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Journal of the Formosan Medical Association (2016) 115, 801–806

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

Tet oncogene family member 2 gene alterations in childhood acute myeloid leukemia

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Received 28 August 2014; received in revised form 4 August 2015; accepted 4 August 2015

KEYWORDS
acute myeloid leukemia;
bioinformatics;
children;
molecular biology;
TET2

Background/Purpose: Mutations in the tet oncogene family member 2 gene (*TET2*) are frequently found in adult patients with acute myeloid leukemia (AML). Reports of *TET2* mutations in children are limited. We assessed the prevalence of *TET2* mutations in Taiwanese children with AML and analyzed their prognosis.
Methods: Between 1997 and 2010, a total of 69 consecutive children with AML were enrolled at the National Taiwan University Hospital. The analysis for *TET2* mutations was performed using direct sequencing. Clinical characteristics and overall survival (OS) were compared between patients with and without *TET2* alterations.
Results: Intronic and missense mutations were identified. No nonsense or frameshift mutations were observed. Two putative disease-causing missense mutations (S609C and A1865G) were identified in one patient. We estimated the prevalence of *TET2* mutations in the current patient population to be 1.4%. The most common polymorphism was I1762V (45%), followed by V218M (12%), P29R (6%), and F868L (6%). Patients with polymorphism I1762V had an increased

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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http://dx.doi.org/10.1016/j.jfma.2015.08.002
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10-year survival rate compared with patients without I1762V (48.4% vs. 25.7%, $p = 0.049$) by Chi-square test; OS was not different when examined using the Kaplan–Meier method ($p = 0.104$).
Conclusion: The prevalence of *TET2* mutations in children with AML compared with adults with AML was lower and less complex. Patient prognosis associated with *TET2* mutations in children requires further investigation.
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Introduction

Acute myeloid leukemia (AML) is a phenotypically and genetically heterogeneous disease affecting 10–15% of pediatric patients with malignancies.[1] In the past decade, molecular studies have identified an increasing panel of genetic markers associated with AML. These markers enhance risk stratification, modify treatment strategy, and improve patient prognosis.[2]

Myeloid leukemogenesis is a multistep process and the classic two-hit model must be modified to account for novel classes of mutant disease alleles, most notably mutations in epigenetic modifiers. These include mutations in the genes encoding tet methylcytosine dioxygenase 2 (*TET2*), isocitrate dehydrogenase 1 (*IDH1*), *IDH2*, additional sex combs-like 1 (*ASXL1*), enhancer of zeste homologue 2 (*EZH2*), and DNA methyltransferase 3A (*DNMT3A*).[3] *TET2* is a DNA methylation regulator that normally converts 5mC to 5hmC, an intermediate event leading to demethylation.[4,5] Deletion of *TET2 in vivo* results in increased hematopoietic stem cell (HSC) self-renewal and development of myeloid skewing in differentiation.[6]

TET2 mutations are found in 7–23% of adults with AML and have demonstrated a correlation with patient prognosis.[7–12] *TET2* mutations appear to be associated with poor prognosis as determined by overall survival in adult Taiwanese patients with AML with intermediate-risk cytogenetics.[8] Other reports similarly found that *TET2* mutations are a poor prognostic indicator in cytogenetically normal AML patients with favorable genotypes.[3,10,13] However, some reports have demonstrated no prognostic effect of *TET2* mutations in patients with primary AML.[9,11] A majority of *TET2* mutations are heterozygous in patients with leukemia where expression of the wild-type allele has been retained, suggesting that *TET2* functions as a haploinsufficient tumor suppressor in most patients.[7]

The prevalence of *TET2* mutations has been demonstrated to be lower in pediatric patients with AML (1.5–6%).[5,14,15] The prognostic value of *TET2* mutations in a pediatric population is not easily determined due to the low incidence of applicable cases. Kutny et al[16] reported that patients with *TET2* mutations had lower event-free survival compared with patients who are nonmutation carriers; this difference was not statistically significant. The same group also reported patients with I1762V polymorphism had better overall survival.[16] The role of *TET2* mutations in AML remains uncertain. Our study aimed to investigate *TET2* mutations in pediatric AML using bioinformatic tools to further evaluate associations with pathogenic effects and disease association.

Methods

Patients and sample collection

Patients with AML who were younger than 18 years of age and who had adequate cryopreserved bone marrow cells for a complete mutation analysis were enrolled. All data were collected between 1997 and 2010 at the National Taiwan University Hospital. Age at onset, sex, initial white blood cell count, blast percentage, hemoglobin level, platelet count, karyotype results, immunophenotyping results, treatment regimens, and outcomes were all retrospectively obtained using patient chart data.

Except for patients with acute promyelocytic leukemia (APL) who received the TPOG APL-97 or APL-2001 protocol, all other patients received the TPOG AML 97A protocol. The institutional ethics committee approved this study (No. DMR100-IRB-035).

DNA sequencing and *TET2* mutation analyses

Genomic DNA was isolated from patients' bone marrow samples at AML diagnosis, and at complete remission (CR) or relapse. Mutation analyses of *TET2* (NM_001127208) were performed using polymerase chain reaction (PCR) followed by direct sequencing.[11] The primer sequences and estimated product lengths are listed in Table S1. Paired samples (diagnosis and either CR or relapse) were compared in a total of 32 patient samples. Missense mutations were regarded as true only if they had been published previously or could be verified in bone marrow samples in patients in remission in the current study. Missense mutations existed both in diagnosis and samples from patients in CR were regarded as a polymorphism. For those novel nonsynonymous mutations without available CR samples, the pathogenic effects were predicted by bioinformatic tools: Polymorphism Phenotyping v2 (PolyPhen-2),[17] MutPred,[18] and Sorting Intolerant From Tolerant (SIFT).[19] When two or more bioinformatics approaches predicted pathological effects, the non-synonymous alterations were considered to be putative mutations. Variants within introns and synonymous variants were not analyzed further.

Table 1 Patient characteristics.

Patient characteristic	
Age, y, median (range)	9 (0–18)
Sex, *n*, (male/female)	38/31
Blasts bone marrow, %, median (range)	71 (1–95)
Blasts peripheral blood, %, median (range)	48 (0–99)
Subtype acute myeloid leukemia, *n*	
M0	2
M1	15
M2	19
M3	5
M4	13
M5	9
M6	2
M7	2
Others	2
Cytogenetics, *n*	
Normal	12
t(8;21)	11
t(15;17)	4
inv(16)/t(16;16)	6
Relapse	31

Statistical analysis

When analyzing patient prognosis, only patients with available active disease were enrolled. Clinical characteristics were compared using a Chi-square test for binary variables and a *t*-test for continuous variables between patients with and without *TET2* alterations. All eligible case data were collected and analyzed to estimate overall survival (OS), which was measured from the start of treatment to death from any cause. Survival curves were estimated using the Kaplan–Meier method. A value of $p < 0.05$ was considered statistically significant.

Results

Patient profile

A total of 69 pediatric patients with AML, including 38 males (55%) and 31 females (45%), were enrolled. The median age was 9 years (range: 0.01–17.5 years). Thirty-one patients (45%) experienced relapse. AM2L and AM1L accounted for most (27.5% and 21.7%, respectively) patient cases. Twelve patients (17%) had a normal karyotype. The French-American-British (FAB) classification and details of cytogenetic changes in patients are listed in Table 1.

TET2 mutation analysis

A total of 103 bone marrow samples were analyzed for mutations. Samples from the active disease stage were collected from 66 patients (59 at diagnosis and 22 at relapse). For the *TET2* mutation analysis, only intronic mutations and missense mutations were analyzed. There were nine missense mutations, distributed across the whole coding sequence, without hot spots (Figure 1). No nonsense or frameshift mutations were identified.

Among 69 patients, 32 patients had samples collected while in CR. Missense mutations P29R, V218M, R814C, F868L, S1039L, and I1762V were classified as polymorphisms. The most common polymorphism was I1762V (n = 31, 45%), followed by V218M (*n* = 8, 12%), P29R (*n* = 4, 6%), and F868L (*n* = 4, 6%). For missense mutations S609C, E1513G, and A1865G, no available sample from a patient in CR was available. Details of published reports describing polymorphisms and missense mutations is shown in Table 2.[10,20–33] According to published reports, E1513G was classified as a polymorphism and A1865G was classified as a true missense mutation. SIFT, Polyphen-2, and MutPred predicted the nonsynonymous mutation S609C as being pathologic; therefore, it was classified as a putative missense mutation. We determined the prevalence of *TET2* mutations in our patient population was 1.4% (1 of 69).

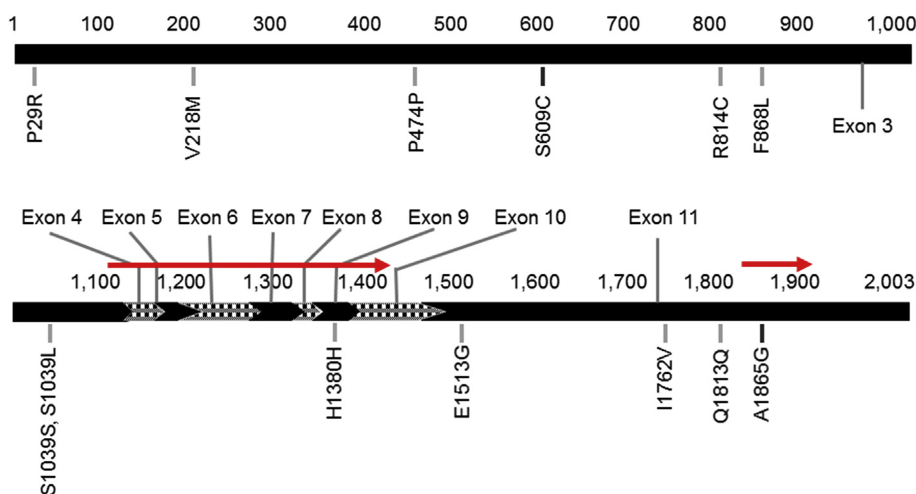

Figure 1 Location of *TET2* missense mutations. Putative missense mutations and polymorphisms are represented by black tick marks and gray tick marks, respectively. Arrows indicate conserved regions (amino acids 1134–1444 and 1842–1921).

Table 2 Published polymorphisms and missense mutations of the *TET2* gene.

	In conserved area	Not in conserved area
Polymorphism	C1211Y,[10] H1219Q,[10] H1219R,[10] L1248T,[8] R1261C,[10] C1263R,[10] C1298Y,[10] G1361D,[10] F1368L,[10] D1376E,[10] H1382Y,[10] I1873T,[10] A1876V,[10] A1882P,[10] L1899P[10]	P29R,[8,15] L34F,[11,13,15] P174H,[13] V218M,[8,15] G355D,[15] P363L,[8,15] N767D,[10] R814C,[8] Y867H,[11,13,15] F868L,[8] P968R,[15] E1010D,[10] S1039L,[8] E1073V,[13] Q1084P,[11,13] A1505T,[10] E1513G,[8] R1543P,[8] G1697R,[10] V1718L,[10] L1721W,[8,15] P1723S,[13,15] I1762V,[8,15] H1778R,[8,15] V1833I[10]
Missense mutations	C1135Y,[13,25] E1137K,[9] Y1148C,[9] H1150D,[20] L1151P,[9,12] R1167T,[21,25] I1175S,[22,25] I1175V,[23,25] C1193W,[22,25] C1193Y,[22,25] I1195V,[24] K1197R,[25] V1199I,[11,25] S1204C,[13,25] L1210P,[25] L1212S,[9,27] R1214W,[13,22,25] R1216Q,[24] H1219N,[8] H1219L,[26] H1219Y,[22,25] C1221Y,[9] W1233G,[26] D1242V,[13,25] Y1245S,[13,25] G1256R,[27] R1261C,[10,13,25,26] R1261H,[9,13,25] R1261L,[25] C1263G,[10] A1264P,[10] T1270A,[28] C1271G,[20] C1271Y,[11] C1271W,[25] C1273S,[10] G1275E,[8,24,25] G1275W,[9] G1282D,[11,24,25] F1287L,[21,25] P1287S,[29] G1288S,[8] G1288V,[13,24] C1289T,[27] C1289W,[27] C1289F,[22,25] C1289V,[25] C1289Y,[10] W1291R,[8,25,29] S1292R,[11,25] N1296G,[27] C1298Y,[10] K1299E,[23,25] K1299G,[30] K1299N,[21,25] F1300C,[26] R1302G,[21,23,25] R1302Y,[29] S1303R,[26] S1303N,[10] E1318G,[21,25,29] L1322Q,[8] L1322P,[8,22,25] L1326S,[8] M1333R,[9] L1340P,[8,25] A1344E,[24] Q1348R,[9] E1352K,[9] A1355P,[24] C1358G,[25] R1359C,[8,14,25] R1359S,[22] R1359H,[8,22,25] L1360R,[24] G1361D,[26] G1361S,[22,25] R1366H,[8,10,25] P1367S,[21,25,29] F1368Y,[11,25] G1370Q,[31] G1370V,[11,25] T1372I,[25] C1374Y,[8] H1380P,[24] H1380Y,[8–10,28] D1384V,[22,25] M1388I,[12] C1396W,[25] L1398R,[25] V1417F,[10,13,25] D1427Y,[8] R1440T,[25] A1443V,[16] D1844G,[24] D1849E,[9] S1853G,[9] G1860R,[22,25] G1861R,[27] A1865G,[8] H1868R,[25] H1868D,[8] H1868P,[10] G1869W,[23,25] S1870L,[26] I1871S,[8] L1872P,[23,25] I1873T,[8,13,21–23,25,26,29,30] C1875R,[25] E1879A,[22,25] H1881R,[13,25,26] H1881Q,[25] T1884A,[9,22,25,26] P1889H,[8] P1894H,[10] R1896G,[26] R1896S,[25] R1896M,[23] S1898F,[23,25] V1900A,[9,13,25] H1904Q,[24] K1905E,[26] G1913D,[21,25,29] G1913V,[22,25] A1919V[13,25]	C25R,[20] L34F,[12,27] P101H,[11,25] S145N,[10,13,25] Q232R,[20] S282F,[11,25] A308T,[21,25] N312S,[13,25] L346P,[32] A394T,[9] P399L,[21,25] K423R,[16] G429R,[9] S460F,[13,15,25] T492S,[11,25] T497S,[26] Q548L,[11,25] H573Y,[20] D666G,[13,25] R686S,[25] D688G,[20] F760Y,[14] N767D,[16] G773V,[11,25] E788L,[28] R814H,[9,16] S817T,[21,25] S826I,[28] Y867H,[12,21,25] F868L,[10,20] P941S,[13,25] E1010D,[16] S1039L,[16] T1114N,[25] T1114I,[33] S1448N,[9] R1467K,[9] A1505T,[9] V1557C,[27] R1572Q,[9] R1572W,[26] P1575F,[25] Y1579S,[8] G1606A,[20] R1660G,[25] Y1696C,[20] V1718L,[9,13,16,25] R1739I,[26] G1754R,[9] H1757D,[13,25] A1794G,[26] L1801F,[8] C1811R,[13,25] Q1828L,[13,25] R1926H,[13,25] P1937S,[24] P1941S,[13,24,25] P1962L,[9,10,24,25] R1966H,[13,25] E1973K,[16] R1974M,[13,25] T1980I,[9] R2000K[13,25]

Clinical manifestations

A comparison of clinical and biological variables between patients with and without I1762V revealed no significant difference between the two groups in terms of age, sex, cytogenetics, blood parameters, and bone marrow blast cells. Patients with polymorphism I1762V demonstrated increased survival rate compared with patients without I1762V (48.4% vs. 25.7%, $p = 0.049$) by Chi-square test. However, OS was not different for patients with and without polymorphism I1762V using the Kaplan-Meier method ($p = 0.104$, Figure 2).

Discussion

The current study analyzed *TET2* mutations in 69 pediatric AML patients—only intronic and missense mutations were identified. Two putative disease-causing missense mutations (S609C and A1865G) were identified in one patient. The overall prevalence of *TET2* mutations in our patients was estimated to be 1.4% (1 of 69). Patients with polymorphism I1762V had better 10-year survival rate compared with patients without I1762V (48.4% vs. 25.7%, $p = 0.049$) using the Chi-square test.

The prevalence of *TET2* mutations in children with AML was lower compared with adults with AML.[5,13,15,23,24,27] In the current study, identifying the *TET2* mutation also proved to be difficult. Moreover, no nonsense or frameshift mutations were identified, which supported the hypothesis that the *TET2* mutation was less complex in children with AML. Similar to the low prevalence of *TET2* mutations in children with AML, functional mutations of other methylation-associated genes, such as *IDH1* or *IDH2* and *DNMT3A* were notably less common (rare or absent) in pediatric patients.[5,34,35] Ho et al[5] reported that 2 IDH2-mutated patients harbored t(8;21), whereas IDH mutations rarely occurred in adult AML patients with favorable cytogenetic abnormalities.[36] Liang et al[14] described that 5.6% of children with AML harbor epigenetic regulator gene mutations and only 1.5% of children had combined with Class I or Class II mutations.

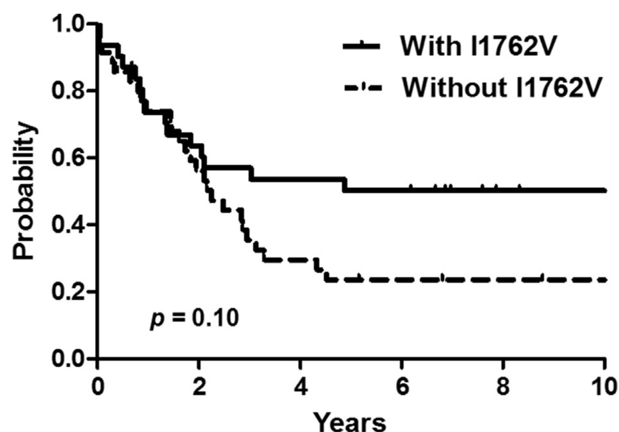

Figure 2 The overall survival (OS) of patients with and without polymorphism I1762V. Patients with polymorphism I1762V had an increased 10-year survival rate compared with patients without I1762V (48.4% vs. 25.7%, $p = 0.049$) using the Chi-square test; OS was not different when examined using the Kaplan–Meier method ($p = 0.104$).

It is notable that methylation-associated somatic mutations arise in a substantially smaller proportion of pediatric AML cases compared with adult AML cases.[5,14] There are likely other significant mechanisms of epigenetic dysregulation in childhood AML patients that have not been elucidated. For example, the MLL gene at 11q23, encoding a histone methyltransferase with putative epigenetic function, is associated with the *IDH2* mutation.[5,37]

To the best of our knowledge, we are currently reporting the first use of bioinformatics tools to estimate the pathogenic value of *TET2* missense mutations. Several studies have applied similar methodology to predict the potentially pathogenic effect of nonsynonymous single nucleotide substitution; the tendency in computational mutation analysis is to use a broad set of prediction methods in order to attain reliable results.[38,39] Published *TET2* mutations and polymorphisms were identified in the literature, which provided the basis for estimated pathogenic effects using bioinformatics tools: Polyphen-2, MutPred, and SIFT. Although two or all of these bioinformatics tools predict this missense mutation pathogenic, we assumed this missense mutation is "true mutation". The assumed results were compared to realistic reported results and the correlation was significant ($p = 0.005$). The positive predictive rate also can achieve 72%. Bioinformatics tools are an economical and practical method to estimate a potential pathogenic effect associated with *TET2* mutations in children with AML.

Published data on the prognostic value of *TET2* mutations in childhood AML are scarce. Only Kutny et al[16] have reported that the I1762V polymorphism was associated with an improved prognosis. We also found patients with polymorphism I1762V tended to have an increased 10-year survival rate compared with patients without polymorphism I1762V using the Chi-square test (48.4% vs. 25.7%, $p = 0.049$); although OS was not different using the Kaplan-Meier method ($p = 0.104$). *TET2* mutations do not appear to be correlated with clinical characteristics or prognosis in childhood AML; however, due to the small number of reported cases, it was difficult to draw any firm conclusions regarding implications for prognosis.[5,15,16]

This current study had several limitations, including the retrospective, single-center, observational study design with a relatively small subject pool. However, considering the patient population it can be considered a pilot study in the pediatric AML space. Future studies should consider focusing on differences in age-specific genetic and epigenetic mechanisms of myeloid leukemogenesis, as these differences may contribute to the development of targeted therapies. Furthermore, using bioinformatics tools to predict a pathogenic effect should be used with caution considering the variable response and lack of a clear association to AML outcomes.

In conclusion, the prevalence of *TET2* mutations in pediatric patients with AML is low and less complex compared with adult AML. The prognostic value of the *TET2* mutation needs further investigation in order to confirm a role in childhood AML.

Acknowledgments

This grant was supported by the China Medical University and Hospital, Taiwan (DMR-100-175). Moreover, part of our research work was done by the research laboratory of pediatrics, Children's Hospital, China Medical University.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jfma.2015.08.002.

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