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# Ethnic Variation of *TET2* SNP rs2454206 and Association with Clinical Outcome in Childhood AML: A Report from the Children's Oncology Group

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Epigenetic deregulation is a common finding in myeloid malignancies, and epigenetic therapies have been used successfully to treat patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Inactivating mutations of *TET2* have been found in



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**Conflict of Interest:**

J.F. is employed by Amgen Inc (Thousand Oaks, CA, USA). The remaining authors declare no competing financial interests.

Supplementary information is available at Leukemia's website.

myeloid cancers and impair the hydroxylation of 5-methylcytosine.[1] A study of 104 pediatric AML patients found only 4 patients (3.8%) with somatic mutations of *TET2*.[2] There is, however, growing evidence that germline single nucleotide polymorphisms (SNPs) may also predict outcomes.[3, 4] Here we demonstrate that somatic mutations of TET2 are rare in pediatric AML, but we present novel evidence that the *TET2* SNP rs2454206 (I1762V) is a prognostic marker for outcome in pediatric AML.

This study included 403 patients treated on Children's Cancer Group study CCG-2961 (N=169) or COG AAML03P1 (N=234). The CCG-2961 cohort was used as a discovery set and the prognostic biomarker (*TET2* SNP rs2454206) was validated in the COG AAML03P1 cohort. Outcomes analyzed included overall survival (OS), event-free survival (EFS), relapse rate (RR) and non-relapse mortality (NRM). Hazard ratios (HRs) were determined in univariate and multivariate analyses including risk group (Supplementary Material).

DNA extracted from Ficoll enriched diagnostic material was subjected to PCR amplification of the entire coding sequence of *TET2* using 17 primer pairs (Supplemental Table 1). Sequence data were analyzed to identify somatic mutations and SNPs (Supplemental Material). Expression quantitative trait loci (eQTL) analysis was performed to evaluate the association between *TET2* SNP rs2454206 and all probes within 1 Mb (Supplemental Material).[5] For replication, the MuTHER study was interrogated.[6] SNPs in strong linkage disequilibrium with SNP rs2454206 were evaluated for effect on regulatory motifs.[7, 8] We performed principal component analysis (PCA) on whole-genome genotype data available for a random subset of the samples (n=69) to quantify their genomic ancestry.[9]

In an initial cohort of 169 patients treated on CCG-2961, 26 germline variants were found in *TET2* exons. (Supplemental Table 2). Sixteen SNPs were too rare (prevalence 0.58%–2.3%) to offer potential of significant correlation with outcome given the cohort size. Of the 10 remaining SNPs with higher prevalence (4%–54%), only the most prevalent SNP, rs2454206 (A>G, I1762V) was associated with survival. OS was significantly higher for patients with minor allele genotypes ($TET2^{AG/GG}$) than those with $TET2^{AA}$ genotype (60±10% vs. 38±11% at 5 years, log-rank $P$=0.013; Supplemental Figure 1a). This finding was validated in an independent cohort of 234 patients treated on COG AAML03P1 (5-year OS 73±8% for $TET2^{AG/GG}$ vs. 57±10% for $TET2^{AA}$; log-rank $P$=0.031; Supplemental Figure 1b).

The prevalence of $TET2^{AG/GG}$ genotypes was similar in both studies (54% on CCG-2961 and 50% on AAML03P1) and to that observed in the general population. Sequence analysis of a subset of remission samples confirmed the rs2454206 genotype as germline. As rs2454206 genotype had similar clinical consequences in both study cohorts, subsequent analyses were conducted on the combined cohort ($n$=403).

The prevalence of somatic mutations was only 1.7% (7/403), and these few mutations were not significantly associated with rs2454206 genotype. Three patients had nonsense mutations (Q917X, R1216X, S1798X), one patient had two nonsense mutations (Q958X and E1323X), and 2 patients had missense mutations (C171F, L1332P). One patient had a heterozygous single base insertion (ins1870-1871) causing a frame shift and early

termination (E637X). Among these 7 patients with *TET2* somatic mutations, at the time of last follow-up 1 patient was alive without relapse and 6 patients had relapsed.

The rs2454206 genotype varied by race. $TET2^{AA}$ genotype was present in 79% of black patients vs. 39% of white patients (p<0.001) (Supplemental Figure 2). This is similar to the frequency reported in healthy individuals (http://browser.1000genomes.org). There was no difference in median age, gender, median WBC, median blast percentage, FAB groups, cytogenetic groups, mutations of *CEBPA* and *WT1*, *FLT3*-ITD or disease risk group between patients with $TET2^{AG/GG}$ and $TET2^{AA}$ genotypes. There was a lower prevalence of *NPM1* mutations with $TET2^{AG/GG}$ compared to $TET2^{AA}$ (2.8% vs. 9.5%, $P$=0.009). Despite decreased prevalence of this favorable prognostic marker, the superior outcome in the $TET2^{AG/GG}$ group suggests this SNP is independent of current risk group markers, and this is supported by the multivariate analysis reported below.

Remission rate and relapse risk were similar for patients with $TET2^{AG/GG}$ and $TET2^{AA}$ genotypes, but OS and NRM differed significantly (Supplemental Table 3 and Figure 3). Five-year OS was significantly lower with $TET2^{AA}$ compared to $TET2^{AG/GG}$ (49±7% vs. 68±7%, log-rank $P$=0.002). The NRM was significantly higher with $TET2^{AA}$ compared to $TET2^{AG/GG}$ (16% vs. 8%, P=0.035). Patient characteristics and outcomes were compared for patients who were homozygous ($TET2^{GG}$; $N$=57) and heterozygous ($TET2^{AG}$; $N$=152) for the minor allele of rs2454206 (Supplemental Material). There was no difference in OS or NRM, and these minor allele genotypes are grouped together for the following analyses.

Multivariate analyses demonstrated that *TET2* SNP genotype was an independent predictor of OS and NRM when analyzed with cytogenetic/molecular risk factors and also a predictor of OS when analyzed with race (Table 1). To further explore the impact of race, patients were stratified into 4 groups by race and rs2454206 genotype. In this comparison, OS and NRM differed significantly (Figure 1). White patients with the $TET2^{AA}$ genotype had a 5-year NRM of 14±7% and OS of 54±10% while those with $TET2^{AG/GG}$ genotypes had NRM of 8±4% ($P$=0.23) and OS of 68±7% ($P$=0.09). Among non-white patients, those with the $TET2^{AA}$ genotype had a NRM of 24±12% and OS of 40±14% while those with $TET2^{AG/GG}$ genotypes had a NRM of 10±14% ($P$=0.17) and OS of 63±22% ($P$=0.08). Further among non-white patients, the relapse rate trended lower at 27±14% for $TET2^{AA}$ compared to 53±26% for $TET2^{AG/GG}$ ($P$=0.066).

Whole-genome data available from 69 patients in the cohort showed high concordance of self-reported race with the corresponding genomic ancestry derived from principal component analyses (PCA).[9] Furthermore, association analyses between rs2454206 and outcome with the first two principal components as covariates showed that the resulting hazard ratios were in the direction and magnitude expected though not significant likely due to the reduced sample size (Supplemental Table 4).

A detailed analysis of the causes of NRM and non-lethal toxicities was performed (Supplemental Material and Tables 5–8). In summary, infections were the major cause of NRM for the entire cohort, but patients with $TET2^{AA}$ genotype experienced a greater proportion of infection related NRM. There was no association between rs2454206 and

organ system toxicities. The *TET2*$^{AA}$ genotype, however, was associated with increased number of ICU days and higher NRM in specific chemotherapy courses.

We sought to functionally characterize rs2454206 using expression quantitative trait loci (eQTL) information derived from a comprehensive transcriptome study of the HapMap3 LCLs.[5] The SNP rs2454206 was found to be a *cis* eQTL (p=0.0004 with Bonferroni significance threshold of 0.007) for CXXC Finger Protein 4 (*CXXC4*) in the MEX samples, with each additional G allele associated with increased expression of the gene (Supplemental Figure 4). Furthermore, the SNP showed consistent direction of effect in all other populations (CEU, CHB, GIH and LWK) although not significant (Supplemental Material). The *cis* eQTL association with *CXXC4* was replicated using data from the MuTHER study (Supplemental Material and Figure 5). The association between the *TET2* SNP and *CXXC4* expression is remarkable given that *CXXC4* is a negative regulator of *TET2*.[10] To further evaluate this long-range interaction, we interrogated Hi-C data (http://www.3dgenome.org) that enables genome-wide three dimensional proximity mapping.[11] We found cell-type specific significant interaction between *CXXC4* and *TET2* in hematologic cells (GM12878 LCL) that was not present in endothelial cells (HUVEC) or epithelial cells (HMEC) (Supplemental Figure 6).

We identified 19 SNPs in strong linkage disequilibrium ($r^2 \geq 0.80$) with SNP rs2454206 in the CEU samples of the 1000 Genomes Project. Alleles at these SNPs alter known regulatory motifs (Supplemental Table 9), showing that these variants are likely to affect transcription.[8] In contrast, in the samples of African descent (YRI), no SNP passed the same $r^2$ threshold for linkage disequilibrium with SNP rs2454206, suggesting that the SNP is likely to be the causal variant at this locus.

Thus, while somatic mutations of *TET2* are rare (1.7%) in our large cohort of over 400 pediatric AML patients, we demonstrate that the minor allele of a common *TET2* SNP (rs2454206) was associated with improved survival in two independent clinical trials. The superior OS was not due to differences in risk of relapse; rather, the *TET2* genotypes were associated with differences in NRM, particularly due to infection.

The association between rs2454206 and NRM was consistent between racial groups. This suggests that the observed genetic association was unlikely to be due to confounding by population stratification. We observed that non-white patients with *TET2*$^{AA}$ genotype showed excess toxicity compared to those with *TET2*$^{AG/GG}$ genotype and white patients, predominantly due to increased infection rates. Access to chemotherapy, differences in supportive care or leukemia phenotype, and reduced compliance were unlikely explanations for the observed differences, as therapy was uniformly delivered in the inpatient setting for all patients according to CCG/COG protocols. Associations of specific host polymorphisms with drug toxicities is well documented, but are generally linked to alterations in function of drug metabolizing genes.[12–14] Our observation cannot be directly accounted for by alterations in drug metabolism, and may suggest that they are associated with alternate mechanisms that confer host susceptibility to non-leukemic complications.[3, 15]

Our functional analysis did link rs2454206 to *CXXC4* expression. *CXXC4* has recently been reported to affect caspase activation and act as a negative regulator of *TET2*.[10] This SNP may further serve as a marker of other polymorphisms that alter *TET2* function as we found that it is in strong linkage disequilibrium with multiple SNPs that alter regulatory motifs. Validation of *TET2* rs2454206 genotype as a marker of increased NRM, especially in the non-white population will allow more targeted monitoring and supportive care in a population that may be at elevated risk of NRM.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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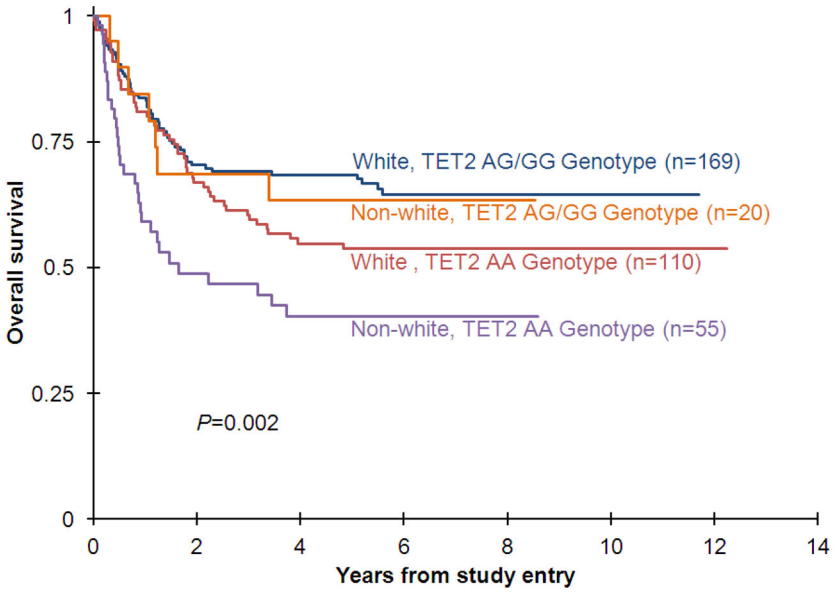


**Figure 1a**

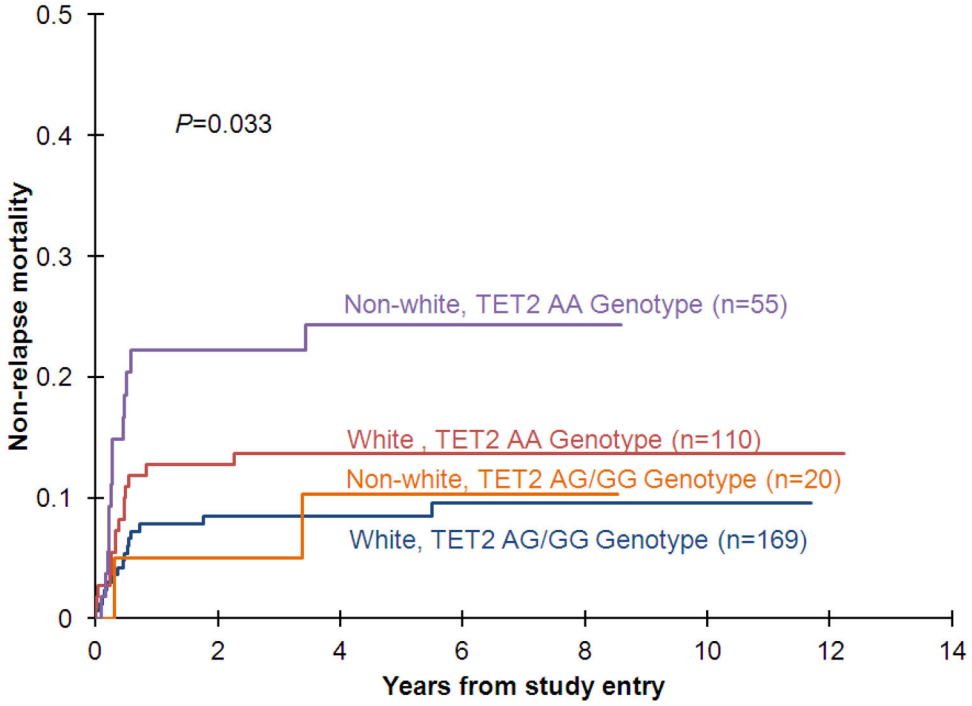


**Figure 1b**

**Figure 1.**

Kaplan-Meier curves of overall survival (a) and non-relapse mortality (b) by race and SNP rs2454206 genotype

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**Table 1**

Multivariate Analyses of SNP rs2454206 Genotype, Risk Groups and Race

| **TET2 SNP and Risk Groups** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | **OS from study entry** | | | **NRM from study entry** | | |
| | **N** | **HR** | **95% CI** | **p** | **HR** | **95% CI** | **p** |
| **TET2 SNP** | | | | | | | |
| ***TET2*** $^{AG/GG}$ | 174 | 1 | | | 1 | | |
| ***TET2*** $^{AA}$ | 170 | 1.65 | 1.18–2.31 | **0.004** | 1.59 | 1.04–2.44 | **0.034** |
| **Risk groups** | | | | | | | |
| **Standard** | 164 | 1 | | | 1 | | |
| **Low** | 127 | 0.44 | 0.29 – 0.66 | **<0.001** | 0.83 | 0.52 – 1.33 | 0.437 |
| **High** | 53 | 1.49 | 0.99 – 2.25 | 0.055 | 1.46 | 0.82 – 2.57 | 0.196 |

| **TET2 SNP and Race** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | **OS from study entry** | | | **NRM from study entry** | | |
| | **N** | **HR** | **95% CI** | **p** | **HR** | **95% CI** | **p** |
| **TET2 SNP** | | | | | | | |
| ***TET2*** $^{AG/GG}$ | 189 | 1 | | | 1 | | |
| ***TET2*** $^{AA}$ | 165 | 1.5 | 1.07 – 2.11 | **0.018** | 1.4 | 0.91–2.16 | 0.122 |
| **Race** | | | | | | | |
| **White** | 279 | 1 | | | 1 | | |
| **Non-white** | 75 | 1.47 | 1.01 – 2.16 | **0.047** | 1.82 | 1.15 – 2.88 | **0.011** |

**Abbreviations:** HR, hazard ratio; CI, confidence interval; OS, overall survival; NRM, non-relapse mortality.

**Risk Group definitions:** Low risk: t(8;21), inv(16), CEBPA or NPM mutation; High risk: monosomy 7, -5/5q- or FLT3/ITD+ with high allelic ratio; Standard risk: All other patients with available cytogenetic data