080/07357900701708385

12 Champeris Tsaniras S, Villiou M, Giannou A, Nikou S, Petropoulos M, Pateras I, Tserou P, Karousi F, Lalioti M, Gorgoulis V, Patmanidi A, Stathopoulos G, Bravou V, Lygerou Z, Taraviras S: Geminin ablation *in vivo* enhances tumorigenesis through increased genomic instability. J Pathol 246(2): 134-140, 2018. DOI: 10.1002/path.5128

13 Richardson C, Jasin M: Frequent chromosomal translocations induced by DNA double-strand breaks. Nature 405(6787): 697-700, 2000. DOI: 10.1038/35015097

14 Khanna K, Jackson S: DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet 27(3): 247-254, 2001. DOI: 10.1038/85798

15 Timson D, Singleton M, Wigley D: DNA ligases in the repair and replication of DNA. Mutat Res 460(3-4): 301-318, 2000. DOI: 10.1016/s0921-8777(00)00033-1

16 Tomkinson A, Mackey Z: Structure and function of mammalian DNA ligases. Mutat Res 407(1): 1-9, 1998. DOI: 10.1016/s0921-8777(97)00050-5

17 Bassing C, Alt F: The cellular response to general and programmed DNA double strand breaks. DNA Repair 3(8-9): 781-796, 2004. DOI: 10.1016/j.dnarep.2004.06.001

18 Pollard J, Gatti R: Clinical radiation sensitivity with DNA repair disorders: an overview. Int J Radiat Oncol Biol Phys 74(5): 1323-1331, 2009. DOI: 10.1016/j.ijrobp.2009.02.057

19 Chistiakov D, Voronova N, Chistiakov P: Genetic variations in DNA repair genes, radiosensitivity to cancer and susceptibility to acute tissue reactions in radiotherapy-treated cancer patients. Acta Oncol 47(5): 809-824, 2008. DOI: 10.1080/02841860801885969

20 O'Driscoll M, Jeggo P: The role of double-strand break repair - insights from human genetics. Nat Rev Genet 7(1): 45-54, 2006. DOI: 10.1038/nrg1746

21 Schwarz K, Ma Y, Pannicke U, Lieber M: Human severe combined immune deficiency and DNA repair. BioEssays 25(11): 1061-1070, 2003. DOI: 10.1002/bies.10344

22 Grawunder U, Zimmer D, Fugmann S, Schwarz K, Lieber M: DNA ligase IV is essential for V(D)J recombination and DNA double-strand break repair in human precursor lymphocytes. Mol Cell 2(4): 477-484, 1998. DOI: 10.1016/s1097-2765(00)80147-1

23 Riballo E, Critchlow S, Teo S, Doherty A, Priestley A, Broughton B, Kysela B, Beamish H, Plowman N, Arlett C, Lehmann A, Jackson S, Jeggo P: Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. Cur Biol 9(13): 699-S2, 1999. DOI: 10.1016/s0960-9822(99)80311-x

24 Assis J: Ovarian cancer and DNA repair: DNA ligase IV as a potential key. World J Clin Oncol 4(1): 14, 2013. DOI: 10.5306/wjco.v4.i1.14

25 Tseng R, Hsieh F, Shih C, Hsu H, Chen C, Wang Y: Lung cancer susceptibility and prognosis associated with polymorphisms in the nonhomologous end-joining pathway genes. Cancer 115(13): 2939-2948, 2009. DOI: 10.1002/cncr.24327

26 Yin M, Liao Z, Liu Z, Wang L, O'Reilly M, Gomez D, Li M, Komaki R, Wei Q: Genetic variants of the nonhomologous end joining gene*LIG4*and severe radiation pneumonitis in nonsmall cell lung cancer patients treated with definitive radiotherapy. Cancer 118(2): 528-535, 2012. DOI: 10.1002/cncr.26214

27 Xu F, Han J, Zhang Y, Zhang Y, Liu X, Qi G, Liu D, Chen Y, Zhao Y, Bai L: Associations of *LIG4* and *HSPB1* genetic polymorphisms with risk of radiation-induced lung injury in lung cancer patients treated with radiotherapy. Biomed Res Int 2015: 1-6, 2015. DOI: 10.1155/2015/860373

28 Wu M, Chen L, Hsia N, Shen Y, Shen T, Wang Z, Yang Y, Wang Y, Chang W, Hsia T, Bau D, Tsai C: Significant contribution of interleukin-18 genotypes to lung cancer risk in Taiwanese. Anticancer Res 42(7): 3381-3387, 2022. DOI: 10.21873/anticanres.15825

29 Li C, Yang Y, Hsia T, Shen T, Shen Y, Chang W, Wang Y, Tsai C, Bau D: Association of *Interleukin-8* promoter genotypes with Taiwan lung cancer risk. Anticancer Res 42(3): 1229-1236, 2022. DOI: 10.21873/anticanres.15590

30 Yang M, Lin K, Lu M, Jeng L, Hsiao C, Yueh T, Fu C, Li H, Yen S, Lin C, Wu C, Pang S, Bau D, Tsai F: Contribution of matrix metalloproteinases-1 genotypes to gastric cancer susceptibility in Taiwan. BioMedicine 7(2): 10, 2017. DOI: 10.1051/bmdcn/2017070203

31 Roos W, Thomas A, Kaina B: DNA damage and the balance between survival and death in cancer biology. Nat Rev Cancer 16(1): 20-33, 2016. DOI: 10.1038/nrc.2015.2

32 Sibanda BL, Critchlow SE, Begun J, Pei XY, Jackson SP, Blundell TL, Pellegrini L: Crystal structure of an Xrcc4-DNA ligase IV complex. Nat Struct Biol 8(12): 1015-1019, 2001. DOI: 10.1038/nsb725

33 Deshpande R, Wilson T: Modes of interaction among yeast Nej1, Lif1 and Dnl4 proteins and comparison to human XLF, XRCC4 and Lig4. DNA Repair 6(10): 1507-1516, 2007. DOI: 10.1016/j.dnarep.2007.04.014

34 Bryans M, Valenzano M, Stamato T: Absence of DNA ligase IV protein in XR-1 cells: evidence for stabilization by XRCC4. Mutat Res 433(1): 53-58, 1999. DOI: 10.1016/s0921-8777(98)00063-9

35 Chuang C, Wang C, Hsu C, Hsiao C, Chen G, Yen S, Li H, Chang W, Tsai C, Wang S, Bau D: Contribution of double-strand break repair gene Nijmegen breakage syndrome 1 genotypes, gender difference and smoking status to Taiwanese lung cancer. Anticancer Res 37(5): 2417-2423, 2017. DOI: 10.21873/anticanres.11581

36 Chen HJ, Chang WS, Hsia TC, Miao CE, Chen WC, Liang SJ, Chen AC, Chang JG, Tsai CW, Hsu CM, Tsai CH, Bau DT: Contribution of genotype of DNA double-strand break repair gene XRCC3, gender, and smoking behavior to lung cancer risk in Taiwan. Anticancer Res 35(7): 3893-3899, 2015.

37 Hsia TC, Tsai CW, Liang SJ, Chang WS, Lin LY, Chen WC, Tu CY, Tsai CH, Bau DT: Effects of ataxia telangiectasia mutated (ATM) genotypes and smoking habits on lung cancer risk in Taiwan. Anticancer Res 33(9): 4067-4071, 2013.

38 Hsu N, Wang H, Wang C, Chang C, Chiu C, Lee H, Tsai C, Bau D: Lung cancer susceptibility and genetic polymorphism of DNA repair gene XRCC4 in Taiwan. Cancer Biomark 5(4-5): 159-165, 2009. DOI: 10.3233/CBM-2009-0617

39 Hsia TC, Liu CJ, Chu CC, Hang LW, Chang WS, Tsai CW, Wu CI, Lien CS, Liao WL, Ho CY, Bau DT: Association of DNA double-strand break gene XRCC6 genotypes and lung cancer in Taiwan. Anticancer Res 32(3): 1015-1020, 2012.

40 Hsia TC, Chang WS, Chen WC, Liang SJ, Tu CY, Chen HJ, Liang JA, Tsai CW, Hsu CM, Tsai CH, Bau DT: Genotype of DNA double-strand break repair gene XRCC7 is associated with lung cancer risk in Taiwan males and smokers. Anticancer Res 34(12): 7001-7005, 2014.

41 rs1805388. Available at https://www.ncbi.nlm.nih.gov/snp/rs1805388 [Last accessed on June 19, 2023]

42 Hang B, Wang Y, Huang Y, Wang P, Langley S, Bi L, Sarker A, Schick S, Havel C, Jacob P, Benowitz N, Destaillats H, Tang X, Xia Y, Jen K, Gundel L, Mao J, Snijders A: Short-term early exposure to thirdhand cigarette smoke increases lung cancer incidence in mice. Clin Sci 132(4): 475-488, 2018. DOI: 10.1042/CS20171521

43 Toyooka T, Ibuki Y: Cigarette sidestream smoke induces phosphorylated histone H2AX. Mutat Res 676(1-2): 34-40, 2009. DOI: 10.1016/j.mrgentox.2009.03.002

44 Girard P, Kysela B, Härer C, Doherty A, Jeggo P: Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. Hum Mol Gen 13(20): 2369-2376, 2004. DOI: 10.1093/hmg/ddh274

45 Pearce C, Australian Cancer Study (Ovarian Cancer), Near A, Van den Berg D, Ramus S, Gentry-Maharaj A, Menon U, Gayther S, Anderson A, Edlund C, Wu A, Chen X, Beesley J, Webb P, Holt S, Chen C, Doherty J, Rossing M, Whittemore A, McGuire V, DiCioccio R, Goodman M, Lurie G, Carney M, Wilkens L, Ness R, Moysich K, Edwards R, Jennison E, Kjaer S, Hogdall E, Hogdall C, Goode E, Sellers T, Vierkant R, Cunningham J, Schildkraut J, Berchuck A, Moorman P, Iversen E, Cramer D, Terry K, Vitonis A, Titus-Ernstoff L, Song H, Pharoah P, Spurdle A, Anton-Culver H, Ziogas A, Brewster W, Galitovskiy V, Chenevix-Trench G, Australian Ovarian Cancer Study Group, on behalf of the Ovarian Cancer Association Consortium: Validating genetic risk associations for ovarian cancer through the international Ovarian Cancer Association Consortium. Br J Cancer 100(2): 412-420, 2009. DOI: 10.1038/sj.bjc.6604820

46 Jakubowska A, Gronwald J, Menkiszak J, Górski B, Huzarski T, Byrski T, Tołoczko-Grabarek A, Gilbert M, Edler L, Zapatka M, Eils R, Lubiński J, Scott R, Hamann U: BRCA1-associated breast and ovarian cancer risks in Poland: no association with commonly studied polymorphisms. Breast Cancer Res Treat 119(1): 201-211, 2010. DOI: 10.1007/s10549-009-0390-5

47 Han J: Polymorphisms in DNA double-strand break repair genes and breast cancer risk in the Nurses' Health Study. Carcinogenesis 25(2): 189-195, 2003. DOI: 10.1093/carcin/bgh002

48 De las Peñas R, Sanchez-Ronco M, Alberola V, Taron M, Camps C, Garcia-Carbonero R, Massuti B, Queralt C, Botia M, Garcia-Gomez R, Isla D, Cobo M, Santarpia M, Cecere F, Mendez P, Sanchez J, Rosell R: Polymorphisms in DNA repair genes modulate survival in cisplatin/gemcitabine-treated non-small-cell lung cancer patients. Ann Oncol 17(4): 668-675, 2006. DOI: 10.1093/annonc/mdj135

49 Kuschel B: Variants in DNA double-strand break repair genes and breast cancer susceptibility. Hum Mol Genet 11(12): 1399-1407, 2002. DOI: 10.1093/hmg/11.12.1399

50 Sobczuk A, Smolarz B, Romanowicz H, Zadrozny M, Baszczynski J, Westfal B, Pertynski T: Analysis of the polymorphisms in non-homologous DNA end joining (NHEJ) gene Ku70 and Ligase IV in sporadic breast cancer in women. Pol J Pathol 61(1): 27-31, 2010.

51 Sakiyama T, Kohno T, Mimaki S, Ohta T, Yanagitani N, Sobue T, Kunitoh H, Saito R, Shimizu K, Hirama C, Kimura J, Maeno G, Hirose H, Eguchi T, Saito D, Ohki M, Yokota J: Association of amino acid substitution polymorphisms in DNA repair genes TP53, POLI, REV1 and LIG4 with lung cancer risk. Int J Cancer 114(5): 730-737, 2005. DOI: 10.1002/ijc.20790

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