The Contribution of MMP-8 Promoter Genotypes to Childhood Leukemia

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Abstract. *Background/Aim: Accumulated evidence has supported the notion that matrix metalloproteinase (MMP) genotypes are associated with the susceptibility of many types of cancers. However, few reports have studied the contribution of MMP genotypes to either diagnostic or prognostic potential in non-solid tumors such as leukemia. In this study, we firstly investigated the contribution of a polymorphism in the promoter region of MMP-8 (-799C/T) and two non-synonymous polymorphisms (Val436Ala and Lys460Thr) to childhood leukemia. Patients and Methods: In this study, 266 patients with childhood acute lymphoblastic leukemia (ALL) and 266 non-cancer control patients were collected and the genomic DNA was isolated from their peripheral blood. MMP-8 -799C/T, Val436Ala and Lys460Thr polymorphic genotypes of each subject were determined by the typical polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: The results showed that the three polymorphisms were not significantly associated with an increased risk of childhood ALL in the overall investigated population.*

Furthermore, when the analyses were stratified by age and gender, no significant association between these genotypes and increased ALL risk was found. Conclusion: Our findings suggest that the polymorphisms at MMP-8 -799C/T, Val436Ala and Lys460Thr may not play a major role in determining the personal susceptibility to childhood ALL in Taiwan.

In recent years, mounting evidence have suggested that genetic factors play a significant role in the development of childhood acute lymphoblastic leukemia (ALL). For example, Down syndrome and Fanconi anemia, which are both inherited genetic human diseases, have been found to be associated with an elevated risk of ALL (1, 2). Additionally, genetic mutations in several cancer-related genes, such as *p53, N-ras*, and *PHF6*, have also been frequently identified among ALL patients (3). Furthermore, only a small fraction of children who are exposed to environmental factors go on to develop ALL, indicating the potential for a genetic predisposition to develop childhood ALL (4).

Matrix metalloproteinases (MMPs), a family of proteins regulating the aggravation of extracellular matrix and basement membranes (5), play an important role in cell proliferation, differentiation, apoptosis, invasion, migration, metastasis, angiogenesis and immune surveillance during carcinogenesis (6). Previous studies reported that functional polymorphisms of *MMPs* may determine the inter-individual differences of susceptibility to several types of cancer (7-11). In 2011, it was reported that patients with relatively over-expressed MMP protein pattern and homozygous for rare genotypes in rs8113877T>G or rs17576A>G of the *MMP-9* gene were at highest risk of chronic lymphocytic leukemia

in European adults (12). In 2014, a whole-exome sequencing analysis identified a novel mutation on *MMP-8*, G189D, and validated its association with acute megakaryoblastic leukemia in an adult patient (13). In 2015, in a study recruiting 778 Danish and German childhood ALL patients, rs3216144 and rs10502001 of *MMP-7* were found to be associated with risk of relapse of childhood ALL (14). The above three papers are the few papers investigating the genetic contribution of MMPs to leukemia, and only the last one is for the childhood ALL.

Among the MMPs, MMP-8 is a collagen cleaver which is present in the connective tissues, and encoded by *MMP-8* (15, 16). In literature, the *MMP-8* -799C/T genotype was associated with breast cancer (17) and the electrophoretic mobility shift assays revealed differences in nuclear protein binding to oligonucleotides representing the -799C/T genotype (18). In addition, the promoter constructs containing the CT and TT genotypes at the -799C/T had a 3-fold greater activity in chorion-like trophoblast cells compared to the constructs containing the C alleles (18). However, the role of *MMP-8* genotypes were never examined in ALL, not to mention childhood ALL. The current study aimed at investigating the contribution of *MMP-8* -799C/T, Val436Ala and Lys460Thr polymorphisms to the susceptibility of childhood ALL in a Taiwanese population.

Materials and Methods

Patients and controls. The protocol of the current study was approved by the Institutional Review Board of China Medical University Hospital, and written informed consent was obtained from one or both parents of all participants. Two hundred and sixty-six patients diagnosed with childhood ALL (all patients under 18 years of age) were recruited between 2005-2010 from the General Surgery outpatient clinics within the Pediatric Departments at the China Medical University Hospital and the National Taiwan University Hospital, Taiwan, Republic of China. All of the clinical characteristics of these patients, including their histological details, were identified by expert surgeons. All children voluntarily participated, completed a questionnaire with the help of their parents or guardians, and provided peripheral blood samples. The questionnaire recorded their disease history, diet and sleeping habit, and the disease history, diet, behavioral lifestyle and socioeconomic status of the parents. An equal number of age-matched non-cancer healthy volunteers were selected for use as a control group following initial random sampling from the Health Examination Cohort established from 2005 to 2010 as previously published (19-21). The registered health practitioners in the hospital provided a multidisciplinary team approach of health assessment for the volunteers. Most of the volunteers underwent health examinations every 5 to 6 months. A total of 457 volunteers aged under 18 years were recruited into this study. They were cancer free by the age at diagnosis with the International Classification of Disease, ninth revision (ICD-9) codes (defined by World Health Organization). Finally, 266 participants were included for analysis in the study to match the population structure (number, age and gender) with our

case population. The overall agreement rate in the study was above 85%. Selected recorded characteristics of the subjects in case and control groups are summarized and compared in Table I.

Methodology of MMP-8 genotyping. Genomic DNA from peripheral blood of each participant was extracted aliquoted and stored as previously described (22-24). The primers for *MMP-8* -799C/T, Val436Ala and Lys460Thr polymorphisms were custom designed by our team (shown in Table II) and the genotyping polymerase chain reaction (PCR) cycling conditions *via* My Cycler (Biorad, Hercules, CA, USA) for *MMP-8* were: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 s, 57°C for 30 s and 72°C for 30 s and a final extension at 72°C for 10 min as previously published (25, 26).

Statistical analysis. Pearson's Chi-square test without Yates' correction or Fisher's exact test was used to compare the distribution of the *MMP-8* genotypes between case and control groups. The associations between the *MMP-8* polymorphisms and childhood ALL risk were estimated by computing odds ratios (ORs) as well as their 95% confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounding factors if needed.

Results

Basic characteristics compared between childhood ALL patient and control groups. The frequency distributions of the characteristics for 266 childhood ALL cases and 266 controls are summarized and compared in Table I. No difference was found between the two groups as for age and gender since we have matched them very well (*p*>0.05) (Table I).

Association analysis of MMP-8 genotypes at -799C/T, Val436Ala and Lys460Thr with childhood ALL risk. The distributions of genetic frequencies for the *MMP-8* polymorphisms for the *childhood ALL* patients and controls are presented and compared in Table III. First, there were no CT or CC genotypes at *MMP-8* Val436Ala among either the cases or the controls. That is to say, all the subjects were of TT genotype at *MMP-8* Val436Ala (Table III, lowest part). Second, the ORs with adjusting those possible confounding factors (age and gender) for the people carrying variant CT and TT genotypes at *MMP-8* promoter -799C/T were 0.88 (95%CI=0.63-1.21, *p*=0.4403) and 0.79 (95%CI=0.49-1.35, *p*=0.5420) respectively, compared to those carrying the CC wild-type genotype (Table II, upper part). The *p*-value for trend was not significant (*p*=0.6832) (Table II). In the dominant model (CT plus TT *versus* CC), the association between *MMP-8* promoter -799C/T polymorphism and the risk for childhood leukemia was still not statistically significant (adjusted OR=0.82, 95%CI=0.61-1.23, *p*=0.3859) (Table III, upper part). Last, a very small percentage of Taiwanese children were of heterozygous variant AC genotype at *MMP-8* Lys460Thr (0.8% and 1.1% in childhood ALL patient and control groups, respectively) and there was

Table I. *Distribution of selected demographics of the 266 childhood ALL patients and the 266 matched controls.*

Characteristics	Controls (n=266)			Patients (n=266)			*p*-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age at onset (years old)			8.3 (4.8)			7.0 (4.4)	0.6483a
Gender							1.0000b
Male	148	55.6%		148	55.6%		
Female	118	44.4%		118	44.4%		

aBased on Student's *t*-test; bbased on Chi-square test; SD: standard deviation.

Table II. *Primer sequences, specific restriction enzymes, fragment sizes of the PCR products after digestion according to their allelic genotypes.*

Polymorphism (rs number)	Primer sequences	Restriction enzyme	SNP sequence	DNA fragment size (bp)
C-799T	F: 5'-CCATCTTCACATAGCCTTGG-3'	*Sfc* I	T	285 bp
(rs11225395)	R: 5'-CCTTGTCTTCTGCCTGTGAA-3'		C	172+113 bp
Lys460Thr	F: 5'-GGATTACAGGCATTAGCCAC-3'	*Nla* III	A	332 bp
(rs35866072)	R: 5'-CGAAAATGCATGCTGAACTTCC-3'		C	245+87 bp
Val436Ala	F: 5'-GGATTACAGGCATTAGCCAC-3'	*Bbs* I	C	264 bp
(rs34009635)	R: 5'-GCCATATCTACAGTTAAGCCAT-3'		T	162+102 bp

*F and R indicate forward and reverse primers, respectively.

no association between *MMP-8* Lys460Thr AC genotypes and the risk for childhood ALL (adjusted OR=0.73, 95%CI=0.22-3.87, *p*=0.6532) (Table III, medium part).

Association of MMP-8 allelic types at -799C/T, Val436Ala and Lys460Thr and childhood ALL risk. Supporting the findings in Table III, there is no differential distribution of allelic frequencies between the childhood ALL patient and control groups as for the *MMP-8* promoter -799C/T or Lys460Thr (Table IV). In detail, the adjusted OR for the subjects carrying the T allele at *MMP-8* promoter -799C/T was 0.87 (95%CI=0.67-1.26, *p*=0.3869), compared to those carrying the C wild-type allele (Table IV, upper part). As for the allelic frequencies at *MMP-8* Val436Ala and Lys460Thr polymorphic sites, there was no association between their genotypes and increased risk of childhood ALL (Table IV, medium and lower parts).

Discussion

MMPs play an important role in regulating the extracellular matrix components and carcinogenesis. In the literature, the genotypes of SNPs at promoter region of many *MMP* genes were found to be associated with the risk of several types of cancer (7-11). However, the papers investigating the

polymorphisms of MMPs as risk factors for childhood ALL are lacking, except of the whole-genome study that found that rs3216144 and rs10502001 of *MMP-7* were associated with risk of relapse of childhood ALL (14). In that work, the samples were composed of 778 Danish and German children, all of whom were Caucasian. The authors examined as many as 25,000 to 34,000 preselected polymorphic sites to identify the relapse risk of these children. Among the polymorphic sites they investigated, rs3216144 and rs10502001 of *MMP-7* were the two with the highest significant association (p=6.0*10^{-6} in combined cohorts) (14). These findings support the idea that MMPs may play an important role in carcinogenesis, and the genomic markers on *MMPs* can serve as valuable predictive biomarkers for the development or reoccurrence of childhood ALL.

MMP-8 plays an important role in the development of ALL. Early in the twentieth century, MMP-8 was found to be highly expressed in Jurkat T leukemia cells, which was closely related to the suppression of invasiveness in T cells and could be down-regulated by genistein (27). In animal models, the mutant mice deficient in MMP-8 were more susceptible to develop skin cancer (28, 29), which strongly suggested that MMP-8 may play a protective role against the development of cancers. From the viewpoint of genomic study, some positive epidemiological findings reported that genotypes of promoter

Table III. *Distributions of matrix metalloproteinase-8 (MMP-8) genotypic frequencies among childhood ALL cases and controls.*

	Cases (%)	Controls (%)	Adjusted OR (95%CI)[a]	*p*-Value[b]
-799C/T				
CC (wildtype)	139 (52.3)	129 (48.5)	1.00 (reference)	
CT	98 (36.8)	105 (39.5)	0.88 (0.63-1.21)	0.4403
TT	29 (10.9)	32 (12.0)	0.79 (0.49-1.35)	0.5420
CT+TT	127 (47.7)	137 (51.5)	0.82 (0.61-1.23)	0.3859
p_{trend}				0.6832
Lys460Thr				
AA (wildtype)	264 (99.2)	263 (98.9)	1.00 (reference)	
AC	2 (0.8)	3 (1.1)	0.73 (0.22-3.87)	0.6532
CC	0 (0.0)	0 (0.0)	--	
Val436Ala				
TT (wildtype)	266 (100.0)	266 (100.0)	1.00 (reference)	
CT	0 (0.0)	0 (0.0)	--	
CC	0 (0.0)	0 (0.0)	--	

OR: Odds ratio; CI: confidence interval. [a]Data has been adjusted with confounding factors include age and gender. [b]Based on Chi-square test without Yates' correction or Fisher's exact test; *$p<0.05$.

Table IV. *Allelic frequencies for matrix metalloproteinase-8 (MMP-8) polymorphisms among childhood ALL cases and controls.*

Polymorphic site Allele	Cases (%) N=532	Controls (%) N=532	Adjusted OR (95%CI)[a]	*p*-Value[b]
C-799T				
Allele C	376 (70.7)	363 (68.2)	1.00 (reference)	0.3869
Allele T	156 (29.3)	169 (31.8)	0.87 (0.67-1.26)	
Lys460Thr				
Allele A	530 (99.6)	529 (99.4)	1.00 (reference)	0.6540
Allele C	2 (0.4)	3 (0.6)	0.68 (0.24-4.31)	
Val436Ala				
Allele T	532 (100.0)	532 (100.0)	1.00 (reference)	
Allele C	0 (0.0)	0 (0.0)	--	

OR: Odds ratio; CI: confidence interval. [a]Data has been adjusted with confounding factors include age, gender and smoking status. [b]Based on Chi-square test without Yates' correction or Fisher's exact test; *$p<0.05$.

regions of other MMPs such as MMP-1 and MMP-9, may serve as promising markers for the prediction of lung cancer susceptibility and prognosis (30-32). From the molecular viewpoint, MMP-8 might increase cell adhesion by rearrangement of cytoskeleton actin, thus decreasing cell invasion (33). However, there were few epidemiological studies investigating the role of polymorphisms in *MMP-8* to the susceptibility of cancers (34). In 2008, the G variant allele at *MMP-8* +17 C/G was found to be associated with a decreased risk of developing lung cancer, while there was no contribution of the particular polymorphism to the overall survival rates for the lung cancer patients (35). From the above information, in the present study, we firstly focused on the genotypes of specific *MMP-8* polymorphisms among a Taiwanese population and assessed whether there was an association between the genotypes of the promoter region of *MMP-8* (-799C/T) and two nonsynonymous polymorphisms (Val436Ala and Lys460Thr) with childhood ALL risk. The results showed that no significant association was observed and our findings suggest that these three *MMP-8* polymorphisms may not play a critical role in mediating susceptibility to childhood ALL (Tables III and IV). Furthermore, when the analyses were stratified by age and gender, no significant association between these genotypes and childhood ALL risk was observed (data not shown). The phenotypic data including the expression levels of MMP-8 at mRNA or protein are not currently available for further analysis. The complete correlation of patient status, genotype and phenotype would be very helpful to understand the role of MMP-8 in childhood ALL development.

In conclusion, this is the first study to investigate the contribution of genotypes at the *MMP-8* promoter and non-synonymous polymorphisms to childhood ALL development. Our results suggest that the genotypes of the promoter region -799C/T and nonsynonymous Val436Ala and Lys460Thr at *MMP-8*, do not significantly confer susceptibility to Taiwan childhood ALL. Further studies elucidating the contribution of the genotypes of other members of *MMPs* to childhood ALL development are urgently needed.

Conflicts of Interest

All Authors declare no conflicts of interest.

Acknowledgements

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