Ethnic Variation of \textit{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 \textit{TET2} have been found in
myeloid cancers and impair the hydroxylation of 5-methylcytosine. A study of 104 pediatric AML patients found only 4 patients (3.8%) with somatic mutations of TET2. There is, however, growing evidence that germline single nucleotide polymorphisms (SNPs) may also predict outcomes. 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). For replication, the MuTHER study was interrogated. SNPs in strong linkage disequilibrium with SNP rs2454206 were evaluated for effect on regulatory motifs.

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

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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<sup>AA</sup> 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 TET<sup>2AG/GG</sup> and TET<sup>2AA</sup> genotypes. There was a lower prevalence of NPM1 mutations with TET<sup>2AG/GG</sup> compared to TET<sup>2AA</sup> (2.8% vs. 9.5%, P=0.009). Despite decreased prevalence of this favorable prognostic marker, the superior outcome in the TET<sup>2AG/GG</sup> 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 TET<sup>2AG/GG</sup> and TET<sup>2AA</sup> genotypes, but OS and NRM differed significantly (Supplemental Table 3 and Figure 3). Five-year OS was significantly lower with TET<sup>2AA</sup> compared to TET<sup>2AG/GG</sup> (49±7% vs. 68±7%, log-rank P=0.002). The NRM was significantly higher with TET<sup>2AA</sup> compared to TET<sup>2AG/GG</sup> (16% vs. 8%, P=0.035). Patient characteristics and outcomes were compared for patients who were homozygous (TET<sup>2GG</sup>; N=57) and heterozygous (TET<sup>2AG</sup>; 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 TET<sup>2AA</sup> genotype had a 5-year NRM of 14±7% and OS of 54±10% while those with TET<sup>2AG/GG</sup> genotypes had NRM of 8±4% (P=0.23) and OS of 68±7% (P=0.09). Among non-white patients, those with the TET<sup>2AA</sup> genotype had a NRM of 24±12% and OS of 40±14% while those with TET<sup>2AG/GG</sup> 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 TET<sup>2AA</sup> compared to 53±26% for TET<sup>2AG/GG</sup> (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). 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 TET<sup>2AA</sup> genotype experienced a greater proportion of infection related NRM. There was no association between rs2454206 and
organ system toxicities. The TET2\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{2} \textgeq 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.\textsuperscript{8} In contrast, in the samples of African descent (YRI), no SNP passed the same r\textsuperscript{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\textsuperscript{AA} genotype showed excess toxicity compared to those with TET2\textsuperscript{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.\textsuperscript{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.\textsuperscript{3, 15}

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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. 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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References

1. Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature. 2010 Dec; 468(7325):839–843. [PubMed: 21057493]
2. Langemeijer SM, Jansen JH, Hooijer J, van Hoogen P, Stevens-Linders E, Massop M, et al. TET2 mutations in childhood leukemia. Leukemia. 2011 Jan; 25(1):189–192. [PubMed: 21042320]
3. Ho PA, Kuhn J, Gerbing RB, Pollard JA, Zeng R, Miller KL, et al. WT1 synonymous single nucleotide polymorphism rs16754 correlates with higher mRNA expression and predicts significantly improved outcome in favorable-risk pediatric acute myeloid leukemia: a report from the children’s oncology group. J Clin Oncol. 2011 Feb; 29(6):704–711. [PubMed: 21189390]
4. Wagner K, Damm F, Göhring G, Görlich K, Heuser M, Schäfer I, et al. Impact of IDH1 R132 mutations and an IDH1 single nucleotide polymorphism in cytogenetically normal acute myeloid leukemia: SNP rs11554137 is an adverse prognostic factor. J Clin Oncol. 2010 May; 28(14):2356–2364. [PubMed: 20368538]
5. Stranger BE, Montgomery SB, Dimas AS, Parts L, Stegle O, Ingle CE, et al. Patterns of cis regulatory variation in diverse human populations. PLoS Genet. 2012; 8(4):e1002639. [PubMed: 22532805]
6. Nica AC, Parts L, Glass D, Nisbet J, Barrett A, Sekowska M, et al. The architecture of gene regulatory variation across multiple human tissues: the MuTHER study. PLoS Genet. 2011; 7(2):e1002003. [PubMed: 21304890]
7. Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, et al. A map of human genome variation from population-scale sequencing. Nature. 2010 Oct 28; 467(7319):1061–1073. [PubMed: 20981092]
8. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 2012 Jan; 40(Database issue):D930–934. [PubMed: 22064851]
9. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006 Aug; 38(8):904–909. [PubMed: 16862161]
10. Ko M, An J, Bandukwala HS, Chavez L, Aijo T, Pastor WA, et al. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXXC domain protein IDAX. Nature. 2013 May 2; 497(7447):122–126. [PubMed: 23563267]

11. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell. 2014 Dec 18; 159(7):1665–1680. [PubMed: 25497547]

12. Shibata T, Minami Y, Mitsuma A, Morita S, Inada-Inoue M, Oguri T, et al. Association between severe toxicity of nilotinib and UGT1A1 polymorphisms in Japanese patients with chronic myelogenous leukemia. International journal of clinical oncology. 2013 Apr 23.

13. Hagleitner MM, Coenen MJ, Aplenc R, Patino-Garcia A, Chiusolo P, Gemmati D, et al. The role of the MTHFR 677C>T polymorphism in methotrexate-induced liver toxicity: a meta-analysis in patients with cancer. The pharmacogenomics journal. 2013 May 7.

14. Zhou F, Gao G, Ren S, Li X, He Y, Zhou C. The association between COX-2 polymorphisms and hematologic toxicity in patients with advanced non-small-cell lung cancer treated with platinum-based chemotherapy. PLoS One. 2013; 8(4):e61585. [PubMed: 23620771]

15. Damm F, Heuser M, Morgan M, Yun H, Grosshennig A, Göhring G, et al. Single nucleotide polymorphism in the mutational hotspot of WT1 predicts a favorable outcome in patients with cytogenetically normal acute myeloid leukemia. J Clin Oncol. 2010 Feb; 28(4):578–585. [PubMed: 20038731]
Figure 1.
Kaplan-Meier curves of overall survival (a) and non-relapse mortality (b) by race and SNP rs2454206 genotype.

Figure 1a

Figure 1b

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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   | 1.5    | 0.018 | 1.4 | 0.91–2.16 | 0.122 |
| TET2 AA                  | 165| 1.5 | 1.07–2.11 | 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/Sq- or FLT3/ITD+ with high allelic ratio; Standard risk: All other patients with available cytogenetic data.