Research Paper

BARD1 Gene Polymorphisms Confer Nephroblastoma Susceptibility

Wen Fu [a,b,1], Jinhong Zhu [c,1], Si-Wei Xiong [d,1], Wei Jia [a,b], Zhang Zhao [a,b], Shi-Bo Zhu [a,b], Jin-Hua Hu [a,b], Feng-Hua Wang [a,b], Huimin Xia [a,b], Jing He [a,b,*], Guo-Chang Liu [a,b,**]

[a] Department of Pediatric Urology, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510623, Guangdong, China
[b] Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510623, Guangdong, China
[c] Molecular Epidemiology Laboratory and Department of Laboratory Medicine, Harbin Medical University Cancer Hospital, Harbin 150040, Heilongjiang, China
[d] Department of Urology, Guangzhou First People's Hospital, Guangzhou Medical University, Guangzhou 510180, Guangdong, China

ARTICLE INFO

ABSTRACT

BRCA1-associated RING domain protein 1 (BARD1) is a tumor suppressor, which forms a heterodimer with BRCA1. Three BARD1 gene polymorphisms (rs7585356 G>A, rs6435862 T>G and rs3768716 A>G) were initially identified as high-risk neuroblastoma susceptibility loci by a previous GWAS. Because of the general tumor-suppressing function of BARD1, we hypothesized that these BARD1 gene polymorphisms might modify the susceptibility to nephroblastoma. We genotyped these polymorphisms in 145 cases and 531 controls using Taqman methods. Out of three polymorphisms, only the rs7585356 G>A polymorphism was significantly associated with increased susceptibility to nephroblastoma [AA vs. GG: adjusted odds ratio (OR) = 1.78, 95% confidence interval (CI) = 1.01–3.12]. Combined analysis of three polymorphisms indicated that subjects with 3 risk genotypes exhibited significantly elevated nephroblastoma risk, when compared with subjects with 0–2 risk genotypes (adjusted OR = 1.72, 95% CI = 1.02–2.89). Stratified analysis revealed that in term of clinical stage, rs7585356 AA carriers were associated with increased risk of developing clinical stage I + II nephroblastoma. The presence of three risk genotypes was significantly associated with nephroblastoma risk in females and clinical stage I + II nephroblastoma. Our results suggested that BARD1 rs7585356 G>A may be associated with nephroblastoma risk.

1. Introduction

Nephroblastoma (Wilms' tumor) is an embryonal kidney malignancy. It is the most commonly diagnosed renal tumor in children, but rarely occurs in adults. The annual incidence rate of nephroblastoma is about 7–10 cases per million in children younger than 15 years old, making up 6–7% of all childhood cancers (Ko and Ritchey, 2009). The incidents vary among the different ethnic groups, with the highest rates found in black Africana and the lowest in Asians (Ko and Ritchey, 2009). The variation in incident rates reflected implication of genetic

factors in the aetiology of the disease. It was estimated that nephroblastoma affected 3.3 in one million children between 2002 and 2010 in China (Bao et al., 2013). Nephroblastoma is highly responsive to treatments, with a relatively favorable prognosis. The long term survival for regional and metastatic disease is over 90% and 75%, respectively (Ko and Ritchey, 2009; Szychot et al., 2014). However, there is still room for improvement in risk prediction and management of the disease.

Approximately, 10%–15% of nephroblastomas are related to germline pathogenic variants or epigenetic alterations formed in the early stage of embryogenesis (Dome and Huff, 1993). Roughly 1%–2% of cases have at least one relative also developed Wilms' tumor. Germline genetic and epigenetic variations are most frequently identified in Wilms' Tumor 1 (WT1) gene and the 11p15.5 locus (Dome and Huff, 1993) in patients with Wilms' tumor. In addition, genetic aberrations in other genes, including VHL, PBRM1, BAP1, may be implicated in the tumorigenesis of kidney cancers (Tan et al., 2015). Although pathogenic genetic alterations are clear in some families, they remain unknown for the majority of individuals. Therefore, additional relevant variants in other genes should be further explored.

BRCA1-associated RING domain protein 1 (BARD1) is encoded by the human BARD1 gene, which is known to interact with breast cancer

susceptibility gene 1 (BRCA1) (Wu et al., 1996; Irminger-Finger and Jefford, 2006; Irminger-Finger et al., 2016). BARD1 shares homologies with BRCA1 in primary structure within the N-terminal RING finger motif and two C-terminal BRCA1 carboxy-terminal (BRCT) domains (Wooster et al., 1994). These two proteins form a heterodimer through their N-terminal RING finger mofis (Meza et al., 1999). BARD1 is able to regulate the tumor-suppressor function of BRCA1 (Greenberg et al., 2006; Kim et al., 2006; Simons et al., 2006; Irminger-Finger et al., 2016). Disruption of the BARD1/BRCA1 interaction may impair BRCA1 tumor suppression functions. BARD1 itself is also a tumor suppressor (Irminger-Finger et al., 2016). Mice with conditional deletion of BARD1 in mammary epithelial cells developed breast cancer (Shakya et al., 2008). Moreover, structure-affecting mutations in the BARD1 gene were frequently identified in breast, ovarian, and uterine cancers (Irminger-Finger and Jefford, 2006). Because of the biological importance of BARD1, single nucleotide polymorphisms (SNPs) that alter its function or expression may modify susceptibility to cancer. A previous genome-wide association study (GWAS) found that BARD1 gene polymorphisms (rs7585356 G>A, rs6435862 T>G and rs3768716 A>G) were associated with neuroblastoma susceptibility (Capasso et al., 2009). Given the importance of BARD1 in cancer, we investigated whether the three SNPs confer nephroblastoma susceptibility in a Southern Chinese population consisting of 145 cases and 531 controls.

2. Materials and Methods

2.1. Study Population

Totally, 145 patients with nephroblastoma and 531 cancer-free controls were recruited for this hospital-based case-control study. All the cases were enrolled from the Guangzhou Women and Children's Medical Center, with newly diagnosed and histopathologically verified neuroblastoma. The cancer-free controls were frequency matched to cases on age and sex, who visited the same hospital for a regular physical examination (He et al., 2016a,b,c,d; Zhang et al., 2016; Zheng et al., 2016). All the participants were ethnic Chinese Han. Patients would be excluded, if bearing other types of tumor, secondary or recurrent tumors, and receiving chemotherapy or radiotherapy previously. Demographic and clinical data on each participant, including age, sex, and clinical characteristics, were acquired by structured questionnaire or archived medical records. This study obtained approval from the Institutional Review Board of Guangzhou Women and Children's Medical Center. Written informed consent was signed by all participants or their guardians in accordance with the Declaration of Helsinki.

2.2. SNP Selection and Genotyping

Three SNPs (rs7585356 G>A, rs6435862 T>G, and rs3768716 A>G) in the BARD1 gene identified in a previous GWAS were chosen for this study (Capasso et al., 2009). We used the TIANamp Blood DNA Kit (TianGen Biotech Co. Ltd., Beijing, China) to isolate genomic DNA from 2 mL venous blood sample, following the manufacturer's instructions (He et al., 2016c). Samples of genomic DNA were processed according to previous protocol (He et al., 2012; Zhu et al., 2015, 2016). Generally, we prepared all the DNA samples in a dilution of 10 ng/μL and added to the 96-well plates until further utilization. Genotyping for the SNPs was performed in the 384-well format and Taqman method was adopted. For the purpose of quality control, 10% of samples were picked randomly and regenotyped, and the two sets of genotyping results were 100% concordant.

2.3. Statistical Analysis

χ^2 test was performed to compare the differences in the demographic variables and distributions of genotypes between cases and controls. We used goodness-of-fit χ^2 test to evaluate whether genotype

distributions of SNPs followed Hardy-Weinberg equilibrium (HWE) in control subjects. Unconditional univariate logistic regression analysis was performed. Furthermore, odds ratios (ORs) and 95% confidence intervals (CIs), with adjustment for age and sex, were computed to determine the strength of the associations between studied SNPs and the risk of nephroblastoma. We also performed false-positive report probability (FPRP) analysis to further explore if the significant findings were just chance or noteworthy observations (He et al., 2016a). SAS software (version 9.1; SAS Institute, Cary, NC) was used to conduct all statistical analyses. All P values were two-sided, and a significance level of 0.05 was adopted for this study.

3. Results

3.1. Characteristics of Study Population

We totally recruited 145 nephroblastoma patients and 531 cancer-free controls with ages ranging from 1 to132 and from 0.07 to 156 months old, respectively (Table 1). There were no significant difference between cases and controls regarding age (26.17 ± 21.48 vs. 29.73 ± 24.86 months old, P = 0.725) and sex (P = 0.956). The clinical stages were also obtained for nephroblastoma patients. There were 4 patients (2.76%) in stage I, 49 (33.49%) in stage II, 50 (34.48%) in stage III, and 33 (22.76%) in stage IV. We failed to determine clinical stage for 9 patients.

3.2. Association Analysis

We performed the goodness-of-fit χ^2 test to test whether the distribution of genotype frequency of SNPs departed from expected pattern (Table 2). All the P values were above 0.05 (rs7585356 G>A: P_{HWE} = 0.948; rs6435862 T>G: P_{HWE} = 0.205; rs3768716 A>G: P_{HWE} = 0.415) suggested that all the analyzed SNPs were in accordance with HWE in the control subjects. Single locus analysis demonstrated that the rs7585356 G>A polymorphism was significantly associated with an increased risk of nephroblastoma (AA vs. GG: adjusted OR = 1.78, 95% CI = 1.01–3.12). There was no association observed for either the rs6435862 T>G or the rs3768716 A>G polymorphism. We next examined the combined effects of risk genotypes. There was a trend showing that the risk of nephroblastoma was increasing with the number of risk genotypes (adjusted OR = 1.26, 95% CI = 1.03–1.53). However, only carriers of three risk genotypes were at significantly higher risk than those without risk genotype (adjusted OR = 2.21, 95% CI = 1.18–4.17). Moreover, when we divided subjects into two groups (0–2 and 3 risk genotypes), we found that subjects with 3 risk genotypes

Table 1
Frequency distribution of selected variables for nephroblastoma cases and cancer-free controls.

Variables	Cases (n = 145)		Controls (n = 531)		P^a
	No.	%	No.	%	
Age range, month	1–132		0.07–156		0.725
Mean ± SD	26.17 ± 21.48		29.73 ± 24.86		
≤18	66	45.52	233	43.88	
>18	79	54.48	298	56.12	
Gender					0.956
Female	64	44.14	233	43.88	
Male	81	55.86	298	56.12	
Clinical stages					
I	4	2.76			
II	49	33.79			
III	50	34.48			
IV	33	22.76			
NA	9	6.21			

[a] Two-sided χ^2 test for distributions between nephroblastoma cases and cancer-free controls.

Table 2
Logistic regression analysis of associations between *BARD1* polymorphisms and nephroblastoma risk.

Genotype	Cases (N = 141)	Controls (N = 531)	P^a	Crude OR (95% CI)	P	Adjusted OR (95% CI)[b]	P^b
rs7585356 (HWE = 0.948)							
GG	53 (37.59)	235 (44.26)		1.00		1.00	
AG	64 (45.39)	237 (44.63)		1.20 (0.80–1.80)	0.385	1.20 (0.80–1.81)	0.372
AA	24 (17.02)	59 (11.11)		**1.80 (1.03–3.16)**	**0.039**	**1.78 (1.01–3.12)**	**0.044**
Additive			0.128	1.31 (1.00–1.71)	0.051	1.30 (1.00–1.71)	0.055
Dominant	88 (62.41)	296 (55.74)	0.153	1.32 (0.90–1.93)	0.156	1.32 (0.90–1.93)	0.155
Recessive	117 (82.98)	472 (88.89)	0.067	1.64 (0.98–2.75)	0.060	1.61 (0.96–2.71)	0.070
rs6435862 (HWE = 0.205)							
TT	112 (79.43)	381 (71.75)		1.00		1.00	
TG	26 (18.44)	133 (25.05)		0.67 (0.42–1.06)	0.089	0.66 (0.41–1.06)	0.085
GG	3 (2.13)	17 (3.20)		0.60 (0.17–2.09)	0.433	0.62 (0.18–2.15)	0.449
Additive			0.171	0.70 (0.47–1.04)	0.076	0.70 (0.47–1.04)	0.077
Dominant	29 (20.57)	150 (28.25)	0.061	0.66 (0.42–1.03)	0.068	0.66 (0.42–1.03)	0.067
Recessive	138 (97.87)	514 (96.80)	0.488	0.66 (0.19–2.28)	0.508	0.68 (0.20–2.35)	0.538
rs3768716 (HWE = 0.415)							
AA	108 (76.60)	364 (68.55)		1.00		1.00	
AG	29 (20.57)	148 (27.87)		0.66 (0.42–1.04)	0.072	0.65 (0.41–1.03)	0.064
GG	4 (2.84)	19 (3.58)		0.71 (0.24–2.13)	0.541	0.73 (0.24–2.19)	0.572
Additive			0.166	0.72 (0.50–1.05)	0.087	0.72 (0.49–1.05)	0.083
Dominant	33 (23.40)	167 (31.45)	0.059	0.67 (0.43–1.03)	0.065	0.66 (0.43–1.02)	0.059
Recessive	137 (97.16)	512 (96.42)	0.660	0.79 (0.26–2.35)	0.668	0.81 (0.27–2.42)	0.703
Combined effect of risk genotypes							
0	27 (19.15)	141 (26.55)		1.00		1.00	
1	8 (5.67)	32 (6.03)		1.31 (0.54–3.14)	0.551	1.27 (0.53–3.07)	0.591
2	82 (58.16)	302 (56.87)		1.42 (0.88–2.29)	0.153	1.43 (0.88–2.31)	0.146
3	24 (17.02)	56 (10.55)		**2.24 (1.19–4.21)**	**0.012**	**2.21 (1.18–4.17)**	**0.014**
Trend			0.021	**1.26 (1.03–1.53)**	**0.022**	**1.26 (1.03–1.53)**	**0.022**
0–2	117 (82.98)	457 (89.45)		1.00		1.00	
3	24 (17.02)	56 (10.55)	0.042	**1.74 (1.04–2.92)**	**0.037**	**1.72 (1.02–2.89)**	**0.042**

The results were in bold if the 95% CI excluded 1 or P < 0.05.
[a] χ^2 test for genotype distributions between nephroblastoma patients and controls.
[b] Adjusted for age and gender.

exhibited significantly elevated nephroblastoma risk in comparison to subjects with 0–2 risk genotypes (adjusted OR = 1.72, 95% CI = 1.02–2.89).

3.3. Stratified Analysis

We then performed stratified analysis to explore how age, gender, and clinical stage influence the association between selected polymorphisms and nephroblastoma susceptibility (Table 3). No association

was observed between individual SNPs and nephroblastoma susceptibility in subgroups defined by age and sex. Interestingly, we found that subjects carrying rs7585356 AA genotype significantly tended to develop clinical stage I + II nephroblastoma, when compared with those carrying G alleles. However, the rs7585356 AA genotype did not appear to increase the risk of clinical stage III + IV nephroblastoma. We further investigated the cumulative effects of these SNPs on nephroblastoma in the stratified analysis. We found that the presence of three risk genotypes was significantly associated with the risk of

Table 3
Stratification analysis for association between *BARD1* genotypes and nephroblastoma risk.

Variables	rs7585356 (case/control) GG/AG	AA	Adjusted OR[a] (95% CI)	P^a	rs6435862 (case/control) TT	TG/GG	Adjusted OR[a] (95% CI)	P^a	rs3768716 (case/control) AA	AG/GG	Adjusted OR[a] (95% CI)	P^a	Risk genotypes (case/control) 0–2	3	Adjusted OR[a] (95% CI)	P^a
Age, month																
≤18	56/205	12/28	1.69 (0.81–3.55)	0.165	49/167	15/66	0.78 (0.41–1.48)	0.447	47/154	17/79	0.71 (0.38–1.32)	0.277	52/206	12/27	1.77 (0.84–3.73)	0.134
>18	65/267	12/31	1.62 (0.79–3.32)	0.193	63/214	14/84	0.56 (0.30–1.05)	0.070	61/210	16/88	0.61 (0.33–1.12)	0.110	65/269	12/29	1.74 (0.84–3.61)	0.134
Gender																
Female	52/210	12/23	2.08 (0.97–4.46)	0.060	52/160	12/73	0.51 (0.26–1.01)	0.054	49/150	13/83	0.55 (0.29–1.05)	0.070	52/211	12/22	**2.19 (1.01–4.71)**	**0.046**
Male	65/262	12/36	1.32 (0.65–2.69)	0.441	60/221	17/77	0.80 (0.44–1.46)	0.474	59/214	18/84	0.76 (0.43–1.37)	0.369	65/264	12/34	1.42 (0.70–2.90)	0.335
Clinical stage																
I + II	40/472	11/59	**2.14 (1.03–4.45)**	**0.041**	41/381	10/150	0.62 (0.30–1.27)	0.190	41/364	10/167	0.51 (0.25–1.06)	0.071	40/475	11/56	**2.29 (1.10–4.77)**	**0.027**
III + IV	68/472	13/59	1.53 (0.80–2.95)	0.200	65/381	16/150	0.63 (0.35–1.12)	0.116	62/364	19/167	0.67 (0.39–1.16)	0.157	68/475	13/56	1.63 (0.84–3.13)	0.147

The results were in bold if the 95% CI excluded 1 or P < 0.05.
[a] Adjusted for age and gender.

Table 4
False-positive report probability values for the association between *BARD1* genotypes and nephroblastoma susceptibility.

Genotype	Crude OR (95% CI)	P^a	Statistical power[b]	Prior probability				
				0.25	0.1	0.01	0.001	0.0001
rs7585356 G>A								
AA vs. GG	1.80 (1.03–3.16)	0.039	0.307	0.276	0.534	0.927	0.992	0.999
AA vs. GG/AG								
Stage I + II	2.20 (1.07–4.52)	0.032	0.162	0.371	0.639	0.951	0.995	0.999
Risk genotypes								
3 vs. 0	2.24 (1.19–4.21)	0.012	0.175	0.175	0.388	0.875	0.986	0.999
3 vs. 0–2	1.74 (1.04–2.92)	0.037	0.289	0.275	0.533	0.926	0.992	0.999
Females	2.21 (1.03–4.76)	0.042	0.161	0.440	0.702	0.963	0.996	1.000
Stage I + II	2.33 (1.13–4.80)	0.022	0.122	0.347	0.614	0.946	0.994	0.999

[a] χ^2 test was used to calculate the genotype frequency distributions.
[b] Statistical power was calculated using the number of observations in the subgroup and the OR and *P* values in this table.

nephroblastoma in females when compared with that of 0–2 risk genotypes (adjusted OR = 2.19, 95% CI = 1.01–4.71). Moreover, carriers of three genotypes had 2.29-fold increase in the risk of developing clinical stage I + II nephroblastoma (95% CI = 1.10–4.77).

Thus, when we performed FPRP analysis, all the significant findings disappeared at the prior probability level of 0.1 and FPRP threshold of 0.2 (Table 4).

4. Discussion

Genetic factors have been known to contribute to the development of nephroblastoma. However, genetic factors related to nephroblastoma are largely unknown, except for some genetic and epigenetic alterations in *WT1* and the 11p15.5 locus (Dome and Huff, 1993). Previous GWAS initially discovered six *BARD1* SNPs significantly associated with high-risk neuroblastoma susceptibility (Capasso et al., 2009). We tested the rs6435862 T>G, rs3768716 A>G, and rs7585356 G>A, because the rs6435862 T>G and rs3768716 A>G were the most significant SNPs in that GWAS study, and the last one is positioned in the 3′ UTR region. Another reason why we studied these SNP was due to the importance of BARD1 in cancer. These three SNPs were also chosen for our previous replication study in Southern Chinese population with 201 neuroblastoma patients and 531 controls (Zhang et al., 2016).

In this study, we investigated the roles of three polymorphisms in the *BARD1* gene in modifying nephroblastoma susceptibility in this case-control study with 145 nephroblastoma patients and 531 controls. Among the three SNPs, only the rs7585356 G>A polymorphism was significantly associated with nephroblastoma susceptibility. Intriguingly, stratified analysis indicated that subjects harboring the rs7585356 A alleles were more likely to have early stage of the disease. The rs7585356 AA homozygotes had a significantly increased risk of nephroblastoma at OR of 1.78. Moreover, these SNP might collectively contribute to the risk. The risk of developing nephroblastoma for subjects with three risk genotypes was significantly higher than those carrying two risk genotypes or less. Moreover, in the stratified analysis, the significant association was observed in female and patients with stage I and II disease. Despite the significant findings in the present study, it is should be noted that our previous study reported that based link disequilibrium (LD), these three SNPs could also secure 10 more polymorphisms, using SNPinfo software (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc. htm) (Zhang et al., 2016). The rs3768716 was in LD with rs17487792, rs17487827, rs17488049, rs2070096, while rs7585356 was in LD with rs6720708, rs12614960, 1374230, 16852600, 1979028, and rs207562. As a result, it remain unclear which SNP exactly modifies the risk of nephroblastoma, rs7585356 or another SNP in high LD with it. Moreover, the underlying mechanism by which the significant SNP alters the expression or function of BARD1 needs to be clarified.

A number of lines of evidence has suggested BARD1 as a tumor suppressor (Irminger-Finger and Jefford, 2006; Irminger-Finger et al., 2016). BRCA1-BARD1 heterodimer plays a crucial role in homologous repair in response to DNA damage (Stark et al., 2004). The knockout of

Bard1 is lethal in mouse (McCarthy et al., 2003). The study of *Bard1* knockout mice demonstrated that BARD1 is indispensible to maintain cell viability and genetic stability (McCarthy et al., 2003). Conditional knockout *Bard1* in mammary epithelia cells induced the development of breast cancer, which mimics the human breast cancer phenotype observed in individuals, harboring *BRCA1* mutation (Shakya et al., 2008). Mechanistic studies indicated that the BARD1-BRCA1 heterodimer is also implicated in ubiquitin dependent protein degradation as an E3 ubiquitin ligase (Hashizume et al., 2001). Mutations that impaired the E3 ubiquitin ligase activity of the heterodimer predispose to breast and ovarian cancer (Brzovic et al., 2001; Hashizume et al., 2001; Ruffner et al., 2001).

Moreover, some non-synonymous polymorphisms (e.g., cys557Ser, Arg378Ser, Val507Met, and Pro24Ser) in the *BARD1* gene have been also frequently investigated for their association with cancer susceptibility, and studies mainly involved breast cancer (Morris et al., 2006; Onay et al., 2006; Stacey et al., 2006; Vahteristo et al., 2006; Guenard et al., 2009; Ding et al., 2011; Sun et al., 2012), neuroblastoma (Capasso et al., 2009, 2013), and cervical cancer (Zhou et al., 2009). Nonetheless, the associations between these *BARD1* SNPs and cancer susceptibility have been often paradoxical. Some studies supported the associations (Huo et al., 2007), while others had opposite results (Liu et al., 2015). The factors that contribute to the inconsistency are, but not limited to differences in genotyping methods, experiment designs (hospital-based design or population-based design), different populations and ethnicities, as well as sample size of studies. Therefore, it is critical that all association studies consider external validity issues and candidly state the populations to which the results can be applied. Several meta-analyses were undertaken to reevaluate such associations (Ding et al., 2011; Liu et al., 2015). Ding et al. reported a lack of association between *BARD1* Cys557Ser polymorphism and breast cancer risk in a pooled analysis comprising 11,870 cases and 7687 controls in 2011 (Ding et al., 2011). Liu et al. collected 10 case-control studies in 2015 (Liu et al., 2015). They found that *BARD1* Val507Met and Pro24Ser polymorphisms were associated with decreased cancer risk independently, but not *BARD1* Arg378Ser (Liu et al., 2015).

A number of limitations of this study should be noted. First, owing to the extremely low incidence of nephroblastoma, the sample size of this case-control was relatively small. Consequently, statistic power of this study was compromised (statistical power no more than 0.307 for significant findings). The significant findings might be chance observations (FPRP values larger than 0.2 at the prior probability level of 0.1). Second, we concentrated on only three *BARD1* SNPs. *BARD1* gene is highly polymorphic, harboring 4941 SNPs as a minimum (http://www.ncbi.nlm. nih.gov/projects/SNP). Other SNPs that potentially affect the expression and function of BARD1 should be investigated in the future. Third, we only included Southern Chinese Children in this study. These findings cannot be generalized from one ethnicity to another before validation study. Fourth, in this retrospective study, some important information (e.g., parental exposures) was not available. Due to differences in genetic backgrounds and environmental exposures among the different ethnicities, these findings should be cross-validated with different populations.

Notwithstanding these limitations, our finding did suggest that the rs7585356 G>A polymorphism may confer genetic susceptibility to nephroblastoma. The studied SNPs collectively may increase the risk of nephroblastoma. Moreover, these SNPs appear to be related to the clinical stages of this disease. Well-designed, large, multi-center studies are warranted to strengthen these findings.

Funding Sources

Conflict of Interest Disclosures

The authors declare no competing financial interests.

Authorship Contributions

W. Fu, J. Zhu, J. He and G.C. Liu designed and performed the study and wrote the manuscript; W. Jia, Z. Zhao, S.B. Zhu, J.H. Hu, F.H. Wang and H. Xia collected the samples and information; S.W. Xiong and J. He participated in analyzing data; W. Fu, J. He and G.C. Li coordinated the study over the entire time. All authors reviewed the final manuscript.

Acknowledgements

References

Bao, P.P., Li, K., Wu, C.X., Huang, Z.Z., Wang, C.F., Xiang, Y.M., Peng, P., Gong, Y.M., Xiao, X.M., Zheng, Y., 2013. Recent incidences and trends of childhood malignant solid tumors in Shanghai, 2002–2010. Zhonghua Er Ke Za Zhi 51, 288–294.

Brzovic, P.S., Meza, J.E., King, M.C., Klevit, R.E., 2001. BRCA1 RING domain cancer-predisposing mutations. Structural consequences and effects on protein-protein interactions. J. Biol. Chem. 276, 41399–41406.

Capasso, M., Devoto, M., Hou, C., Asgharzadeh, S., Glessner, J.T., Attiyeh, E.F., Mosse, Y.P., Kim, C., Diskin, S.J., Cole, K.A., Bosse, K., Diamond, M., Laudenslager, M., Winter, C., Bradfield, J.P., Scott, R.H., Jagannathan, J., Garris, M., McConville, C., London, W.B., Seeger, R.C., Grant, S.F., Li, H., Rahman, N., Rappaport, E., Hakonarson, H., Maris, J.M., 2009. Common variations in BARD1 influence susceptibility to high-risk neuroblastoma. Nat. Genet. 41, 718–723.

Capasso, M., Diskin, S.J., Totaro, F., Longo, L., De Mariano, M., Russo, R., Cimmino, F., Hakonarson, H., Tonini, G.P., Devoto, M., Maris, J.M., Iolascon, A., 2013. Replication of GWAS-identified neuroblastoma risk loci strengthens the role of BARD1 and affirms the cumulative effect of genetic variations on disease susceptibility. Carcinogenesis 34, 605–611.

Ding, D.P., Zhang, Y., Ma, W.L., He, X.F., Wang, W., Yu, H.L., Guo, Y.B., Zheng, W.L., 2011. Lack of association between BARD1 Cys557Ser variant and breast cancer risk: a meta-analysis of 11,870 cases and 7,687 controls. J. Cancer Res. Clin. Oncol. 137, 1463–1468.

Dome, J.S., Huff, V., 1993–2017. Wilms tumor predisposition. In: Pagon, R.A., Adam, M.P., Ardinger, H.H., Wallace, S.E., Amemiya, A., Bean, L.J.H., Bird, T.D., Ledbetter, N., Mefford, H.C., Smith, R.J.H., Stephens, K. (Eds.), GeneReviews®. Seattle (WA): University of Washington, Seattle PMID:20301471.

Greenberg, R.A., Sobhian, B., Pathania, S., Cantor, S.B., Nakatani, Y., Livingston, D.M., 2006. Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. Genes Dev. 20, 34–46.

Guenard, F., Labrie, Y., Ouellette, G., Beauparlant, C.J., Durocher, F., 2009. Genetic sequence variations of BRCA1-interacting genes AURKA, BAP1, BARD1 and DHX9 in French Canadian families with high risk of breast cancer. J. Hum. Genet. 54, 152–161.

Hashizume, R., Fukuda, M., Maeda, I., Nishikawa, H., Oyake, D., Yabuki, Y., Ogata, H., Ohta, T., 2001. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. J. Biol. Chem. 276, 14537–14540.

He, J., Qiu, L.X., Wang, M.Y., Hua, R.X., Zhang, R.X., Yu, H.P., Wang, Y.N., Sun, M.H., Zhou, X.Y., Zhang, Y., Wang, Y.J., Wang, J.C., Jin, L., Wei, Q.Y., Li, J., 2012. Polymorphisms in the XPG gene and risk of gastric cancer in Chinese populations. Hum. Genet. 131, 1235–1244.

He, J., Wang, F., Zhu, J., Zhang, R., Yang, T., Zou, Y., Xia, H., 2016a. Association of potentially functional variants in the XPG gene with neuroblastoma risk in a Chinese population. J. Cell. Mol. Med. 20, 1481–1490.

He, J., Yang, T., Zhang, R., Zhu, J., Wang, F., Zou, Y., Xia, H., 2016b. Potentially functional polymorphisms in the LIN28B gene contribute to neuroblastoma susceptibility in Chinese children. J. Cell. Mol. Med. 20, 1534–1541.

He, J., Zhang, R., Zou, Y., Zhu, J., Yang, T., Wang, F., Xia, H., 2016c. Evaluation of GWAS-identified SNPs at 6p22 with neuroblastoma susceptibility in a Chinese population. Tumour Biol. 37, 1635–1639.

He, J., Zhong, W., Zeng, J., Zhu, J., Zhang, R., Wang, F., Yang, T., Zou, Y., Xia, H., 2016d. LMO1 gene polymorphisms contribute to decreased neuroblastoma susceptibility in a Southern Chinese population. Oncotarget 7, 22770–22778.

Huo, X., Hu, Z., Zhai, X., Wang, Y., Wang, S., Wang, X., Qin, J., Chen, W., Jin, G., Liu, J., Gao, J., Wei, Q., Shen, H., 2007. Common non-synonymous polymorphisms in the BRCA1 Associated RING Domain (BARD1) gene are associated with breast cancer susceptibility: a case-control analysis. Breast Cancer Res. Treat. 102, 329–337.

Irminger-Finger, I., Jefford, C.E., 2006. Is there more to BARD1 than BRCA1? Nat. Rev. Cancer 6, 382–391.

Irminger-Finger, I., Ratajska, M., Pilyugin, M., 2016. New concepts on BARD1: regulator of BRCA pathways and beyond. Int. J. Biochem. Cell Biol. 72, 1–17.

Kim, H.S., Li, H., Cevher, M., Parmelee, A., Fonseca, D., Kleiman, F.E., Lee, S.B., 2006. DNA damage-induced BARD1 phosphorylation is critical for the inhibition of messenger RNA processing by BRCA1/BARD1 complex. Cancer Res. 66, 4561–4565.

Ko, E.Y., Ritchey, M.L., 2009. Current management of Wilms' tumor in children. J. Pediatr. Urol. 5, 56–65.

Liu, X., Zhang, X., Chen, Y., Yang, X., Xing, Y., Ma, L., 2015. Association of three common BARD1 variants with cancer susceptibility: a system review and meta-analysis. Int. J. Clin. Exp. Med. 8, 311–321.

McCarthy, E.E., Celebi, J.T., Baer, R., Ludwig, T., 2003. Loss of Bard1, the heterodimeric partner of the Brca1 tumor suppressor, results in early embryonic lethality and chromosomal instability. Mol. Cell. Biol. 23, 5056–5063.

Meza, J.E., Brzovic, P.S., King, M.C., Klevit, R.E., 1999. Mapping the functional domains of BRCA1. Interaction of the ring finger domains of BRCA1 and BARD1. J. Biol. Chem. 274, 5659–5665.

Morris, J.R., Pangon, L., Boutell, C., Katagiri, T., Keep, N.H., Solomon, E., 2006. Genetic analysis of BRCA1 ubiquitin ligase activity and its relationship to breast cancer susceptibility. Hum. Mol. Genet. 15, 599–606.

Onay, V.U., Briollais, L., Knight, J.A., Shi, E., Wang, Y., Wells, S., Li, H., Rajendram, I., Andrulis, I.L., Ozcelik, H., 2006. SNP-SNP interactions in breast cancer susceptibility. BMC Cancer 6, 114.

Ruffner, H., Joazeiro, C.A., Hemmati, D., Hunter, T., Verma, I.M., 2001. Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. Proc. Natl. Acad. Sci. U. S. A. 98, 5134–5139.

Shakya, R., Szabolcs, M., McCarthy, E., Ospina, E., Basso, K., Nandula, S., Murty, V., Baer, R., Ludwig, T., 2008. The basal-like mammary carcinomas induced by Brca1 or Bard1 inactivation implicate the BRCA1/BARD1 heterodimer in tumor suppression. Proc. Natl. Acad. Sci. U. S. A. 105, 7040–7045.

Simons, A.M., Horwitz, A.A., Starita, L.M., Griffin, K., Williams, R.S., Glover, J.N., Parvin, J.D., 2006. BRCA1 DNA-binding activity is stimulated by BARD1. Cancer Res. 66, 2012–2018.

Stacey, S.N., Sulem, P., Johannsson, O.T., Helgason, A., Gudmundsson, J., Kostic, J.P., Kristjansson, K., Jonsdottir, T., Sigurdsson, H., Hrafnkelsson, J., Johannsson, J., Sveinsson, T., Myrdal, G., Grimsson, H.N., Bergthorsson, J.T., Amundadottir, L.T., Gulcher, J.R., Thorsteinsdottir, U., Kong, A., Stefansson, K., 2006. The BARD1 Cys557Ser variant and breast cancer risk in Iceland. PLoS Med. 3, e217.

Stark, J.M., Pierce, A.J., Oh, J., Pastink, A., Jasin, M., 2004. Genetic steps of mammalian homologous repair with distinct mutagenic consequences. Mol. Cell. Biol. 24, 9305–9316.

Sun, G., Wang, J.T., Ma, B.L., Geng, Z.L., Ren, G.H., Shan, M.H., Ma, B., Ma, L.L., Wang, Y., 2012. Association between single nucleotide polymorphisms of BARD 1 gene and susceptibility of early-onset breast cancer in Uygur women in Xinjiang. Zhonghua Zhong Liu Za Zhi 34, 341–347.

Szychot, E., Apps, J., Pritchard-Jones, K., 2014. Wilms' tumor: biology, diagnosis and treatment. Transl. Pediatr. 3, 12–24.

Tan, H., Bao, J., Zhou, X., 2015. Genome-wide mutational spectra analysis reveals significant cancer-specific heterogeneity. Sci. Rep. 5, 12566.

Vahteristo, P., Syrjakoski, K., Heikkinen, T., Eerola, H., Aittomaki, K., von Smitten, K., Holli, K., Blomqvist, C., Kallioniemi, O.P., Nevanlinna, H., 2006. BARD1 variants Cys557Ser and Val507Met in breast cancer predisposition. Eur. J. Hum. Genet. 14, 167–172.

Wooster, R., Neuhausen, S.L., Mangion, J., Quirk, Y., Ford, D., Collins, N., Nguyen, K., Seal, S., Tran, T., Averill, D., et al., 1994. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 265, 2088–2090.

Wu, L.C., Wang, Z.W., Tsan, J.T., Spillman, M.A., Phung, A., Xu, X.L., Yang, M.C., Hwang, L.Y., Bowcock, A.M., Baer, R., 1996. Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nat. Genet. 14, 430–440.

Zhang, R., Zou, Y., Zhu, J., Zeng, X., Yang, T., Wang, F., He, J., Xia, H., 2016. The association between GWAS-identified BARD1 gene SNPs and neuroblastoma susceptibility in a Southern Chinese population. Int. J. Med. Sci. 13, 133–138.

Zheng, J., Zhang, R., Zhu, J., Wang, F., Yang, T., He, J., Xia, H., 2016. Lack of associations between XPC gene polymorphisms and neuroblastoma susceptibility in a Chinese population. Biomed. Res. Int. 2016, 2932049.

Zhou, X., Han, S., Wang, S., Chen, X., Dong, J., Shi, X., Xia, Y., Wang, X., Hu, Z., Shen, H., 2009. Polymorphisms in HPV E6/E7 protein interacted genes and risk of cervical cancer in Chinese women: a case-control analysis. Gynecol. Oncol. 114, 327–331.

Zhu, J., Wang, M., Zhu, M., He, J., Wang, J.C., Jin, L., Wang, X.F., Xiang, J.Q., Wei, Q., 2015. Associations of PI3KR1 and mTOR polymorphisms with esophageal squamous cell carcinoma risk and gene-environment interactions in Eastern Chinese populations. Sci. Rep. 5, 8250.

Zhu, J., Wang, M., He, J., Zhu, M., Wang, J.C., Jin, L., Wang, X.F., Yang, Y.J., Xiang, J.Q., Wei, Q., 2016. Polymorphisms in the AKT1 and AKT2 genes and oesophageal squamous cell carcinoma risk in an Eastern Chinese population. J. Cell. Mol. Med. 20, 666–677.