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1 **Prominence of *NUDT15* genetic variation associated with 6-mercaptopurine tolerance in a**
2 **genome-wide association study of Japanese children with acute lymphoblastic leukemia**

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37 **Summary**

38 Inherited genetic variation is associated with 6-mercaptopurine (6-MP) dose reduction and
39 frequent 6-MP induced toxicities. However, tolerable dose for 6-MP is not completely predicted by
40 the known variation in *NUDT15* and *TPMT* among Asian children with acute lymphoblastic leukemia
41 (ALL). We performed a genome-wide association study (GWAS) related to 6-MP dose among
42 Japanese children with ALL. This GWAS comprised 224 patients previously enrolled in Tokyo
43 Children's Cancer Study Group clinical studies with replication attempted in 55 patients. Genome-
44 wide single nucleotide polymorphism (SNP) genotypes were evaluated for association with 6-MP
45 average dose during initial 168 days of maintenance therapy. Possible associations were observed
46 across 5 gene coding regions, among which only variants at 13q14.2 were genome-wide significant
47 and replicated (rs116855232, *NUDT15*, $\beta=-10.99$, $P=3.7\times 10^{-13}$). Notable findings were observed for
48 variants in *AFF3* (rs75364948, $P=2.05\times 10^{-6}$) and *CHST11* (rs1148407, $P=2.09\times 10^{-6}$), but were not
49 replicated possibly due to small numbers. A previously reported candidate SNP in *MTHFR* was
50 associated with higher 6-MP average dose (rs1801133, $P=0.045$), and *FOLH1* (rs12574928) was
51 associated in a candidate region evaluation ($P_{adjust}=0.013$). This study provides strong evidence that
52 rs116855232 in *NUDT15* is the prominent genetic factor associated with 6-MP tolerable dose in
53 Japanese.

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61 **Introduction**

62 A survival probability of 80-90% in children with acute lymphoblastic leukemia (ALL) has
63 been achieved due largely in part to advances in combination chemotherapy¹. 6-mercaptopurine (6-
64 MP) is a main component for improving therapeutic outcomes, but tolerability is different in each
65 patient. Failure to minimize incidents of therapy interruption may affect prognostic outcomes for
66 childhood ALL patients². Response to 6-MP has been associated with variants in genes participating
67 in the 6-MP metabolism pathway³. It is well known that genetic variation of the *TPMT* gene is
68 associated with 6-MP intolerance³. However, the *TPMT* variant was not shown to be associated with
69 thiopurine induced toxicities in Japanese⁴, and the frequencies of TPMT poor metabolizer was lower
70 in East Asians compared with other races. In 2014, the *NUDT15* rs116855232 variant was reported to
71 influence 6-MP intolerance identified through a genome-wide association study (GWAS), and the
72 frequency of that variant is higher in Asians compared to populations of European and African
73 ancestries⁵. Subsequently, *NUDT15* genotype was shown to affect 6-MP tolerability among Asians
74 undergoing childhood ALL therapy⁴.

75 To date, several candidate gene studies have shown that genetic variation in *NUDT15* is
76 associated with 6-MP dose reduction and frequent 6-MP-induced toxicities in childhood ALL^{4,6-13}.
77 *NUDT15* enzyme dephosphorylates thio-guanosine triphosphate (GTP) and deoxy thio-GTP to thio-
78 guanosine monophosphate (GMP) and deoxy thio-GMP, respectively. Lower *NUDT15* enzyme leads
79 to increased thioguanosine incorporation ratio to 6-MP dose and 6-MP related toxicities. In some
80 instances, severe toxicities requiring significant 6-MP dose reduction have been observed in patients
81 who do not carry the known genetic risk factors for 6-MP tolerability. Thus, tolerable dose for 6-MP
82 is not completely predicted by the variation in *NUDT15* and *TPMT*.

83 The aim of this study was to perform a GWAS to examine the role of inherited genetic variation
84 related to 6-MP dose, with objectives to both identify newly associated variation, as well as to

85 characterize the variants previously reported in diverse populations, among Japanese childhood ALL
86 patients. Moreover, we pursued a targeted examination of genetic variation across the candidate gene
87 regions to identify additional associations with 6-MP dose.

88

89 **Methods**

90 Patients and sample collection

91 This GWAS comprised patients previously enrolled in a Tokyo Children's Cancer Study Group
92 (TCCSG) clinical study including L89-12^{14,15}, L95-14^{15,16}, L99-15¹⁷ and L04-16¹⁸ from 23 clinical
93 centers collaborating on this genomic study. Patients were recruited at the time of routine outpatient
94 follow-up visit between 2013 and 2015, as previously described¹⁹. Briefly, patients were considered
95 eligible if they were aged 19 years or younger at the time of ALL diagnosis and self-identified as
96 Japanese. Saliva samples were collected at the time of follow-up visit after remission. DNA was
97 extracted using the Oragene prepIT DNA extraction kit (DNA Genotek, Ottawa, Canada), and stored
98 at -80 °C. The study protocol was approved by the institutional review boards of all collaborating
99 institute and hospital involved in patient recruitment. Written informed consent was obtained from the
100 parents of each participant together with a written informed consent or assent by the child depend on
101 age.

102

103 Genotyping and quality control

104 Whole genome single nucleotide polymorphism (SNP) microarray genotyping was performed
105 using the Illumina HumanCoreExome-12 v1.1 BeadChip (San Diego, CA) and has been described
106 previously ¹⁹. Briefly, quality control (QC) procedures comprised excluding samples if the genotype
107 call-rate was below 95% and samples that exhibited relatedness based on an identity-by-descent
108 analysis. SNPs were excluded if genotype call-rate was less than 99%, genotype distribution deviated

109 from that expected based on Hardy-Weinberg equilibrium ($P > 1 \times 10^{-6}$), or the minor allele frequency
110 (MAF) was less than 0.01. Additionally, principal components (PC) analysis based on a subset of the
111 post-QC genome-wide SNPs in low linkage disequilibrium (LD) was performed using EIGENSTRAT
112 2.0 software together with HapMap data from Japanese, and sample outliers were excluded based on
113 a plot of the leading PCs.

114 Genome-wide SNP imputation was performed using ShapeIT2 and Minimac4 with reference
115 population from the 1000 Genomes Project Phase III Version 5. SNP imputation QC comprised
116 excluding poorly imputed SNPs defined by an R^2 of less than 0.5, resulting in a total of 6,236,137
117 SNPs available for analysis.

118

119 Clinical data

120 The clinical data and details of the administration of 6-MP doses during maintenance therapy were
121 available for 289 Japanese patients. In TCCSG protocols, maintenance therapy is initiated with 40
122 mg/m²/day of 6-MP and 25 mg/m²/week of oral methotrexate. These dosages were adjusted to
123 maintain the target leucocyte count at 1,500 – 3,000/mm³. We excluded patients who started at more
124 than 125% or less than 75% of protocol 6-MP dose, as therapeutic dose for 6-MP and MTX were
125 adjusted for drug induced toxicities before initiation of maintenance therapy. In total, we included
126 224 ALL patients (discovery cohort) who started maintenance therapy using the normal protocol
127 dose (30–50 mg/m²/day). The outcome variable for this GWAS was defined as 6-MP average dose
128 for initial 168 days of maintenance therapy.

129

130 Replication series

131 The replication series included 55 patients who started maintenance therapy by normal protocol
132 recruited previously through TCCSG as part of a separate study⁴. DNA were extracted from peripheral

133 blood obtained at remission, and SNP genotyping was performed using Taqman real-time PCR assays
134 (Applied Biosystems, Waltham, MA).

135

136 Statistical analysis

137 We performed genome-wide association analyses of SNPs in relation to 6-MP average dose for
138 initial 168 days of maintenance therapy among the discovery cohort using linear regression assuming
139 a log-additive genetic model of inheritance and adjusting for age at diagnosis. Association analysis
140 assuming dominant and recessive genetic inheritance models were also performed. Results showing a
141 $P < 5 \times 10^{-8}$ were considered statistically significance at the genome-wide level, and a $P < 1 \times 10^{-5}$
142 was considered as showing a suggestive association. In the replication series, association analysis was
143 conducted similarly using linear regression assuming a log-additive genetic model and adjusting for
144 age at diagnosis. We defined a Bonferroni corrected $P < 0.05$ as statistically significant in the
145 replication. Additionally, among the top SNPs, the difference of 6-MP average dose across the three
146 possible genotypes was evaluated using the Kruskal-Wallis test. Normality test of 6-MP distribution
147 was evaluated using the D'Agostino-Pearson test.

148 The association of specific candidate SNPs reported previously, as well as the variants in coding
149 regions of those candidate genes were examined. Association results with a nominal p-value of less
150 than 0.05 was considered statistically significant for specific candidate SNPs. For regional
151 examination of association results across candidate genes, SNPs with a nominal p-value of less than
152 0.05 were considered noteworthy, and a condensed list of SNPs pruned on LD ($r^2 > 0.50$) within each
153 gene were adjusted for multiple testing based on 10,000 permutation of the data on the 6-MP dose
154 outcome in which a p-value below a family-wise type I error rate threshold of 0.05 was considered
155 statistically significant. Statistical analyses were performed using PLINK version 1.9, R software
156 (version 3.6.1) and Prism 9 (GraphPad Software, San Diego, CA).

157

158 **Results**

159 Genome-wide association analysis of 6-MP average dose during 168 days of maintenance therapy

160 A total of 224 patients were included in the genome-wide association analysis. No patients
161 needed to be excluded due to outlying genetic ancestry based on PC analysis evaluations. The
162 characteristics of patients were shown in Table 1. Age at diagnosis was significantly correlated with
163 6-MP average dose ($\beta = -0.88$, $P = 6.96 \times 10^{-5}$). The median 6-MP average doses for the initial 168
164 days were 41.1 (interquartile range: IQR 25% – 75%, 32.8 – 48.6), and did not appear to deviate from
165 a normal distribution ($P > 0.05$).

166 In the genome-wide analysis of the discovery cohort, linear regression of the 6-MP average
167 dose adjusted for age at diagnosis showed minimal evidence of genomic inflation ($\lambda = 1.01$)
168 (Supplementary Figure S1). The Manhattan plot of the results showed potential association of variants
169 representing 5 genetic coding regions ($P < 1 \times 10^{-5}$) with 6-MP average dose (Figure 1, Table 2 and
170 Supplementary Table S1). Genome-wide significant associations with 6-MP average dose were
171 observed for variants at the chromosome 13q14.2 region in which the leading SNP was rs116855232
172 located in *NUDT15* SNP ($\beta = -11.45$, $P_{additive} = 8.5 \times 10^{-10}$, $P_{dominant} = 2.27 \times 10^{-9}$). Other variants
173 within this cluster of associated SNPs were in LD with this SNP (Supplementary Figure S2, $r^2 > 0.8$).
174 Suggestive associations were observed at chromosome 2q11.2 (rs75364948, *AFF3*), 2p21
175 (rs14452634, *THADA*), 12q23.3 (rs1148407, *CHST11*) and 16q23.2-3 (rs12934986 and rs10153053,
176 *CMIP*) (Table 2). In a sensitivity analysis, the results excluding outlier values of 6-MP (defined as the
177 values of more than the third IQR + $1.5 \times$ IQR or less than the first IQR + $1.5 \times$ IQR) showed a
178 persistent association with the chromosome 13q14.2 region, and slight attenuation of association for
179 the other loci (Supplementary Figure S3).

180 To identify associations in the absence of the effect of the well-known loci for 6-MP intolerance,

181 we excluded patients who were carriers of the *TPMT* rs1142345 and *NUDT15* rs116855232 variants.
182 In this cohort, 7 and 41 patients were carriers of the *TPMT* and *NUDT15* variants, respectively. Only
183 one patient carried both the *TPMT* and *NUDT15* variants. Among the remaining 178 patients, the
184 genome-wide analysis of 6-MP average dose showed potential associations with variants representing
185 13 genetic coding regions ($P < 1 \times 10^{-5}$) (Supplementary Table S2). The results of the suggestive
186 variants for *CHST11*, *CMIP* and *THADA* were similar to that of the full analysis.

187

188 Replication analysis

189 For the replication series, we selected three potentially associated SNPs that reside within genes
190 that have been previously implicated in 6-MP tolerability or progression of hematological malignancy
191 (Table 2). The association with *NUDT15* rs116855232 strongly replicated in this series ($P = 5.43 \times$
192 10^{-5}); however, while the direction of association was consistent, rs75364948 (*AFF3*) and rs1148407
193 (*CHST11*) were not significantly associated with 6-MP average dose (Table 2). Combining the
194 estimates from the discovery and replication cohorts, a strong genome-wide significant association
195 between rs116855232 (*NUDT15*) and 6-MP average dose was observed ($\beta = -10.99$, $P = 3.66 \times 10^{-13}$).

196 Variant rs116855232 in the *NUDT15* gene represents the prominent association with 6-MP
197 average dose within our Japanese study. Among the combined discovery and replication cohorts, 51
198 patients were heterozygous for rs116855232 (C/T), 5 patients were homozygous for the variant (T/T).
199 The median of the 6-MP average dose for patients with CT and TT were 31.6 and 13.1 mg/m²/day,
200 respectively; these 6-MP doses were significantly lower compared to that of patients with the CC
201 genotype (41.9 mg/m²/day, $P = 3.74 \times 10^{-11}$, Figure 2A).

202

203 Previously reported candidate genes and 6-MP tolerable dose

204 As a secondary aim, we selected candidate loci based on review of the literature and the

205 PharmGKB database (<https://www.pharmgkb.org>) and performed targeted examination of these
206 candidate regions. SNPs rs1142345 (*TPMT*)⁵, rs3765534 (*ABCC4*)²⁰, rs1142345 (*ITPA*)^{21,22},
207 rs4149056 (*SLCO1B1*)²³, rs1801133 (*MTHFR*)²⁴, rs61886492 (*FOLHI*)²⁵, rs12199316 (*NHLRC1*)²⁶
208 and rs72846714 (*NT5C2*)²⁷ have been studied previously as candidate loci for 6-MP tolerable dose
209 and/or toxicities. Among these, *MTHFR* rs1801133 showed a significant association with 6-MP
210 average dose in our study ($\beta = 2.26$, $P = 0.045$). However, the other reported genetic variants were not
211 related with 6-MP dose in this analysis (Table 3). In addition, other variants in LD with specific
212 candidate SNPs were not associated. A range of genetic variation across these candidate genes were
213 targeted to examine whether other SNPs may be associated with 6-MP average dose in Japanese. SNP
214 associations showing nominal p-values of less than 0.05 were observed for *MTHFR*, *FOLHI*,
215 *ABCC4*, and *ITPA* (Table 3). Among these, rs12574928 in *FOLHI* showed a statistically
216 significant association with 6-MP average dose after adjusting for multiple testing ($\beta = -8.02$, $P =$
217 0.013).

218

219 **Discussion**

220 We observed strong evidence showing that genetic variation at the chromosome 13q14.2 region
221 is significantly associated with 6-MP average dose for initial 168 days of maintenance therapy. Among
222 the cluster of association signals, *NUDT15* rs116855232 was the leading SNP, and the other variants
223 spanning the *SUCLA2* and *MED4* genes were in strong LD with this SNP. *NUDT15* genetic variation
224 has been shown to effect thiopurine tolerance in our previous studies, as well as others from Asian
225 countries^{4,12,28} In this study, 19 of 279 patients experienced a reduction of average 6-MP dose during
226 the 168 days to a level less than 20 mg/m²/day. Five of 19 patients (26%) who needed a dose reduction
227 were carriers of the rs116855232 variant. Lower *NUDT15* enzyme leads to increased thioguanosine
228 incorporation ratio to 6-MP dose and 6-MP related toxicities. Based on the characteristics of the

229 current study comprising an ethnically homogeneous group of Japanese children with ALL, *NUDT15*
230 was observed to have the strongest effect on 6-MP tolerance, and the associated variant rs116855232
231 is more frequent in Asians than other races/ethnicities.

232 The *TPMT* variant is a well-known genetic risk factor for 6-MP tolerance³. In this population,
233 only 10 patients (1.8%) carried the *TPMT* rs1142345 variant, and their 6-MP average dose was 40.0
234 (19.1 – 57.4) mg/m²/day. The MAF of rs1142345 in this Japanese cohort was lower than populations
235 of European and African ancestry (MAF; 4 – 6%), and there were no patients who were homozygous
236 carriers. While not significant possibly due to limitations in statistical power, the effect size of this
237 variant was also weaker compared to reports from other races/ethnicities.

238 Interestingly, 2 patients with *TPMT* rs1142345 requiring 6-MP average dose of less than 20
239 mg/m², were carriers of the *NUDT15* rs116855232 variant. Yang et al. reported that patients with
240 heterozygous genotypes for both *TPMT* rs1142345 and *NUDT15* rs116855232 had 30-60% of 6-MP
241 standard dose during 6-months of maintenance therapy among the multi-ethnic patient cohort⁵. Choi
242 et al. reported *NUDT15* rs116855232 was significantly associated with high 6-thioguanine nucleotide
243 levels in erythrocytes, 6-MP dose ratio, and frequency of leukopenia, but *TPMT* and other variants
244 were not associated among Korean children with ALL⁹. In our population, the average 6-MP dose for
245 heterozygous carriers of *TPMT* rs1142345 was similar to wild-type patients, but the average dose for
246 patients with both *TPMT* rs1142345 and *NUDT15* rs116855232 was 50% of tolerable dose of the wild-
247 type patients. Moreover, the standard dose of 6-MP on protocol for childhood ALL in Asian countries²⁹
248 is set at 70% of the dose (40-60 mg/m²) applied to protocols in Europe and US (75 mg/m²). Although
249 there does appear to be an effect of *TPMT* rs1142345 on 6-MP tolerability in Asians in the presence
250 of *NUDT15* variation, these effects may be small due to low prevalence of the variant and a low
251 standard dose. Results suggest that heterozygous variants of both *NUDT15* and *TPMT* may induce less
252 tolerability for 6-MP than *NUDT15* heterozygous genotype alone.

253 Other genetic variants identified through candidate gene approaches have been reported to be
254 associated with thiopurine tolerable dose. In our study, *MTHFR* rs1801133 was associated with 6-MP
255 dose, but the direction of association is in contrast to other reports²⁴. *MTHFR* is involved in folate
256 metabolism and rs1801133 has been associated with hepatotoxicity. However, our study showed
257 rs1801133 to be associated with high 6-MP average dose. Other previously reported candidate genetic
258 variants were not associated with 6-MP average dose in our study.

259 In maintenance therapy, 6-MP dose and therapeutic duration is different by country, and allele
260 frequencies of candidate variants may be different. This circumstance makes validation of SNP
261 associations and consistency a challenge. The candidate missense SNP rs61886492 in *FOLHI* could
262 not be evaluated in the current study due to its absence in the Japanese population. This variant was
263 previously reported to be associated with 6-MP mediated toxicity²⁵. Interestingly, regional evaluation
264 of genetic variation of this gene in our study identified a significant association signal led by SNP
265 rs12574928 located in an intronic region. This SNP has been described as an expression quantitative
266 trait locus (eQTL) for *FOLHI* in multiple tissues as documented in the Genotype-Tissue Expression
267 (GTEx) Portal. Due to LD broadly spread across this relatively short gene, localizing a defined causal
268 region will require additional studies.

269 We showed that *NUDT15* rs116855232 is the most prominently associated genetic loci for 6-
270 MP tolerance in Japanese children with ALL. However, 6 of 20 patients who required significant 6-
271 MP dose reduction to a level below 20 mg/m²/day did not carry the known risk variants for 6-MP
272 tolerance. Moreover, 17 patients needed to increase 6-MP dose to a level greater than 60 mg/m²/day.
273 Five previously unreported variants located in four genes showed suggestive associations with 6-MP
274 tolerance, including loci residing in *AFF3*, *CHST11*, *THADA* and *CMIP*. However, these suggestive
275 variants are located in intronic regions, and their functional significance has not been reported. The
276 associated *AFF3* rs75364948 and surrounding SNPs in strong LD showed to be potential eQTL for

277 *AFF3* and *KIAA1211L*. *AFF3* encodes a tissue-restricted nuclear transcriptional activator, and *AFF3*
278 upregulation was reported to be high in tamoxifen resistant breast cancer³⁰. While the function of
279 *KIAA1211L* is not clear, a previous study of molecular markers among childhood leukemia patients
280 showed this gene to be differentially expressed between patients with and without central nervous
281 system involvement³¹. *CHST11* was reported previously in relation to the efficacy of methotrexate
282 treatment in rheumatoid arthritis³². *THADA* and *CMIP* genetic variants were identified in GWAS as
283 a susceptibility locus for type 2 diabetes mellitus^{33,34}, and other diseases^{35,36}. These suggestive findings
284 could not be replicated in our small patient series in the present study. Future activities will comprise
285 securing additional patient numbers for a statistically robust opportunity to confirm these findings in
286 further replication attempts.

287 In this study, 6-MP average dose for initial 168 days of maintenance therapy was negatively
288 correlated with age at diagnosis. Therefore, the association of 6-MP and genetic variant was adjusted
289 by age. Although there have been no reports on the relationship between 6-MP tolerable dose and age,
290 some reports have shown that age was negatively correlated with TPMT activity³⁷.

291 There are some limitations of this study to acknowledge. Due the retrospective nature of this
292 study, we decided to exclude patients who started at more than 125% or less than 75% of protocol 6-
293 MP dose. These patients were not suitable for evaluation for 6-MP response to standard therapy
294 in maintenance therapy because therapeutic dose for 6-MP and MTX were adjusted for drug
295 induced toxicities before maintenance therapy. Therefore, it is possible that our study cohort
296 underrepresented patients who deviated from the normal 6-MP tolerance. Despite this limitation, our
297 study is meaningful in describing the responsible genetic factors of 6-MP tolerance among patients
298 starting with standard protocol dose.

299

300 **Conclusion**

301 We provide strong evidence that *NUDT15* rs116855232 is the prominent genetic factor
302 associated with 6-MP tolerable dose, and it currently represents the most meaningful clinically
303 predictive marker in Japanese. These findings indicated that *NUDT15* genotyping should be
304 considered prior to initiation of 6-MP treatment. Prospects for the identification of additional loci of
305 6-MP tolerance are high after further validation of suggestive loci observed in this Japanese study.

306

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310 Children's Cancer Association of Japan, and the Japan Leukemia Research Fund.

311

312 **Conflict of interest**

313 There are no conflicts of interest to declare.

314

315 **Authorship contributions**

316 Y.T., K.Y.U., M.T. and A.T. designed the experiments. Y.T., K.Y.U. and M.H. analyzed data. M.M.,
317 Y.A., D.H., Y.N., M.Y., D.K., S.O., K.A., T.I., M.T. K.K., and A.M. collected the clinical data. T.K.
318 and F.M. contributed to perform genotyping. Y.T. and K.Y.U. wrote the paper. All authors approved
319 the manuscript and the interpretation of the data.

320

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435

436 **Figure legends**

437 Figure 1. Manhattan plot of results of the GWAS using linear regression adjusting for age at diagnosis.

438

439 Figure 2. Average 6-MP dose for 168 days from initial maintenance therapy in the combined Discovery

440 and replication cohort (N = 279). Difference between genotypes were analyzed by the Kruskal-Wallis

441 test. (A) *NUDT15* rs116855232; (B) *AFF3* rs75364948; (C) *CHST11* rs1148407.

Table 1. Demographic and clinical characteristics of patients

	Discovery	Replication
Patient number	224	55
Gender		
Female	99 (44.2%)	24 (43.6%)
Male	125 (55.8%)	31 (56.4%)
Age at diagnosis (years)		
0-1	14 (6.3%)	3 (5.5%)
2-4	104 (46.4%)	19 (34.5%)
5-10	79 (35.3%)	26 (47.3%)
11-19	27 (12.0%)	7 (12.7%)
Median (range)	4.7 (0.9 – 15.7)	5.0 (1 – 17)
6-mercaptopurine initial dose (mg/m ² , median)	39.9 (30.2 - 50.0)	40.1 (30.8 – 49.5)
6-mercaptopurine average dose for 168 days (mg/m ² , median)	41.1 (13.3 – 78.5)	37.2 (5.5 – 60.0)
methotrexate dose (mg/m ² /week, median)	24.5 (0 – 31.5)	24.7 (0 – 30)

Table 2. Results of the genome-wide association analysis of 6-mercaptopurine dose and replication (age adjusted)

Chr	Position (GRCh37)	Gene	SNP	MAF in discovery	Discovery (N = 224)		Replication (N = 55)		Combined (N = 279)	
					β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
13	48619855	<i>NUDT15</i>	rs116855232	0.094	-11.45 (-14.95, -7.95)	8.46×10^{-10}	-9.13 (-13.29, -4.96)	5.43×10^{-5}	-10.99 (-13.82, -8.16)	3.66×10^{-13}
2	100399866	<i>AFF3</i>	rs75364948	0.203	6.49 (3.88, 9.10)	2.05×10^{-6}	0.84 (-4.76, 6.44)	0.766	5.83 (3.42, 8.24)	3.12×10^{-6}
12	105058593	<i>CHST11</i>	rs1148407	0.138	8.08 (4.83, 11.3)	2.09×10^{-6}	2.22 (-3.28, 7.74)	0.421	6.52 (3.56, 9.49)	2.03×10^{-5}
2	43501170	<i>THADA</i>	rs144526347	0.015	21.37 (12.74, 30.00)	2.28×10^{-6}	--	--	--	--
16	81553070	<i>CMIP</i>	rs12934986	0.277	5.63 (3.24, 8.01)	6.43×10^{-6}	--	--	--	--
16	81659083	<i>CMIP</i>	rs10153053	0.234	-5.91 (-8.40, -3.14)	5.79×10^{-6}	--	--	--	--

Abbreviations; Chr, chromosome; SNP, single nucleotide polymorphism; MAF, minor allele frequency.

Table 3. Results of the relationship between previously reported candidate genes and average 6-mercaptopurine dose for 168 days (age adjusted)

Chr	Position (GRCh37)	Gene	SNP	Function	Alleles	MAF	Discovery (N = 224)			Reference
							β (95% CI)	P (nominal)	P (adjusted)	
Previous reported SNPs										
1	11856378	<i>MTHFR</i>	rs1801133	Missense	G/A	0.375	2.26 (0.06, 4.46)	0.0454	--	24
6	18123502	<i>NHLRC1</i>	rs12199316	Upstream	C/G	0.394	-0.69 (-3.06, 1.69)	0.553	--	26
6	18130918	<i>TPMT</i>	rs1142345	Missense	T/C	0.0156	-2.38 (-11.45, 6.70)	0.608	--	2
10	104878454	<i>NT5C2</i>	rs72846714	Intron	G/A	0.024	3.75 (-6.00, 13.5)	0.452	--	27
11	49186274	<i>FOLH1</i>	rs61886492	Missense	G/A	--	--	--	--	25
12	21331549	<i>SLCO1B1</i>	rs4149056	Missense	T/C	0.147	-2.45 (-5.47, 0.57)	0.113	--	23
13	95815415	<i>ABCC4</i>	rs3765534	Missense	C/T	0.143	-1.66 (-4.88, 1.55)	0.310	--	20
20	3193842	<i>ITPA</i>	rs1127354	Missense	C/A	0.152	1.01 (-2.05, 4.07)	0.520	--	21, 22
Genetic variants across previously reported candidate gene regions (nominal P-value<0.05)										
11	49213504	<i>FOLH1</i>	rs12574928	Intron	C/T	0.056	-8.02 (-12.9, -3.21)	1.59×10^{-3}	0.013	25
13	95707834	<i>ABCC4</i>	rs9561773	Intron	C/T	0.228	3.69 (1.02, 6.37)	7.35×10^{-3}	0.577	20
13	95953517	<i>ABCC4</i>	rs11568681	Missense	G/T	0.018	10.91 (2.54, 19.29)	0.011	0.707	20
20	3200778	<i>ITPA</i>	rs959815466	Intron	C/T	0.013	-11.55 (-21.2, -1.90)	0.019	0.126	21,22

Abbreviations; Chr, chromosome; SNP, single nucleotide polymorphism; MAF, minor allele frequency.



