in vivo *38*: 127-133 (2024)
doi:10.21873/invivo.13419

The Contribution of DNA Ligase 4 Polymorphisms to Colorectal Cancer

YANG DENG[1,2,3], TAO-WEI KE[4,5], TE-CHENG YUEH[5,6,7], YU-TING CHIN[5,6], YUN-CHI WANG[5,6], YI-CHIH HUNG[5,6], MEI-CHIN MONG[8], YA-CHEN YANG[8], WEN-TZU WU[8], WEN-SHIN CHANG[3,5,6], JIAN GU[3*], DA-TIAN BAU[5,6,9*] and CHIA-WEN TSAI[3,5,6*]

[1]*Department of General Surgery, Wuxi Branch of Shanghai Ruijin Hospital, Wuxi, P.R. China;*
[2]*Department of General Surgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, P.R. China;*
[3]*Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX, U.S.A.;*
[4]*Department of General Surgery, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[5]*Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[6]*Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, R.O.C.;*
[7]*National Defense Medical Center, Taipei, Taiwan, R.O.C.;*
[8]*Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan, R.O.C.;*
[9]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: While numerous biomarkers associated with genetic susceptibility to colorectal cancer (CRC) have been identified and validated through epidemiological studies, the specific influence of DNA ligase 4 (Lig4) genotypes remains unexplored. This study aimed to elucidate the hitherto unexamined relationship between Lig4 genotypes and CRC risk. Materials and Methods: The genotypes of Lig4 rs1805388 were determined applying the polymerase chain reaction-restriction fragment length polymorphism methodology. The potential association between these genotypes and CRC risk was assessed in a Taiwanese population comprising 362 CRC cases and an equal number of age- and sex-matched controls. Results: In the genotypic analysis, the distribution of CC, CT, and TT genotypes for Lig4 rs1805388 among CRC cases was 54.7%, 38.1%, and 7.2%, respectively. This distribution was not significantly different from the controls, which exhibited genotypic frequencies of 57.2%, 36.7%, and 6.1%, respectively (p for trend=0.7314). Analysis of allelic distribution indicated that individuals carrying the T allele of Lig4 rs1805388 displayed a slightly elevated CRC risk compared to those carrying the C allele (odds ratio=1.10, 95% confidence interval=0.87-1.39, p=0.4685). Conclusion: The variant genotypes of Lig4 rs1805388 may not serve as predictive markers for CRC risk in the Taiwanese population.*

*These Authors contributed equally to this study.

Correspondence to: Jian Gu, Da-Tian Bau, and Chia-Wen Tsai, 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: jiangu@mdanderson.org (Gu J); artbau2@gmail.com (Bau DT); wenwen816@gmail.com (Tsai CW)

Key Words: Colorectal cancer, DNA ligase 4, genotypes, non-homologous end-joining, single nucleotide polymorphism.

Colorectal cancer (CRC) constitutes approximately 11% of all newly diagnosed cancer cases and stands as the third most prevalent cancer worldwide, leading to the second highest number of cancer-related fatalities (1). The pathogenesis of CRC encompasses a myriad of factors that contribute to intricate genetic and epigenetic mechanisms, culminating in the conversion of normal colonic mucosa into malignant tissues (2). Deficient DNA repair capacity is closely linked with genomic instability and heightened susceptibility to cancer (3-5). Furthermore, emerging evidence underscores the strong correlation between genetic variations in the DNA damage responses, microsatellite instability status and mitochondrial copy numbers and other pathways, serving as crucial determinants for CRC patients (6-11).

The human genome is incessantly subjected to attacks from both endogenous and exogenous DNA damaging agents. Rapid repair of DNA adducts is imperative to avert genomic instability and prevent the emergence of malignant changes. To achieve this, the DNA repair machinery promptly initiates corrective actions upon lesion detection, aiming to rectify altered bases or any other anomalies. In the literature, several well-defined DNA repair pathways exist, encompassing direct reversal, excision repair [including base excision repair (BER) and nucleotide excision repair (NER)], mismatch repair (MMR), and double-strand break (DSB) repair pathways [including homologous recombination repair (HR) and non-homologous end joining (NHEJ)] (12, 13). Among these pathways, DSBs represent the most severe form of DNA damage, stemming from both endogenous and exogenous factors, such as replication errors, ionizing radiation exposure, free radicals, and telomere dysfunction (14, 15). Timely repair of DSBs is crucial, as failure to do so in cells can lead to severe human disorders, including various types of cancer.

The *Lig4* gene, located on chromosome 13q33, encodes a nuclear protein which plays a role in NHEJ (16, 17). Clinically, the significance of the Lig4 protein in maintaining genomic stability is underscored by the fact that deficiency in this gene is responsible for Lig4 deficiency syndrome (OMIM 606593 or ORPHA99812), characterized by severe disorders including combined immunodeficiency, bird-head-like facial features, bony deformations, skin conditions, and susceptibility to malignancy (18). Cells from Lig4-deficient patients display increased radiosensitivity and exhibit defective NHEJ repair capacity (19-21). Recently, this syndrome has been effectively corrected through allogeneic hematopoietic stem cell transplantation (22). In a mouse model, Lig4 deficiency leads to late embryonic lethality with numerous neuronal cells undergoing apoptosis, along with developmental arrest of lymphocytes (23, 24). Collectively, this evidence underscores the critical role of Lig4 in maintaining the integrity of the human genome. Furthermore, subtle genomic variations in Lig4 may be associated with an increased risk of human disorders.

In comparison to other members of the NHEJ sub-pathway, studies focusing on *Lig4* genotyping have been relatively rare. Among the various cancer types studied, breast cancer has received the most frequent attention (25-29). Within the realm of polymorphic sites, *Lig4* rs1805388 has emerged as the most investigated variant, involving a C to T shift. This polymorphic site has also undergone scrutiny for its potential associations with glioma (30-33), prostate cancer (34, 35), lung cancer (36-39), nasopharyngeal carcinoma (3). However, its potential role in CRC has yet to be explored. Thus, we are motivated to initiate a preliminary hospital-based case-control study aimed at unraveling the potential contribution of *Lig4* rs1805388 to CRC.

Materials and Methods

Recruitment of colorectal cancer cases and matched controls. The selection of CRC cases and healthy controls adhered to the methodology delineated in our previous publications (40, 41). In brief, CRC cases were sourced from patients seeking care at the Department of General Surgery within China Medical University Hospital (CMUH), with comprehensive pathological data meticulously recorded for each individual. Control subjects were meticulously matched with a 1:1 ratio to the cases, taking age and sex into consideration. Informed consent was obtained from all participants, who contributed blood samples for the study. The research protocols were granted approval and oversight by the Institutional Review Board of CMUH (approval code: DMR99-IRB-108), and the study was carried out in alignment with the principles outlined in the Declaration of Helsinki. Notably, both cases and controls were of Taiwanese origin, and Table I concisely presents the salient characteristics of the study population.

Genotyping procedure for Lig4 rs1805388. Genomic DNA was isolated from peripheral blood leukocytes of each participant within 24 h of blood collection, utilizing the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC), as detailed in standard biomedical practices (40, 42, 43). The extracted DNA was quantified, preserved at -80˚C for long-term storage, and apportioned as working stock at -20˚C for genotyping purposes. The genotyping protocol for *Lig4* rs1805388 was developed by the Terry Fox Cancer Research Lab, Taichung, Taiwan. The primer sequences used were as follows: forward 5'-TCTGTATTCGTTCTAAAGTT-3' and reverse 5'-TGCTTTACTAGTTAAACGAG-3'. The polymerase chain reaction (PCR) cycling conditions consisted of an initial denaturation step at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 s, annealing at 52˚C for 30 s, extension at 72˚C for 30 s, and a final extension at 72˚C for 10 min. Following PCR amplification, the products were digested with the *HpyCH4* III restriction endonuclease and resolved using 3% agarose gel electrophoresis for a duration of 25 min. The *Lig4* rs1805388 genotypes were distinguished as follows: wild-type CC genotype with a 121-bp product, heterozygous variant CT genotype with products of 121 bp, 65 bp, and 56 bp, and homozygous variant TT genotype with products of 65 bp and 56 bp. Genotyping was independently repeated by at least two researchers, who are acknowledged contributors, in a double-blind fashion. Notably, all genotyping outcomes achieved a 100% success rate and demonstrated complete concordance among the results.

Statistical analysis. The assessment of Hardy-Weinberg equilibrium among the distinct *Lig4* rs1805388 genotypes in 362 non-cancer controls was conducted using the goodness-of-fit chi-square test. The distributions of age, sex, personal habits, BMI, different *Lig4* rs1805388 genotypes, and alleles within subgroups were evaluated using Pearson's chi-square test. Logistic regression analysis was employed to compute odds ratios (ORs) along with their corresponding 95% confidence intervals (CIs) for discerning associations between *Lig4* rs1805388 genotypes and CRC risk. A *p*-value below 0.05 was deemed statistically significant for all comparisons.

Results

The demographic profiles of the 362 CRC patients and their corresponding 362 matched controls are presented in Table I. As the controls were meticulously paired with the cases at a 1:1

Table I. *Selected characteristics of the 362 patients with colorectal cancer and 362 non-cancer controls,*

Characteristic	Controls, n=362		Cases, n=362		*p*-Value[a]
	n	%	n	%	
Age (years)					
≤60	95	26.2%	95	26.2%	1.0000
>60	267	73.8%	267	73.8%	
Sex					
Male	203	56.1%	203	56.1%	1.0000
Female	159	43.6%	159	43.9%	
Smoking					
Yes	84	23.2%	91	25.1%	0.5434
No	278	76.8%	271	74.9%	
Alcohol drinking					
Yes	51	14.1%	44	12.2%	0.4410
No	311	85.9%	318	87.8%	
BMI					
<24	175	48.3%	193	53.3%	0.1809
≥24	187	51.7%	169	46.7%	
Tumor size (cm)					
<5			195	53.9%	
≥5			167	46.1%	
Location					
Colon			257	71.0%	
Rectum			105	29.0%	
Lymph node involvement					
Negative			210	58.0%	
Positive			152	42.0%	

SD: Standard deviation; BMI: body mass index; [a]based on Chi-square test with Yates' correction.

ratio, based on age and sex, noteworthy dissimilarities in the distribution of these two variables between the case and control cohorts were absent. Furthermore, notable disparities in the prevalence of smoking (p=0.543), alcohol consumption (p=0.441), and individuals categorized with lower (<24) or higher (≥24) BMI (p=0.181) did not manifest between the case and control groups.

Table II illustrates the distribution of *Lig4* rs1805388 genotypes within the cohort of 362 non-cancer controls as well as among an equivalent number of CRC patients. The genotypic frequencies of *Lig4* rs1805388 among the control subjects were determined to be in adherence with the Hardy-Weinberg equilibrium (p=0.9175, Table II). Moreover, the genotypic distributions of *Lig4* rs1805388 were found to be equivalently distributed between the CRC patients and the non-cancer control group (p for trend=0.7314, Table II). Additionally, neither the heterozygous CT nor the homozygous variant TT genotypes of *Lig4* rs1805388 exhibited associations with an altered risk of CRC (OR=1.08 and 1.24, 95%CI=0.80-1.48 and 0.68-2.25, p=0.6601 and 0.5900, respectively; Table II). Similar assessments were carried out in both recessive and dominant models. In the recessive model, a non-significant 1.20-fold increased CRC risk was observed for carriers of the *Lig4* rs1805388 TT genotype in comparison to those possessing CC+CT genotypes (95%CI=0.66-2.15, p=0.6541, Table II). In the dominant model, carriers of the CT+TT genotypes of *Lig4* rs1805388 exhibited a non-significant 1.11-fold increased CRC risk in comparison to those harboring the CC genotypes (95%CI=0.82-1.48, p=0.5493, Table II).

To corroborate the findings presented in Table II, an analysis of allelic frequency distribution for *Lig4* rs1805388 was conducted, with the outcomes presented in Table III. The results of the allelic frequency distribution analysis further reinforce the notion that the *Lig4* rs1805388 genotype is not associated with CRC risk. This assertion is substantiated by the observation that the prevalence of the variant T allele was 26.2% among the CRC patients and 24.4% within the cancer-free control group (OR=1.10, 95%CI=0.87-1.39, p=0.4685).

Discussion

Genotypes of DNA repair genes are frequently regarded as crucial markers, given their potential involvement in genomic stability alterations that can instigate or advance carcinogenesis (44-46). In the human DNA repair system, the principal mechanisms encompass direct reverse, BER, NER, MMR, and

Table II. *Distribution of Lig4 rs1805388 genotypes among the 362 patients with colorectal cancer and 362 non-cancer controls.*

Genotypes	Controls, N	%	Patients, N	%	OR (95%CI)	*p*-Value[a]
Lig4 rs1805388						
CC	207	57.2	198	54.7	1.00 (Reference)	
CT	133	36.7	138	38.1	1.08 (0.80-1.48)	0.6601
TT	22	6.1	26	7.2	1.24 (0.68-2.25)	0.5900
p_{trend}						0.7314
p_{HWE}						0.9175
Carrier comparison						
CC+CT	340	93.9	336	92.8	1.00 (Reference)	
TT	22	6.1	26	7.2	1.20 (0.66-2.15)	0.6541
CC	207	57.2	198	54.7	1.00 (Reference)	
CT+TT	155	42.8	164	45.3	1.11 (0.82-1.48)	0.5493

N: Number; OR: odds ratio; CI: Confidence interval; [a]Based on Chi-square test with Yates' correction; p_{HWE}: whether the frequencies in controls are consistent with Hardy-Weinberg Equilibrium.

Table III. *Distribution of Lig4 rs1805388 allelic frequencies among the 362 colorectal cancer cases and the 362 non-cancer controls.*

Allele	Controls, N	%	Patients, N	%	OR (95%CI)	*p*-Value[a]
Lig4 rs1805388						
C	547	75.6%	534	73.8%	1.00 (Reference)	
T	177	24.4%	190	26.2%	1.10 (0.87-1.39)	0.4685

N: Number; OR: odds ratio; CI: Confidence interval; [a]Based on Chi-square with Yates' correction test.

Table IV. *Variant (T) allele frequencies of Lig4 rs1805388 in different populations.*

SNP	Population	Sample size of healthy controls, n	Variant (T) allele frequency
rs1805388	European	265,276	0.162
	African	15,706	0.108
	African American	15,166	0.109
	Asian	3,818	0.212
	East Asian	2,450	0.206
	Other Asian	1,368	0.222
	Taiwanese (current study)	362	0.244

Data were extracted from https://www.ncbi.nlm.nih.gov/snp/

DSB repair. Genotypes within these DNA repair genes can potentially dictate the cellular DNA repair capacity and, consequently, exert an influence on an individual's genetic predisposition to specific types of cancer. Among these various genes, Lig4 potentially holds a pivotal role in mediating the repair of DSBs through the NHEJ pathway. However, to date, the role of *Lig4* genotypes in the context of colorectal carcinogenesis remains unexplored (47). In the literature, the Lig4 protein has been shown to interact with another NHEJ protein, XRCC4, culminating in the final ligation of the break through an ATP-dependent step (48, 49). In recent years, several studies have reported significant associations between genotypes in DSB repair genes and CRC, such as ataxia telangiectasia mutated (*ATM*) (50, 51), and X-ray repair cross-complementing group 4 (*XRCC4*) (52-56). Nonetheless, comprehensive investigations into the association between *Lig4* genotypes and CRC risk remain rare.

Consequently, we embarked on the pioneering investigation of *Lig4* rs1805388 genotypes within the Taiwanese population. Notably, we conducted a comparative analysis between the distribution of *Lig4* rs1805388 genotypes among our cancer-free controls and the data documented on the National Center

for Biotechnology Information (NCBI) website, encompassing diverse populations worldwide. With a dataset involving 3,818 Asian subjects and 2,450 East Asian subjects, the minor allelic frequencies at *Lig4* rs1805388 were calculated to be 0.212 and 0.206, respectively (Table IV, updated as of 2023/08/15). Within our dataset, the variant T allele exhibited a frequency of 0.244, a representation of the Taiwanese population. It is evident that Asians exhibit distinct *Lig4* rs1805388 genotypic patterns compared to Caucasians (Table IV), despite the vital role of *Lig4* in NHEJ.

Subsequently, an exploration of the correlation between *Lig4* rs1805388 genotypes and CRC risk transpired in a genetically homogeneous population in Taiwan, encompassing 362 healthy individuals and 362 CRC cases. Our findings unveiled that neither the CT nor the TT genotypes of *Lig4* rs1805388 were associated with statistically significant modifications in CRC risk (Table II and Table III). Almost two decades ago, reports indicated that the T allele at *Lig4* rs1805388 is linked with diminished adenylation and ligation activities of the Lig4 protein (57). Our current data implies that *Lig4* rs1805388 might not be the principal determinant influencing the quantity and/or quality of Lig4 protein, thereby potentially leading to a considerably reduced capability for DSB repair and an increased CRC risk. Consequently, there could exist other polymorphic sites, such as *Lig4* rs1805386, that warrant thorough investigation in order to uncover genetic markers pertinent to the prediction of CRC risk.

In conclusion, this study has illuminated that the presence of the variant T allele at *Lig4* rs1805388 might not serve as a reliable indicator for predicting susceptibility to CRC. Subsequent investigations should be directed towards comprehending the ramifications of other polymorphic sites within *Lig4*, while also exploring the interplay between genotypes and phenotypes. Furthermore, a reassessment of the correlation between genotypes and/or the overall capacity of the NHEJ pathway is imperative. Above all, the role of *Lig4* in the context of colorectal carcinogenesis warrants thorough and continued exploration.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Conceptualization: D.Y., D.T.B., C.W.T. and W.S.C.; Collection: T.W.K. and Y.C.H.; Data curation: M.C.M. and C.W.T.; Genotyping: Y.C.W., Y.T.C. and W.S.C.; Statistics: W.T.W., Y.C.Y. and C.W.T.; Phenotyping: D.T.B. and W.S.C.; Project administration: T.C.Y. and D.T.B.; Supervision: D.T.B., W.S.C. and C.W.T.; Validation: T.W.K. and J.G.; Writing—original draft: D.Y., J.G., and C.W.T.; Writing—review and editing, D.T.B., D,Y. and C.W.T.; All Authors have read and agreed to the published version of the manuscript.

Acknowledgements

The Authors would like to acknowledge the Tissue-Bank of China Medical University Hospital for their invaluable technical support. Furthermore, the authors would like to extend their gratitude to all the study participants, as well as the doctors, nurses, and colleagues who contributed to the study. The technical assistances from Yu-Hsin Lin, Yi-Wen Hung and Hou-Yu Shih are appreciated by all the Authors. This study was supported by the grant from China Medical University and Asia University (CMU111-ASIA-03 and CMU112-ASIA-02).

References

1 Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, Vignat J, Ferlay J, Murphy N, Bray F: Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Gut 72(2): 338-344, 2023. DOI: 10.1136/gutjnl-2022-327736

2 Roelands J, van der Ploeg M, Ijsselsteijn ME, Dang H, Boonstra JJ, Hardwick JCH, Hawinkels LJAC, Morreau H, de Miranda NFCC: Transcriptomic and immunophenotypic profiling reveals molecular and immunological hallmarks of colorectal cancer tumourigenesis. Gut 72(7): 1326-1339, 2023. DOI: 10.1136/gutjnl-2022-327608

3 Tsai CW, Shih LC, Chang WS, Hsu CL, He JL, Hsia TC, Wang YC, Gu J, Bau DT: Non-homologous end-joining pathway genotypes significantly associated with nasopharyngeal carcinoma susceptibility. Biomedicines 11(6): 1648, 2023. DOI: 10.3390/biomedicines11061648

4 Bau DT, Mau YC, Ding SL, Wu PE, Shen CY: DNA double-strand break repair capacity and risk of breast cancer. Carcinogenesis 28(8): 1726-1730, 2007. DOI: 10.1093/carcin/bgm109

5 Bau DT, Fu YP, Chen ST, Cheng TC, Yu JC, Wu PE, Shen CY: Breast cancer risk and the DNA double-strand break end-joining capacity of nonhomologous end-joining genes are affected by BRCA1. Cancer Res 64(14): 5013-5019, 2004. DOI: 10.1158/0008-5472.CAN-04-0403

6 Huang W, Li W, Xu N, Li H, Zhang Z, Zhang X, He T, Yao J, Xu M, He Q, Guo L, Zhang S: Differences in DNA damage repair gene mutations between left- and right-sided colorectal cancer. Cancer Med 12(9): 10187-10198, 2023. DOI: 10.1002/cam4.5716

7 Liu W, Mahdessian H, Helgadottir H, Zhou X, Thutkawkorapin J, Jiao X, Wolk A, Lindblom A, Swedish Low-risk Colorectal Cancer Study Group: Colorectal cancer risk susceptibility loci in a Swedish population. Molecular Carcinogenesis 61(3): 288-300, 2022. DOI: 10.1002/mc.23366

8 Guan X, Li M, Bai Y, Feng Y, Li G, Wei W, Fu M, Li H, Wang C, Jie J, Meng H, Wu X, Deng Q, Li F, Yang H, Zhang X, He M, Guo H: Associations of mitochondrial DNA copy number with incident risks of gastrointestinal cancers: A prospective case–cohort study. Mol Carcinog 62(2): 224-235, 2023. DOI: 10.1002/mc.23478

9 Cacina C, Ulu E, Arikan S, Surmen ST, Yaylim I: The investigation of MAPK7 gene variations in colorectal cancer risk. In Vivo 37(2): 644-648, 2023. DOI: 10.21873/invivo.13123

10 Wu MH, Yueh TC, Chang WS, Tsai CW, Fu CK, Yang MD, Yu CC, Bau DT: Contribution of matrix metalloproteinase-1 genotypes to colorectal cancer in Taiwan. Cancer Genomics Proteomics 18(3): 245-251, 2021. DOI: 10.21873/cgp.20255

11 Yueh TC, Hung YW, Shih TC, Wu CN, Wang SC, Lai YL, Hsu SW, Wu MH, Fu CK, Wang YC, Ke TW, Chang WS, Tsai CW, Bau DT: Contribution of murine double minute 2 genotypes to colorectal cancer risk in Taiwan. Cancer Genomics Proteomics 15(5): 405-411, 2018. DOI: 10.21873/cgp.20099

12 Alhegaili AS: Role of DNA repair deficiency in cancer development. Pak J Biol Sci 26(1): 15-22, 2023. DOI: 10.3923/pjbs.2023.15.22

13 Qin S, Kitty I, Hao Y, Zhao F, Kim W: Maintaining genome integrity: Protein kinases and phosphatases orchestrate the balancing act of DNA double-strand breaks repair in cancer. Int J Mol Sci 24(12): 212, 2023. DOI: 10.3390/ijms241210212

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

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

16 Timson DJ, Singleton MR, Wigley DB: 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

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

18 Huang M, Dong G, Lu X, Xiao F, Zhou Q, Zhang S: DNA ligase IV dificiency with elevated serum IgG levels suspected to have myelodysplastic syndrome: a case report. BMC Pediatr 22(1): 588, 2022. DOI: 10.1186/s12887-022-03655-x

19 Pollard JM, Gatti RA: 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

20 O'Driscoll M, Jeggo PA: 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 MR: Human severe combined immune deficiency and DNA repair. Bioessays 25(11): 1061-1070, 2003. DOI: 10.1002/bies.10344

22 He J, Tian X, Luo T, Zou R, Yin Z, Chen K, Zhu C, He X: Allogeneic hematopoietic stem cell transplantation corrects ligase IV deficiency. Transpl Immunol 80: 101897, 2023. DOI: 10.1016/j.trim.2023.101897

23 Grawunder U, Zimmer D, Fugmann S, Schwarz K, Lieber MR: 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

24 Riballo E, Critchlow SE, Teo SH, Doherty AJ, Priestley A, Broughton B, Kysela B, Beamish H, Plowman N, Arlett CF, Lehmann AR, Jackson SP, Jeggo PA: Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. Curr Biol 9(13): 699-S2, 1999. DOI: 10.1016/s0960-9822(99)80311-x

25 Zhou LP, Luan H, Dong XH, Jin GJ, Man DL, Shang H: Lack of association between LIG4 gene polymorphisms and the risk of breast cancer: a HuGE review and meta-analysis. Asian Pac J Cancer Prev 13(7): 3417-3422, 2012. DOI: 10.7314/apjcp.2012.13.7.3417

26 Acevedo N, Mercado D, Vergara C, Sánchez J, Kennedy MW, Jiménez S, Fernández AM, Gutiérrez M, Puerta L, Caraballo L: Association between total immunoglobulin E and antibody responses to naturally acquired Ascaris lumbricoides infection and polymorphisms of immune system-related LIG4, TNFSF13B and IRS2 genes. Clin Exp Immunol 157(2): 282-290, 2009. DOI: 10.1111/j.1365-2249.2009.03948.x

27 Fu YP, Yu JC, Cheng TC, Lou MA, Hsu GC, Wu CY, Chen ST, Wu HS, Wu PE, Shen CY: Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: a multigenic study on cancer susceptibility. Cancer Res 63(10): 2440-2446, 2003.

28 Garcia-Closas M, Egan KM, Newcomb PA, Brinton LA, Titus-Ernstoff L, Chanock S, Welch R, Lissowska J, Peplonska B, Szeszenia-Dabrowska N, Zatonski W, Bardin-Mikolajczak A, Struewing JP: Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses. Hum Genet 119(4): 376-388, 2006. DOI: 10.1007/s00439-006-0135-z

29 Han J, Hankinson SE, Ranu H, De Vivo I, Hunter DJ: 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

30 Liu Y, Zhou K, Zhang H, Shugart YY, Chen L, Xu Z, Zhong Y, Liu H, Jin L, Wei Q, Huang F, Lu D, Zhou L: Polymorphisms of LIG4 and XRCC4 involved in the NHEJ pathway interact to modify risk of glioma. Hum Mutat 29(3): 381-389, 2008. DOI: 10.1002/humu.20645

31 Zhao P, Zou P, Zhao L, Yan W, Kang C, Jiang T, You Y: Genetic polymorphisms of DNA double-strand break repair pathway genes and glioma susceptibility. BMC Cancer 13: 234, 2013. DOI: 10.1186/1471-2407-13-234

32 Su Y, Qi S, Dou C, Shuang L, Yan H: Association of LIG4 and XRCC4 gene polymorphisms with the risk of human glioma in a Chinese population. Int J Clin Exp Pathol 8(2): 2057-2062, 2015.

33 Jiao K, Qin J, Zhao Y, Zhang H: Genetic effects of XRCC4 and ligase IV genes on human glioma. Neuroreport 27(14): 1024-1030, 2016. DOI: 10.1097/WNR.0000000000000649

34 Fachal L, Gómez-Caamaño A, Peleteiro P, Carballo A, Calvo-Crespo P, Sánchez-García M, Lobato-Busto R, Carracedo A, Vega A: Association of a XRCC3 polymorphism and rectum mean dose with the risk of acute radio-induced gastrointestinal toxicity in prostate cancer patients. Radiother Oncol 105(3): 321-328, 2012. DOI: 10.1016/j.radonc.2012.09.013

35 Henríquez-Hernández LA, Valenciano A, Foro-Arnalot P, Alvarez-Cubero MJ, Cozar JM, Suárez-Novo JF, Castells-Esteve M, Ayala-Gil A, Fernández-Gonzalo P, Ferrer M, Guedea F, Sancho-Pardo G, Craven-Bartle J, Ortiz-Gordillo MJ, Cabrera-Roldán P, Herrera-Ramos E, Lara PC: Polymorphisms in DNA-repair genes in a cohort of prostate cancer patients from different areas in Spain: heterogeneity between populations as a confounding factor in association studies. PLoS One 8(7): e69735, 2013. DOI: 10.1371/journal.pone.0069735

36 Tseng RC, Hsieh FJ, Shih CM, Hsu HS, Chen CY, Wang YC: 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

37 Yin M, Liao Z, Liu Z, Wang LE, 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

38 Xu F, Han JC, Zhang YJ, Zhang YJ, Liu XC, Qi GB, Liu D, Chen YZ, Zhao YX, 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: 860373, 2015. DOI: 10.1155/2015/860373
39 Lee YH, Wang SC, Li CH, Chen LH, Shen TC, Liu YF, Wang YC, Tsai CW, Hsia TC, Bau DT, Chang WS: The contribution of DNA Ligase 4 genetic variations to Taiwanese lung cancer. Anticancer Res 43(8): 3447-3453, 2023. DOI: 10.21873/anticanres.16520
40 Yueh TC, Wang YC, Chin YT, Hung YC, Mong MC, Yang YC, Pei JS, Gu J, Tsai CW, Bau DT, Chang WS: Impact of Mir196a-2 genotypes on colorectal cancer risk in Taiwan. Int J Mol Sci 24(14): 11613, 2023. DOI: 10.3390/ijms241411613
41 Yueh TC, Hung YC, Lee HT, Yang MD, Wang ZH, Yang YC, Ke TW, Pei JS, Tsai CW, Bau DT, Chang WS: Role of Matrix Metallopeptidase-2 genotypes in Taiwanese patients with colorectal cancer. Anticancer Res 42(11): 5335-5342, 2022. DOI: 10.21873/anticanres.16040
42 Yang MD, Lin KC, Lu MC, Jeng LB, Hsiao CL, Yueh TC, Fu CK, Li HT, Yen ST, Lin CW, Wu CW, Pang SY, Bau DT, Tsai FJ: Contribution of matrix metalloproteinases-1 genotypes to gastric cancer susceptibility in Taiwan. Biomedicine (Taipei) 7(2): 10, 2017. DOI: 10.1051/bmdcn/2017070203
43 Tsai CW, Chang WS, Xu Y, Huang M, Bau DT, Gu J: Associations of genetically predicted circulating insulin-like growth factor-1 and insulin-like growth factor binding protein-3 with bladder cancer risk. Mol Carcinog 60(11): 726-733, 2021. DOI: 10.1002/mc.23334
44 Vodicka P, Vodenkova S, Horak J, Opattova A, Tomasova K, Vymetalkova V, Stetina R, Hemminki K, Vodickova L: An investigation of DNA damage and DNA repair in chemical carcinogenesis triggered by small-molecule xenobiotics and in cancer: Thirty years with the comet assay. Mutat Res Genet Toxicol Environ Mutagen 885: 503564, 2023. DOI: 10.1016/j.mrgentox.2022.503564
45 de Almeida LC, Calil FA, Machado-Neto JA, Costa-Lotufo LV: DNA damaging agents and DNA repair: From carcinogenesis to cancer therapy. Cancer Genet 252-253: 6-24, 2021. DOI: 10.1016/j.cancergen.2020.12.002
46 Moraes MC, Neto JB, Menck CF: DNA repair mechanisms protect our genome from carcinogenesis. Front Biosci (Landmark Ed) 17(1): 1362, 2012. DOI: 10.2741/3992
47 Liu J, Zheng B, Li Y, Yuan Y, Xing C: Genetic polymorphisms of DNA repair pathways in sporadic colorectal carcinogenesis. J Cancer 10(6): 1417-1433, 2019. DOI: 10.7150/jca.28406
48 van Gent DC, van der Burg M: Non-homologous end-joining, a sticky affair. Oncogene 26(56): 7731-7740, 2007. DOI: 10.1038/sj.onc.1210871
49 Grawunder U, Wilm M, Wu X, Kulesza P, Wilson TE, Mann M, Lieber MR: Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. Nature 388(6641): 492-495, 1997. DOI: 10.1038/41358
50 Beggs AD, Domingo E, McGregor M, Presz M, Johnstone E, Midgley R, Kerr D, Oukrif D, Novelli M, Abulafi M, Hodgson SV, Fadhil W, Ilyas M, Tomlinson IP: Loss of expression of the double strand break repair protein ATM is associated with worse prognosis in colorectal cancer and loss of Ku70 expression is associated with CIN. Oncotarget 3(11): 1348-1355, 2012. DOI: 10.18632/oncotarget.694
51 Gao Y, Hayes RB, Huang WY, Caporaso NE, Burdette L, Yeager M, Chanock SJ, Berndt SI: DNA repair gene polymorphisms and tobacco smoking in the risk for colorectal adenomas. Carcinogenesis 32(6): 882-887, 2011. DOI: 10.1093/carcin/bgr071
52 Bau DT, Yang MD, Tsou YA, Lin SS, Wu CN, Hsieh HH, Wang RF, Tsai CW, Chang WS, Hsieh HM, Sun SS, Tsai RY: Colorectal cancer and genetic polymorphism of DNA double-strand break repair gene XRCC4 in Taiwan. Anticancer Res 30(7): 2727-2730, 2010.
53 Wang Y, Wang L, Li X, Liu B, Zhao Q, Chen P, Wang T, Li T, Ji J, Yang F, Wang Q, Wang J, Xiao Y, Xu Y, Feng G, Peng Z, He L, He G: Polymorphisms of XRCC4 are involved in reduced colorectal cancer risk in Chinese schizophrenia patients. BMC Cancer 10: 523, 2010. DOI: 10.1186/1471-2407-10-523
54 Emami N, Saadat I, Omidvari S: Susceptibility to colorectal cancer and two genetic polymorphisms of XRCC4. Pathol Oncol Res 21(4): 881-885, 2015. DOI: 10.1007/s12253-015-9905-z
55 Kabziński J, Majsterek I, Dziki A, Mik M: The role of the XPF gene polymorphism (Xrcc4) ser835ser in the risk of malignant transformation of cells in the colorectal cancer. Pol Przegl Chir 87(2): 83-85, 2015. DOI: 10.1515/pjs-2015-0023
56 Jin D, Zhang M, Hua H: Impact of polymorphisms in DNA repair genes XPD, hOGG1 and XRCC4 on colorectal cancer risk in a Chinese Han Population. Biosci Rep 39(1): BSR20181074, 2019. DOI: 10.1042/BSR20181074
57 Girard PM, Kysela B, Härer CJ, Doherty AJ, Jeggo PA: Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. Hum Mol Genet 13(20): 2369-2376, 2004. DOI: 10.1093/hmg/ddh274

Received August 15, 2023
Revised September 12, 2023
Accepted September 13, 2023