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Genetic Polymorphism of Inosine Triphosphate Pyrophosphatase Is a Determinant of Mercaptopurine Metabolism and Toxicity During Treatment for Acute Lymphoblastic Leukemia

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Abstract

The influence of genetic polymorphism in inosine triphosphate pyrophosphatase (ITPA) on thiopurine-induced adverse events has not been investigated in the context of combination chemotherapy for acute lymphoblastic leukemia (ALL). This study investigated the effects of a common *ITPA* variant allele (rs41320251) on mercaptopurine metabolism and toxicity during treatment of children with ALL. Significantly higher concentrations of methyl mercaptopurine nucleotides were found in patients with the nonfunctional *ITPA* allele. Moreover, there was a significantly higher probability of severe febrile neutropenia in patients with a variant *ITPA* allele among patients whose dose of mercaptopurine had been adjusted for *TPMT* genotype. In a cohort of patients whose mercaptopurine dose was not adjusted for TPMT phenotype, the *TPMT* genotype had a greater effect than the *ITPA* genotype. In conclusion, genetic polymorphism of *ITPA* is a significant determinant of mercaptopurine metabolism and of severe febrile neutropenia, after combination chemotherapy for ALL in which mercaptopurine doses are individualized on the basis of *TPMT* genotype.

Approximately 80% of children with acute lymphoblastic leukemia (ALL) can be cured with combination chemotherapy.[1,2] However, treatment-related toxicity can be life threatening and is the primary cause of interruption or discontinuation of chemotherapy, leading to an increase in relapse risk.[1,3,4] Germline polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters, and drug targets can significantly influence the pharmacokinetics and pharmacological effects of medications and can be significant determinants of the efficacy and toxicity of antileukemic therapy.[5–7] Indeed, the influence of genetic polymorphism in thiopurine S-methyltransferase (TPMT) on the pharmacokinetics and toxicity of mercaptopurine is one of the clearest examples of a clinically important pharmacogenetic trait.[8–10] Mercaptopurine, an analog of hypoxanthine, is widely used in leukemia therapy;[1,2,11]

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CONFLICT OF INTEREST

WE Evans is a co-inventor on a patent awarded for the molecular diagnosis of the major *TPMT* variant alleles. The other authors declared no conflict of interest.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

mercaptopurine and its prodrug azathioprine are also used as immunosuppressive therapy in inflammatory bowel disease and other autoimmune disorders.[12,13] Mercaptopurine requires intracellular activation to thiopurine nucleotides to exert its pharmacological effects. Intracellularly, mercaptopurine is converted into thioinosine monophosphate by hypoxanthine guanine phosphoribosyltransferase and is subsequently converted into thioguanosine monophosphate through a two-step process involving inosine monophosphate dehydrogenase and guanosine monophosphate synthetase.[14] This process is in competition with methylation by TPMT, which is influenced by a common genetic polymorphism in the *TPMT* gene.[9] TPMT converts mercaptopurine into inactive methyl mercaptopurine but also metabolizes thioinosine monophosphate into methyl thioinosine monophosphate, a molecule that can inhibit *de novo* purine synthesis.[15] The molecular mechanisms of mercaptopurine's antileukemic effects are related to interference with the activity of DNA-processing enzymes due to subtle structural changes in the DNA after incorporation of thioguanine nucleotides (TGNs).[14,16] The importance of inhibition of *de novo* purine synthesis (mainly by methyl mercaptopurine nucleotides, MMPNs) is less well defined in the treatment of ALL.[9]

It is well established that nonfunctional variant alleles of the *TPMT* gene encode proteins that are rapidly degraded, resulting in low enzymatic activity. Inheritance of these variant alleles is associated with a marked increase in the concentration of TGNs and a significantly higher risk of hematopoietic toxicity after mercaptopurine treatment.[10] However, it is less clear whether polymorphisms in genes encoding other enzymes involved in mercaptopurine metabolism (Figure 1) also influence its efficacy and toxicity. Indeed, some patients with wild-type *TPMT* alleles develop mercaptopurine-related adverse events, for reasons that are not fully understood.

Among various possible candidate genes, inosine triphosphate pyrophosphatase (*ITPA*) has been associated, in some studies, with adverse events from azathioprine and mercaptopurine treatment of inflammatory bowel disease,[17–19] whereas other studies have failed to show any significant association between *ITPA* polymorphism and adverse events from the use of these agents in treating inflammatory bowel disease;[20–22] as such, this issue remains unresolved.[23,24] ITPA is an enzyme that catalyzes the hydrolysis of inosine triphosphate (ITP) to inosine monophosphate (IMP).[24] IMP is a central intermediate in purine metabolism and can be converted to ITP, to ATP through adenosine monophosphate, or to guanosine triphosphate through guanosine monophosphate. The putative role of ITPA is to protect cells from the accumulation of potentially harmful nucleotides, such as ITP or deoxyinosine triphosphate, that may be incorporated into the RNA and DNA.[25] The single-nucleotide polymorphism (SNP) rs41320251 is a C>A transversion (minor allele frequency: 0.083 in Caucasians, 0.033 in Africans, and 0.11 in Asians[26]) located in exon 2 of the gene.[25] It causes an amino acid change (P32T) that abolishes ITPA enzymatic activity in homozygous individuals and reduces the activity to 25% in heterozygous subjects;[25,27,28] this pattern is consistent with impaired assembly of the dimeric structure of the enzyme resulting from the P32T amino acid change.[29] is genetic polymorphism, leading to lower ITPA enzyme activity, gives rise to the physiological effect of abnormal accumulation of ITP in cells, which by itself is a clinically benign condition.[25,30] Characterization of the *ITPA* haplotype structure has shown that the SNP rs41320251 is the most relevant polymorphism in determining low ITPA enzymatic activity.[28]

The influence of *ITPA* genetic polymorphism on mercaptopurine toxicity has not been defined in the context of combination chemotherapy for ALL. This study assessed the influence of nonfunctional variant alleles of *TPMT* and *ITPA* on mercaptopurine metabolism and toxicity in patients with ALL whose mercaptopurine dosages had been adjusted on the basis of *TPMT* genotype.

RESULTS

Patients and *TPMT/ITPA* genotyping

For patients enrolled in the Total 13B protocol, *TPMT* and *ITPA* genotypes were determined in 244 of the 246 children included; in 2 patients *ITPA* genotype was not evaluable because the SNP call rate was <95% and failed replicate analysis by TaqMan assay. Among the 244 patients with both *TPMT* and *ITPA* genotypes, 11 (4.5%) were found to have one variant allele of the *TPMT* gene and one wild-type *ITPA* allele, 33 (13.5%) had one variant *ITPA* allele and one wild-type *TPMT* allele, and 1 patient (0.41%) had one variant allele for both *TPMT* and *ITPA*; all of the other 199 patients (81.6%) were wild type for both *TPMT* and *ITPA*. Because only 1 patient had a variant allele for both *TPMT* and *ITPA*, we performed parallel analyses in which data from this patient were excluded or included. The significant findings did not differ between these two analyses. Results presented in this article are those attained excluding this patient; those obtained when this patient was included are reported in the Supplementary Data S1 online.

For patients enrolled in the Total 12 protocol, *TPMT* and *ITPA* genotypes were determined in 101 of the 188 patients: the other patients were not studied, either because DNA was not collected or a sufficient quantity was no longer available. Among the 101 patients studied, 12 (11.9%) were found to have one variant allele of the *TPMT* gene and one wild-type *ITPA* allele, 19 (18.8%) had one variant *ITPA* allele and one wild-type *TPMT* allele, and no patient had one variant allele for both *TPMT* and *ITPA*; all of the other 70 patients (69.3%) were wild type for both *TPMT* and *ITPA*. The demographic characteristics of the patients enrolled are reported in Table 1.

The genotype distributions were in Hardy–Weinberg equilibrium in both cohorts of patients.

Effect of *TPMT/ITPA* genotype on rate of *de novo* purine synthesis (Total 13B)

De novo purine synthesis was measured in ALL cells from 196 patients; 47 patients were not evaluable because of insufficient ALL cell yields from the bone marrow aspirate. No significant difference was found in the rate of *de novo* purine synthesis at diagnosis between the patients with wild-type *ITPA* (median 90.8 fmol/nmol/h, range 0–5,018, $n = 171$) and variant *ITPA* (median 52.8 fmol/nmol/h, range 0–1,441, $n = 25$) or between those with wild-type *TPMT* (median 89.6 fmol/nmol/h, range 0–5,018, $n = 186$) and variant *TPMT* (median 33.8 fmol/nmol/h, range 0.82–819.1, $n = 10$); no significant effect was found even when analyzed by the multilocus genotypes. The lack of an effect of *TPMT* and *ITPA* genotype was confirmed by multivariate analysis after adjusting for age, race, sex, and treatment arm.

Effect of *TPMT*/*ITPA* genotypes on mercaptopurine metabolites in bone marrow leukemia cells after initial therapy with mercaptopurine (Total 13B)

These studies were performed in the subgroup of patients treated with mercaptopurine alone: estimations were performed in 56 patients; TGN was measurable in only 49 patients because for 7 patients the assay failed for technical reasons. Neither *ITPA* nor *TPMT* genotypes were significantly related to TGN levels in bone marrow ALL cells from patients treated with an initial single dose of intravenous high-dose mercaptopurine, even after adjusting for the potential confounders: age, race, and sex (Figure 2). MMPN concentrations were significantly higher in patients with a variant *ITPA* allele and lower in those with a variant *TPMT* allele, both as per univariate analysis (*ITPA* variant vs. *ITPA* wild type, $P = 0.0038$; *TPMT* variant vs. *TPMT* wild type, $P = 0.026$) and in the pairwise analysis for the multilocus genotype (*TPMT* wild type/*ITPA* variant vs. *TPMT* wild type/*ITPA* wild type, $P = 0.0056$; *TPMT* variant/*ITPA* wild type vs. *TPMT* wild type/*ITPA* wild type, $P = 0.030$, Figure 3). Multivariate analysis adjusted for age, race, sex, and treatment arm confirmed, after initial treatment with

mercaptopurine, the significant effect of the *ITPA* genotype ($P = 0.00030$), but not the *TPMT* genotype ($P = 0.060$), on MMPN concentrations. Boxplots showing mercaptopurine metabolite concentrations independently for *ITPA* and *TPMT* genotypes are available in Supplementary Data S1 online (Figures 1S and 2S, respectively).

Effect of *TPMT*/*ITPA* genotypes on mercaptopurine metabolites in erythrocytes during continuation therapy that includes mercaptopurine (Total 13B)

Concentrations of thiopurine metabolites were measured in erythrocytes obtained during continuation therapy with chronic oral mercaptopurine (75 mg/m^2 daily). For TGN, 534 estimations were made in 113 patients (median of five measurements per patient, range 1–9); the median concentration measured for each patient was 267.5 pmol/8 × 10^8 erythrocytes (range 58.4–1,023.0 pmol/8 × 10^8) and, as a measure of intrapatient variability, the median interquartile range across patients was 82.2 (range 0.10–499.80 pmol/8 × 10^8). For MMPN, 278 measurements were made in 107 patients (median of two measurements per patient, range 1–8); the median concentration measured for each patient was 10,440.0 pmol/8 × 10^8 erythrocytes (range 54.5–37,780.0 pmol/8 × 10^8) and, as a measure of intrapatient variability, the median interquartile range across patients was 2,776.0 (range 21.0–13,730.0 pmol/8 × 10^8). TGN concentrations (Figure 4) were higher in patients with the variant *TPMT* genotype (*TPMT* variant vs. *TPMT* wild-type, $P = 0.0077$); no effect of *ITPA* genotype on TGN was found (*ITPA* variant vs. *ITPA* wild type, $P = 0.95$). Pairwise analysis of the multilocus genotype confirmed the effect of *TPMT* only on TGN concentrations. MMPN concentrations (Figure 5) were higher in patients with a variant *ITPA* allele and lower in those with a variant *TPMT* allele, both as per univariate analysis (*ITPA* variant vs. *ITPA* wild type, $P = 0.0057$; *TPMT* variant vs. *TPMT* wild type, $P = 0.032$) and in the pairwise analysis for the multilocus genotype (*TPMT* wild type/*ITPA* variant vs. *TPMT* wild type/*ITPA* wild type, $P = 0.0086$; *TPMT* variant/*ITPA* wild type vs. *TPMT* wild type/*ITPA* wild type, $P = 0.048$). No significant effect of age, race, or sex on any of these correlations was found. Boxplots showing mercaptopurine metabolite concentrations independently for *ITPA* and *TPMT* genotypes are available in Supplementary Data S1 online (Figure 3S and 4S, respectively).

TPMT and *ITPA* genotypes and mercaptopurine dose adjustment during continuation therapy (Total 13B)

The dose of mercaptopurine was adjusted for patients enrolled in the Total 13B protocol, in accordance with patient tolerance and *TPMT* genotype. Among the 205 patients who completed treatment, 8 (3.9%) had a variant *TPMT* genotype. Among the 197 patients with wild-type *TPMT*, 6 (3.0%) were on a mercaptopurine dose that had been reduced by 30% or more by completion of therapy. Among the 8 patients with variant *TPMT*, 2 (25.0%) were taking a dose reduced by 30% or more; the probability of being prescribed a lower dose of mercaptopurine was significantly higher for those with a *TPMT* variant allele than for those with a wild-type allele (odds ratio (OR) = 10.6; 95% confidence interval (CI) = 1.8–63.9; $P = 0.0099$). This was confirmed in a multivariate analysis adjusted for age, race, sex, and treatment arm. None of the patients with a variant *ITPA* genotype was found to be on a reduced dose of mercaptopurine at the end of the treatment, and there was no statistical evidence for a relationship between *ITPA* genotype and mercaptopurine dose reduction during continuation therapy.

Effect of *TPMT*/*ITPA* genotypes on the incidence of toxicity

During continuation therapy in the Total 13B protocol, in which mercaptopurine doses were adjusted on the basis of *TPMT* genotype and mercaptopurine metabolite concentrations, children with an *ITPA* variant allele experienced a higher incidence of grade 3/4 febrile neutropenia as compared with children with a homozygous wild-type *ITPA* genotype (OR = 3.0; 95% CI = 1.2–7.3; $P = 0.018$, Table 2). There was no significant difference in the incidence

of grade 3/4 febrile neutropenia in children with the *TPMT* variant allele as compared to those with a homozygous wild-type *TPMT* genotype (OR = 1.4; 95% CI = 0.3–6.9; $P = 0.71$). Cumulative incidence curves for grade 3/4 febrile neutropenia during continuation therapy, in relation to *ITPA* genotype, are shown in Figure 6.

A significantly higher proportion of children with grade 4 febrile neutropenia have an ITPA variant allele when compared with children with no febrile neutropenia or children with grade 1–2 febrile neutropenia (excluding grade 3) (OR = 5.2; 95% CI = 1.3–20.9; $P = 0.021$).

There was no significant effect of age, ethnicity, or sex on any correlation of toxicity with the *TPMT* or *ITPA* genotypes described. None of the other toxicities that were evaluated (e.g., gastrointestinal toxicity and infection) was significantly associated with the *ITPA* or *TPMT* genotype in patients enrolled in the Total 13B protocol.

During continuation therapy in the Total 12 protocol, in which mercaptopurine doses were not adjusted on the basis of *TPMT* genotype, children with a *TPMT* variant allele experienced a higher incidence of grade 3/4 infection as compared with children with a homozygous wild-type *TPMT* genotype (OR = 4.1; 95% CI = 1.2–14.0; $P = 0.026$). There was no significant difference in the incidence of grade 3/4 infection in children with the *ITPA* variant allele as compared to those with a homozygous wild-type genotype (OR = 0.90; 95% CI = 0.34–2.4; $P = 0.84$). Age, ethnicity, and sex had no significant effect on the incidence of grade 3/grade 4 infection. Gastrointestinal toxicity, which was the other evaluated toxicity, was not significantly associated with the *ITPA* or *TPMT* genotype in patients enrolled in the Total 12 protocol.

Effect of *TPMT*/*ITPA* genotypes on the efficacy of treatment in patients treated according to Total 13B protocol

With aggressive supportive care of treatment-related toxicities, no significant effect of the *TPMT* or *ITPA* genotypes was found on the long-term efficacy of treatment, measured as event-free survival at 5, 8, or 10 years after diagnosis.

DISCUSSION

This study has documented that inheritance of a nonfunctional variant allele for either *TPMT* or *ITPA* is associated with significant modification in the metabolism of mercaptopurine during treatment of ALL. Although the importance of the *TPMT* genetic polymorphism is very well known and characterized,[8–10,32] this is the first report showing a significant effect of the *ITPA* genetic polymorphism in the context of mercaptopurine therapy individualized on the basis of *TPMT* genotype. Here we document significantly higher concentrations of the methylated nucleotide metabolites of mercaptopurine in leukemia cells and erythrocytes of patients who have inherited a nonfunctional *ITPA* allele. In contrast, inheritance of a variant *ITPA* allele was not associated with differences in TGN concentrations in either leukemia cells or erythrocytes. Although *ITPA* is known to be involved in mercaptopurine metabolism (Figure 1), the mechanism by which *ITPA* variant alleles influence the accumulation of methylated thionucleotides has not been fully elucidated.

It is known that individuals with reduced activity of ITPA have physiologically higher concentrations of the endogenous nucleotide, ITP.[25] The methylated nucleotide metabolites of mercaptopurine can be produced by direct methylation of thioITP by TPMT;[15] alternatively, methylthioITP can be produced by conversion of methylthioIMP to methylthioITP, as is known to occur with the physiological nucleotides.[33] Interestingly, it has been recently shown that methylthioITP has less affinity for ITPA than does thioITP, and this could account for the greater accumulation of methylthioITP in patients with ITPA deficiency (i.e., *ITPA*

heterozygotes).[24] The assay we used to measure these nucleotide concentrations does not discriminate among the mono-, di-, and triphosphate nucleotides,[33] and therefore it is not known whether one or all of these nucleotides were affected by the reduction in ITPA activity; however, because ITPA primarily cleaves thioITP to thioIMP, patients with reduced ITPA activity (i.e., patients with the *ITPA* variant) should have an increased concentration of primarily the triphosphate nucleotide (thioITP), leading to an accumulation of methylthioITP, documented in this study as an increase in MMPN.

The level of *de novo* purine synthesis can influence mercaptopurine pharmacology, but our studies revealed no relationship between *de novo* purine synthesis in ALL cells and *ITPA* genotype.

These studies reveal that the cumulative incidence of febrile neutropenia in patients receiving a chemotherapy protocol that includes mercaptopurine individualized for *TPMT* is significantly greater among those who have inherited an *ITPA* variant allele. The association was particularly evident during the second half of continuation therapy (Figure 6), when the treatment was predominantly chronic (i.e., daily mercaptopurine and weekly methotrexate treatment[31]), and remained significant when the analysis was limited to only life-threatening events (i.e., grade 4 fever and neutropenia). Febrile neutropenia in cancer patients is a serious complication of cytotoxic chemotherapy, and it generally leads to hospitalization for evaluation and treatment. Even when properly treated it can be associated with significant morbidity, mortality, and costs.[34–37] Moreover, febrile neutropenia leads to treatment delays and reductions in chemotherapy dosage, which may negatively affect the long-term outcome of the treatment for malignancy.[34–37] In the current analysis, *ITPA* genotype was shown to have a significant influence on the risk of fever and neutropenia (and the morbidity and costs associated with its treatment), but fortunately this did not influence event-free survival in the patients. We postulate that, although the *ITPA* polymorphism significantly influences the risk of toxicity (febrile neutropenia), this did not influence the efficacy of the treatment because we immediately and aggressively treated febrile neutropenia with antibiotics, assuming it to be a result of infection until proven otherwise. By avoiding toxicity-related deaths associated with the higher frequency of fever and neutropenia, there was no adverse effect of *ITPA* polymorphism on the efficacy of ALL therapy. Given the higher risk of this toxicity, patients with variant *ITPA* and wild-type *TPMT* should be closely monitored for the risk of febrile neutropenia during treatment with mercaptopurine.

The higher frequency of febrile neutropenia observed in patients with a variant *ITPA* allele may have been caused by the higher concentration of methylated thiopurine nucleotides that we documented in patients with a variant nonfunctional *ITPA* allele; these metabolites are known to have cytotoxic properties, and their accumulation may contribute to a more persistent neutropenia, increasing the likelihood of febrile neutropenia events.[9,38]

It is also noteworthy that in a prior cohort of St Jude patients with ALL whose mercaptopurine doses were not adjusted on the basis of *TPMT* genotype or TGN concentrations (St Jude Protocol Total 12, 1988–1991)[39] we documented a higher probability of grade 3/4 infections in patients who had inherited *TPMT* deficiency, but that this was not significantly associated with *ITPA* genotype (Figure 7). That is, if mercaptopurine doses are not individualized on the basis of *TPMT* genotype, then *TPMT* will be the predominant determinant of severe hematopoietic toxicity; whereas, if doses are adjusted for *TPMT*, then *ITPA* has a significant influence on the risk of febrile neutropenia.

All previous studies that evaluated the role of *ITPA* polymorphism in the toxicity of thiopurine have been carried out in patients with inflammatory bowel disease, with contradictory results.[17–24] Most of these studies involved patients on doses of mercaptopurine that were not

systematically adjusted on the basis of *TPMT* genotype, and our findings indicate that this is probably the reason for the inconsistent results relating to the effect of *ITPA* in these earlier studies.

In summary, we have shown that genetic polymorphism of *ITPA* (rs41320251) is a significant determinant of mercaptopurine metabolism and of severe, life-threatening febrile neutropenia during treatment in patients with ALL who are treated with combination chemotherapy involving mercaptopurine doses individualized on the basis of *TPMT* genotype and the concentration of TGNs. This illustrates the evolution of pharmacogenetics in clinical practice; as treatment is individualized for one genetic determinant of drug response, the importance of other genetic polymorphisms emerges.

METHODS

Patients

We studied children enrolled as patients in two single-institution clinical protocols for the treatment of newly diagnosed ALL. All the patients and/or their parents provided informed consent for the institutional review board–approved protocols.

The study was initially focused on patients receiving treatment according to the Total 13B protocol to assess the associations between different clinical and pharmacological phenotypes related to mercaptopurine treatment. For patients enrolled in the Total 12 protocol, only toxicity was analyzed for its relation to *TPMT* and *ITPA* genotypes.

For both the Total 12 and Total 13B protocols, the therapy schedule has been previously described in detail (refs. [31,39]; see Supplementary Data S1 online). Of the 188 patients enrolled in the Total 12 protocol, 101 had genomic DNA samples available for inclusion in this analysis. Of the 247 patients who were enrolled in the St Jude Total 13B protocol, 246 had genomic DNA samples available for inclusion in this analysis.[3]

Genotyping

The major nonfunctional variant alleles of *TPMT* (*TPMT**2, *TPMT**3A, and *TPMT**3C, defined by SNPs rs1142345, rs1800460, and rs1800462) were determined using methods that we have previously described in detail;[32,40] the major nonfunctional variant allele of ITPA (SNP rs41320251) was genotyped on the basis of a specific probe (SNP_A-1646349) in the Affymetrix chip array (Mapping50K_Hind240) and confirmed using a TaqMan assay (Prometheus Labs, San Diego, CA). All genotyping was done on germline DNA extracted from patients' blood samples.

Rate of *de novo* purine synthesis in leukemic blasts

In patients enrolled in the Total 13B protocol, the rate of *de novo* purine synthesis in treatment-naive bone marrow ALL cells was determined by quantifying unlabeled and radio-labeled purine bases (adenine and guanine) after acid hydrolysis of a 2-h *ex vivo* incubation of lymphoblasts with ^{14}C-formate using methods that we have previously described in detail.[41]

Measurement of mercaptopurine metabolites in leukemia cells

In patients enrolled in the Total 13B protocol, concentrations of mercaptopurine metabolites (TGN and MMPN) were measured in bone marrow ALL cells using methods that we have previously described in detail.[33] These analyses were performed on leukemia cells obtained after initial therapy with mercaptopurine in a subgroup of patients whose initial therapy consisted of a single intravenous infusion of mercaptopurine (1,000 mg/m^2 infused IV over 6 h); the other patients received initial therapy with a combination of mercaptopurine and

methotrexate and were therefore not included in the analysis of mercaptopurine metabolism in ALL cells.[31]

Measurement of mercaptopurine metabolites in erythrocytes

The concentrations of the two principal metabolites (TGN and MMPN) were measured in patients' erythrocytes using previously described methods.[42,43] Samples were collected during continuation therapy from patients who were compliant with their mercaptopurine therapy and who had taken a stable dose for at least 12 of the prior 14 days; the median value of multiple measurements relating to each patient was used in the analyses.

Mercaptopurine dose adjustment during continuation chemotherapy

For patients enrolled in the Total 12 protocol, the dose of mercaptopurine was 75 mg/m^2, and it was not adjusted prospectively for *TPMT* genotype. Rather, the mercaptopurine dose was modified only if patients developed dose-limiting toxicity attributable to mercaptopurine.[10] For those enrolled in the Total 13B protocol, the standard dose of mercaptopurine was 75 mg/m^2, but the dose was adjusted prospectively according to each patient's *TPMT* genotype and tolerance to therapy (see Supplementary Data S1 online).

Toxicity

Of the 246 patients enrolled in this study, 240 were evaluable for the toxicity analysis: 5 patients with Down syndrome and 1 patient with cystic fibrosis were excluded because their underlying conditions could influence toxicity. During continuation therapy, adverse events were documented and graded prospectively using the National Cancer Institute Common Toxicity Criteria version 1.0, as previously described.[3] For the analyses, the estimation of toxicity was dichotomized as "present" (grades 3–4) or "absent" (grades 0–2). The toxicities considered in this study were febrile neutropenia, gastrointestinal toxicity, and infection in those on the Total 13B protocol; and gastrointestinal toxicity and infection for those on the Total 12 protocol. Febrile neutropenia events were not recorded independently of infection for the Total 12 protocol patients. For patients enrolled in the Total 13B protocol, a separate analysis was carried out to compare the incidence of grade 4 febrile neutropenia with the incidence of nil febrile neutropenia and of grades 1 and 2 febrile neutropenia, separately from grade 3 events.

Efficacy of treatment

Efficacy of treatment was defined as event-free survival and overall survival at 5, 8, and 10 years after the diagnosis of leukemia.

Statistical analysis

The associations between the genotypes and the pharmacological or metabolic phenotypes were evaluated using Wilcoxon test for two-group comparisons and pairwise comparisons among multiple groups defined by the multilocus genotypes.

Logistic regression was used to assess the association between mercaptopurine dose reductions at the end of treatment and *TPMT* and *ITPA* genotypes.

A weighted logistic regression model that takes time at risk of each patient into account[3] was used to test the association between genotype and incidence of the toxicity events.

Event-free survival rates were estimated using the Kaplan–Meier method and were compared between genotypes using the log rank test (see Supplementary Data S1 online).

To test for potential confounders, all associations between the considered phenotypes and the genotypes were confirmed by multivariate analyses using linear or generalized linear models (see Supplementary Data S1 online).

Statistical analyses were performed using SAS version 9.1.3 Service Pack 4 (SAS institute, Cary, NC) and R version 2.6.1 (http://www.r-project.org).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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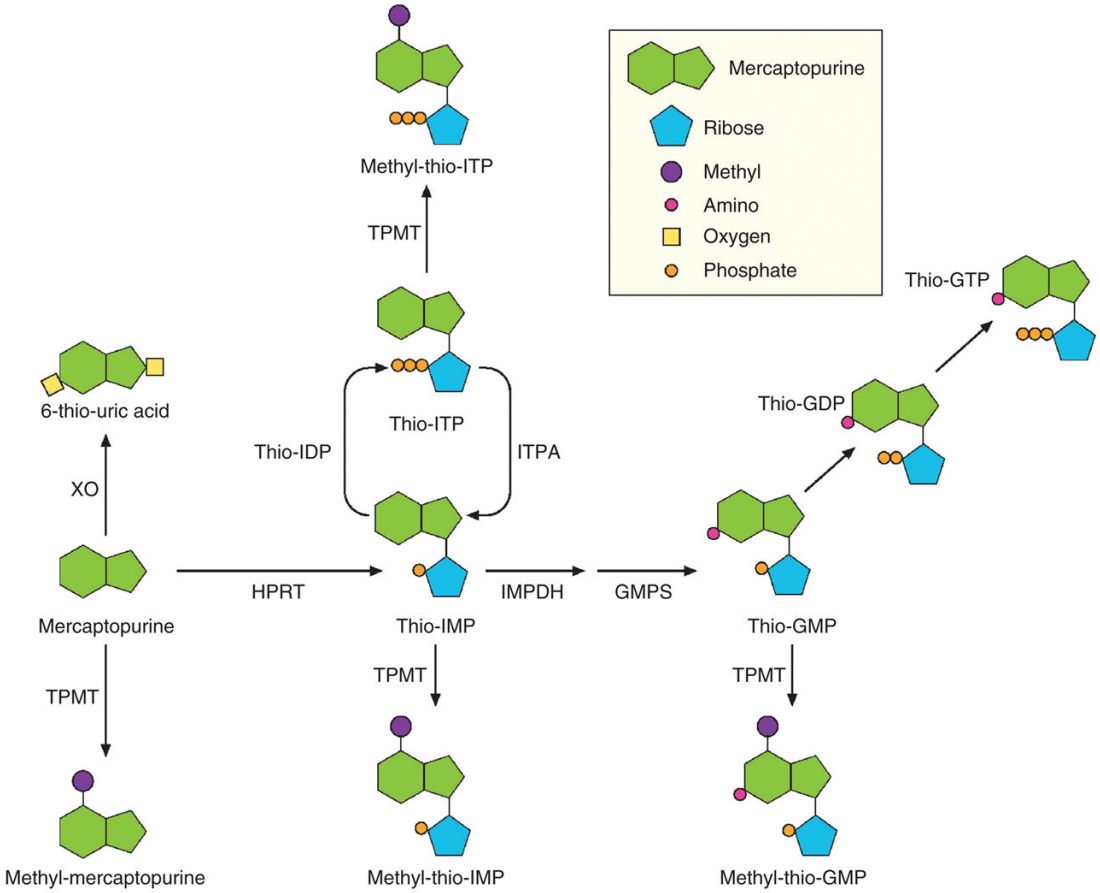

Figure 1.

Schematic representation of the metabolism of mercaptopurine and the enzymes involved. GDP, guanosine diphosphate; GMP, guanosine monophosphate; GMPS, guanosine monophosphate synthase; GTP, guanosine triphosphate; HPRT, hypoxanthine phosphoribosyltransferase; IMPDH, inosine monophosphate dehdrogenase; IDP, inosine diphosphate; IMP, inosine monophosphate; ITP, inosine triphosphate; ITPA, inosine triphosphate pyrophosphatase; K, kinase; TPMT, thiopurine S-methyltransferase; XO, xanthine oxidase.

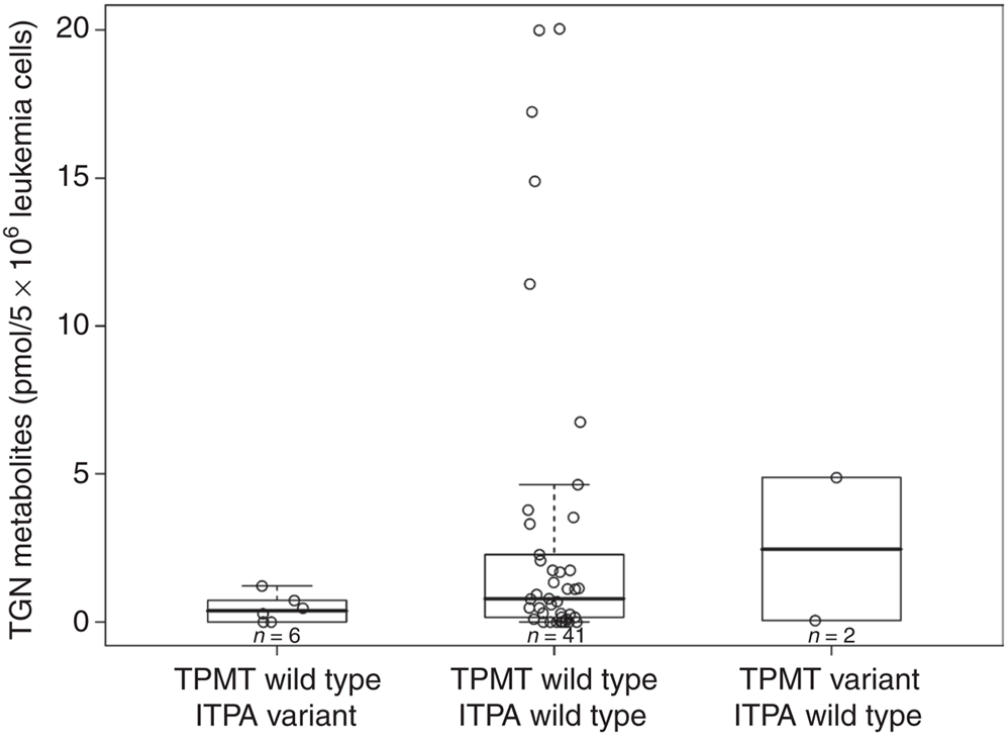

Figure 2.

TPMT/ITPA multilocus genotype and concentrations of mercaptopurine thioguanine nucleotide metabolite (TGN) measured in bone marrow leukemia cells after initial treatment with mercaptopurine alone; concentrations did not differ significantly by *TPMT/ITPA* genotype. ITPA, inosine triphosphate pyrophosphatase; TPMT, thiopurine S-methyltransferase.

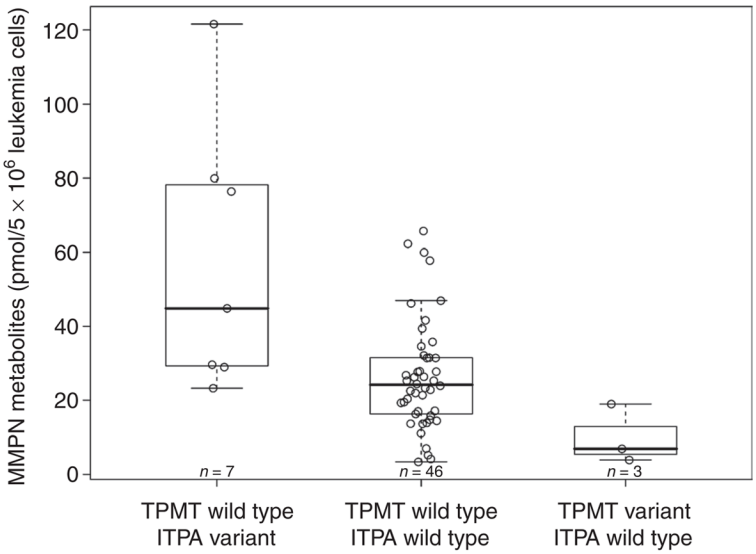

Figure 3.

TPMT/ITPA multilocus genotype and concentrations of mercaptopurine metabolite methylmercaptopurine nucleotide (MMPN) measured in bone marrow leukemia cells after initial treatment with mercaptopurine alone; concentrations were higher in patients with a multilocus genotype *TPMT* wild type/*ITPA* variant ($P = 0.0056$, Wilcoxon test) and lower in patients with a multilocus genotype *TPMT* variant/*ITPA* wild type ($P = 0.030$, Wilcoxon test) as compared to patients with a wild-type genotype for both genes. Boxes include data between the 25th and 75th percentiles, and whiskers indicate the minimal and maximal values excluding the outliers. ITPA, inosine triphosphate pyrophosphatase; TPMT, thiopurine S-methyltransferase.

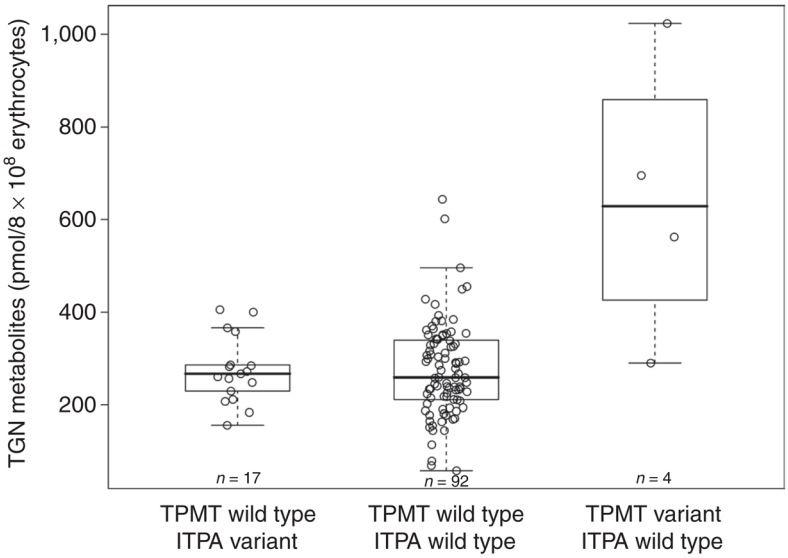

Figure 4.

Multilocus *TPMT/ITPA* genotype and median concentrations of mercaptopurine metabolite thioguanine nucleotide (TGN) measured during chronic treatment with mercaptopurine in accordance with the St Jude Total 13B protocol. Mercaptopurine doses were adjusted for *TPMT* genotype so as to avoid toxic TGN concentrations. Red-blood-cell TGN concentrations differed by *TPMT* genotype, resulting in higher concentrations ($P = 0.0095$, pairwise Wilcoxon test) in patients with a multilocus genotype of *TPMT* variant/*ITPA* wild type than in patients with a wild-type genotype for both genes. ITPA, inosine triphosphate pyrophosphatase; TPMT, thiopurine S-methyltransferase.

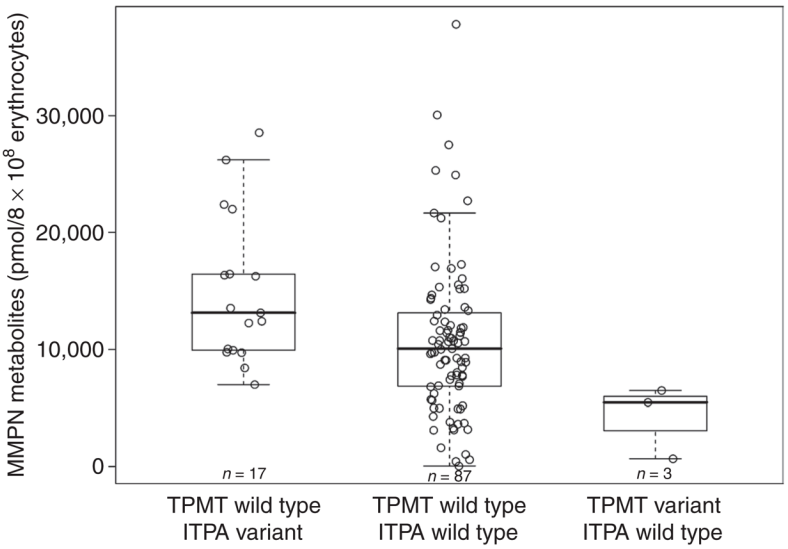

Figure 5.

Multilocus *TPMT/ITPA* genotype and median concentrations of mercaptopurine metabolite methylmercaptopurine nucleotide (MMPN) measured during chronic treatment with mercaptopurine in accordance with the St Jude Total 13B protocol. Mercaptopurine doses were adjusted for *TPMT* genotype so as to avoid toxic TGN concentrations. Red-blood-cell MMPN concentrations differed among patients according to *TPMT/ITPA* genotypes, showing higher concentrations in patients with a multilocus genotype *TPMT* wild type/*ITPA* variant (P = 0.0086, pairwise Wilcoxon test) and lower concentrations in patients with a multilocus genotype of *TPMT* variant/*ITPA* wild type (P = 0.048, pairwise Wilcoxon test) as compared to patients who were wild type for both loci. Boxes include data between the 25th and 75th percentiles, and whiskers indicate the minimal and maximal values excluding the outliers. ITPA, inosine triphosphate pyrophosphatase; TPMT, thiopurine S-methyltransferase.

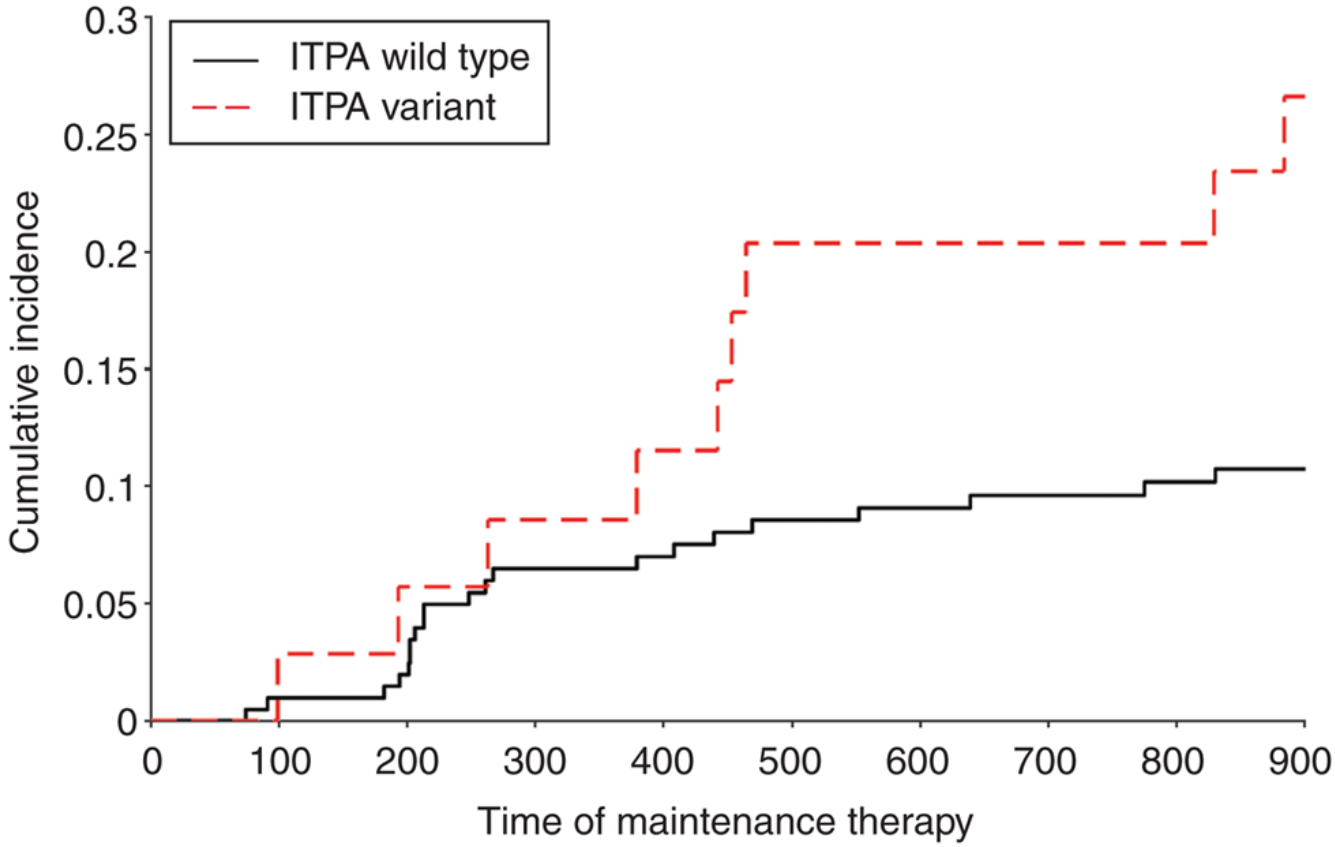

Figure 6.

ITPA genotype and cumulative incidence curves for the risk of grade 3/4 febrile neutropenia in patients with acute lymphoblastic leukemia during chronic continuation therapy that included mercaptopurine in accordance with the St Jude Total 13B protocol. At day 900 of maintenance therapy, the estimate of incidence of these adverse events was 10.7% ± 2.2 in patients with wild-type *ITPA* and 26.6% ± 7.8 in patients with variant *ITPA*. ITPA, inosine triphosphate pyrophosphatase; TPMT, thiopurine S-methyltransferase.

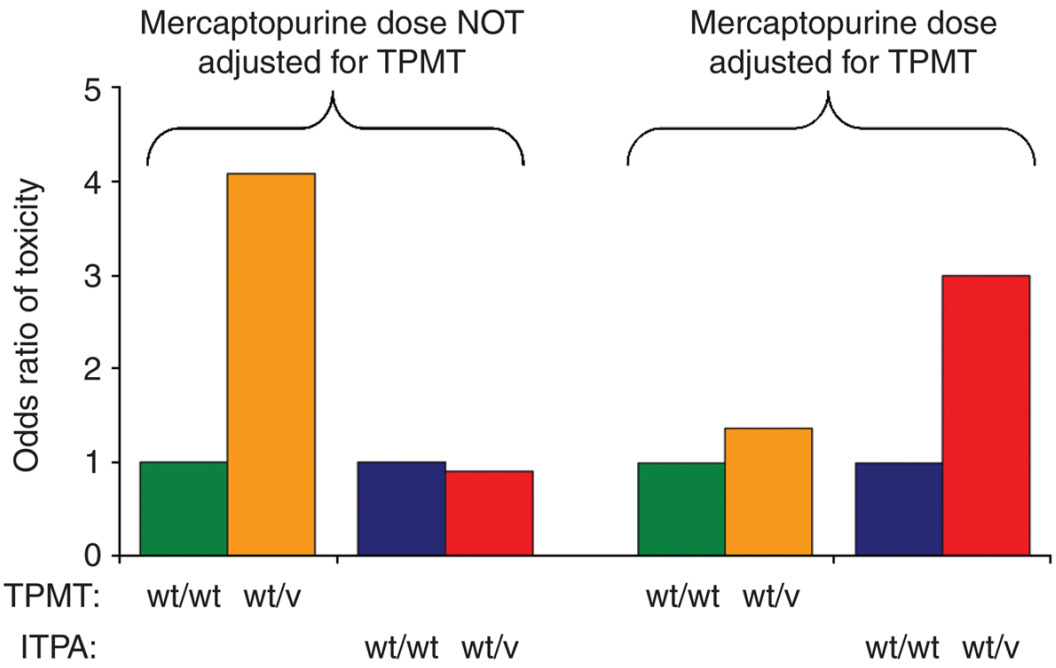

Figure 7.

TPMT genotype, *ITPA* genotype, and the odds ratio of severe toxicity during continuation therapy of children with acute lymphoblastic leukemia for whom the dose of mercaptopurine was not adjusted on the basis of *TPMT* genotype (St Jude Total 12) and in those for whom the dose was adjusted on the basis of *TPMT* genotype (St Jude Total 13B). Toxicity measured prospectively according to the Total 12 protocol (i.e., no mercaptopurine dose adjustment on the basis of *TPMT*) was grade 3/4 infection, whereas the comparable toxicity measured according to the Total 13B (i.e., mercaptopurine dose adjusted for *TPMT*) was grade 3/4 febrile neutropenia. Odds ratios are from a weighted logistic regression model and are adjusted for treatment arm and patient's age, race, and sex. ITPA, inosine triphosphate pyrophosphatase; TPMT, thiopurine S-methyltransferase. v, variant allele; wt, wild-type allele.

Table 1

Demographic and clinical characteristics of patients with acute lymphoblastic leukemia enrolled in each treatment protocol

Protocol	**Total 12**	**Total 13B**
Total number of patients	101	244
Age in years (median and range)	4.3 (0.60–18.7)	5.9 (0.08–18.8)
Gender (*n* (%))		
Female	46 (45.5)	101 (41.4)
Male	55 (54.4)	143 (58.6)
Ethnic group (*n* (%))		
White	91 (90.1)	187 (76.6)
Black	9 (8.9)	45 (18.4)
Other	1 (0.99)	19 (7.8)
Treatment arm/risk group[a] (*n*(%))		
Standard/high	—	128 (52.5)
Low	—	116 (47.5)

[a] Risk group classification was done only for patients in Total 13B protocol according to criteria previously described in detail.[31]

Table 2

Logistic regression model for the incidence of grade 3/4 febrile neutropenia in patients with ALL during continuation therapy that includes mercaptopurine according to the St Jude Total 13B protocol and *ITPA* genotype

	OR (95% CI)	***P* value**
ITPA variant vs. *ITPA* wild type	2.98 (1.21–7.35)	0.018
Age		
<1 Year vs. 1–10 years	0.57 (0.06–5.49)	0.063
More than 10 vs. 1–10 years	1.05 (0.39–2.84)	0.92
Race		
Black vs. white	1.20 (0.41–3.50)	0.74
Other vs. white	0.52 (0.18–1.51)	0.23
Sex		
Female vs. male	1.23 (0.56–2.73)	0.61
Treatment arm		
High/standard risk vs. low risk	0.86 (0.34–2.17)	0.74

ALL, acute lymphoblastic leukemia; CI, confidence interval; ITPA, inosine triphosphate pyrophosphatase; OR, odds ratio.