TET2 Single Nucleotide Polymorphism in Myeloid Neoplasms Among Egyptian Patients

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Abstract Acute myelogenous leukemia (AML) is a heterogeneous disease characterized by myeloid progenitor cells uncontrolled proliferation gradually replacing normal hematopoiesis. To evaluate Ten Eleven Translocation 2 gene (TET2) single nucleotide polymorphism (SNP) (rs2454206, rs34402524, rs61744960) in AML, and chronic myeloid leukemia (CML) in relation to their disease prognostic criteria. The study included 136 subjects; 52 AML, 54 CML and 30 subjects as control group matched for age and sex. Routine investigations including CBC, bone marrow aspiration, flow cytometry biochemical investigations and cytogenetics and molecular study were performed accordingly. DNA extraction and SNP assay for TET2 gene polymorphism was done using (Thermo-Fisher predesigned SNP, USA) PCR prism 7500. The mean age was 43.4 ± 14.0 years in AML patients, 45.98 ± 15.7 years in CML patients and 39.3 ± 6.587 years in control group ($p > 0.05$). The frequency of TET2 SNP rs 34402524 ranged from heterozygous to homozygous in both AML (46%, 54%) and CML (48%, 46.2%) groups but was mainly homozygous among the control (80%) group ($p = 0.012$). TET2 SNP rs 2454206 was mainly wild in CML (65.4%) and control (63.3%) groups compared to

AML as wild was only in (46%) and heterozygous in (44%) with only 10% being homozygous ($p = 0.046$). TET2 SNP rs 61744960 showed a homozygous pattern among all three group (AML CML and control) showing no statistical significance ($p = 0.528$). Eventhough, higher non responders to treatment were among homozygous and heterozygous groups yet, response to therapy as respect to specific TET2 SNP showed no significant variation ($p > 0.05$). TET2 SNP in CML cases did not alter the prognostic criteria as no statistical significance was noted ($p > 0.05$) except for TET2 SNP rs 34402524 where homozygous cases had larger spleen size ($p = 0.019$). TET2 SNP is common in Egyptian myeloid neoplasm. This is the first study in this field and further studies are recommended to investigate TET2 and relation to other hematological malignancies and leukemogenesis transformation.

Keywords Acute myeloid leukemia · Chronic myeloid leukemia · Ten Eleven Translocation 2 gene

Introduction

Acute myelogenous leukemia (AML) is a heterogeneous disease characterized by myeloid progenitor cells uncontrolled proliferation replacing normal hematopoiesis in the bone marrow [1]. The genetic changes arising in the neoplastic clone lead to cascades of molecular events that cause abnormal proliferation, aberrant differentiation, and inhibition of normal hematopoiesis by the malignant cells [2, 3]. Genetic events transformation are increasingly important and characterize the diagnosis, define prognosis, and plan of therapy in AML [4].

Ten Eleven Translocation gene2 (TET-2) has pleiotropic roles during hematopoiesis, including stem cells self-

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renewal, lineage commitment, and terminal differentiation of monocytes [5]. TET2 gene is located in chromosome 4q24 region [6] and has 11 exons [7], resulting in three messenger RNA (mRNA) isoforms differing due to alternative splicing [8].

The TET proteins includes TET-1, TET-2, TET-3 which are α -ketoglutarate and Fe^{2+} dependent enzymes capable of modifying DNA methylation status [9]. TET family enzymes and 5-hmC are critical in epigenetic regulation during development [10]. Polymorphisms arise through mutations. It can be inherited as a DNA sequence thus making it possible to be tracked from parent to child [11]. Mutations in Ten-Eleven-Translocation-2 (TET2) among myeloid malignancies were first detected by high-resolution single nucleotide polymorphism (SNP) and comparative genomic hybridization arrays [12]. Homozygous and heterozygous mutations in *TET-2* gene are recurrent events in human hematopoietic malignancies. Most of these mutations decrease TET-2 enzymatic activity by truncating the protein or affecting its catalytic activity. TET-2 deletion is sufficient to initiate myeloid and lymphoid transformation [13]. The role of TET2 polymorphism is not fully established according to the prognostic and responsiveness to treatment in context of myeloid malignancies. In this study the variation in TET2 polymorphism expression will be investigated to compare between acute myeloid and chronic myeloid leukemia suggesting a possible role in the leukemogenesis transformation.

Aim of Work

The aim of this study is to evaluate the frequency of the TET2 single nucleotide polymorphism (SNP) (rs2454206, rs34402524, rs61744960) in Acute Myeloid Leukemia patients compared to chronic myeloid leukemia and healthy controls in relation to the disease prognostic criteria.

Materials and Methods

The study included 136 subjects; 52 cases were diagnosed as AML, 54 cases were diagnosed as CML and 30 subjects as control group matched for age and sex. Cases were selected from Alexandria Main University Hospital Internal Medicine Department (Hematology Unit) to determine the selected SNPs genotype frequency. Written Informed consent was taken from every patient and approval of the Ethical committee (IRB No. 00008699, FWA No. 00015712) was provided. Routine investigations including CBC, bone marrow aspiration, flow cytometry biochemical investigations and cytogenetics and molecular study were performed accordingly. A 3 ml blood collected via EDTA tubes from either peripheral blood or bone marrow aspirate was done.

DNA extraction was performed using Invitrogen purelink genomic DNA minikit (Cat No. k1820-01, Lot No 1510617). The SNP assay for TET2 gene polymorphism was done using (Thermo-Fisher predesigned SNP, USA) PCR prism 7500 device included three polymorphisms that are

c-25996719-10, rs 34402524, lot p161221-001 H06, PN*40 (intron missense).

c-11566753-20, rs 2454206, lot p161221-001 H08 PN*40 (intron missense).

c-25746528, rs 61744960, lot p161221-001 H05 PN*40 (intron missense).

Two samples among each AML and CML failed to yield sufficient DNA amount for the PCR run. AML cases were given 3 + 7 protocol and were followed up for 2 months to compare to outcome while CML cases started Tyrosine kinase inhibitors.

Statistical Analysis

The software of IBM SPSS 20 was used. Data were tested for normality using Kolmogorov–Smirnov test, Shapiro–Wilk test. Measurement data were displayed in the form of minimum, maximum, mean $\pm$ SD. *t* test was used for comparing means in parametric data. Qualitative data were displayed in percentages and tested by Pearson's Chi-Square and Fisher Exact Test according to the categories and cells estimation %. If the distribution was nonparametric in distribution, measurement data were displayed in the form of median value and range and nonparametric test (Mann–Whitney *U*) was used for comparing median value. Spearman bivariate correlation analysis was used for analyzing correlation. *p* < 0.05 indicated statistical significance.

Results

The mean age was 43.4 ± 14.0 years in AML patients, 45.98 ± 15.7 years in CML patients and 39.3 ± 6.587 years in control group (*p* > 0.05). The frequency of TET2 SNPs rs 34402524 ranged from heterozygous to homozygous in both AML (46%, 54%) and CML (48%, 46.2%) groups but was mainly homozygous among the control (80%) group (*p* = 0.012). TET2 SNP rs 2454206 was wild among CML (65.4%) and control (63.3%) groups compared to AML which was wild in (46%) and heterozygous in (44%) with only 10% being homozygous (*p* = 0.046). TET2 SNP rs 61744960 showed a homozygous pattern among all three group (AML CML and control) showing no statistical significance (*p* = 0.528) (Table 1, Fig. 1). TET2 SNP in AML was not significantly influenced by gender, FAB classification, mortality, cytogenetic findings *p* > 0.05 (Table 2). AML patients were started on 3 + 7 protocol and were followed up for

Table 1 TET2 single polymorphism patterns for the 3 positions among the three studied groups (AML, CML, and control)

TET2 SNP	AML		CML		Control		Test of significance
	No. (n = 52)	%	No. (n = 54)	%	No. (n = 30)	%	
rs 34402524*							
Wild	0	0	3	5.8	0	0	$p = 0.012$
Heterozygous	23	46	25	48.0	6	20	
Homozygous	27	54	24	46.2	24	80	
rs 2454206*							
Wild	23	46	34	65.4	19	63.3	$p = 0.046$
Heterozygous	22	44	18	34.6	11	36.7	
Homozygous	5	10	0	0	0	0	
rs 61744960*							
Heterozygous	2	4	2	3.8	0	0	$p = 0.528$
Homozygous	48	96	50	96.2	30	100	

Bold values indicate $p < 0.05$ considered significant

Percentages by column * 2 cases failure

Fig. 1 Distribution of single nucleotide polymorphism among the studied group **Table 2** Cytogenetic findings in relation to the 3 single nucleotide polymorphism of TET2 gene among the acute myeloid leukemia patients in the studied group

SNP	Cytogenetic findings				Test of significance
	Normal karyotype		Abnormal		
	No.	%	No.	%	
RS 2454206*					
Wild (n = 23)	18	36	5	10	$p = 0.868$
Mutant (n = 27)	18	36	9	18	
RS 34402524*					
Heterozygous (n = 23)	17	34	6	12	$p = 0.227$
Homozygous (n = 27)	19	38	8	16	
RS 61744960*					
Heterozygous (n = 2)	2	4	0	0	$p = 0.999$
Homozygous (n = 48)	34	68	14	28	

F Fisher test

$p < 0.05$ significant * 2 failure

$p < 0.05$ significant * 2 cases failure

Table 3 Outcome among TET2 single polymorphism patterns for the TET2 SNP in 3 positions within the AML studied group

		Outcome	AML		Test of significance
			No.	%	
RS 34402524*					
Heterozygous (n = 23)	Responders		10	20	<i>p</i> = 0.438
	Non-responders		13	26	
Homozygous (n = 27)	Responders		12	24	
	Non-responders		15	30	
RS 2454206*					
Wild (n = 23)	Responders		11	24	<i>p</i> = 0.615
	Non-responders		11	24	
Heterozygous (n = 22)	Responders		10	20	
	Non-responders		12	24	
Homozygous (n = 5)	Responders		1	2	
	Non-responders		4	8	
RS 61744960*					
Heterozygous (n = 2)	Responders		1	2	<i>p</i> = 0.861
	Non-responders		1	2	
Homozygous (n = 48)	Responders		23	46	
	Non-responders		25	50	
<i>p</i> < 0.05 considered significant * 2 cases failure					

$p < 0.05$ considered significant * 2 cases failure

Table 4 Comparison between prognostic factors in CML cases in chronic phase and TET2 polymorphism

SNP	CML			
	Spleen size		WBC	Sokal score
	No.	Mean $\pm$ SD	Median	Mean $\pm$ SD
Rs 34402524*				
Wild	3	18.75 $\pm$ 3.2	16,000	1.01 $\pm$ 0.057
Heterozygous	25	20.4 $\pm$ 3.6	79,000	1.33 $\pm$ 0.28
Homozygous	24	24.3 $\pm$ 4.1	66,500	1.45 $\pm$ 0.26
Test of significance		$p = 0.019$	$p = 0.07$	$p = 0.87$
Rs 2454206*				
Wild	34	21.47 $\pm$ 4.41	67,000	1.31 $\pm$ 0.29
Heterozygous	18	24.05 $\pm$ 3.68	70,000	1.44 $\pm$ 0.25
Test of significance		$p = 0.108$	$p = 0.224$	$p = 0.221$
Rs 61744960*				
Heterozygous	2	23.5 $\pm$ 1.5	455,500	1.4
Homozygous	50	22.27 $\pm$ 4.34	67,000	1.366 $\pm$ 0.28
Test of significance		$p = 0.54$	$p = 0.132$	$p = 0.907$

Bold values indicate $p < 0.05$ is considered significant

* 2 cases failure

2 months. The outcome was classified as responders vs non responders. Despite that higher frequency were non responders within both homozygous and heterozygous groups yet, response to therapy as compared to specific TET2 SNP showed no significant variation ($p > 0.05$) (Table 3). TET2

SNP in CML cases did not alter the prognostic criteria as no statistical significance was noted ($p > 0.05$) except for TET2 SNP rs 34402524 where homozygous cases had larger splenic size ($p = 0.019$) (Table 4).

Discussion

In our study, there is no statistical significant difference between the three groups as regard age and gender ($p = 0.261$). The frequency TET2 SNP rs 34402524 showed heterozygous expression along with homozygous in AML and CML but was mainly homozygous among the control group ($p = 0.012$). TET2 SNP rs 2454206 was mainly wild among CML and control groups compared to wild, heterozygous and 10% homozygous expression among AML ($p = 0.046$). TET2 SNP rs 61744960 showed a homozygous pattern among all three group ($p = 0.528$) (Table 1, Fig. 1).

Hamed et al. [14] reported that TET2 polymorphism SNP rs 6843141 was detected in all AML cases with 83.9% patients showing homozygous pattern and 16.1% patients showing heterozygous pattern while the control group was mostly wild type 44% and heterozygous group 44% subjects ($p < 0.001$).

Kutny et al. [15] in a study involving 403 patients enrolled in Children's Cancer Group reported that TET2 SNPs prevalence was (4–54%), the most common was SNP, rs2454206 (A > G, I1762V).

Hamed et al. [16] reported that TET2 was under-expression in a total of 87.9% de-novo AML patients and in 12.1% chronic myeloid leukemia in blastic crisis. In all, 66.6% of cases had normal cytogenetics. This could be attributed to single nucleotide polymorphism or mutations occurring that did not show gene expression by PCR.

Li et al. from Taiwan in 2011 [17] reported that about 78.6% of patients were presented with TET2 SNP (single nucleotide polymorphism). All SNPs were heterozygous, only 4 SNP were homozygous; all were in SNP rs2454206 (I1762V) [17]. The most prevalent polymorphism was I1762V (45%), V218M (12%), P29R (4.6%), and F868L (4.6%).

Chou et al. [12] reported a total of 486 patients, consisting of 212 females and 274 males; median age of 51.5 years were recruited for TET2 mutation analysis. Seventy-six different TET2 mutations were detected in 64 patients (13.2%). Double mutations of TET2 were detected in 27 patients (42.2%), and 6 of them had homozygous TET2 mutation.

TET2 mutation may be a secondary event during initial leukemogenesis, and leukemic cells with this mutation can be selected away by chemotherapy, leaving TET2-wild residual leukemia cells to grow subsequently into major population at relapse. Alternatively, TET2 mutation may be important for initiation of leukemogenesis, but not necessary for maintenance of the phenotype [12].

In our study, TET2 SNP did not showed any statistical difference between polymorphism genotype and age,

gender FAB and cytogenetics status in AML patients ($p > 0.05$). The majority of cases were of normal karyotyping either within wild, heterozygous or homozygous expression ($p > 0.05$) (Table 2). As for outcome among AML patients in regard to specific TET2 SNPs did not show statistical significance ($p > 0.05$) (Table 3).

Hamed et al. [14] reported that 55.5% of AML patients were cytogenetically with TET2 polymorphism ($p > 0.05$). Kutny et al. [15] stated that TET2 SNP rs2454206 genotype is influenced by racial variations. *TET2^{AA}* genotype occurred in 79% of black versus 39% of white patients ($p < 0.001$). There was no significant relation between SNP genotype and median age, gender, median WBC, median blast percentage, FAB groups, cytogenetic groups, between patients with *TET2^{AG/GG}* and *TET2^{AA}* genotypes ($p > 0.05$). OS was higher in minor allele genotypes (*TET2^{AG/GG}*) than in *TET2^{AA}* genotype at 5 years ($p = 0.013$). An independent cohort of 234 patients treated on COG AAML03P1 (5-year OS $73 \pm 8\%$ for *TET2^{AG/GG}* vs. $57 \pm 10\%$ for *TET2^{AA}*; ($p = 0.031$).

Li et al. from Taiwan in 2011 [17] reported that clinical characteristics including gender, age, WBC at diagnosis, cytogenetics, treatment outcome (death, relapse) were not different between SNP polymorphism groups. Kaplan–Meier survival analysis did not show difference in overall survival and event free survival among SNP groups ($p > 0.05$). Chou et al. [12] reported that chromosome data available in 467 patients related TET2 mutation to patients with intermediate risk cytogenetics according to SWOG criteria ($p < 0.001$) were normal karyotype ($p = 0.0064$), or isolated trisomy 8 ($p = 0.017$).

Niboural et al. [18] studied denovo AML patients achieving CR to focus on TET2 mutations from potential polymorphisms. He reported no significant difference between patients' characters: gender, age, hemoglobin level, WBC, platelet count, FAB, and cytogenetics and TET2 genotypes ($p > 0.05$).

As for CML in chronic phase, it is clear that TET2 polymorphism is common yet did not alter the prognostic criteria for these patients in regard to sokal score and WBC count, and spleen size ($p > 0.05$) except for TET2 SNP Rs 34402524 where homozygous group had a huger spleen size compared to wild type ($p < 0.05$) (Table 4). This can reflect the role of TET2 polymorphism in either CML transformation or resistance to Tyrosine kinase inhibitors.

Conclusions and Recommendations

TET2 SNP is a common finding among Egyptian myeloid neoplasm patients. It may have a potential role in leukemogenesis as a racial variation. This is the first paper to compare it to prognostic parameters and further studies are

recommended to investigate TET2 and relation to other hematological malignancies.

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Author's Contribution ED corresponding author funded, selected patients and data, participated in the lab work for molecular study, and followed up cases after induction/remission cycle. NAMH and NAE suggested the field of research, supervised, monitored and guided the research. HSK Medical Genetics Center performed the molecular study on TET2 polymorphism. MWA Clinical Pathology performed the routine diagnostic tests including biochemical tests bone marrow aspiration immunophenotyping and cytogenetics analysis.

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Availability of Data and Materials All data are in the possession of corresponding author and were presented in figure and tables and materials of study were executed in both Medical Genetics Center and Clinical Pathology in Faculty of Medicine, Alexandria University.

Compliance with Ethical Standards

Conflict of interests All authors declare that they have no conflict of interest.

Ethical Approval All procedures in this research was subjected to the ethical regulation of ethical committee and approval of the Ethical committee of the Faculty Of Medicine–Alexandria University according to ICH GCP guidelines and applicable local and institutional regulations and guidelines governing EC operation (IRB No. 00008699, FWA No. 00015712) full-filling the 1964 Helsinki declaration.

Informed Consent Written Informed consent was taken from every patient. All authors certify that they have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere. There is no conflict of interest and the corresponding author is the main author whom research was done by. There is no image, video or details concerning patients requiring consent to publish.

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