Unraveling Novel Genetic Determinants of Thiopurine Response Via TWAS

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Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. Thiopurines such as 6-mercaptopurine (6MP) are essential in ALL maintenance therapy. However, dose-limiting toxicities can significantly disrupt treatment. While genetic variants in *TPMT* and *NUDT15* are known to affect thiopurine response, many patients with normal function genotypes in these genes still experience adverse effects, suggesting that additional genes might be involved. We analyzed 663 pediatric ALL patients enrolled in the AALL03N1 trial to identify novel genetic determinants of 6MP sensitivity, focusing on individuals with normal function *TPMT* and *NUDT15* genotypes. A transcriptome-wide association study (TWAS) was conducted to focus on expression quantitative trait loci (eQTLs). Findings were validated in two independent cohorts: St. Jude Total Therapy XV ($n = 390$) and XVI ($n = 552$). TWAS identified 31 genes associated with 6MP dose intensity (q-value < 0.90). Of these, the imputed *GNAQ* expression was positively correlated with 6MP dose intensity and passed multiple testing thresholds in the validation cohorts. The rs60561071 variant, the eQTL in the *GNAQ* TWAS model, was associated with reduced gene expression and lower 6MP dose intensity. This study identifies *GNAQ* as a novel gene associated with thiopurine tolerance in ALL patients lacking known risk alleles in *TPMT* and *NUDT15*. Moreover, this research highlighted the innovative use of TWAS, providing deeper insights into the molecular mechanisms that explain drug response variability.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Thiopurines, including 6-mercaptopurine (6MP), are essential in pediatric ALL therapy. Variants in *TPMT* and *NUDT15* are known to influence thiopurine response; however, many patients without these variants still experience toxicities that limit the treatment.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study aimed to identify novel genetic variants influencing dose tolerance in ALL patients who do not have *TPMT* and *NUDT15* variants.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study identified GNAQ as a novel gene associated with 6MP dose intensity. One variant (rs60561071) was linked to lower GNAQ expression and potentially reduced drug tolerance. These findings were validated in two independent ALL cohorts.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ These findings expand the pharmacogenomic knowledge about thiopurine response, identifying GNAQ as a potential biomarker for 6MP dose adjustment. Incorporating GNAQ into clinical practice would enable more accurate dosing, reduce toxicities, and thus improve better outcomes in ALL treatment.

Acute lymphoblastic leukemia (ALL) represents the most common pediatric cancer and a major cause of cancer-related death before 20 years of age.[1] Treatment typically involves multiagent chemotherapy over 2–3 years, with thiopurines, including 6-mercaptopurine (6MP), forming the backbone of maintenance treatment.[2] This approach has raised the 5-year overall survival rate to over 90% in children with ALL.[3] However, thiopurine use is limited by adverse drug reactions (ADRs)—particularly dose-dependent myelosuppression[4]—occurring in 10%–30% of patients.[5] These ADRs often lead to dose reduction or interruption of therapy,[5] impacting treatment efficacy. Genetic polymorphisms in thiopurine-metabolizing genes are key drivers of

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interindividual variability in thiopurine toxicity.[6] Among them, thiopurine S-methyltransferase (TPMT) is a well-established pharmacogenomic marker,[7] with three single nucleotide polymorphisms (SNPs) (TPMT*2, TPMT*3A, and TPMT*3C) accounting for over 90% of reduced or absent activity alleles.[8] In addition, Nudix Hydrolase 15 (NUDT15) also impacts 6MP metabolism. Loss of NUDT15 enzymatic activity led by SNP rs116855232 has shown to cause severe myelosuppression at standard doses.[9] According to these genotype–phenotype associations, the Clinical Pharmacogenetics Implementation Consortium (CPIC) published dosing guidelines for 6MP incorporating *TPMT* and *NUDT15* genotypes.[8]

However, some patients without loss-of-function variants in TPMT or NUDT15 still cannot tolerate thiopurine treatment,[10] indicating that other genetic factors contribute to variability in response to 6MP. These biomarkers likely have weaker effect sizes than the known variants, and integrating biological knowledge may increase the power to discover them from association studies.

METHODS

Patients

A cohort of 663 patients with acute lymphoblastic leukemia (ALL) was considered who participated in the Children's Oncology Group (COG) clinical trial AALL03N1 (https://clinicaltrials.gov/ identifier NCT00268528) to investigate variability in 6MP dose tolerance in ALL patients.[10] Written informed consent was obtained from patients and/or parents/legal guardians. Eligibility criteria included: diagnosis of ALL at age ≤ 21 years; in first continuous remission; belonging to one of four ethnic categories (i.e., African-American, Asian, European-American, or Hispanic). Patients were enrolled during 6 months of maintenance therapy that included a daily 75 mg/m^2 dose of 6MP that could be adjusted based on the degree of myelosuppression or occurrence of infections. 6MP dose intensity is a marker for drug tolerance and toxicities. It was assessed as the ratio between clinician-prescribed MP dose and planned protocol dose (%) and was measured monthly for the 6-month duration of the study.[10]

Validation cohorts include 390 and 552 children with ALL treated on the St Jude Children's Research Hospital Total Therapy XV (TOTXV, https://clinicaltrials.gov/study/NCT00137111) and XVI protocols (TOTXVI, https://clinicaltrials.gov/study/NCT00549848), respectively. 6MP dose intensity was determined over the period of maintenance therapy.[11]

Genotyping

Germline DNA was extracted from peripheral blood or bone marrow samples and genotyped using Affymetrix Genome-Wide Human SNP Array 6.0. Genotype calling was performed using Birdseed. Missing genotypes and genotypes at additional variants were later imputed using TOPMed Imputation Server.[12,13] Genotypes were coded as 0, 1, 2 for AA, AB, and BB genotypes, assuming an additive genetic model. Principal component analysis (PCA) was applied to determine the genetic ancestral composition of patients using Plink. In addition, genotypes of *TPMT* and *NUDT15* were determined to later subset the population for patients carrying normal function alleles of both *TPMT* and *NUDT15* genes.

Statistical analysis

Association analysis between 6MP dose intensity and demographical traits was performed using the Wilcoxon rank test for dichotomic variables, the Kruskal–Wallis test for multi-groups categorical variables, and Spearman's correlation for continuous variables. The analysis between phenotype and genotype was performed using the prediGT pipeline described below.

prediGT pipeline

A custom pipeline was developed to test the association between genotypes and the phenotype of interest, incorporating their potential to affect gene expression. In particular, an initial GWAS analysis is performed to generate summary statistics for variants as to their association with traits as described above; subsequently, post-GWAS analysis transcriptome-wide association study (TWAS) allows us to detect significant expression quantitative trait loci (eQTLs)–trait associations by imputing transcription levels from the GWAS results.[14]

GWAS analysis of genotype data is performed using the association analysis tool from the *plink 2.0* software (https://www.cog-genomics.org/plink/2.0/).[15] In general, the *glm* tool fits a linear or logistic regression model for each variant to find association with quantitative or binary phenotypes, respectively. In our study, the imputed genotypes were tested for genetic association with total dose intensity of 6MP using the general linear model with gender and, for ancestry, the top five ranked principal components from PCA as covariates. Only SNPs with minor allele frequency greater than 5% were considered.

TWAS analysis aims to integrate gene expression level data to test the association with the trait of interest. The TWAS analysis is computed in the pipeline by *MetaXcan* scripts *S-PrediXcan* and *S-MulTiXcan*[14] (https://github.com/hakyimlab/MetaXcan), with the former focusing on single tissue and the latter extending to multi-tissue analysis. In our analysis, we focused on S-PrediXcan in whole blood ($n = 9500$ genes), as neutropenia is one of the most common toxicities induced by 6MP. The pipeline is available on GitHub (https://github.com/charliexbrown/prediGT).

TWAS significance was corrected using the false discovery rate (FDR) method evaluated through the *q*-value package. *Q*-value distribution was visually evaluated in relation to the *p*-value distribution (**Figure S1**), and consequentially, genes with a *q*-value < 0.90 in the TWAS in the discovery cohort were selected to be evaluated in validation cohorts. The selection of this threshold was to limit the false negatives associated with small sample sizes, along with the availability of validation cohorts to further reduce false positives. For validation, a one-tailed test was used to check the concordance of direction of effects, and the significance threshold was set based on Bonferroni correction according to the number of genes selected for validation.

RESULTS

To discover novel genomic determinants of 6MP dose tolerance, we limited our analysis to patients who had normal function genotypes of *TPMT* and *NUDT15* genes. We applied a two-stage process of discovery and validation in the TWAS analysis (**Figure 1**). The discovery cohort included 663 patients with newly diagnosed ALL enrolled in AALL03N1, with 587 patients with normal function *TPMT* and *NUDT15*; and the validation cohorts included patients enrolled in St Jude TOTXV ($n = 408$) and TOTXVI ($n = 552$) cohorts, with 389 and 480 patients with normal function *TPMT* and *NUDT15* genotypes, respectively.

TWAS analysis in the discovery cohort using whole blood as the tissue for the predictive model identified 31 genes (**Table S1**, *p*-value < 0.0025, *q*-value < 0.90) that are associated with 6MP dose intensity in patients with normal function TPMT/NUDT15. Two genes among the 31 genes with *q*-value < 0.9, namely *GNAQ* and *ZNF562*, passed the nominal significance threshold (*p*-value < 0.05, one-tailed) in the validation cohorts (TOTXV

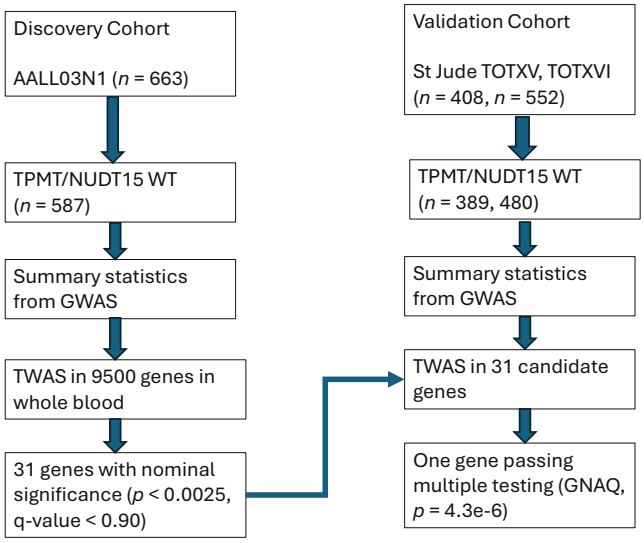

Figure 1 Overall workflow of TWAS analysis.

and TOTXVI). Only one gene, G Protein Subunit Alpha Q (GNAQ), passed the multiple testing threshold (calculated as 0.05/31 = 0.0016) in the validation cohort (p-value = 0.00062, one-tailed). In a meta-analysis including all three cohorts, the p-value for GNAQ is 4.3e-6, reaching genome-wide significance adjusting for Bonferroni correction (0.05/9500 = 5.3e-6) considering the total 9500 genes tested in the whole blood by *S-PrediXcan*. A significant positive correlation of higher expression of this gene with higher dose intensity in the ALL003N1 cohort (p-value = 0.0007, z score = 3.37) was concordant among St. Jude TOTXV (p-value = 0.01, z score = 2.53) and TOTXVI cohorts (p-value = 0.04, z score = 2.04). GNAQ remained nominally significantly associated with dose intensity in the TWAS considering the ALL003N1 cohort including TPMT/NUDT15 variant patients (p = 0.009). Neither TPMT nor NUDT15 was significant from this analysis (p = 0.31 and 0.2 respectively). To further explore the relationship between GNAQ expression and 6MP response, we analyzed GNAQ expression in primary leukemia samples with 6MP drug sensitivity data from Lee et al.[16] GNAQ expression was positively associated with 6MP resistance (coefficient = 0.034, p = 0.0023) in a linear model adjusting for lineage and subtype.

Intronic variant rs60561071, represented by a deletion of thymine (T), was the only eQTL for GNAQ TWAS model in whole blood according to S-PrediXcan. In all cohorts, 6MP dose intensity was lower for patients with T allele deletions (**Figure 2**). In GTEx (https://gtexportal.org/), the deletion of the T allele of rs60561071 was nominally associated with lower expression of GNAQ in whole blood (p = 0.00041, normalized effect size = −0.048). In the AALL03N1 cohort, the minor allele C had frequencies of 0.23 in Europeans (EUR), 0.29 in Africans (AFR), 0.188 in Admixed Americans, and 0.35 in Asians. No significant interaction between ancestry and GNAQ genotype was observed (p = 0.49) (**Figure S2**, **Table S2**). Lastly, additional covariates, including age and WBC, were considered in association with 6MP dose intensity and GNAQ eQTL but did not impact significantly (**Table S3**).

DISCUSSION

Thiopurines, including 6MP, are essential for ALL treatment, but are limited by a narrow therapeutic window, especially in pediatric patients, and often lead to dose-limiting toxicities in hematopoietic tissues. The ongoing need for MP dose adjustments poses a challenge in clinical practice and may impact the treatment outcome for ALL. TPMT and NUDT15 variants are known to impair thiopurine metabolism,[17] leading to the need to reduce doses to mitigate toxicity.

In this study, 663 patients with ALL were genotyped and analyzed using GWAS and TWAS to identify biomarkers that could contribute to variability in 6MP response. We identified 31 TWAS genes that could be associated with 6MP dose intensity and validated *GNAQ* gene expression in additional validation cohorts. The limited number of genes that could be validated may be due to a limited sample size, differences in phenotype preparation, and population composition.

GNAQ, which encodes a G-protein alpha subunit class q (Gαq) involved in GPCR signaling,[18] showed a positive correlation with 6MP dose. GNAQ signaling leads to YAP activation through Trio and its downstream GTPases, Rho and Rac.[18] Thioguanine metabolites, such as TGTP, have been shown to inhibit Rac1 activity, inducing T-cell apoptosis.[19] Similar to Rac, GNAQ acts as a molecular switch, shifting between its GTP-bound active form and GDP-bound inactive form.[20] In our study, GNAQ was overexpressed in correlation with higher 6MP doses in whole blood. Accordingly, GNAQ expression was positively correlated with 6MP resistance (LC50) in leukemia patients from Lee et al.[16] This correlation suggests that GNAQ might play a role in mediating the effects of 6MP. It is possible that TGTP could inhibit GNAQ, thereby reducing cellular responses such as proliferation, similar to how Rac1 suppression causes the apoptosis of T cells. Previous studies have shown that Gαq deficiency in B cells leads to significantly enhanced B-cell survival and resistance to apoptosis,[21] consistently with previous studies in NK cell[22] and T cell.[23] Conversely, in solid tumors such as neuroblastoma and uveal melanoma, *GNAQ* promotes proliferation via Akt-Bcl2 and Rho/Rac-YAP axes, respectively.[24,25] Overall, GNAQ appears to have a dual role in regulating survival and apoptosis pathways. However, the positive correlation between GNAQ expression and 6MP dose in our study may suggest that in the context of leukemia, GNAQ may promote cell survival, thus contributing to reduced sensitivity to 6MP-induced cytotoxicity. Therefore, individuals with higher GNAQ expression might require higher 6MP doses to reach the same therapeutic effect. Future longitudinal gene expression studies during thiopurine treatment can further elucidate the mechanisms of how GNAQ influences thiopurine response.

In AALL03N1, patients with CT/C or C/C genotypes rs60561071 received lower median doses (62.4 and 60.8 mg/m^2 respectively), compared to CT/CT patients (67.4 mg/m^2). In contrast, patients with heterozygous or homozygous TPMT/NUDT15 genotypes received median doses of 48.8 and 5.0 mg/m^2, respectively.[10] We further examined the average ANC levels in TOTXV and TOTXVI cohorts; no significant association was observed (p > 0.19). This is likely in part confounded by the dose adjustment during the maintenance therapy but is consistent with weaker effects of rs60561071 SNP alone. Even though the individual effect size for GNAQ was smaller, polygenic risk including

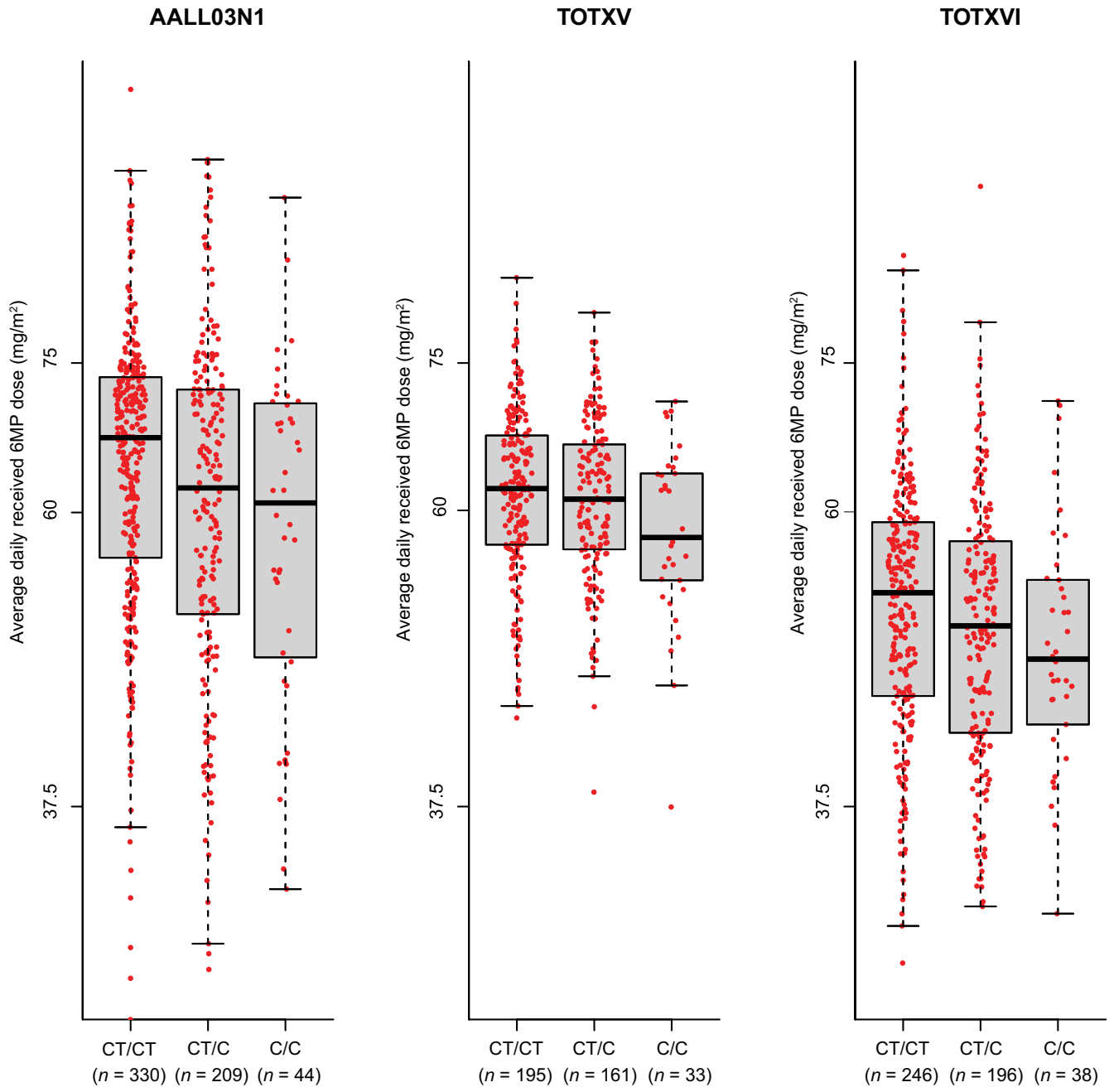

Figure 2 Association between average daily received 6MP dose and *GNAQ* SNP rs60561071 genotypes. 6MP dose intensity was significantly lower for patients carrying additional risk alleles (–) in all cohorts using general linear model analysis.

multiple variants of small effects could still potentially be clinically relevant in the future.

In conclusion, this study highlights the complexity of thiopurine metabolism and underscores the importance of pharmacogenomics in optimizing ALL therapy outcomes. *GNAQ* emerges as a novel gene potentially modulating 6MP efficacy. Further research could investigate its potential predictive biomarker alongside *TPMT* and *NUDT15*. In addition, incorporating gene expression profiling during treatment may provide a more complete understanding of how genetic variation shapes thiopurine response. Further validation of these genetic markers in diverse populations and their integration into clinical practice could help to mitigate dose-related toxicities and improve therapeutic outcomes.

AUTHOR CONTRIBUTIONS

C.B., W.Y., J.J.Y., G.S., S.E.K., and M.L. wrote the manuscript; J.J.Y. designed the research; C.B. and W.Y. performed the research; C.B. and W.Y. analyzed the data.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST
The authors declared no competing interests for this work.

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