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Novel Contribution of Long Non-coding RNA *MEG3* Genotype to Prediction of Childhood Leukemia Risk

JEN-SHENG PEI[1*], WEN-SHIN CHANG[2,3*], CHAO-CHUN CHEN[1*], MEI-CHIN MONG[4], SHIH-WEI HSU[5], PEI-CHEN HSU[1], YUAN-NIAN HSU[6], YUN-CHI WANG[2,3], CHIA-WEN TSAI[2,3] and DA-TIAN BAU[2,3,7]

[1]*Department of Pediatrics, Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan, R.O.C.;*
[2]*Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, R.O.C.;*
[3]*Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[4]*Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan, R.O.C.;*
[5]*Taichung Armed Forces General Hospital, Taichung, Taiwan, R.O.C.;*
[6]*Department of Family Medicine, Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan, R.O.C.;*
[7]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: Acute lymphoblastic leukemia (ALL) is frequent among children. Few studies have researched the relationship between maternally expressed gene 3 (MEG3) and cancer risk. We hypothesized long non-coding RNA MEG3 polymorphisms might influence the risk of childhood ALL. Materials and Methods: In a total of 266 patients with childhood ALL and 266 healthy controls, genotypes of MEG3 rs7158663, rs3087918, rs11160608 and rs4081134 single nucleotide polymorphisms were investigated for their associations with childhood ALL. Results: MEG3 rs7158663 AG and AA genotypes were significantly associated with ALL [odds ratio=1.61 (95% confidence interval=1.12-2.31) and 2.21 (1.16-4.22), respectively]. The A allele also exhibited a statistical association with higher risk of ALL (p=0.0015). There was no positive association as for rs3087918, rs11160608 or rs4081134. Interestingly, a significant interaction between MEG3 rs7158663 and age (≥3.5 years) and gender (male) was found. Conclusion: MEG3 rs7158663 AG/AA genotypes were associated with higher susceptibility to childhood ALL. These novel findings should be validated in larger populations and different ethnicities.*

Childhood acute lymphoblastic leukemia (ALL) is a malignant disorder commonly found among children and frequently in children less than 15 years of age. The peak ages of early onset are 2-5 years old (1, 2), accounting for approximately one-third of childhood tumors (3). The etiology and pathogenesis remain unclear, and it is believed that childhood ALL is the consequence of a combinative influence of genetic variations and lifestyle/environmental factors (4, 5). In recent years, there is mounting evidence that single nucleotide polymorphisms (SNPs) may play an important role in determining the risk for childhood ALL (6-11). However, the role of many genetic factors is unclear, and further exploration of the complex genomic understanding about childhood ALL is urgently needed.

Long non-coding RNAs (lncRNA) are defined as RNAs longer than 200 nucleotides and form a novel group of non-coding RNAs, with microRNA-like features, which are not translated into functional proteins (12). Mounting evidence has shown that lncRNAs play a critical role in controlling a variety of biological processes, including leukemia progression, immune regulation, carcinogenesis, tumor metastasis and drug resistance (13-15). The maternally expressed gene 3 (*MEG3*) encodes a myelocyte-related lncRNA and is believed to be involved in the process of carcinogenesis and responsiveness to chemotherapy (16, 17). *MEG3* is considered a tumor suppressor (15). It has been reported to play a role in several

*These Authors contributed equally to this study.

Correspondence to: 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: datian@mail.cmuh.org.tw (D-TB); artbau2@gmail.com (D-TB)

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types of cancer, including bladder (18), gastric (19), and lung (20), as well as hepatocellular carcinoma (21). Accumulating evidence has suggested that genetic variants in the *MEG3* gene predispose to cancer. However, the impacts of *MEG3* polymorphisms in childhood ALL remain unclear. According to the information above, we were interested in investigating the contribution of *MEG3* genotypes to childhood ALL. We also summarize the literature on the associations of *MEG3* genotypes with various types of cancer to provide the reader with a better understanding of the contribution of *MEG3* genotype to cancer risk.

Materials and Methods

Recruitment of childhood leukemia cases and healthy controls. Childhood ALL cases were ascertained by pediatric oncologists with pathological confirmation. All the recruited cases completed a questionnaire with help from their parents or guardians, and donated blood samples. Healthy controls without prior history of any tumor were recruited in the same period. Healthy controls were matched to each case by age (±2 years) and gender. All the participants were Taiwanese. This study was approved by the Institutional Review Board of China Medical University Hospital (DMR103-IRB-153).

Genotyping methodology for MEG3 genotype. Peripheral blood was collected from each participant and the genomic DNA was extracted within 24 h (22). The genotypes at *MEG3* SNP rs7158663, rs3087918, rs11160608 and rs4081134 were determined using a TaqMan assay with an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). For quality control, two negative controls (distilled water without DNA sample) were included in each 96-well plate. Furthermore, genotyping of about 3% of randomly selected samples was repeated in a blind fashion to confirm the genotyping results.

Statistical methodology. The good-of-fit chi-square test was used to tests for deviation from Hardy–Weinberg equilibrium of the selected polymorphic sites. Student's *t*-test was used to check the distributions of ages. Pearson's chi-square methodology was used in checking the distribution pattern of *MEG3* genotypes and the interaction of *MEG3* genotype with age and gender. The contribution of *MEG3* genotypes to childhood ALL were also validated with the corresponding odds ratios (ORs) and 95% confidence intervals (CIs). Any association with a *p*-value less than 0.05 was considered statistically significant

Results

Comparison of basic and clinical demographics. The distributions of age of ALL onset, gender and white blood cell counts of childhood ALL cases and matched healthy controls are shown in Table I. In addition, the immunophenotype, risk classification and survival time of the childhood leukemia cases are also shown. There was no difference in distributions of age and gender between the cases and controls ($p>0.05$) since the two groups were matched for these indices (Table I). The mean white blood cell count of childhood ALL cases was significantly higher than those of healthy controls ($p<0.0001$). Among the patients, 48.9% (130 in number) were at standard risk, 25.2% (67 in number) were at high risk, and 25.9% (69 in number) were at very high risk. Lastly, 25.9% of the patients survived less than 5 years (Table I).

Table I. *Distribution of some basic and clinical demographics of the 266 patients with childhood acute lymphoblastic leukemia and the 266 matched controls*.

Characteristic	Controls (n=266)	Cases (n=266)	*p*-Value
Age at onset age, years			
Mean±SD	8.3±4.8	7.0±4.4	0.6483[a]
Gender, n (%)			
Male	148 (55.6%)	148 (55.6%)	
Female	118 (44.4%)	118 (44.4%)	>0.99[b]
White blood cell count ($\times 10^9$/l)			
Mean±SD	7.5±2.0	54.3±75.9	**<0.0001**
Immunophenotype, n (%)			
B Subtype		227 (85.3%)	
T Subtype		39 (14.7%)	
Risk classification, n (%)			
Standard risk		130 (48.9%)	
High risk		67 (25.2%)	
Very high risk		69 (25.9%)	
Survival, years			
<5 Years		69 (25.9%)	
≥5 Years		197 (74.1%)	

SD: Standard deviation. [a]Based on Student's *t*-test; [b]based on chi-square test without Yates' correction. Statistically significant *p*-values are shown in bold.

Significant association between MEG3 rs7158663 genotypes and childhood ALL. The genotypic distributions of *MEG3* rs7158663 among the controls and the childhood ALL patients are presented and analyzed in Table II. Firstly, the genotypic frequencies of *MEG3* rs7158663 among the control individuals were in Hardy–Weinberg equilibrium ($p=0.7108$). Secondly, the genotypes of *MEG3* rs7158663 were differently distributed between the childhood ALL and healthy control groups (p for trend=0.0064) (Table II). Carrying *MEG3* rs7158663 heterozygous AG or homozygous AA genotype was associated with an increased childhood ALL risk, compared with the wild-type GG genotype (OR=1.61 and 2.21, 95% CI=1.12-2.31 and 1.16-4.22; $p=0.0097$ and 0.0142, respectively). Thirdly, in the recessive model, compared with those carrying GG and AG genotypes, risk of ALL for homozygous variant AA genotype carriers at *MEG3* rs7158663 did not reach a significant level (OR=1.79, 95% CI=0.96-3.35; $p=0.0641$). Fourthly, in the dominant model, there was an increased risk of childhood ALL for

Table II. *Maternally expressed gene 3 (MEG3) rs7158663 genotypes among the 266 patients with childhood acute lymphoblastic leukemia and 266 healthy controls.*

		Controls, n (%)	Patients, n (%)	OR (95% CI)	*p*-Value[a]
Genotype	GG	153 (57.5%)	118 (44.4%)	1.00 (Reference)	
	AG	96 (36.1%)	119 (44.7%)	1.61 (1.12-2.31)	**0.0097**
	AA	17 (6.4%)	29 (10.9%)	2.21 (1.16-4.22)	**0.0142**
	p_{trend}				**0.0064**
	p_{HWE}				0.7108
Carrier comparison	GG+AG	249 (93.6%)	237 (89.1%)	1.00 (Reference)	
	AA	17 (6.4%)	29 (10.9%)	1.79 (0.96-3.35)	0.0641
	GG	153 (57.5%)	118 (44.4%)	1.00 (Reference)	
	AG+AA	113 (42.5%)	148 (55.6%)	1.70 (1.21-2.39)	**0.0024**

CI: Confidence interval; OR: odds ratio; p_{trend}: *p*-value for trend analysis; p_{HWE}: *p*-value for Hardy–Weinberg equilibrium analysis. [a]Based on chi-square test without Yates' correction. Statistically significant *p*-values are shown in bold.

Table III. *Maternally expressed gene 3 (MEG3) rs3087918 genotypes among the 266 patients with childhood acute lymphoblastic leukemia and 266 healthy controls.*

		Controls, n (%)	Patients, n (%)	OR (95% CI)	*p*-Value[a]
Genotype	TT	98 (36.9%)	105 (39.5%)	1.00 (Reference)	
	GT	128 (48.1%)	129 (48.5%)	0.94 (0.65-1.36)	0.7445
	GG	40 (15.0%)	32 (12.0%)	0.75 (0.44-1.28)	0.2885
	p_{trend}				0.5672
	p_{HWE}				0.8647
Carrier comparison	TT+GT	226 (85.0%)	234 (88.0%)	1.00 (Reference)	
	GG	40 (15.0%)	32 (12.0%)	0.77 (0.47-1.27)	0.3106
	TT	98 (36.9%)	105 (39.5%)	1.00 (Reference)	
	GT+GG	168 (63.1%)	161 (60.5%)	0.89 (0.63-1.27)	0.5321

CI: Confidence interval; OR: odds ratio; ptrend: *p*-value for trend analysis; p_{HWE}: *p*-value for Hardy–Weinberg equilibrium analysis. [a]Based on chi-square test without Yates' correction.

those carrying an A allele at *MEG3* rs7158663, compared with those with homozygous GG genotype (OR=1.70, 95% CI=1.21-2.39, *p*=0.0024). These results showed that AG and AA genotype carriers at *MEG3* rs7158663 had a significantly elevated risk for childhood ALL.

Non-significant association for other MEG3 SNPs examined. The genotypic distributions of *MEG* rs3087918, rs11160608, and rs4081134 are presented and analyzed in Table III, Table IV and Table V, respectively. The genotypic frequencies of *MEG3* rs3087918, rs11160608, and rs4081134 among the control individuals all fit the Hardy–Weinberg equilibrium (all $p>0.05$). However, none of these SNPs seemed to be significantly associated with ALL in any model analyzed (all $p>0.05$).

Allelic frequency distribution analysis supported the findings. To validate the results shown in Table II to Table V, analysis of the allelic frequency distribution for *MEG3* rs7158663, rs3087918, rs11160608, and rs4081134 SNPs were conducted, and the results are shown in Table VI. Most importantly, the variant A allele at *MEG3* rs7158663 was found in 33.3% in the childhood ALL group and in 24.4% in the control group (OR=1.54, 95% CI=1.18-2.01, *p*=0.0015, Table VI). This supports the finding that the A allele was associated with an increased risk of childhood ALL in Taiwanese. For other SNPs, including rs3087918, rs11160608, and rs4081134, none of the variant alleles seemed to be significantly associated with childhood ALL risk.

Interaction of MEG3 rs7158663 genotypes with age and gender. We were interested in examining whether there was any difference of *MEG3* rs7158663 genotypes according to age and gender. Table VII shows that among children aged less than 3.5 years, those with *MEG3* rs7158663 AG and AA variant genotypes had non-significant odds of having childhood ALL (95% CI=0.97-2.70 and 0.54-3.43, *p*=0.0627 and 0.5048, respectively). Even after adjustment for gender, the level was similar and did not reach the significance level (adjusted OR=1.78 and 1.43, 95% CI=0.92-2.62 and 0.52-

Table IV. *Maternally expressed gene 3 (MEG3) rs11160608 genotypes among the 266 patients with childhood acute lymphoblastic leukemia and 266 healthy controls.*

		Controls, n (%)	Patients, n (%)	OR (95% CI)	*p*-Value[a]
Genotype	AA	85 (32.0%)	80 (30.1%)	1.00 (Reference)	
	AC	131 (49.2%)	137 (51.5%)	1.11 (0.75-1.64)	0.5944
	CC	50 (18.8%)	49 (18.4%)	1.04 (0.63-1.71)	0.8737
	p_{trend}				0.8625
	p_{HWE}				0.9699
Carrier comparison	AA+AC	216 (81.2%)	217 (81.6%)	1.00 (Reference)	
	CC	50 (18.8%)	49 (18.4%)	0.98 (0.63-1.51)	0.9113
	AA	85 (32.0%)	80 (30.1%)	1.00 (Reference)	
	AC+CC	181 (68.0%)	186 (69.9%)	1.09 (0.76-1.58)	0.6393

CI: Confidence interval; OR: odds ratio; ptrend: *p*-value for trend analysis; p_{HWE}: *p*-value for Hardy–Weinberg equilibrium analysis. [a]Based on chi-square test without Yates' correction.

Table V. *Maternally expressed gene 3 (MEG3) rs4081134 genotypes among the 266 patients with childhood acute lymphoblastic leukemia and 266 healthy controls.*

		Controls, n (%)	Patients, n (%)	OR (95% CI)	*p*-Value[a]
Genotype	GG	149 (56.0%)	141 (53.0%)	1.00 (Reference)	
	AG	101 (38.0%)	107 (40.2%)	1.12 (0.78-1.60)	0.5345
	AA	16 (6.0%)	18 (6.8%)	1.19 (0.58-2.42)	0.6335
	p_{trend}				0.7744
	p_{HWE}				0.8381
Carrier comparison	GG+AG	250 (94.0%)	248 (93.2%)	1.00 (Reference)	
	AA	16 (6.0%)	18 (6.8%)	1.13 (0.57-2.27)	0.7230
	GG	149 (56.0%)	141 (53.0%)	1.00 (Reference)	
	AG+AA	117 (44.0%)	125 (47.0%	1.13 (0.80-1.59)	0.4861

CI: Confidence interval; OR: odds ratio; p_{trend}: *p*-value for trend analysis; pHWE: *p*-value for Hardy–Weinberg equilibrium analysis. [a]Based on chi-square test without Yates' correction.

Table VI. *Distribution of allelic frequencies for rs7158663, rs3087918, rs11160608 and rs4081134 single nucleotide polymorphisms (SNPs) of maternally expressed gene 3 (MEG3) among the 266 patients with childhood acute lymphoblastic leukemia and 266 healthy controls.*

SNP	Allele	Controls, n (%)	Cases, n (%)	OR (95% CI)	*p*-Value[a]
rs7158663	G	402 (75.6%)	355 (66.7%)	1.00 (Reference)	
	A	130 (24.4%)	177 (33.3%)	1.54 (1.18-2.01)	**0.0015**
rs3087918	T	324 (60.9%)	339 (63.7%)	1.00 (Reference)	
	G	208 (39.1%)	193 (36.3%)	0.89 (0.69-1.14)	0.3427
rs11160608	A	301 (56.6%)	297 (55.8%)	1.00 (Reference)	
	C	231 (43.4%)	235 (44.2%)	1.03 (0.81-1.31)	0.8048
rs4081134	G	399 (75.0%)	389 (73.1%)	1.00 (Reference)	
	A	133 (25.0%)	143 (26.9%)	1.10 (0.84-1.45)	0.4843

CI: Confidence interval; OR: odds ratio. [a]Based on chi-square test without Yates' correction. Statistically significant *p*-values are shown in bold.

3.34, respectively) (Table VII). Among children aged 3.5 years and over, those with *MEG3* rs7158663 AA genotype were at 3.49-fold odds of having childhood ALL (95% CI=1.36-8.92, *p*=0.0066). The difference was even more significant after adjusting for gender (adjusted OR=3.52, 95% CI=1.42-8.75, 0.0066; Table VII).

Table VIII shows that among boys, those with *MEG3* rs7158663 AG and AA genotypes had 1.75- and 3.11-fold of

Table VII. *Maternally expressed gene 3 (MEG3) rs7158663 genotype in childhood acute lymphoblastic leukemia risk after stratification by age.*

	Age <3.5 years					Age ≥3.5 years, n (%)				
Genotype	Controls, n (%)	Cases, n (%)	OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value	Controls, n (%)	Cases, n (%)	OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value
GG	77 (57.9)	62 (46.6)	1.00 (ref)	1.00 (ref)		76 (57.1)	56 (42.1)	1.00 (ref)	1.00 (ref)	
AG	46 (34.6)	60 (45.1)	1.62 (0.97-2.70)	1.78 (0.92-2.62)	0.0627	50 (37.6)	59 (44.4)	1.60 (0.96-2.67)	1.65 (0.93-2.72)	0.0702
AA	10 (7.5)	11 (8.3)	1.37 (0.54-3.43)	1.43 (0.52-3.34)	0.5048	7 (5.3)	18 (13.5)	3.49 (1.36-8.92)	3.52 (1.42-8.75)	**0.0066**
Total	133	133				133	133			
p_{trend}					0.1724					**0.0135**

CI: Confidence interval; aOR, adjusted odds ratio; OR: odds ratio; p_{trend}, *p*-value for trend analysis. [a]By multivariate logistic regression analysis; [b]by multivariate logistic regression analysis after adjusting for gender. Statistically significant *p*-values are shown in bold.

Table VIII. *Maternally expressed gene 3 (MEG3) rs7158663 genotype in childhood acute lymphoblastic leukemia risk after stratification by gender.*

	Male					Female				
Genotype	Controls, n (%)	Cases, n (%)	OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value	Controls, n (%)	Cases, n (%)	OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value
GG	87 (58.8)	63 (42.6)	1.00 (ref)	1.00 (ref)		66 (55.9)	55 (46.6)	1.00 (ref)	1.00 (ref)	
AG	53 (35.8)	67 (45.3)	1.75 (1.08-2.83)	1.63 (1.02-2.07)	**0.0238**	43 (36.4)	52 (44.1)	1.45 (0.85-2.49)	1.37 (0.73-2.06)	0.1756
AA	8 (5.4)	18 (12.1)	3.11 (1.27-7.59)	2.59 (1.34-4.49)	**0.0101**	9 (7.7)	11 (9.3)	1.47 (0.57-3.80)	1.41 (0.49-2.77)	0.4281
Total	148	148				118	118			
p_{trend}					**0.0095**					0.3583

CI: Confidence interval; aOR, adjusted odds ratio; OR: odds ratio; p_{trend}, *p*-value for trend analysis. [a]By multivariate logistic regression analysis; [b]by multivariate logistic regression analysis after adjusting for age. Statistically significant *p*-values are shown in bold.

odds of having childhood ALL (95% CI=1.08-2.83 and 1.27-7.59, *p*=0.0238 and 0.0101), respectively. Even after adjustment for age, the significance remained (adjusted OR=1.63 and 2.59, 95% CI=1.02-2.07 and 1.34-4.49, respectively) (Table VIII). Among girls, these differences were not as obvious as for the boys, no matter their genotype, nor before and after adjusting for their age (Table VIII).

Discussion

lncRNAs play a critical role in leukemia development and scientists are devoted to elucidating their various biological functions involved in leukemia carcinogenesis (15). Among these, *MEG3* is a tumor suppressor, and *via* various signaling pathways, it is highly involved in the regulation of the p53 expression and p53-dependent transcription (23). In a panel of cancer types, protein expression of lncRNA *MEG3* was found to be down-regulated in gastric, liver, colorectal, breast and ovarian cancer (24). However, (childhood) leukemia is not in this list. SNPs of *MEG3* have been associated with cell phenotype alteration, cancer susceptibility, and chemotherapy toxicity in patients with solid tumors (24, 25). To our knowledge, the current study is the first to investigate the association of *MEG3* genotypes with childhood leukemia.

In the current study, we revealed that the frequencies of GG, AG, and AA genotypes at *MEG3* rs7158663 were 57.5%, 36.1%, and 6.4% in healthy Taiwanese controls (Table II). Among the childhood ALL cases, *MEG3* rs7158663 variants AG and AA were both statistically significantly more frequent (44.7% and 10.9%) than in the corresponding controls (Table II). We confirmed the findings *via* carrier comparisons and allelic frequency analysis, revealing that variant AA genotype and A allele contribute to elevated childhood ALL risk (Table II and Table VI). On the contrary, no positive association was found for *MEG3* rs3087918, rs11160608, or rs4081134 (Table III, Table IV, Table V and Table VI). In addition, the present study is the first to reveal a significant interaction of *MEG3* rs7158663 genotypes with age and gender for childhood ALL risk (Table VII and Table VIII). Our studies demonstrated that older children (≥3.5 years old) with AA genotype at *MEG3* rs7158663 and boys carrying one or more A alleles tended to be more likely to have childhood ALL (Table VII and Table VIII). The detailed mechanisms of how *MEG3*

Table IX. *Summary of findings from literature (until 31/10/2021) on the association of single nucleotide polymorphisms of maternally expressed gene 3 (MEG3) genotype among different types of cancer.*

First author (Ref)	Year published	Cohort ethnicity	Cancer type	Controls, n	Cases, n	Highlight of genotyping findings
Cao *et al*. (26)	2016	Han	Colorectal	527	518	rs7158663 AA genotype contributed to higher risk rs3087918 did not contribute to altered risk rs11160608 did not contribute to altered risk rs4081134 did not contribute to altered risk rs10144253 did not contribute to altered risk
Yang *et al*. (27)	2018	Han	Lung	526	526	rs4081134 AA genotype contributed to lower risk rs7158663 did not contribute to altered risk
Zhuo *et al*. (28)	2018	Han	Neuroblastoma	783	392	rs4081134 AA/AG genotypes contributed to higher risk rs7158663 did not contribute to altered risk
Hou *et al*. (29)	2019	Han	Oral	984	444	rs11160608 AC genotype contributed to higher risk rs3783355 did not contribute to altered risk rs4378559 did not contribute to altered risk rs4906024 did not contribute to altered risk rs4081134 did not contribute to altered risk rs2281511 did not contribute to altered risk rs12431658 did not contribute to altered risk
Ali *et al*. (24)	2020	Egyptian	Breast	154	150	rs7158663 AA genotype contributed to higher risk
Zheng *et al*. (25)	2020	Han	Breast	700	434	rs3087918 GG genotype contributed to lower risk rs7158663 did not contribute to altered risk rs3087918 did not contribute to altered risk
Xu *et al*. (30)	2021	Han	Prostate	200	165	rs11627993 did not contribute to altered risk rs7158663 did not contribute to altered risk
Shaker *et al*. (31)	2021	Egyptian	Breast	150	180	rs7158663 AG/AA genotypes contributed to higher risk
Pei *et al*.	Current	Taiwanese	Childhood ALL	266	266	rs7158663 AG/AA genotypes contributed to higher risk rs3087918 did not contribute to altered risk rs11160608 did not contribute to altered risk rs4081134 did not contribute to altered risk

ALL: Acute lymphocytic leukemia.

genotype contributes to age and gender differences in the etiology of childhood ALL need further investigation.

In literature, there are very few articles reporting the association of *MEG3* genotypes with cancer risk, not to mention (childhood) leukemia. This does not mean the *MEG3* genotype is irrelevant to cancer development, on the contrary, it means that genotyping investigations focusing on *MEG3* genotypes and their contribution to all types of cancer are urgently needed. To provide a clearer picture of how important *MEG3* genotypes are in determining and predicting cancer risk, we summarize the contribution of *MEG3* genotypes to cancer risk as given in literature.

In 2016, Cao *et al*. explored the contributions of five *MEG3* polymorphic variants, rs3087918, rs11160608, rs7158663, rs4081134 and rs10144253, to colorectal cancer risk. They found that only the AA genotype of *MEG3* rs7158663 significantly increased colorectal cancer risk, especially in those aged less than 60 years and with a family history of cancer (26). In 2018, Yang *et aI*. examined the associations of *MEG3* rs7158663 and rs4081134 with lung cancer risk with a 526-case and 526-control Han population. They found that the AA genotype of *MEG3* rs4081134 may be associated with a reduced lung cancer risk, while no association between the *MEG3* rs7158663 genotype and lung cancer risk was found (27). In the same year, Zhuo and colleagues explored the role of *MEG3* rs7158663 and rs4081134 in childhood neuroblastoma. Theirs was a very valuable in case–control study since the research in childhood neuroblastoma are quite rare, and the sample size was relatively large, with 392 cases and 783 controls. They found AG and AA genotypes of *MEG3* rs4081134 tended to develop neuroblastoma among subgroups aged >18 months and with clinical stage III/IV disease. No association of *MEG3* rs7158663 with childhood neuroblastoma was found (28). In 2019, Hou and colleagues investigated the contributions of the genotypes of seven polymorphic sites on *MEG3*, rs4378559, rs3783355, rs11160608, rs4906024, rs4081134, rs2281511 and rs12431658, to oral cancer risk. The polymorphic sites are all intronic sites, while they omitted *MEG3* rs7158663. They found a critical role of *MEG3* rs11160608 in oral carcinogenesis within a Han population of 984 controls and 444 cases. They also conducted a phenotypic luciferase assay, reporting that the activity of *MEG3*

rs11160608 A allele was lower than that of the rs11160608 C allele (29). In 2020, Ali *et al*. attempted to determine the influence of *MEG3* rs7158663 on the serum *MEG3* level and breast cancer risk in an Egyptian population of 154 controls and 150 patients with breast cancer. They found the AA genotype at *MEG3* rs7158663 not only correlated with a lower level expression of *MEG3* in serum, but also with an elevated breast cancer risk (24). In 2020, Zheng and colleagues examined the contribution of *MEG3* rs3087918, rs7158663 and rs11160608 to breast cancer risk with 434 cases and 700 controls. They found the GG genotype of *MEG3* rs3087918 was associated with a reduced risk of breast cancer (25). In 2021, Xu *et al*. explored the association of *MEG3* rs11627993 and rs7158663 with prostate cancer risk in a Han population of 165 cases and 200 controls. They had no positive finding (30). Recently, Shaker and colleagues investigated the impact of genotypes of *MEG3* rs7158663 on the expression levels of microRNA-182 and microRNA-29, and on breast cancer risk in a moderate Egyptian population of 150 controls, 120 fibroadenoma and 180 breast cancer cases. The AG and AA genotypes contributed to a higher risk of breast cancer. Moreover, the A allele of *MEG3* rs7158663 was associated with a significantly lower serum level of *MEG3*, which correlated with higher TMN staging and larger tumor size among the breast cancer cases (31) (Table IX).

Several limitations and potential improvements of the present study should be noted. Firstly, these findings were based on genomic analysis of a single gene with a relatively moderate sample size of childhood ALL cases which need to be validated using more independent and larger investigations. Secondly, our study was a hospital-based study, although two well-known and outstanding medical centers were involved. Therefore, potential sources of selection bias may exist. Thirdly, the current study had incomplete information on lifestyle, environmental and familial factors which may contribute to childhood ALL. Further studies are needed to reveal the correlation of lifestyle/environmental information and family history with genetic variants such as *MEG3* in childhood ALL. The mRNA level of *MEG3* was reported to be lower in patients with breast cancer than in healthy controls, and those with *MEG3* rs7158663 AA genotype had the lowest level among the various genotypes (24). Therefore, the phenotype of *MEG3* rs7158663 AA genotype should be validated among patients with childhood ALL, and the consequences of a lower serum *MEG3* level should be further investigated.

In conclusion, this study provides first-hand evidence that the A allele at *MEG3* rs7158663 may serve as a predictor for childhood ALL. Moreover, there was an obvious interaction of age (≥3.5 years) and gender (boys) with *MEG3* rs7158663 genotype on personal susceptibility to childhood ALL. Further studies are needed to validate the clinical practicability of this novel biomarker.

Conflicts of Interest

All the Authors declare no conflicts of interest in this study.

Authors' Contributions

Research Design: Pei JS, Chang WS, Bau DT and Tsai CW; Patient and Questionnaire Summarize: Chen CC, Pei JS and Hsu PC; Experiment Data Clearing and Checking: Mong MC and Hsu SW; Statistical Analysis: Hsu YN and Wang YC Manuscript Writing: Chang WS, Tsai CW and Bau DT; Reviewing and Revising: Bau DT.

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