Contribution of DNA Double-strand Break Repair Gene *XRCC3* Genotypes to Triple-negative Breast Cancer Risk

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Abstract. *Aim: The DNA-repair gene X-ray repair cross-complementing group 3 (XRCC3) is important in DNA double-strand break repair and plays a critical part in initiation of carcinogenesis. Triple-negative breast cancer (TNBC) is the most difficult breast cancer subtype with no existing gene-targeting drugs and little knowledge on its genetic etiology. This study aimed to investigate the contribution of the XRCC3 genotype to individual TNBC susceptibility. Materials and Methods: A total of 2,464 Taiwan citizens consisting of 1,232 breast cancer cases and 1,232 controls were enrolled in this case–control study, and genotyping of XRCC3 rs1799794, rs45603942, rs861530, rs3212057, rs1799796, rs861539 and rs28903081 were performed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). We also conducted risk-stratified sub-group analyses to determine the association between the genotype and age- and hormone-related characteristics of breast cancer sub-groups. Results: There was no significant difference between breast cancer and control groups in the distributions of the genotypic or allelic frequencies as for the XRCC3 rs1799794 (p=0.5195 and 0.9545), rs45603942 (p=0.3478 and 0.1449), rs861530 (p=0.4567 and 0.5081), rs3212057 (p=1.0000 and 1.0000), rs1799796 (p=0.8487 and 0.7315) and rs28903081 (p=1.0000 and 1.0000), respectively. However, the XRCC3 rs861539 TT genotype was more prevalent in patients with breast cancer [odds ratio (OR)=2.99, 95% confidence interval (CI)=1.62-5.55; p=0.0002], and especially among those who were younger than 55 years (OR=2.61, 95% CI=1.82-3.73; p=0.0001), with first menarche earlier than 12.2 years (OR=2.47, 95% CI=1.74-3.52; p=0.0001), with menopause at 49.0 years old or later (OR=2.53, 95% CI=1.76-3.62; p=0.0001), or with TNBC (OR=2.05, 95% CI=1.46-4.28; $p=4.63*10^{-4}$). Conclusion: XRCC3 rs861539 TT is a potential predictive marker for TNBC in Taiwanese women and investigations in other populations are warranted for further universal application in cancer detection and prediction.*

Breast cancer is one of the most common worldwide malignancies among women, and its morbidity and mortality have not decreased with the development of anticancer drugs (1-3). Among the sub-types of breast cancer, the most dangerous is triple-negative breast cancer (TNBC), which refers to breast tumors that do not express the proteins for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) (4). TNBC is characterized by highly invasive tumors, with poor prognosis, likely to recur locally and metastasize distantly. Clinically, endocrinotherapy and anti-HER2 target treatment are ineffective. However, no standard therapy is available at present. In Taiwan, breast cancer ranks second among all cancers, noted for high incidence, high mortality, and early onset, and about 10% of breast cancer cases are TNBC (5, 6).

Platinum drugs may crosslink with DNA double strands, leading to DNA double-strand breaks (DSBs), inhibiting DNA replication and transcription and eventually causing cell-cycle arrest, DNA repair and cell death. Patients with TNBC are abnormal in a variety of genes and signal

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transduction pathways and have defects in DNA repair. Consequently, platinum drugs might be more efficacious in treatment of TNBC compared to alternative agents (7). Mounting evidence has shown that genetic polymorphisms in DNA repair genes may affect overall DNA repair ability, thereby impacting the efficacy of individualized chemotherapy (8-11).

The X-ray repair cross-complementing group 3 (*XRCC3*) gene, that is located on human chromosomes 14q32.3, encodes for a DNA-repair protein XRCC3. XRCC3 is a member of the DNA repair protein RAD51 homolog 1 (RAD51)-related protein family that plays a role in homologous recombination (HR) to repair DSBs and maintain the overall integrity of the human genome (12). In literature, several studies have been performed to evaluate the relationship between the rs861539 C/T polymorphism (also named Thr241Met, T241M, C18067T and C722T) of the *XRCC3* gene and cancer risk, making it the most commonly studied polymorphism of the *XRCC3* gene (13-16). In literature, there are some reports investigating the contribution of *XRCC3* rs861539 polymorphism to breast cancer but the findings are controversial and no article discusses the subgroup of TNBC (17-25). Some studies have identified the T variant of XRCC3 rs861539 as being associated with increased risk for breast cancer (17-21), while others did not (22-25). Pooled and meta-analyses tended to show a small but significant increase in breast cancer risk for this polymorphism (26-29). However, none of the previous literature discussed the contribution of *XRCC3* rs861539 to TNBC. The lack of analysis of TNBC may be the limited sample size of patients with TNBC and lack of detection and recording for triple-negative markers.

Following the central dogma of molecular biology, variants of the *XRCC3* rs861539 polymorphism may affect the function of the encoded protein, leading to altered DNA-repair capacity, that increased the level of bulky DNA adducts in leukocytes of healthy individuals (13). Among patients with bladder cancer, it was also found that variants of *XRCC3* rs861539 polymorphism affected the function of the encoded protein and consequently altered the DNA repair capacity of their cells (30). Thus, the rs861539 C/T polymorphism and other polymorphic sites may also play a role in the pathogenesis and development of breast cancer, especially of TNBC.

As far as we are aware of, *XRCC3* genotype among Taiwanese has never been investigated in association with breast cancer, let alone TNBC. This study's genotyping work ascertained correlation between *XRCC3* polymorphisms and breast cancer risk in a large population of Taiwanese women, with 1,232 cases and 1,232 controls. Additional analyses evaluated the contribution of *XRCC3* genotypes to breast cancer with specific clinicopathological features, such as those of TNBC.

Materials and Methods

Investigated population and sample collection. A total of 1,232 patients diagnosed with breast cancer were recruited at the outpatient clinics of general surgery at the China Medical University Hospital in Taichung, Taiwan. Female patients were included and males were excluded. Clinical characteristics of patients (including histological details) were all defined by expert surgeons. The questionnaire included questions on history and frequency of alcohol consumption and smoking habits, and "ever" having such a habit was defined as more than twice a week for a period of more than half year. Self-reported alcohol consumption and smoking habits were evaluated and classified as categorical variables. Staining of protein expression on slides was performed, reviewed and scored by two independent pathologists. For ER, PR, and p53 immunoassaying (Santa Cruz Biotechnology, Santa Cruz, CA, USA), nuclear staining in 10% of neoplastic cells served as the positive cutoff; a Ki-67 labelling index of >30% was considered positive. HER2/neu immunochemistry (Santa Cruz Biotechnology) results were derived according to the package insert and guidelines of the American Society of Clinical Oncology and College of American Pathologists (31). All patients voluntarily participated, completing self-administered questionnaires and supplying peripheral blood samples. An equal number of age-matched healthy volunteers without breast cancer as controls were selected after initial random sampling from the hospital's Health Examination Cohort. Exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic disease.

Genotyping conditions. Genomic DNA was extracted from peripheral blood leucocytes using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC). In this study, a total of seven polymorphic sites were analyzed for all the participants of both the control and case groups. Briefly, all seven polymorphic sites were genotyped by means of a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was performed on a BioRad Mycycler (BioRad, Hercules, CA, USA) following the manufacturer's instructions. Each PCR reaction consisted of 5 min initial cycle at 94˚C for 5 min; 40 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s; and a final extension at 72˚C for 10 min. Then the SNP-containing DNA amplicons were subjected to individual overnight digestion by restriction endonucleases following the manufacturer's instructions (see Table I for details). Following digestion, each sample was immediately analyzed by 3% agarose gel electrophoresis. Details of the primer sequences, and enzymatic digestion conditions for each SNP analyzed in this study are summarized in Table I.

Statistical analyses. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotypeic frequencies of *XRCC3* SNPs in the controls from those expected under the Hardy–Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's chi-square test was used to compare the distribution of the *XRCC3* genotypes between cancer cases and non-cancer controls. Cancer risk associated with the genotypes was estimated as odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. Data differences were recognized as significant when the statistical *p*-value was less than 0.05.

Table I. *Summary of the rs numbers, primers, amplicon length before and after enzyme digestion, and restriction enzymes for all the X-ray repair cross complementing protein 3 single nucleotide polymorphisms investigated in this study.*

rs number	Primer sequence	Restriction enzyme	Amplicon length	Allele and enzymatic fragment sizes
rs1799794	F: 5'-CACACTGCGGTCTTGCAGTG-3'	*Bts*CI	505 bp	G: 505 bp
	R: 5'-CAGGCTGGGTCTGGATACAA-3'			A: 289 + 216 bp
rs45603942	F: 5'-GGGATGCAGGTTCAACTGAC-3'	*Alu*I	352 bp	C: 352 bp
	R: 5'-AACTTGGACTGTGTCAAGCA-3'			T: 187 + 165 bp
rs861530	F: 5'-CCGAGGAACGTGCTGAACTT-3'	*Fat*I	497 bp	G: 497 bp
	R: 5'-CTCCCTAACAGCCTCCATGT-3'			A: 293 + 204 bp
rs3212057	F: 5'-CCATGACCGCAGGCACTTGT-3'	*HpyCH4*III	455 bp	G: 455 bp
	R: 5'-AGAACGCGACAAGGATGGTA-3'			A: 235 + 220 bp
rs1799796	F:5'-GG AACCAGTTGT GTGAGCCT-3'	*Alu*I	430 bp	G: 430 bp
	R: 5'-CCTGGTTGATGCACAGCACA-3'			A: 226 + 204 bp
rs861539	F: 5'-GACACCTTGT TGGAGTGTGT-3'	*Fat*I	358 bp	C: 358 bp
	R: 5'-GTCTTCTCGATGGTTAGGCA-3'			T: 200 + 158 bp
rs28903081	F: 5'-CTGCTTCCTGTTTCTCAGGT-3'	*BstU*I	198 bp	A: 198 bp
	R: 5'-GCACTGATCGTGTAGGAACA-3'			G: 102 + 96 bp

Table II. *Distribution of demographic and lifestyle characteristics of patients with breast cancer and matched controls.*

Characteristic	Controls (n=1232)			Patients (n=1232)			*p*-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)							
<40	359	29.1%		362	29.4%		0.89[a]
40-55	558	45.3%		547	44.4%		
>55	315	25.6%		323	26.2%		
Age at menarche (years)			12.4 (0.7)			12.1 (0.6)	0.79[b]
Age at birth of first child (years)			29.4 (1.2)			29.8 (1.4)	0.63[b]
Age at menopause (years)			48.8 (1.8)			49.3 (2.0)	0.59[b]
Site							
Unilateral				1,198	97.2%		
Bilateral				34	2.8%		
Family history							
First degree (mother, sister and daughter)	12	1.0%		55	4.5%		<0.0001[a]
Second degree	3	0.2%		6	0.5%		
No history	1,217	98.8%		1,171	95.0%		
Lifestyle habit							
Cigarette smokers	86	7.0%		170	13.8%		<0.0001[a]
Alcohol drinkers	91	7.4%		162	13.1%		<0.0001[a]

Statistical results based on [a]Chi-square or [b]unpaired Student's *t*-test.

Results

A total of 1,232 patients diagnosed with breast cancer and 1,232 matched controls were enrolled, as compared and summarized in Table I. Patients and controls were well-matched regarding their ages, ages at menarche, and ages when bearing their first child ($p>0.05$) (Table II). The patient group had significantly more smokers and alcohol drinkers than did the control group ($p<0.05$) (Table II).

The frequencies of the genotypes of the *XRCC3* polymorphisms in the breast cancer and control groups are shown in Table III. The results of genotyping analysis revealed the distribution of *XRCC3* rs861539 was significantly different between breast cancer and control groups ($p=1.10\times10^{-6}$), while the distributions of *XRCC3* rs1799794, rs45603942, rs861530, rs3212057, rs1799796 and rs28903081 genotypes were not ($p>0.05$) (Table III). The ORs associated with CT and TT genotypes were 1.74

Table III. *Distribution of X-ray repair cross complementing protein 3 (XRCC3) genotypes among patients with breast cancer and controls.*

Genotype	Controls (n=1232)	%	Patients (n=1232)	%	*p*-Value[a]	Odds ratio (95% CI)
rs1799794						
GG	310	25.2%	297	24.1%		1.00 (Ref)
AG	668	54.2%	696	56.5%	0.4069	1.09 (0.90-1.32)
AA	254	20.6%	239	19.4%	0.9035	0.98 (0.77-1.25)
p for trend					0.5195	
rs45603942						
CC	1,157	93.9%	1,139	92.5%		1.00 (Ref)
CT	62	5.0%	78	6.3%	0.1647	1.28 (0.91-1.80)
TT	13	1.1%	15	1.2%	0.7077	1.17 (0.56-2.47)
p for trend					0.3478	
rs861530						
AA	384	31.2%	358	29.1%		1.00 (Ref)
AG	649	52.7%	678	55.0%	0.2162	1.12 (0.94-1.34)
GG	199	16.1%	196	15.9%	0.6633	1.06 (0.83-1.35)
p for trend					0.4567	
rs3212057						
GG	1,232	100.0%	1,232	100.0%		1.00 (Ref)
AG	0	0.0%	0	0.0%		
AA	0	0.0%	0	0.0%		
p for trend					1.0000	
rs1799796						
AA	558	45.3%	563	45.6%		1.00 (Ref)
AG	612	49.7%	613	49.8%	0.9342	0.99 (0.84-1.17)
GG	62	5.0%	56	4.6%	0.6286	0.90 (0.61-1.31)
p for trend					0.8487	
rs861539						
CC	1,131	91.8%	1,052	85.4%		1.00 (Ref)
CT	87	7.1%	141	11.4%	0.0001*	1.74 (1.32-2.31)*
TT	14	1.1%	39	3.2%	0.0002*	2.99 (1.62-5.55)*
p for trend					1.10×10^{-6}*	
rs28903081						
GG	1,232	100.0%	1,232	100.0%		1.00 (Ref)
AG	0	0.0%	0	0.0%		
AA	0	0.0%	0	0.0%		
p for trend					1.0000	

Ref., Reference; CI: confidence interval. [a]Based on Chi-square test. *Statistically significant.

and 2.99 (95% CI=1.32-2.31 and 1.62-5.55; p=0.0001 and 0.0002) compared to the CC wild-type genotype.

The distributions of alleles for each of the *XRCC3* genotypic polymorphisms in the breast cancer and control groups are shown in Table IV. The results of the analysis revealed that the distribution of *XRCC3* rs861539 was significantly different between breast cancer and control groups ($p=3.77\times10^{-9}$), while the distribution of *XRCC3* rs1799794, rs45603942 and rs861530 was not ($p>0.05$) (Table IV). Those for *XRCC3* rs3212057 and rs28903081 were not analyzed since they lack genetic polymorphism in the Taiwanese population. The OR for carrying the minor T allele at *XRCC3* rs861539 was 1.99 (95% CI=1.58-2.52; $p=3.77\times10^{-9}$) compared to carrying the major C allele (Table IV).

It is well-known that distinct sub-types of breast cancer may have different mechanisms of carcinogenesis, we therefore analyzed the association among *XRCC3* rs861539 genotypes with age-related and clinicopathological characteristics of patients with breast cancer (Tables V and VI). For the analysis of age of onset of breast cancer, the results showed that CT and TT genotypes for *XRCC3* rs861539 were related to increased risk of diagnosed with breast cancer onset when patients were younger than 55 years (OR=2.19 and 5.61, 95% CI=1.48-3.23 and 2.31-13.63, respectively; $p=8.84\times10^{-8}$), but not in those aged 55 years or older (p=0.3073). Carrying a T allele-bearing genotype (CT or TT) was also associated with 2.61-fold higher risk than carrying wild-type CC in those younger than 55 years (OR=2.61, 95% CI=1.82-3.73; p=0.0001) (Table V). For the analysis of age at menarche, the results showed that those with CT and TT genotypes at *XRCC3* rs861539 were at higher risk of breast cancer among those

Table IV. *Distribution of X-ray repair cross complementing protein 3 (XRCC3) alleles among patients with breast cancer and controls.*

Allele	Controls	%	Patients	%	*p*-Value[a]	OR (95% CI)
rs1799794						
Allele G	1,288	52.3%	1,290	52.3%		1.00 (Ref)
Allele A	1,176	47.7%	1,174	47.7%	0.9545	1.00 (0.89-1.11)
rs45603942						
Allele C	2,376	96.4%	2,356	95.6%		1.00 (Ref)
Allele T	88	3.6%	108	4.4%	0.1449	1.24 (0.93-1.65)
rs861530						
Allele A	1,417	57.5%	1,394	56.6%		1.00 (Ref)
Allele G	1,047	42.5%	1,070	43.4%	0.5081	1.04 (0.93-1.16)
rs1799796						
Allele A	1,728	70.1%	1,739	70.6%		1.00 (Ref)
Allele G	736	29.9%	725	29.4%	0.7315	0.98 (0.87-1.11)
rs861539						
Allele C	2,349	95.3%	2,245	91.1%		1.00 (Ref)
Allele T	115	4.7%	219	8.9%	3.77×10^{-9}*	1.99 (1.58-2.52)*

Ref., Reference; OR, odds ratio; CI: confidence interval; [a]Based on Chi-square test. *Statistically significant.

who had first menarche at age younger than 12.2 years (OR=2.12 and 4.66, 95% CI=1.45-3.12 and 2.02-10.74, respectively; $p=4.71\times10^{-7}$), but not in those whose menarche started later ($p=0.2250$). Those with CT and TT genotypes were also at 2.47-fold higher risk than those with wild-type CC among those who were younger than 12.2 years (OR=2.47, 95% CI=1.74-3.52; $p=0.0001$) (Table V).The results showed that women with T allele-carrying genotypes were associated with higher risk of breast cancer regardless of age at their birth of their first child (Table V). Women with T allele-carrying genotypes at *XRCC3* rs861539 in whom menopause started age 49 years or more were at higher risk of breast cancer (OR=2.13, 4.81 and 2.53, 95% CI=1.44-3.18, 2.09-11.05 and 1.76-3.62, respectively; $p=4.18\times10^{-7}$), but not those who were younger at menopause ($p=0.2061$) (Table V). After adjustment for family history, smoking and alcohol drinking habits, all the significant findings in Table V retained the same trends.

Most interestingly, the role of variant genotypes (CT plus TT) serving as a genetic early detector for breast cancer was specifically focused on patients with TNBC (OR=2.05, 95% CI=1.46-4.28; $p=4.63*10^{-4}$), but not for non-TNBC patients (OR=2.05, 95% CI=1.46-4.28; $p=0.3666$) (Table VI). On the contrary, the difference in distribution of the variant genotypes among patients with breast cancer when stratified by Ki-67 status was not statistically significant ($p=0.2503$ and 0.1413 for Ki-67-negative and -positive subgroup comparisons) (Table VI). After the adjustment for individual characteristics including family history, smoking and alcohol drinking habits, the variant genotypes (CT plus TT) remained associated with TNBC, but not non-TNBC or Ki67 status.

Discussion

TNBC is frequently observed in young patients and is associated with larger, higher-grade tumors (4), in addition to higher recurrence rates of metastasis and death (32). Among breast cancer cases, 5-10% are believed to be hereditary and associated with certain gene mutations, such as mutations in *BRCA1* and *BRCA2* (33). Scientists believe that TNBC must have subtle and distinct genomic differences in the common pathways of breast carcinogenesis and are devoted to the search for useful markers, but positive findings are very limited. In 2014, we found that cyclin D1 (*CCND1*) A870G GG genotype was especially less prevalent in Taiwanese patients with TNBC (34). In 2015, Li and her colleagues found that the A allele of *TNFA*-308 was associated with higher metastasis risk among Caucasian and Asian patients with TNBC (35). In 2013, Xie and his colleagues found that the G allele of 8-oxoguanine glycosylase 1 (*hOGG1*) gene was associated with increased risk of TNBC in Chinese Han women (36). Investigating the responses of 60 patients with TNBC to platinum-based chemotherapy, Lu and his colleagues found that the genotype of one important nucleotide excision repair gene, excision repair cross-complementing group 2 (*ERCC2*), would determine the outcome of platinum-based chemotherapy. Patients with GG genotype had better response towards platinum-based chemotherapy than those with GA genotype at *ERCC2* rs1799793 (37). These two genomic markers are both closely involved in excision repair systems.

In the current study, we found a novel biomarker for TNBC in the DSB repair system, *XRCC3* rs861539. The results showed that T allele (or CT and TT genotypes) of *XRCC3* rs861539 is a breast cancer marker for Taiwanese

Table V. *Association of X-ray repair cross complementing protein 3 (XRCC3) rs861539 genotypes with age-related characteristics.*

	XRCC3 rs861539				
Characteristic	Controls, n (%)	Cases, n (%)	p-Value[a]	Crude OR (95% CI)[b]	Adjusted OR (95% CI)[c]
Age of onset					
<55.0 Years					
CC	584 (92.3)	503 (82.1)		1.00 (Ref.)	1.00 (Ref.)
CT	43 (6.8)	81 (13.2)	0.0001*	2.19 (1.48-3.23)*	2.27 (1.55-3.68)*
TT	6 (0.9)	29 (4.7)	0.0001*	5.61 (2.31-13.63)*	5.83 (2.43-12.94)*
CT+TT	49 (7.7)	110 (17.9)	0.0001*	2.61 (1.82-3.73)*	2.84 (1.84-3.95)*
p for trend			8.84×10^{-8}*		
≥55.0 Years					
CC	547 (91.3)	549 (88.7)		1.00 (Ref.)	1.00 (Ref.)
CT	44 (7.3)	60 (9.7)	0.1512	1.36 (0.90-2.04)	1.28 (0.79-1.95)
TT	8 (1.3)	10 (1.6)	0.8130	1.25 (0.49-3.18)	1.21 (0.55-2.98)
CT+TT	52 (8.7)	70 (11.3)	0.1520	1.34 (0.92-1.96)	1.27 (0.83-1.89)
p for trend			0.3073		
Age at menarche					
<12.2 Years					
CC	565 (91.7)	502 (81.8)		1.00 (Ref.)	1.00 (Ref.)
CT	44 (7.1)	83 (13.5)	0.0001*	2.12 (1.45-3.12)*	1.97 (1.33-3.21)*
TT	7 (1.1)	29 (4.7)	0.0001*	4.66 (2.02-10.74)*	4.33 (2.13-11.25)*
CT+TT	51 (8.3)	112 (18.2)	0.0001*	2.47 (1.74-3.52)*	2.28 (1.23-4.01)*
p for trend			4.71×10^{-7}*		
≥12.2 Years					
CC	566 (91.9)	550 (89.0)		1.00 (Ref.)	1.00 (Ref.)
CT	43 (7.0)	58 (9.4)	0.1204	1.39 (0.92-2.09)	1.27 (0.87-2.11)
TT	7 (1.1)	10 (1.6)	0.4728	1.47 (0.56-3.89)	1.39 (0.48-3.85)
CT+TT	50 (8.1)	68 (11.0)	0.0996	1.40 (0.95-2.05)	1.33 (0.93-2.12)
p for trend			0.2250		
Age at birth of first child					
<29.6 Years					
CC	563 (91.4)	531 (86.6)		1.00 (Ref.)	1.00 (Ref.)
CT	47 (7.6)	65 (10.6)	0.0596	1.47 (0.99-2.17)	1.53 (0.96-2.31)
TT	6 (1.0)	17 (2.8)	0.0194*	3.00 (1.18-7.68)*	2.87 (1.23-8.14)*
CT+TT	53 (8.6)	82 (13.4)	0.0081*	1.64 (1.14-2.36)*	1.67 (1.34-2.52)*
p for trend			0.0107*		
≥29.6 Years					
CC	568 (92.2)	521 (84.2)		1.00 (Ref.)	1.00 (Ref.)
CT	40 (6.5)	76 (12.3)	0.0003*	2.07 (1.39-3.09)*	2.08 (1.43-3.24)*
TT	8 (1.3)	22 (3.5)	0.0085*	3.00 (1.32-6.79)*	3.16 (1.44-7.01)*
CT+TT	48 (7.8)	98 (15.8)	0.0001*	2.23 (1.54-3.21)*	2.38 (1.65-3.48)*
p for trend			5.20×10^{-5}*		
Age at menopause					
<49.0 years					
CC	563 (91.4)	546 (88.3)		1.00 (Ref.)	1.00 (Ref.)
CT	46 (7.5)	63 (10.2)	0.1079	1.41 (0.95-2.10)	1.29 (0.91-1.89)
TT	7 (1.1)	9 (1.5)	0.6224	1.33 (0.49-3.58)	1.24 (0.46-3.38)
CT+TT	53 (8.6)	72 (11.7)	0.0892	1.40 (0.96-2.04)	1.26 (0.90-1.97)
p for trend			0.2061		
≥49.0 years					
CC	568 (92.2)	506 (82.4)		1.00 (Ref.)	1.00 (Ref.)
CT	41 (6.7)	78 (12.7)	0.0001*	2.13 (1.44-3.18)*	2.23 (1.52-3.34)*
TT	7 (1.1)	30 (4.9)	0.0001*	4.81 (2.09-11.05)*	4.97 (2.33-10.65)*
CT+TT	48 (7.8)	108 (17.6)	0.0001*	2.53 (1.76-3.62)*	2.65 (1.83-3.39)*
p for trend			4.18×10^{-7}*		

Ref., Reference; OR, odds ratio; CI, confidence interval. [a]Based on chi-square test. [bc]Difference in the trend in statistical significance before ([b]) and after ([c]) adjustment for individual characters including family history, smoking and alcohol drinking habits. *Statistically significant.

Table VI. *Association of X-ray repair cross complementing protein 3 (XRCC3) rs861539 variant genotypes with breast cancer risk stratified by clinicopathological characteristics compared to non-cancer healthy controls.*

Characteristics	Genotype, number (%)[a]		Crude OR (95% CI)	Adjusted OR (95% CI)[b]	*p*-Value[c]
	CC	CT+TT			
Control	1,131 (91.8)	101 (8.2)	1.00 (Ref)	1.00 (Ref)	
Triple-negative status					
No	498 (90.1)	55 (9.1)	1.24 (0.88-1.75)	1.17 (0.83-1.81)	0.3666
Yes	85 (81.7)	19 (18.3)	2.05 (1.46-4.28)*	2.14 (1.55-4.63)*	4.63×10^{-4}*
Ki67 status					
Negative	247 (89.2)	30 (10.8)	1.36 (0.88-2.09)	1.33 (0.84-1.88)	0.2503
Positive	301 (89.1)	37 (10.9)	1.38 (0.93-2.05)	1.28 (0.91-2.10)	0.1413

Ref., Reference. OR, odds ratio; CI, confidence interval. [a]Triple-negative and Ki67 status data were available for 657 and 615 patients with breast cancer, respectively. [b]Difference in the trend in statistical significance after adjustment for individual characters including family history, smoking and alcohol drinking habits. [c]p for trend based on chi-square test. *Statistically significant.

women (Tables III and IV). In addition, T allele carriers of *XRCC3* rs861539 were more prevalent in patients with breast cancer who were younger at onset (<55 years), with earlier first menarche (<12.2 years), with later menopause (≥49 years) (Table V), or with TNBC (Table VI). There was no difference for women with different ages of first full pregnancy, or Ki-67 status (Tables V and VI).

We have collected the largest breast cancer population in Taiwan, 1,232 cases and age-matched controls, to investigate the contribution of *XRCC3* genotypes to breast cancer risk (Table II). We found that the genotype of *XRCC3* rs861539 was associated with breast cancer susceptibility while other polymorphisms were not (Tables III and IV). This significance is consistent with previous findings in Pakistani (20), Polish (21), UK (17), Portuguese (18) and Thai (19) populations. Since we have collected the largest breast cancer population in Taiwan, the strategy of enlarging the number of investigated subjects is not an urgent need.

In the literature, it is well-believed that estrogen exposure is closely related to breast carcinogenesis, and there is no denying that age is the strongest demographic risk factor for most malignancies, since statistically, 75% of malignancies occurred in patients older than 55 years (38). With a large enough sample size, we are confident for the evaluation of the contribution of this SNP to breast cancer with specific clinicopathological features by stratification analysis. The estrogen- and age-related indices included age at onset, age at menarche, age at birth of first child, and age at menopause (Table V). As mentioned above, we are also interested in evaluating the contribution of *XRCC3* rs861539 to TNBC.

In the current study, 104 patients were identified as having TNBC, so named for its negative expression of ER, PR, and HER2/neu (39), and characterized by aggressiveness and higher rates of recurrence and metastasis. It is of great value to determine potential oncotargets for TNBC from analyzing patients' genotypes or phenotypes since the existing targeted therapies which are effective in other sub-types of breast cancer are not effective in dealing with TNBC. Typically, TNBC occurs in young patients whose disease is associated with genetic variations in *BRCA1* and other hereditary genes, such as *hOGG1* and *EGFR2* (31, 36, 40). All our findings show that the longer the estrogen exposure, the higher the risk of TNBC is for women carrying CT and TT genotypes of *XRCC3* rs861539. We also found that the full pregnancy for child was not a protective factor for TNBC among Taiwanese women (Table V). Although the up-regulation of Ki-67 was reported to be a potential indicator for TNBC (41), the expression of Ki-67 has no interaction with *XRCC3* rs861539 genotype in determining the TNBC risk in this study (Table VI).

The XRCC3 protein plays an important in the homologous recombination (HR) repair system, that is the most common pathway for repairing the radiation-induced DNA DSBs together with non homologous end-joining (NHEJ) pathway (42). In addition to affecting DNA-repair capacity in patients with bladder cancer (30), the T variant of *XRCC3* rs861539 was also reported to be associated with elevated levels of DNA adducts (13), chromosomal deletions (43), and sensitivity to ionizing radiation and cross-linking agents (44, 45). In the current study, although we did not measure DNA adducts or repair capacity of the cells from tumor sites of the patients and non-tumor sites from the patients and controls, our findings support the concept that individuals carrying CT and TT genotypes of *XRCC3* rs861539 are at higher risk of breast cancer (17-21) and other cancer types (11, 46) than those carrying the wild-type CC genotype.

In conclusion, the current study indicated that the T allele of *XRCC3* rs861539 may be regarded as a predictive marker for breast cancer and TNBC. The determination of *XRCC3* genotypes among Taiwanese without cancer and those with

breast cancer may contribute to a better understanding of the mechanisms of breast cancer by evaluating possible interactions between *XRCC3* genotypes and well-established risk factors. Population-based association studies in other countries are warranted to validate the possibility of *XRCC3* being a novel gene target for anticancer drug development for breast cancer, especially TNBC.

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References

1 DeSantis C, Ma J, Bryan L and Jemal A: Breast cancer statistics, 2013. CA Cancer J Clin *64*: 52-62, 2014.

2 Kohler BA, Sherman RL, Howlader N, Jemal A, Ryerson AB, Henry KA, Boscoe FP, Cronin KA, Lake A, Noone AM, Henley SJ, Eheman CR, Anderson RN and Penberthy L: Annual report to the nation on the status of cancer, 1975-2011, featuring incidence of breast cancer subtypes by race/ethnicity, poverty, and state. J Natl Cancer Inst *107*: djv048, 2015.

3 Sineshaw HM, Gaudet M, Ward EM, Flanders WD, Desantis C, Lin CC and Jemal A: Association of race/ethnicity, socioeconomic status, and breast cancer subtypes in the National Cancer Data Base (2010-2011). Breast Cancer Res Treat *145*: 753-763, 2014.

4 Bauer KR, Brown M, Cress RD, Parise CA and Caggiano V: Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer *109*: 1721-1728, 2007.

5 Cheng SH, Tsou MH, Liu MC, Jian JJ, Cheng JC, Leu SY, Hsieh CY and Huang AT: Unique features of breast cancer in Taiwan. Breast Cancer Res Treat *63*: 213-223, 2000.

6 Kuo WH, Yen AM, Lee PH, Chen KM, Wang J, Chang KJ, Chen TH and Tsau HS: Cumulative survival in early-onset unilateral and bilateral breast cancer: an analysis of 1907 Taiwanese women. Br J Cancer *100*: 563-570, 2009.

7 Krockenberger M, Engel JB, Hausler S, Dietl J and Honig A: Prolonged clinical benefit from platinum-based chemotherapy in a patient with metastatic triple negative breast cancer. Eur J Gynaecol Oncol *30*: 449-451, 2009.

8 Hsieh YH, Chang WS, Tsai CW, Tsai JP, Hsu CM, Jeng LB and Bau DT: DNA double-strand break repair gene *XRCC7* genotypes were associated with hepatocellular carcinoma risk in Taiwanese males and alcohol drinkers. Tumour Biol, 2015.

9 Hsia TC, Chang WS, Chen WC, Liang SJ, Tu CY, Chen HJ, Liang JA, Tsai CW, Hsu CM, Tsai CH and 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*: 7001-7005, 2014.

10 Hsu CM, Chang WS, Hwang JJ, Wang JY, Hsiao YL, Tsai CW, Liu JC, Ying TH and Bau DT: The role of apurinic/apyrimidinic endonuclease DNA repair gene in endometriosis. Cancer Genomics Proteomics *11*: 295-301, 2014.

11 Tsai CW, Chang WS, Liu JC, Tsai MH, Lin CC and Bau DT: Contribution of DNA double-strand break repair gene *XRCC3* genotypes to oral cancer susceptibility in Taiwan. Anticancer Res *34*: 2951-2956, 2014.

12 Brenneman MA, Weiss AE, Nickoloff JA and Chen DJ: *XRCC3* is required for efficient repair of chromosome breaks by homologous recombination. Mutat Res *459*: 89-97, 2000.

13 Matullo G, Palli D, Peluso M, Guarrera S, Carturan S, Celentano E, Krogh V, Munnia A, Tumino R, Polidoro S, Piazza A and Vineis P: *XRCC1, XRCC3, XPD* gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. Carcinogenesis *22*: 1437-1445, 2001.

14 Qian B, Zhang H, Zhang L, Zhou X, Yu H and Chen K: Association of genetic polymorphisms in DNA repair pathway genes with non-small cell lung cancer risk. Lung Cancer *73*: 138-146, 2011.

15 Ke HG, Li J, Shen Y, You QS, Yan Y, Dong HX, Liu JH and Shen ZY: Prognostic significance of *GSTP1, XRCC1* and *XRCC3* polymorphisms in non-small cell lung cancer patients. Asian Pac J Cancer Prev *13*: 4413-4416, 2012.

16 Guo S, Li X, Gao M, Li Y, Song B and Niu W: The relationship between *XRCC1* and *XRCC3* gene polymorphisms and lung cancer risk in northeastern Chinese. PLoS One *8*: e56213, 2013.

17 Kuschel B, Auranen A, McBride S, Novik KL, Antoniou A, Lipscombe JM, Day NE, Easton DF, Ponder BA, Pharoah PD and Dunning A: Variants in DNA double-strand break repair genes and breast cancer susceptibility. Hum Mol Genet *11*: 1399-1407, 2002.

18 Costa S, Pinto D, Pereira D, Rodrigues H, Cameselle-Teijeiro J, Medeiros R and Schmitt F: DNA repair polymorphisms might contribute differentially on familial and sporadic breast cancer susceptibility: a study on a Portuguese population. Breast Cancer Res Treat *103*: 209-217, 2007.

19 Sangrajrang S, Schmezer P, Burkholder I, Boffetta P, Brennan P, Woelfelschneider A, Bartsch H, Wiangnon S, Cheisilpa A and Popanda O: The *XRCC3* Thr241Met polymorphism and breast cancer risk: a case-control study in a Thai population. Biomarkers *12*: 523-532, 2007.

20 Qureshi Z, Mahjabeen I, Baig R and Kayani M: Correlation between selected *XRCC2, XRCC3* and *RAD51* gene polymorphisms and primary breast cancer in women in Pakistan. Asian Pac J Cancer Prev *15*: 10225-10229, 2014.

21 Smolarz B, Makowska M, Samulak D, Michalska MM, Mojs E, Wilczak M and Romanowicz H: Association between single nucleotide polymorphisms (SNPs) of *XRCC2* and *XRCC3* homologous recombination repair genes and triple-negative breast cancer in Polish women. Clin Exp Med *15*: 151-157, 2015.

22 Loizidou MA, Michael T, Neuhausen SL, Newbold RF, Marcou Y, Kakouri E, Daniel M, Papadopoulos P, Malas S, Kyriacou K and Hadjisavvas A: Genetic polymorphisms in the DNA repair genes *XRCC1, XRCC2* and *XRCC3* and risk of breast cancer in Cyprus. Breast Cancer Res Treat *112*: 575-579, 2008.

23 Thyagarajan B, Anderson KE, Folsom AR, Jacobs DR Jr., Lynch CF, Bargaje A, Khaliq W and Gross MD: No association between *XRCC1* and *XRCC3* gene polymorphisms and breast cancer risk: Iowa Women's Health Study. Cancer Detect Prev *30*: 313-321, 2006.

24 Popanda O, Tan XL, Ambrosone CB, Kropp S, Helmbold I, von Fournier D, Haase W, Sautter-Bihl ML, Wenz F, Schmezer P and Chang-Claude J: Genetic polymorphisms in the DNA double-strand break repair genes *XRCC3, XRCC2*, and *NBS1* are not associated with acute side effects of radiotherapy in breast cancer patients. Cancer Epidemiol Biomarkers Prev *15*: 1048-1050, 2006.

25 Jacobsen NR, Nexo BA, Olsen A, Overvad K, Wallin H, Tjonneland A and Vogel U: No association between the DNA repair gene *XRCC3* T241M polymorphism and risk of skin cancer and breast cancer. Cancer Epidemiol Biomarkers Prev *12*: 584-585, 2003.

26 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 and 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*: 376-388, 2006.

27 Han S, Zhang HT, Wang Z, Xie Y, Tang R, Mao Y and Li Y: DNA repair gene *XRCC3* polymorphisms and cancer risk: a meta-analysis of 48 case–control studies. Eur J Hum Genet *14*: 1136-1144, 2006.

28 Lee KM, Choi JY, Kang C, Kang CP, Park SK, Cho H, Cho DY, Yoo KY, Noh DY, Ahn SH, Park CG, Wei Q and Kang D: Genetic polymorphisms of selected DNA repair genes, estrogen and progesterone receptor status, and breast cancer risk. Clin Cancer Res *11*: 4620-4626, 2005.

29 Breast Cancer Association Consortium. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. J Natl Cancer Inst *98*: 1382-1396, 2006.

30 Matullo G, Guarrera S, Carturan S, Peluso M, Malaveille C, Davico L, Piazza A and Vineis P: DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. Int J Cancer *92*: 562-567, 2001.

31 Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM and Hayes DF: American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med *131*: 18-43, 2007.

32 Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P and Narod SA: Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer *Res 13*: 4429-4434, 2007.

33 Meindl A, Ditsch N, Kast K, Rhiem K and Schmutzler RK: Hereditary breast and ovarian cancer: new genes, new treatments, new concepts. Dtsch Arztebl Int *108*: 323-330, 2011.

34 Liu LC, Su CH, Wang HC, Chang WS, Tsai CW, Maa MC, Tsai CH, Tsai FJ and Bau DT: Contribution of personalized cyclin D1 genotype to triple negative breast cancer risk. Biomedicine (Taipei) *4*: 3, 2014.

35 Li HH, Zhu H, Liu LS, Huang Y, Guo J, Li J, Sun XP, Chang CX, Wang ZH and Zhai K: Tumour necrosis factor-alpha gene polymorphism is associated with metastasis in patients with triple negative breast cancer. Sci Rep *5*: 10244, 2015.

36 Xie H, Xia K, Rong H and Chen X: Genetic polymorphism in hOGG1 is associated with triple-negative breast cancer risk in Chinese Han women. Breast *22*: 707-712, 2013.

37 Lu J, Zhao H, Li S, Tian Z, Zhu X, Wang H and Fu H: Correlation of rs1799793 polymorphism in *ERCC2* and the clinical response to platinum-based chemotherapy in patients with triple negative breast cancer. Int J Clin Exp Med *8*: 2934-2938, 2015.

38 Edwards BK, Howe HL, Ries LA, Thun MJ, Rosenberg HM, Yancik R, Wingo PA, Jemal A and Feigal EG: Annual report to the nation on the status of cancer, 1973-1999, featuring implications of age and aging on U.S. cancer burden. Cancer *94*: 2766-2792, 2002.

39 Lee E, McKean-Cowdin R, Ma H, Spicer DV, Van Den Berg D, Bernstein L and Ursin G: Characteristics of triple-negative breast cancer in patients with a *BRCA1* mutation: results from a population-based study of young women. J Clin Oncol *29*: 4373-4380, 2011.

40 Liu X, Xiao N, Guo W, Wu Y, Cai Z, He Q, Zhang L, Chen X, Sun C, Wang J, Zhu C, Jin H and Wang Y: The *hOGG1* gene 5'-UTR variant c.-53G>C contributes to the risk of gastric cancer but not colorectal cancer in the Chinese population: the functional variation of *hOGG1* for gastric cancer risk. J Cancer Res Clin Oncol *137*: 1477-1485, 2011.

41 Li XR, Liu M, Zhang YJ, Wang JD, Zheng YQ, Li J, Ma B and Song X: CK5/6, EGFR, Ki-67, cyclin D1, and NM23-H1 protein expressions as predictors of pathological complete response to neoadjuvant chemotherapy in triple-negative breast cancer patients. Med Oncol *28(Suppl 1)*: S129-134, 2011.

42 Altieri F, Grillo C, Maceroni M and Chichiarelli S: DNA damage and repair: from molecular mechanisms to health implications. Antioxid Redox Signal *10*: 891-937, 2008.

43 Au WW, Salama SA and Sierra-Torres CH: Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. Environ Health Perspect *111*: 1843-1850, 2003.

44 Fuller LF and Painter RB: A Chinese hamster ovary cell line hypersensitive to ionizing radiation and deficient in repair replication. Mutat Res *193*: 109-121, 1988.

45 Caldecott K and Jeggo P: Cross-sensitivity of gamma-ray-sensitive hamster mutants to cross-linking agents. Mutat Res *255*: 111-121, 1991.

46 Liu JC, Tsai CW, Hsu CM, Chang WS, Li CY, Liu SP, Shen WC and Bau DT: Contribution of double-strand break repair gene *XRCC3* genotypes to nasopharyngeal carcinoma risk in Taiwan. Chin J Physiol *58*: 64-71, 2015.

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