Significant Associations of IncRNA H19 Genotypes with Susceptibility to Childhood Leukemia in Taiwan

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Abstract: The purpose of our study was to investigate whether genetic variations in IncRNA H19 were associated with susceptibility to childhood leukemia. Two hundred and sixty-six childhood leukemia patients and 266 healthy controls were enrolled in Taiwan, and two single nucleotide polymorphisms (SNPs), rs2839698 and rs217727, in H19 were genotyped and analyzed. There was a significant difference in the genotypic distribution of rs2839698 between patients and healthy controls (p = 0.0277). Compared to the wild-type CC genotype, the heterozygous variant CT and homozygous variant TT genotypes were associated with significantly increased risks of childhood leukemia with an adjusted odd ratio (OR) of 1.46 (95% confidence interval (CI), 1.08–2.14, p = 0.0429) and 1.94 (95%CI, 1.15–3.31, p = 0.0169), respectively (log trend = 0.0277). The difference in allelic frequencies between childhood leukemia patients and controls was also significant (T versus C, adjusted OR = 1.53, 95%CI, 1.13–1.79, p = 0.0077). There were no significant differences in the genotypic and allelic distributions of rs217727 between cases and controls. Interestingly, the average level of H19 rs2839698 was statistically significantly higher for patients with CT and TT genotypes than from those with the CC genotype (p < 0.0001). Our results indicate that H19 SNP rs2839698, but not rs217727, may serve as a novel susceptibility marker for childhood leukemia.

Keywords: IncRNA; H19; SNP; childhood leukemia; genetic susceptibility; Taiwan

1. Introduction

Acute lymphoblastic leukemia is the most common type of malignancy among children worldwide. The contributions of inherited genetic factors to the etiology of childhood leukemia have been reported by a few candidate gene association studies, but more remain to be found [1–10]. Long non-coding RNAs (lncRNAs) are defined as non-protein-coding transcripts [11]. Although the functions of most lncRNAs are still not well understood, the number of functionally characterized lncRNAs keeps increasing. LncRNAs play important roles in the regulation of gene expression at transcriptional and post-transcriptional level and are involved in development, differentiation, and human diseases [12–17]. In recent years, the contributions of lncRNAs to carcinogenesis have been documented and have attracted the interest of scientists [18–20].

H19 is a lncRNA of 2.3 kb [21], which is known to be abundantly expressed in embryonal tissue and dramatically down-regulated after birth [22]. Abnormal over-expression of
H19 has been noted in many types of cancer [23], and accumulating evidence indicated that H19 is involved in cancer initiation, progression, and metastasis [24]. Given the oncogenic roles of H19 and the functions of genetic variants in modulating the expression or structure of H19, increasing studies have been performed to examine the associations of single nucleotide polymorphisms (SNPs) in H19 with genetic susceptibility to cancers [25–27]. Two SNPs, rs2839698 and rs217727, have been shown to be associated with the risks of different cancers, such as bladder cancer and breast cancer [25–27]. However, no study has been conducted in childhood leukemia. We hypothesize that H19 rs2839698 and rs217727 SNPs may confer genetic susceptibility to childhood leukemia and thus conduct this case-control study to examine the association of these two SNPs with childhood leukemia in a Taiwan population.

2. Results

2.1. Comparisons of Basic Characters between the Case and Control Groups

The investigated population in this study contained 266 Taiwan childhood leukemia cases and 266 age- and gender-matched healthy children. The age was frequency-matched, and gender was one-on-one matched. The mean age ± standard deviation (SD) was 7.0 ± 4.4 for the cases and 8.3 ± 4.8 for the controls (p = 0.6483). There were 148 boys and 118 girls in cases and controls (Table 1).

Table 1. Distribution of onset age and gender of the 266 childhood leukemia patients and 266 healthy controls.

| Characteristics                  | Cases, n = 266 | Controls, n = 266 | p-Value         |
|----------------------------------|----------------|-------------------|-----------------|
| Onset age, year                  |                |                   |                 |
| Gender                           |                |                   |                 |
| Boy                              | 148 (55.6%)    | 148 (55.6%)       | 0.6483<sup>a</sup> |
| Girl                             | 118 (44.4%)    | 118 (44.4%)       | 1.0000<sup>b</sup> |
| While blood cell counts (10<sup>9</sup>/L) | 7.0 ± 4.4      | 8.3 ± 4.8         |                 |
| t(1;19)                          | 11 (4.1%)      | 148 (55.6%)       | <0.0001         |
| t(4;11)                          | 4 (1.5%)       | 148 (55.6%)       |                 |
| t(9;22)                          | 6 (2.3%)       | 118 (44.4%)       |                 |
| ETV6-RUNX1<sup>c</sup>          | 28 (10.5%)     | 148 (55.6%)       |                 |
| Immunophenotype                  |                |                   |                 |
| B subtype                        | 227 (85.3%)    | 148 (55.6%)       |                 |
| T subtype                        | 39 (14.7%)     | 118 (44.4%)       |                 |
| Risk classification              |                |                   |                 |
| Standard risk                    | 130 (48.9%)    | 148 (55.6%)       |                 |
| High risk                        | 67 (25.2%)     | 118 (44.4%)       |                 |
| Very high risk                   | 69 (25.9%)     | 118 (44.4%)       |                 |
| Survival time, year              |                |                   |                 |
| <5                               | 69 (25.9%)     | 148 (55.6%)       |                 |
| ≥5                               | 197 (74.1%)    | 118 (44.4%)       |                 |

Abbreviations: SD, standard deviation; Notes: <sup>a</sup> Based on Student's t-test; <sup>b</sup> based on chi-square test without Yates’ correction; <sup>c</sup> available for only some of the patients.

2.2. The Relationships between H19 rs2839698 Polymorphism and Risk of Childhood Leukemia

Table 2 shows the genotypic frequencies of H19 rs2839698 SNP in cases and controls. The genotypic distribution was consistent with the Hardy–Weinberg equilibrium (HWE) in the controls (p = 0.8781). The genotype distribution was significantly different between cases and controls (p = 0.0277). In multivariate logistic regression analysis, compared to the wild-type CC genotype, the heterozygous variant CT and homozygous variant TT genotypes were associated with significantly increased risks of childhood leukemia with an adjusted OR of 1.46 (95% CI, 1.08–2.14, p = 0.0429) and 1.94 (95% CI, 1.15–3.31, p = 0.0169), respectively. In the dominant model, individuals carrying the variant genotypes (CT+TT) had an elevated risk of childhood leukemia (adjusted OR = 1.68, 95% CI = 1.12–
2.23, \( p = 0.0130 \), Table 2) compared to the CC genotype. In the allelic test, the “T” allele was associated with a significantly increased risk of childhood leukemia compared to the “C” allele (adjusted OR = 1.53, 95%CI = 1.13–1.79, \( p = 0.0077 \), Table 3).

Table 2. Distributions of H19 rs2839698 genotypes between childhood leukemia and control groups.

| Index | Genotype | Cases          | Controls        | \( p \)-Value | Crude OR (95%CI) |
|-------|----------|----------------|-----------------|---------------|-----------------|
| rs2839698 | CC       | 91 (34.2%)     | 119 (44.7%)     | 1.00 (Ref)   |                 |
|        | CT       | 131 (49.3%)    | 117 (44.0%)     | 0.0429 *     | 1.46 (1.08–2.14) |
|        | TT       | 44 (16.5%)     | 30 (11.3%)      | 0.0169 *     | 1.94 (1.15–3.31) |
|        | CT+TT    | 175 (65.8%)    | 147 (55.3%)     | 0.0130 *     | 1.68 (1.12–2.23) |
|        | \( P_{\text{trend}} \) |            |                 | 0.0277 *     |                 |
|        | \( P_{\text{HWE}} \) |            |                 | 0.8781       |                 |

Abbreviations: OR, odds ratio; CI, confidence interval; Notes: \( p \)-values are calculated by chi-square without Yates’ correction; * Bold values show significance.

Table 3. Allelic frequencies for H19 rs2839698 polymorphism among childhood leukemia and control groups.

| Allele | Cases, \( n \) (%) \((n = 532)\) | Controls, \( n \) (%) \((n = 532)\) | Adjusted OR (95%CI) \( a \) | \( p \)-Value \( b \) |
|--------|----------------------------------|-------------------------------------|-------------------------------|------------------------|
| C      | 355 (66.7)                       | 355 (66.7)                         | 1.00 (Reference)             | 0.0077 *               |
| T      | 177 (33.3)                       | 355 (66.7)                         | 1.53 (1.13–1.79)             |                        |

Abbreviations: OR, odds ratio; CI, confidence interval. \( a \) Data adjusted for age. \( b \) Based on chi-square test without Yates’ correction; * Bold values show significance.

2.3. The Relationships between H19 rs217727 Polymorphism and Risk of Childhood Leukemia

The genotypic frequencies of the H19 rs217727 SNP among childhood leukemia patients and controls are shown in Table 4. The genotypic distribution of H19 rs217727 polymorphism among the control group was consistent with HWE \( (p = 0.9610) \). There was no significant difference in genotypic distribution of the rs217727 SNP between childhood leukemia patients and controls \( (p = 0.9165) \). There were no altered risks of childhood leukemia associated with rs217727 in both genotypic (Table 4) and allelic tests (Table 5).

Table 4. Distributions of H19 rs217727 genotypes between childhood leukemia and control groups.

| Index | Genotype | Cases          | Controls        | \( p \)-Value | Crude OR (95%CI) |
|-------|----------|----------------|-----------------|---------------|-----------------|
| rs217727 | CC       | 111 (41.7%)    | 114 (42.9%)     | 1.00 (Ref)   |                 |
|        | CT       | 120 (45.1%)    | 120 (45.1%)     | 0.8857       | 1.04 (0.80–1.41) |
|        | TT       | 35 (13.2%)     | 32 (12.0%)      | 0.6763       | 1.10 (0.72–1.92) |
|        | CT+TT    | 155 (58.3%)    | 152 (57.1%)     | 0.7923       | 1.06 (0.74–1.45) |
|        | \( P_{\text{trend}} \) |            |                 | 0.9165       |                 |
|        | \( P_{\text{HWE}} \) |            |                 | 0.9610       |                 |

Abbreviations: OR, odds ratio; CI, confidence interval; Notes: \( p \)-values are calculated by Chi-square without Yates’ correction.

Table 5. Distributions of H19 rs217727 genotypes between childhood leukemia and control groups.

| Allele | Cases, \( n \) (%) \((n = 532)\) | Controls, \( n \) (%) \((n = 532)\) | Adjusted OR (95%CI) \( a \) | \( p \)-Value \( b \) |
|--------|----------------------------------|-------------------------------------|-------------------------------|------------------------|
| C      | 342 (64.3)                       | 348 (65.4)                         | 1.00 (Reference)             | 0.7000                 |
| T      | 190 (35.7)                       | 184 (34.6)                         | 1.06 (0.84–1.39)             |                        |

Abbreviations: OR, odds ratio; CI, confidence interval. \( a \) Data adjusted for age. \( b \) Based on chi-square test without Yates’ correction.

2.4. The Relationships between H19 rs2839698 Polymorphism with Clinical Features (Immunophenotypes, Risk Classification, and Survival Time)

The association between the H19 rs2839698 genotype with immunophenotypes, risk classification, and survival time of childhood leukemia are shown in Table 6. No statistically
significant correlation was observed between H19 rs2839698 genotypic distributions and immunophenotypes (Table 6). Interestingly, the percentages of CT + TT genotypes of H19 rs2839698 were statistically higher among the patients of in the high risk and very high risk groups, with an adjusted OR of 1.46 (95% CI = 1.04–1.87) and 1.38 (95% CI = 1.02–1.79), respectively (Table 6, middle panel). The association between the H19 rs2839698 genotype and childhood leukemia was significant for survival time <5 years (adjusted OR = 1.43, 95% CI = 1.03–1.84), but not for those ≥5 years (Table 6, lower panel).

### Table 6. Association between the H19 rs2839698 genotype with clinical index of childhood leukemia.

| Index                        | CC          | CT+TT        | Adjusted OR (95%CI) |
|------------------------------|-------------|--------------|---------------------|
| Immunophenotype              |             |              |                     |
| B subtype                    | 77          | 150          | 1.26 (0.91–1.38)    |
| T subtype                    | 14          | 25           | 1.22 (0.83–1.85)    |
| Risk classification          |             |              |                     |
| Standard risk                | 47          | 83           | 1.28 (0.92–1.45)    |
| High risk                    | 21          | 46           | 1.46 (1.04–1.87)    |
| Very high risk               | 23          | 46           | 1.38 (1.02–1.79)    |
| Survival time (years)        |             |              |                     |
| <5                           | 22          | 47           | 1.43 (1.03–1.84)    |
| ≥5                           | 69          | 128          | 0.83 (0.64–1.23)    |

Abbreviations: OR, odds ratio; CI, confidence interval; * ORs, 95% CI were calculated after adjusting for age and gender status; Bold values show significance.

2.5. The Genotype–Phenotype Correlation of H19 rs2839698 Polymorphism

To investigate the genotype–phenotype correlation, we extracted 30 mRNA from the serum of childhood leukemia patients. These samples were obtained from the children before any chemotherapy. The frequencies of the H19 rs2839698 CC, CT, and TT genotypes were 11 (36.7%), 13 (43.3%), and 6 (20.0%), respectively. The influences of various genotypes on the transcriptional expression of mRNA were evaluated by quantitative RT-PCR (Figure 1). As shown in Figure 1, the average level of mRNA for CT and TT genotypes of the H19 rs2839698 was 1.22- and 1.52-fold, compared with that of the CC genotype, respectively. It is of statistically significantly higher level for patients with CT and TT genotypes than from those with the CC genotype (p < 0.0001) (Figure 1).

Figure 1. Comparison of H19 mRNA expression levels with different H19 rs2839698 genotypes. Quantitative real-time polymerase chain reaction (RT-PCR) for three genotypes of H19 rs2839698 from childhood leukemia patients was conducted and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal control. Fold changes were normalized by the GAPDH expression. * p-values < 0.05.
3. Discussion

Previous studies demonstrated that H19 was over-expressed in some types of tumors, such as hepatocellular carcinoma, lung, esophageal, and bladder cancer [28–31]. Therefore, H19 has been suggested to play a role as an oncogene. The signaling network has not yet been fully established. Li and colleagues reported that H19 could bind directly to ISM1 and could also encode miR-675, which promotes gastric cell proliferation and metastasis by targeting CALN1 [32]. H19 could also act as a molecular sponge for let-7, which is a well-known tumor suppressor miRNA capable of targeting and inhibiting oncogenic HMGA2, a mediator of epithelial–mesenchymal transition (EMT) in pancreatic ductal adenocarcinoma (PDAC). H19 inhibited let-7 and reversed its suppression on HMGA2, resulting in increased HMGA2-mediated EMT and metastasis in PDAC cells [33]. The “T” allele at H19 rs2839698 was reported to be associated with bladder cancer [25], renal cell carcinoma [34], ovarian cancer [35], hepatoblastoma [36], hepatoma cell carcinoma [37,38], gastric cancer [39], colorectal cancer [40,41], and breast cancer [42]. Meanwhile, H19 rs2839698 was reported not to be associated with oral cancer [43], lung cancer [44], cervical cancer [45], glioma [46], and neuroblastoma [47,48]. On the contrary, H19 rs217717 was reported to be associated with altered risks of hepatoblastoma [36], gastric cancer [39], oral cancer [43], and lung cancer [44], while whether the “T” allele is a risk or protective is still controversial [26,42,49]. We have summarized the related literature in Tables 7 and 8 for the genotypic findings for rs2839698 and rs217717 polymorphisms, respectively. There were some meta-analysis studies reporting that the “T” allele of rs2839698 is the risk allele for several types of cancer [50,51], while others reported null results [52–55]. Our current study is the first report of these two H19 SNPs in childhood leukemia.

Table 7. The summary of genotypic findings for H19 rs2839698 polymorphism among cancer risk.

| Author | Year | Cancer | Case/Control, n | Population | Highlight Findings |
|--------|------|--------|-----------------|------------|-------------------|
| Verhaegh | 2008 | Bladder cancer | 177/204 | Netherlands | CT but not TT genotype is associated with decreased bladder cancer risk |
| Cao | 2020 | Renal cell carcinoma | 1094/1027 | China | TT but not CT genotype is associated with increased renal cell carcinoma risk |
| Zhang | 2020 | Ovarian cancer | 219/203 | China | TT but not CT genotype is associated with increased ovarian cancer risk |
| Tan | 2021 | Hepatoblastoma | 213/958 | China | TT but not CT genotype is associated with increased hepatoblastoma risk |
| Wu | 2019 | Hepatocellular carcinoma | 359/1190 | Taiwan | CT but not TT genotype is associated with increased hepatocellular carcinoma risk |
| Yang | 2018 | Hepatocellular carcinoma | 472/472 | China | TT but not CT genotype is associated with increased hepatocellular carcinoma risk |
| Yang | 2015 | Gastric cancer | 500/500 | China | TT but not CT genotype is associated with increased gastric cancer risk |
| Yu | 2020 | Colorectal cancer | 315/441 | China | CT plus TT genotype is associated with increased colorectal cancer risk |
| Li | 2016 | Colorectal cancer | 1147/1203 | China | TT but not CT genotype is associated with increased colorectal cancer risk |
| Safari | 2019 | Breast cancer | 111/130 | Iron | CT and TT genotypes are associated with increased breast cancer risk |
| Lin | 2017 | Breast cancer | 1005/1020 | China | No association |
| Guo | 2017 | Oral cancer | 362/741 | China | No association |
| Wang | 2019 | Lung cancer | 564/1536 | China | No association |
| Huang | 2019 | Cervical cancer | 235/325 | Taiwan | No association |
| Deng | 2020 | Glioma | 605/1300 | China | No association |
| Li | 2019 | Neuroblastoma | 700/1516 | China | No association |
| Hu | 2019 | Child neuroblastoma | 393/812 | China | No association |
We found that individuals carrying the variant genotypes (CT and TT) and allele (T allele) of rs2839698 had significant increased risks of childhood leukemia in a Taiwan population (Tables 2 and 3). In contrast, no such association was observed for rs217727 (Tables 4 and 5). A previous genotype–phenotype correlation study showed that cancer-free controls carrying the variant genotypes (CT and TT) of H19 rs2839698 had a higher expression of H19 mRNA in serum than those with the wild-type CC genotype among 80 healthy controls [39].

Our pilot study found CT and TT of H19 rs2839698 had a higher expression of H19 mRNA in serum than those with CC genotype among 30 childhood leukemia patients (Figure 1). This is consistent with the findings among 74 gastric cancer patients [32]. More than that, the polymorphic variation of H19 rs2839698 may be affecting the binding capacity between H19 and its target miRNAs. A previous study indicated that the oncogenic effect of H19 was partially mediated through the up-regulation of ISM1, a binding protein of H19 [32]. It is possible that the alterations in H19 structure via rs2839698 variation may affect the binding affinity of H19 to ISM1, which consequently promotes proliferation, migration, invasion, and metastasis [32]. However, the precise mechanisms of H19 action remain unclear, and further investigations are needed to verify the hypothesis.

The current study has a few limitations. First, the sample size was modest, and we could not perform stratified analyses. Second, since this was a hospital-based case-control study with all participants being recruited from the same hospital, there might be potential selection bias. However, the genotypic distribution in the control group was compatible

| Author   | Year | Cancer               | Case/Control, n | Population | Highlight Findings                                                                 |
|----------|------|----------------------|-----------------|------------|-----------------------------------------------------------------------------------|
| Verhaegh | 2008 | Bladder cancer       | 177/204         | Netherlands| No association                                                                     |
| Cao      | 2020 | Renal cell carcinoma | 1094/1027       | China      | No association                                                                     |
| Tan      | 2021 | Hepatoblastoma       | 213/958         | China      | CT but not TT genotype is associated with decreased hepatoblastoma risk           |
| Wu       | 2019 | Hepatocellular       | 359/1190        | China      | No Association                                                                    |
| Yang     | 2015 | Gastric cancer       | 500/500         | China      | TT but not CT genotype is associated with increased gastric cancer risk           |
| Li       | 2016 | Colorectal cancer    | 1147/1203       | China      | No association                                                                     |
| Safari   | 2019 | Breast cancer        | 111/130         | Iron       | CT and TT genotypes are associated with decreased breast cancer risk              |
| Lin      | 2017 | Breast cancer        | 1005/1020       | China      | CT and TT genotypes are associated with increased breast cancer risk              |
| Xia      | 2016 | Breast cancer        | 464/467         | China      | No association                                                                     |
| Guo      | 2017 | Oral cancer          | 362/741         | China      | TT but not CT genotype is associated with increased oral cancer risk             |
| Wang     | 2019 | Lung cancer          | 564/1536        | China      | TT but not CT genotype is associated with increased lung cancer risk             |
| Huang    | 2019 | Cervical cancer      | 235/325         | Taiwan     | No association                                                                     |
| Cao      | 2020 | Renal cell carcinoma | 1027/1094       | China      | No association                                                                     |
| Deng     | 2020 | Glioma               | 605/1300        | China      | No association                                                                     |
| Li       | 2019 | Neuroblastoma        | 700/1516        | China      | No association                                                                     |
| Hu       | 2019 | Child neuroblastoma  | 393/812         | China      | No association                                                                     |
with the Hardy–Weinberg expectations. Third, our study was conducted in a Taiwan population and our results need to be validated in other populations.

4. Materials and Methods

4.1. Recruitment of Childhood Leukemia and Control Participants

Childhood leukemia patients were identified and ascertained by a pediatric oncologist with pathologic confirmation. All basic and clinical characteristics of the recruited patients, including their histological details, were collected by physicians. All investigated subjects voluntarily participated this study, completed a questionnaire form with the help of their parents or guardians, and donated up to 5 mL blood. Healthy controls without prior history of any cancer were recruited through random sampling over the same period of 2005 to 2010 as we previously described [6–8]. Controls were matched to cases by age (±2 years) and gender. Finally, 532 participants (266 cases and 266 controls) under 18 years old were included in this study. All the participants were Taiwanese. This study was approved by the Institutional Review Board of China Medical University Hospital (DMR103-IRB-153, approved from 1 August 2018 to 31 July 2021).

4.2. DNA Extraction and Genotyping

Genomic DNA from the peripheral blood leukocytes was extracted (Qiagen, CA, USA). The genotypes of lncRNA H19 rs2839698 and rs217727 were determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) methodology. The PCR-RFLP genotyping methodology of H19 rs2839698 and rs217727 polymorphisms were designed by the Terry Fox Cancer Research Lab. The contig near H19 rs2839698 was amplified with forward primer 5'-AAG-GAG-CAC-CTT-GGA-CAT-CT-3' and reverse primer 5'-CTG-CCA-CGT-CCT-GTA-ACC-AA-3'. The contig near H19 rs217727 was amplified with forward primer 5'-GTC-GCT-ATC-TCT-AGG-TGA-AG-3' and reverse primer 5'-GTG-GAG-GCT-TTG-AAT-CTC-TC-3'. DNA contig is amplified in a 25 µL reaction mixture containing 100 ng of genomic DNA of each subject, 20 µM of each primer, 5 µL of 10X PCR buffer with 1.5 mM MgCl₂, and 1 unit of taq DNA polymerase. The PCR cycle was performed in a PCR Thermocycler (Bio-RAD, Hercules, CA, USA) using the following conditions: initial denaturation 94 ºC for 5 min, followed by denaturation at 94 ºC for 30 s, annealing at 64 ºC for 40 s, and extension at 72 ºC for 45 s. After completion of 40 PCR cycles, a final extension step was carried out at 72 ºC for 10 min. The PCR amplicons were checked by 3% agarose gel electrophoresis. Then the H19 rs2839698 and rs217727 PCR amplicons were digested by Kas I and Eci I, and the product sizes were verified with 3% agarose gel electrophoresis again. The H19 rs2839698 product presented 3 different patterns: an intact single 226 bp fragment for the TT genotype; full-digested fragments of 106 and 120 bp for the homologous variant CC genotype; and fragments of 106, 120, and 226 bp for the heterozygous variant CT genotype, respectively. The H19 rs2839698 product presented 3 different patterns: an intact single 226 bp fragment for the TT genotype; full-digested fragments of 106 and 120 bp for the homologous variant CC genotype; and fragments of 106, 120, and 226 bp for the heterozygous variant CT genotype, respectively.

4.3. Quantitative RT-PCR Assay of H19 mRNA Expression

The transcriptional expression level of H19 was measured using RT-PCR analyzing RNA extracted from the serum of 30 childhood leukemia patients (Qiagen, Redwood, CA, USA). The sequences of H19 forward and reverse primers were 5'-CCCACAACATGAAA GAAATGTTGC-3' and 5'-CACCTTCGAGAGCGATTCC-3', respectively. The sequences of internal control, GAPDH, were 5'-GAAATCCCAATCCATCTTCAGG-3' and 5'-GAGCCCCAGCTTCCTCCATG-3'. Real-time PCR was performed, and fold changes were normalized by the level of GAPDH. Each experiment was carried out blindly by two researchers and at least three.
4.4. Statistical Analysis

The Student’s t-test was used to compare the age between the cases and controls. The Pearson’s chi-square test was used to examine Hardy–Weinberg equilibrium (HWE) in the controls and compare the distribution of H19 rs2839698 and rs217727 genotypes between cases and controls. Logistic regression analyses were used to determine the associations between these two SNPs and childhood leukemia risks by calculating the odds ratios (OR) and 95% confidence intervals (CI). Age was adjusted in multivariate logistic regression analysis (Tables 3 and 5). Any p-value less than 0.05 was considered statistically significant.

5. Conclusions

In conclusion, our results reported for the first time that the variant genotypes and alleles of H19 rs2839698 were significantly associated with increased risks of childhood leukemia in Taiwan. Our study adds another piece of evidence that the H19 rs2839698 polymorphism may modulate the susceptibility to cancers. We also provided a summary of H19 genotype associations with cancer risks. Further studies in all types of cancer across different populations are warranted to clarify the role of H19 polymorphisms in carcinogenesis.

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References

1. Trevino, L.R.; Yang, W.; French, D.; Hunger, S.P.; Carroll, W.L.; Devidas, M.; Willman, C.; Neale, G.; Downing, J.; Raimondi, S.C.; et al. Germline genomic variants associated with childhood acute lymphoblastic leukemia. Nat. Genet. 2009, 41, 1001–1005. [CrossRef]

2. Pei, J.S.; Lee, Y.M.; Lo, H.H.; Hsu, Y.N.; Lin, S.S.; Bau, D.T. Association of X-ray repair cross-complementing-6 genotypes with childhood leukemia. Anticancer Res. 2013, 33, 5395–5399.

3. Pei, J.S.; Hsu, C.M.; Tsai, C.W.; Chang, W.S.; Ji, H.X.; Hsiao, C.L.; Miao, C.E.; Hsu, Y.N.; Bau, D.T. The association of methylenetetrahydrofolate reductase genotypes with the risk of childhood leukemia in Taiwan. PLoS ONE 2015, 10, e0119776. [CrossRef]

4. Pei, J.S.; Chang, W.S.; Hsu, P.C.; Tsai, C.W.; Hsu, C.M.; Ji, H.X.; Hsiao, C.L.; Hsu, Y.N.; Bau, D.T. The Association of Flap Endonuclease 1 Genotypes with the Risk of Childhood Leukemia. Cancer Genom. Proteom. 2016, 13, 69–74.

5. Pei, J.S.; Hsu, P.C.; Chou, A.K.; Tsai, C.W.; Chang, W.S.; Hsiao, C.L.; Hsu, Y.N.; Cheng, S.P.; Bau, D.T. Matrix Metalloproteinase-1 Genotype Contributes to the Risk of Non-solid Tumor in Childhood Leukemia. Anticancer Res. 2016, 36, 5127–5132. [CrossRef] [PubMed]

6. Pei, J.S.; Chou, A.K.; Hsu, P.C.; Tsai, C.W.; Chang, W.S.; Wu, M.F.; Wu, M.H.; Hsia, T.C.; Cheng, S.P.; Bau, D.T. Contribution of Matrix Metalloproteinase-7 Genotypes to the Risk of Non-solid Tumor, Childhood Leukemia. Anticancer Res. 2017, 37, 6679–6684. [PubMed]
7. Pei, J.S.; Chang, W.S.; Hsu, P.C.; Chen, C.C.; Cheng, S.P.; Wang, Y.C.; Tsai, C.W.; Shen, T.C.; Bau, D.T. The contribution of XRCC3 genotypes to childhood acute lymphoblastic leukemia. Cancer Manag. Res. 2018, 10, 5677–5684. [CrossRef] [PubMed]

8. Hsu, P.C.; Pei, J.S.; Chen, C.C.; Chang, W.S.; Kuo, C.C.; Cheng, S.P.; Tsai, C.W.; Bau, D.T.; Gong, C.L. Association of Matrix Metalloproteinase-2 Promoter Polymorphisms With the Risk of Childhood Leukemia. Anticancer Res. 2019, 39, 1185–1190. [CrossRef] [PubMed]

9. Hsu, P.C.; Pei, J.S.; Tseng, H.E.; Hsu, Y.N.; Kuo, C.C.; Lin, M.L.; Chang, W.S.; Wang, Y.C.; Tsai, C.W.; Pei, J.S.; et al. HOGG1 rs105233 Genotypes and Risk of Childhood Acute Lymphoblastic Leukemia in a Taiwanese Population. In Vivo 2019, 33, 1081–1086. [CrossRef] [PubMed]

10. Pei, J.S.; Chang, W.S.; Hsu, P.C.; Chen, C.C.; Chin, Y.T.; Huang, T.L.; Hsu, Y.N.; Kuo, C.C.; Wang, Y.C.; Tsai, C.W.; et al. Significant Association Between the MiR146a Genotypes and Susceptibility to Childhood Acute Lymphoblastic Leukemia in Taiwan. Cancer Genom. Proteom. 2020, 17, 175–180. [CrossRef]

11. Erdmann, V.A.; Szymanski, M.; Hochberg, A.; Groot, N.; Barciszewski, J. Non-coding, mRNA-like RNAs database Y2K. Nucleic Acids Res. 2000, 28, 197–200. [CrossRef]

12. Wilusz, J.E.; Sunwoo, H.; Spector, D.L. Long non-coding RNAs: Functional surprises from the RNA world. Genes Dev. 2009, 23, 1494–1504. [CrossRef] [PubMed]

13. Taft, R.J.; Pang, K.C.; Mercer, T.R.; Dinger, M.; Mattick, J.S. Non-coding RNAs: Regulators of disease. J. Pathol. 2010, 220, 126–139. [CrossRef] [PubMed]

14. Mercer, T.R.; Qureshi, I.A.; Gokhan, S.; Dinger, M.E.; Li, G.; Mattick, J.S.; Mehler, M.F. Long noncoding RNAs in neuronal-glial fate specification and oligodendrocyte lineage maturation. BMC Neurosci. 2010, 11, 14. [CrossRef]

15. Chen, L.L.; Carmichael, G.G. Decoding the function of nuclear long non-coding RNAs. Curr. Opin. Cell Biol. 2010, 22, 357–364. [CrossRef]

16. Dinger, M.E.; Amaral, P.P.; Mercer, T.R.; Pang, K.C.; Bruce, S.J.; Gardiner, B.B.; Askarian-Amiri, M.E.; Ru, K.; Solda, G.; Simons, C.; et al. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome. Res. 2008, 18, 1433–1445. [CrossRef] [PubMed]

17. Loewer, S.; Cabili, M.N.; Guttman, M.; Loh, Y.H.; Thomas, K.; Park, I.H.; Garber, M.; Curran, M.; Onder, T.; Agarwal, S.; et al. Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. Nat. Genet. 2010, 42, 1113–1117. [CrossRef] [PubMed]

18. Li, J.; Wang, X.; Wang, Y.; Yang, Q. H19 promotes the gastric carcinogenesis by sponging miR-29a-3p: Evidence from lncRNA-miRNA network analysis. Epigenomics 2020, 12, 989–1002. [CrossRef]

19. Zhang, J.; Han, C.; Unglerieder, N.; Chen, W.; Song, K.; Wang, Y.; Kwon, H.; Ma, W.; Wu, T. A Transforming Growth Factor-beta and H19 Signaling Axis in Tumor-Initiating Hepatocytes That Regulates Hepatic Carcinogenesis. Hepatology 2019, 69, 1549–1563. [CrossRef]

20. Yoshimura, H.; Matsuda, Y.; Yamamoto, M.; Kamiya, S.; Ishiwata, T. Expression and role of long non-coding RNA H19 in carcinogenesis. Front. Biosci. 2018, 23, 614–625.

21. Brannan, C.I.; Dees, E.C.; Ingram, R.S.; Tilghman, S.M. The product of the H19 gene may function as an RNA. Mol. Cell Biol. 1990, 10, 28–36. [CrossRef]

22. Gabory, A.; Jammes, H.; Dandolo, L. The H19 locus: Role of an imprinted non-coding RNA in growth and development. Bioessays 2010, 32, 473–480. [CrossRef] [PubMed]

23. Gabory, A.; Ripoche, M.A.; Le Digarcher, A.; Watrin, F.; Ziyaya, A.; Forne, T.; Jammes, H.; Ainscough, J.F.; Surani, M.A.; Journot, L.; et al. H19 acts as a trans regulator of the imprinted gene network controlling growth in mice. Development 2009, 136, 3413–3421. [CrossRef] [PubMed]

24. Raveh, E.; Matouk, I.J.; Gilion, M.; Hochberg, A. The H19 Long non-coding RNA in cancer initiation, progression and metastasis—A proposed unifying theory. Mol. Cancer 2015, 14, 184. [CrossRef]

25. Verhaegh, G.W.; Verkleij, L.; Vermeulen, S.H.; den Heijer, M.; Witjes, J.A.; Kiememey, L.A. Polymorphisms in the H19 gene and the risk of bladder cancer. Eur. Urol. 2008, 54, 1118–1126. [CrossRef] [PubMed]

26. Xia, Z.; Yan, R.; Duan, F.; Song, C.; Wang, P.; Wang, K. Genetic Polymorphisms in Long Noncoding RNA H19 Are Associated With Susceptibility to Breast Cancer in Chinese Population. Medicine 2016, 95, e2771. [CrossRef]

27. Hua, Q.; Lv, X.; Gu, X.; Chen, Y.; Chu, H.; Du, M.; Gong, W.; Wang, M.; Zhang, Z. Genetic variants in lncRNA H19 are associated with the risk of bladder cancer in a Chinese population. Mutagenesis 2016, 31, 531–538. [CrossRef]

28. Zhang, L.; Yang, F.; Yuan, J.H.; Yuan, S.X.; Zhou, W.P.; Hua, X.S.; Xu, D.; Bi, H.S.; Wang, F.; Sun, S.H. Epigenetic activation of the MiR-200 family contributes to H19-mediated metastasis suppression in hepatocellular carcinoma. Carcinogenesis 2013, 34, 577–586. [CrossRef]

29. Kondo, M.; Suzuki, H.; Ueda, R.; Osada, H.; Takagi, K.; Takahashi, T.; Takahashi, T. Frequent loss of imprinting of the H19 gene is often associated with its overexpression in human lung cancers. Oncogene 1995, 10, 1193–1198.

30. Hibi, K.; Nakamura, H.; Hirai, A.; Fujikake, Y.; Kasai, Y.; Akiyama, S.; Ito, K.; Takagi, H. Loss of H19 imprinting in esophageal cancer. Cancer Res. 1996, 56, 480–482.

31. Luo, M.; Li, Z.; Wang, W.; Zeng, Y.; Liu, Z.; Qiu, J. Upregulated H19 contributes to bladder cancer cell proliferation by regulating ID2 expression. FEBS J. 2013, 280, 1709–1716. [CrossRef]
32. Li, H.; Yu, B.; Li, J.; Su, L.; Yan, M.; Zhu, Z.; Liu, B. Overexpression of IncRNA H19 enhances carcinogenesis and metastasis of gastric cancer. *Oncotarget* 2014, 5, 2318–2329. [CrossRef]

33. Ma, C.; Nong, K.; Zhu, H.; Wang, W.; Huang, X.; Yuan, Z.; Ai, K. H19 promotes pancreatic cancer metastasis by derepressing let-7’s suppression on its target HMGA2-mediated EMT. *Tumour Biol.* 2014, 35, 9163–9169. [CrossRef]

34. Cao, Q.; Li, P.; Cao, P.; Qian, J.; Du, M.; Li, L.; Wang, M.; Qin, C.; Shao, P.; Zhang, Z.; et al. Genetic Variant in Long Non-Coding RNA H19 Modulates Its Expression and Predicts Renal Cell Carcinoma Susceptibility and Mortality. *Front. Oncol.* 2020, 10, 785. [CrossRef]

35. Zhang, H.B.; Zeng, Y.; Li, T.L.; Wang, G. Correlation between polymorphisms in IGF2/H19 gene locus and epithelial ovarian cancer risk in Chinese population. *Genomics* 2020, 112, 2510–2515. [CrossRef]

36. Tan, T.; Li, J.; Wen, Y.; Zou, Y.; Yang, J.; Pan, J.; Hu, C.; Yao, Y.; Zhang, J.; Xin, Y.; et al. Association between IncRNA H19 polymorphisms and hepatoblastoma risk in an ethnic Chinese population. *J. Cell Mol. Med.* 2020, 25, 742–750. [CrossRef] [PubMed]

37. Wu, E.R.; Chou, Y.E.; Li, Y.F.; Hsueh, K.C.; Lee, H.L.; Yang, S.F.; Su, S.C. Association of lncRNA H19 Gene Polymorphisms with the Occurrence of Hepatocellular Carcinoma. *Genes* 2019, 10, 506. [CrossRef] [PubMed]

38. Yang, M.L.; Huang, Z.; Wang, Q.; Chen, H.H.; Ma, S.N.; Wu, R.; Cai, W.S. The association of polymorphisms in IncRNA-H19 with hepatocellular cancer risk and prognosis. *Biosci. Rep.* 2018, 38, BSR20171652. [CrossRef] [PubMed]

39. Yang, C.; Tang, R.; Ma, X.; Wang, Y.; Luo, D.; Xu, Z.; Zhu, Y.; Yang, L. Tag SNPs in long non-coding RNA H19 contribute to susceptibility to gastric cancer in the Chinese Han population. *Oncotarget* 2015, 6, 15311–15320. [CrossRef] [PubMed]

40. Yu, B.; Chen, J.; Hou, C.; Zhang, L.; Jia, J. LncRNA H19 gene rs2839698 polymorphism is associated with a decreased risk of colorectal cancer in a Chinese Han population: A case-control study. *J. Clin. Lab. Anal.* 2020, 34, e23311. [CrossRef] [PubMed]

41. Li, S.; Hua, Y.; Jin, J.; Wang, H.; Du, M.; Zhu, L.; Chu, H.; Zhang, Z.; Wang, M. Association of genetic variants in IncRNA H19 with risk of colorectal cancer in a Chinese population. *Oncotarget* 2016, 7, 25470–25477. [CrossRef] [PubMed]

42. Safari, M.R.; Mohammad Rezaei, F.; Dehghan, A.; Noroozi, R.; Taheri, M.; Ghafouri-Fard, S. Genomic variants within the long non-coding RNA H19 gene polymorphisms and hepatocellular cancer risk and prognosis. *Biosci. Rep.* 2018, 38, BSR20171652. [CrossRef] [PubMed]

43. Guo, Q.Y.; Wang, H.; Yang, P.; Wang, Y. LncRNA H19 polymorphisms associated with the risk of OSCC in Chinese population. *Eur. Rev. Med. Pharmacol. Sci.* 2017, 21, 3770–3774.

44. Wang, G.; Liu, Q.; Cui, K.; Ma, A.; Zhang, H. Association between H19 polymorphisms and NSCLC risk in a Chinese Population. *J. BUON* 2019, 24, 913–917.

45. Huang, M.C.; Chou, Y.H.; Shen, H.P.; Ng, S.C.; Lee, Y.C.; Sun, Y.H.; Hsu, C.F.; Yang, S.F.; Wang, P.H. The clinicopathological characteristic associations of long non-coding RNA gene H19 polymorphisms with uterine cervical cancer. *J. Cancer* 2019, 10, 6191–6198. [CrossRef] [PubMed]

46. Deng, Y.; Zhou, L.; Yao, J.; Liu, Y.; Zheng, Y.; Yang, S.; Wu, Y.; Li, N.; Xu, P.; Lyu, L.; et al. Associations of IncRNA H19 Polymorphisms at MicroRNA Binding Sites with Glioma Susceptibility and Prognosis. *Mol. Ther. Nucleic Acids* 2020, 20, 86–96. [CrossRef] [PubMed]

47. Li, Y.; Zhuo, Z.J.; Zhou, H.; Liu, J.; Zhang, J.; Zhou, H.; Li, S.; Li, M.; He, J.; et al. H19 gene polymorphisms and neuroblastoma susceptibility in Chinese children: A six-center case-control study. *J. Cancer* 2019, 10, 6358–6363. [CrossRef] [PubMed]

48. Hu, C.; Yang, T.; Pan, J.; Zhang, J.; Yang, J.; He, J.; Zou, Y. Associations between H19 polymorphisms and neuroblastoma risk in Chinese children. *Biosci. Rep.* 2019, 39, BSR20181582. [CrossRef]

49. Lin, Y.; Fu, F.; Chen, Y.; Qiu, W.; Lin, S.; Yang, P.; Huang, M.; Wang, C. Genetic variants in long noncoding RNA H19 contribute to the risk of breast cancer in a southeast China Han population. *Oncol Targets Ther.* 2017, 10, 4369–4378. [CrossRef]

50. Chu, M.; Yuan, W.; Wu, S.; Wang, Z.; Mao, L.; Tian, T.; Lu, Y.; Zhu, B.; Yang, Y.; Wang, B.; et al. Quantitative assessment of polymorphisms in H19 IncRNA and cancer risk: A meta-analysis of 13,392 cases and 18,893 controls. *Oncotarget* 2016, 7, 78631–78639. [CrossRef] [PubMed]

51. Liu, C.; Chen, L.; You, Z.; Wu, Y.; Wang, C.; Zhang, G.; Xu, B.; Chen, M. Association between IncRNA H19 polymorphisms and cancer susceptibility based on a meta-analysis from 25 studies. *Gene* 2020, 729, 144317. [CrossRef]

52. Li, W.; Jiang, X.; Jin, X.; Yan, W.; Liu, Y.; Li, D.; Zhao, Z. Significant association between long non-coding RNA H19 polymorphisms and cancer susceptibility: A PRISMA-compliant meta-analysis and bioinformatics prediction. *Medicine* 2020, 99, e19322. [CrossRef]

53. Liu, X.; Zhao, Y.; Li, Y.; Zhang, J. Quantitative assessment of IncRNA H19 polymorphisms and cancer risk: A meta-analysis based on 48,166 subjects. *Artif. Cells Nanomed. Biotechnol.* 2020, 48, 15–27. [CrossRef] [PubMed]

54. Li, X.F.; Yin, X.H.; Cai, J.W.; Wang, M.J.; Zeng, Y.Q.; Li, M.; Niu, Y.M.; Shen, M. Significant association between IncRNA H19 polymorphisms and cancer susceptibility: A meta-analysis. *Oncotarget* 2017, 8, 45143–45153. [CrossRef]

55. Hashemi, M.; Moazzeni-Roodi, A.; Sarabandi, S.; Karami, S.; Ghavami, S. Association between genetic polymorphisms of long non-coding RNA H19 and cancer risk: A meta-analysis. *J. Genet.* 2019, 98, 81. [CrossRef] [PubMed]