Increased Risk of Thai Childhood Acute Lymphoblastic Leukemia with the MiR196a2 T>C Polymorphism

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Abstract

Objectives: This study assessed associations of the miR196a2 (rs11614913) T>C polymorphism with susceptibility to childhood acute lymphoblastic leukemia (ALL) and clinical outcomes. Materials and Methods: Blood DNA samples from 104 childhood ALL patients and 180 healthy children were studied for the miR-196a2 (rs11614913) polymorphism using a polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) approach. Results: The frequency of the miR-196a2 (rs11614913) T allele in controls was 0.51 compared with 0.33 in ALL cases. In this study, CC, TC heterozygote and CC/TC genotypes were significantly associated with increased childhood ALL susceptibility compared with the TT wild type (OR =4.321, 95% CI = 2.091-8.930 p=0.000, OR = 2.248, 95% CI =1.103-4.579, p=0.024, OR = 2.921, 95% CI = 1.504-5.673 p=0.001, respectively). However, the miR-196a2 (rs11614913) T>C polymorphism was not associated with demographic data or clinico-pathological data in ALL cases. Conclusion: CC, TC and CC+TC genotypes of miR-196a2 (rs11614913) was significantly associated with increased susceptibility in Thai childhood ALL but not with clinical variables.

Keywords: Acute lymphoblastic leukemia- miRNA-196a2- polymorphism

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. The long term survival rate of ALL is nearly 90% in developed countries (Tasian et al., 2015; Pui et al., 2016). However, in Thailand, the long term survival rate is lower (Wongmeerit et al., 2016). The pathogenesis of ALL includes environmental risk factors and genetic susceptibility (Deepa et al., 2015). MicroRNA (miRNA or miR), a noncoding RNA, is the novel epigenetic biomarkers. Previous studies have reported the role of miRNA in several biological process including cell proliferation, differentiation and apoptosis. (Bartel, 2004; Hwang and Mendell, 2006). Recently, it was found that the dysregulation of miRNA expression contributes to multistep processes of carcinogenesis (Jansson and Lund, 2012; Hayes et al., 2014). Single nucleotide polymorphisms (SNPs) in the miRNA gene region have effects on the function of miRNA and contribute to cancer susceptibility (Mishra et al., 2008).

Recently, several studies have investigated the association of miR-196a2 (rs11614913) polymorphism with cancer susceptibility. (Srivastava K and Srivastava A, 2012; Ma et al., 2013). However, there are very few studies focus on the association of miR196-a2 (rs 11614913) polymorphism in childhood ALL. In Caucasian, there is no association between miR-196a2 (rs11614913) polymorphism and risk of childhood ALL (Trevino et al., 2009). However, this significant association was found in Chinese population (Tong N. et al., 2014).

This research aims to investigate the association of miR-196a2 (rs 11614913) polymorphism and childhood ALL susceptibility in Thailand. Moreover, the association of miR-196a2 (rs 11614913) polymorphism and clinical outcomes was also studied.

Materials and Methods

Study Subjects

This research was 284 case-control study including 104 childhood ALL patients and 180 healthy cancer-free children at the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Thailand. Patients were classified by risk-based assignment protocol (Smith et al., 1996), assessed by initial white blood cell counts, French-American-British morphology and lymphomatous disease. Moreover clinical and demographic data including age at diagnosis, sex, immunophenotype and chromosome abnormalities were retrospectively studied.

This research was approved by the Ethics Committee,
Faculty of Medicine Ramathibodi Hospital, Mahidol University and informed consent was obtained from all the participants.

**DNA Extraction**

Genomic DNA extraction from EDTA blood of 194 healthy controls and 107 ALL patients and extracted by salting-out method (Miller SA et al., 1988). DNA was quantitated by a measurement of nano drop spectrophotometer and kept at -20°C prior to use.

**Genotyping of the miR-196a2 (rs11614913) (T>C) polymorphism**

The single nucleotide polymorphism of miR-196a2 (rs11614913) (T>C) was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis. Genotyping of miR-196a2 (rs11614913) (T>C) polymorphism was done using forward primer 5’ CCC-CTT-CCC-TTC-TCC-AGATA 3’ and reverse primer 5’ CGA-AAA-CCG-CTG-GAT GTA-CTT-CAG 3’ as described previously. (Shuma et al., 2015). DNA was amplified in 25 µL reaction mixture containing 100 ng of genomic DNA, 20 µM of each primer, 5 µL of 10 X PCR buffer with 1.5 mM MgCl2 and 1 unit of Taq (i-Taq, European Biotech network, Belgium). The PCR cycle was carried out in a Thermocycler (Bio-RAD, Hercules, CA, USA). The PCR conditions were the initial denaturation of 95°C for 5 minute followed by 35 cycles of 94°C for 35 second, 61°C for 35 second, and 72°C for 40 second, with a final elongation at 72°C for 5 minute. The PCR products were found to be 149 bp in length by electrophoresis on 2% agarose gel, stained with 0.5 µg/ml ethidium bromide and then visualized using the gel documentation.

**Statistical analysis**

Comparisons of genotypes between cases and controls group were analyzed by binary logistic regression analysis. The associations between the clinical data and the distribution of the miR-196a2 genotypes in ALL patients were calculated by Chi-square test. Crude odds ratios (OR) and 95% confidence intervals (CI) were also calculated. P values less that 0.05 were considered statistically significant. All of the statistical analysis using SPSS version 17.0 program (SPSS Inc., Chicago, USA).

**Results**

**Characteristics of the study population**

The demographic data of ALL cases and control subjects as well as clinical data of ALL patients are summarized in Table 1. There were 104 ALL cases and 180 cancer-free controls. The demographic data shown that there was no significant difference in the frequency of sex distribution between ALL cases and controls (p=0.193). There was significant difference in the frequency of age distribution between ALL cases and control (p=0.009). Among ALL patients, there were 101 cases with known immunophenotype including 12 cases with T-ALL (11.88%), 78 cases with Early pre-B ALL (77.23%), 10 cases with pre-B ALL (9.90%) and 1 case of Mixed cell type ALL. The data of treatment protocol collected in 91 cases including 41 cases (45.05%) of low risk, 41 cases (45.05%) of standard risk and 9 cases (9.90%) of high risk ALL.

**Association between miR-196a2 (rs11614913) T>C polymorphism and the susceptibility of childhood ALL**

The allele frequency of miR-196a2 T allele in control was 0.51 compared with 0.33 in ALL cases (Table 2). The frequencies of the miR-196a2 (rs11614913) genotypes was 29.45% for TT, 43.33 % for CT and 27.22 % for CC in controls, whereas the data was 12.50%, for TT, 41.35% for CT and 46.15% for CC in ALL cases. There were significant differences between the distribution of genotype frequencies of miR-196a2 (rs11614913) between ALL cases and controls. It was found that miR-196a2 (rs11614913) variant CC, TC heterozygote and CC/TC genotypes were significantly associated with increase childhood ALL susceptibility compared with TT wild type.(OR =4.321, 95% CI = 2.091-8.930 p=0.000, OR =2.248, 95% CI =1.103-4.579, p=0.024, OR = 2.921, 95% CI =, 1.504-5.673 p=0.001, respectively).

**Association between genotypes of the miR-196a2 (rs11614913) T>C polymorphism and clinicopathological data in childhood ALL patients**

**Table 1. Frequency Distribution of Selected Variables in Cases and Controls**

| Characteristic     | ALL (%) | Controls (%) |
|--------------------|---------|--------------|
| **Demographic**    |         |              |
| Sex                |         |              |
| Males              | 65 (62.50) | 98 (54.44) |
| Females            | 39 (37.50) | 82 (45.56) |
| Age at diagnosis   |         |              |
| < 6 years          | 64 (61.54) | 137 (76.11) |
| >6 years           | 40 (38.46) | 43 (23.89) |
| Immunophenotype    |         |              |
| (n=101)            |         |              |
| T cell             | 12 (11.88) | -            |
| Non T cell         | 89 (88.12) | -            |
| Early pre-B        | 78 (87.64) | -            |
| Pre-B              | 10 (11.24) | -            |
| Mixed              | 1 (1.12) | -            |
| Risk classification|         |              |
| low risk           | 41 (45.05) | -            |
| Standard risk      | 41 (45.05) | -            |
| High Risk          | 9 (9.90) | -            |
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MiR-196a2 T>C Polymorphisms in Thai Childhood Acute Lymphoblastic Leukemia

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Table 2. MiR-196a2 T>C Genotypes Distribution and Allele Frequency in ALL And Controls

| Genotypes | ALL (%) n=104 | Controls (%) n=180 | OR (95%CI)* | p-value** |
|-----------|---------------|-------------------|-------------|-----------|
| TT        | 13 (12.50)    | 53 (29.45)        | 1.00 (Reference) | 0.024 |
| TC        | 43 (41.35)    | 78 (43.33)        | 2.248 |
| CC        | 48 (46.15)    | 49 (27.22)        | 4.321 |
| TC+CC     | 91 (87.50)    | 127 (70.55)       | 2.921 |

Allele frequency
- MiR196a2 T allele: 0.33, 0.51
- MiR196a2 C allele: 0.67, 0.49

* Odds ratio and p-value were calculated by comparison of control and ALL for TC, CC and CC+CT versus TT; ** significant at the 0.05 level of significance

Table 3. The Association between MiR196a2 T>C Genotypes and Clinico-Pathological Data of ALL Patients

| Demographic data | MiR-196a2 T/T | MiR-196a2 CT or CC | p-value |
|------------------|---------------|--------------------|---------|
| Sex (n=104)      |               |                    |         |
| Male             | 9             | 56                 | 0.592   |
| Female           | 4             | 35                 |         |
| Age at diagnosis (n=104) |       |                    |         |
| <6 years         | 7             | 57                 | 0.542   |
| >6 years         | 6             | 34                 |         |
| Initial WBC count (n=97) | |                    |         |
| <50,000 cell /µl | 6             | 73                 | 0.625   |
| >50,000 cell /µl | 2             | 16                 |         |
| Immunophenotype (n=101) | |                    |         |
| T cell           | 2             | 10                 | 0.676   |
| Non T cell       | 11            | 78                 |         |
| Early –pre-B     | 10            | 68                 |         |
| Pre-B            | 1             | 9                  |         |
| Mixed            | 0             | 1                  |         |
| Risk classification (n=91) | |                    |         |
| High Risk        | 2             | 7                  | 0.399*  |
| Low risk or Standard risk | 10 | 72 |         |
| Low risk         | 5             | 36                 |         |
| Standard risk    | 5             | 36                 |         |

*, p-value were calculated by comparison of genotypes between high risk and low risk or standard risk

In this study, there was no association between miR-196a2 (rs11614913) polymorphism and clinical data including gender, age at diagnosis, initial WBC count, immunophenotype and risk classification of ALL patients (Table 3).

Discussion

MiRNAs are non coding RNA that inhibit the expression of protein coding genes by either mRNA degradation or translational repression. MiRNAs regulate cell proliferation, differentiation and apoptosis. Polymorphism of miRNA precursor may affect mature microRNA function (Ryan BM et al., 2010). Recently, several investigators study the association of miR-196a2 (rs11614913) polymorphism and cancer susceptibility. It was found that miR-196a2 (rs11614913) T allele was significantly associated with decreased susceptibility to breast cancer in Caucasian (Hoffman, et al., 2009; Dai et al., 2016). In contrast, TT genotype of miR-196a2 (rs 11614913) was associated with an increased risk of gastric cancer in Chinese (Gu and Tu, 2016). Moreover, it was shown that CC genotype of miR-196a2 (rs11614913) was significantly associated with oral squamous cell carcinoma in Indian (Sushma et al., 2015) and lung cancer in Chinese (Tian et al., 2009 and Yuan et al., 2013).

To evaluate the association between miR-196a2 (rs11614913) polymorphism and susceptibility to Thai childhood ALL, case-control study was investigated in Thai children whose frequency of gender match between cases and controls. However, there is significant difference of age distribution between cases and controls. The previous study shown that the major allele of miR-196a2 (rs11614913) variant CC, TC heterozygote and CC/TC genotypes were significantly associated with increase childhood ALL susceptibility compared with TT wild type. The association of miR-196a2 (rs11614913) genotypes with susceptibility to Thai childhood ALL in this study is similar to Chinese study (Tong N et al., 2014). There were significant differences in the distribution of miR-196a2 (rs11614913) genotypes between ALL cases and controls. The miR-196a2 (rs11614913) variant CC, TC heterozygote and CC/TC genotypes were significantly associated with increase childhood ALL susceptibility compared with TT wild type. The association of miR-196a2 (rs11614913) genotypes with susceptibility to Thai childhood ALL in this study is similar to Chinese study (Tong N et al., 2014). They revealed that TC heterozygote and CC/TC associated with an increased risk of childhood ALL in Chinese. However, in this study, the frequencies of miR-196a2 (rs11614913) T>C polymorphism were not associated with demographic data and clinical outcomes in ALL cases.

There are several target genes of miR-196a2. MiR-196a2 may regulate different genes in different cell type or under different condition in a specific cell type. Genotype-phenotype correlation analysis revealed that CC homozygote in miR-196a2 was associated with significantly increased miR-196a2 expression (Hu et al., 2008). Recently, it was found that the target genes of miR-196a2 are homeobox family of genes which is called Hox genes. They involve in hematopoietic cell growth and differentiation. There was some evidences revealed that...
HOX C8, which suppressed cell migration and metastasis, is the target gene of miR-196a2 (Tong N et al., 2014). Another possible target genes of miR-196a2 are LSP1 (lymphocyte specific protein 1) and GDF3 (Growth/differentiation factor 3 precursor) (Hu et al.,2008). However, there is still no study about the target genes of miR-196a2 in ALL patients. Further study should be done to investigate the pathogenesis about the interaction pathway between miR-196a2 and its target genes in acute lymphoblastic leukemia.

In conclusion, miR-196a2 (rs11614913) T>C polymorphism was significantly associated with susceptibility to Thai childhood ALL and was not significantly associated with clinico-pathological variables.

Conflict of interest

The study presented no conflict of interest.

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References

Bartel DP (2004). MicroRNA: genomics, biogenesis and function. Cell, 116, 281-97.
Chen C, Zhang Y, Zhang L, Weakly SM, Yao Q (2011). MicroRNA-196: critical roles and clinical applications in development and cancer. J cell Mol Med, 15, 14-23.
Dai ZM, Kang HF, Zhang WG, et al (2016). The associations of single nucleotide polymorphisms in miR196a2, miR-499, and miR-608 with breast cancer susceptibility. Medicine, 95, e2826.
Deepa B, Yang JJ, Pui CH (2015). Biology of childhood acute lymphoblastic leukemia. Pediatr Clin North Am, 62, 47-60.
Gu JY, Tu L (2016). Investigating the role of polymorphisms in miR-146a, -149, and 196a2 in the development of gastric cancer. Genet Mol Res, 15, 1-7.
Hayes J, Peruzzi P, Lawler S (2014). MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med, 20, 460-9.
Hoffman AE, Zheng T, Yi C, et al (2009). MicroRNA miR-196a-2 and breast cancer: a genetic and epigenetic association study and functional analysis. Cancer Res, 69, 5970-7.
Hwang H-W, Mendell JT (2006). MicroRNAs in cell proliferation, cell death and tumorigenesis. Br J Cancer, 94, 776-80.
Hu Z, Chen J, Tien T, et al (2008). Genetic variants of miRNA sequences and non–small cell lung cancer survival. J Clin Invest, 118, 2600-8.
Jansson MD, Lund AH (2012). MicroRNA and cancer. Mol Oncol, 6, 590-610.
Ma XP, Zhang T, Peng B, Yu L, Jiang DK (2013). Association between miRNA polymorphism and cancer risk on the findings of 66 case-control studies. PloS One, 8, e79584.
Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cell. Nucleic Acids Res, 16, 1215.

danrakmanee et al  Asian Pacific Journal of Cancer Prevention, Vol 18, 1183–7.

Sarinthorn Rakmanee et al

1120