의학박사 학위논문

Analysis of genetic polymorphism related to toxicity of mercaptopurine in pediatric acute lymphoblastic leukemia

한국인 소아 급성림프모구백혈병에서 mercaptopurine 의 독성에 관련된 유전자 다형성 분석

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Analysis of genetic polymorphism related to toxicity of mercaptopurine in pediatric acute lymphoblastic leukemia

by

Hyery Kim

A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Medical Science (Molecular Oncology) at Seoul National University College of Medicine

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Abstract

Analysis of genetic polymorphism related to toxicity of mercaptopurine in pediatric acute lymphoblastic leukemia

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Introduction: Despite of marked improvement in survival rates of pediatric acute lymphoblastic leukemia (ALL), drug resistance and recurrence are remaining problems. Genetic polymorphism has been known to be an important factor affecting the efficacy and toxicity of antileukemic agents, thus pharmacogenetic researches have been actively conducted in pediatric ALL. Mercaptopurine (MP) is one of the main chemotherapeutics for ALL, and constant MP dose titration is essential to maintain steady drug exposure, while minimizing myelosuppression. Throughout previous studies, thiopurine

methyltransferase (*TPMT*) is proved to be one of the most responsible genes in the pharmacogenetics of MP. Recently, mutations of nucleoside diphosphate linked moiety X-type motif 15 (*NUDT15*) have been shown to be a powerful genetic factor affecting the toxicity and intolerance of MP. In this study, the genetic factors affecting the toxicity of MP were investigated in Korean children with ALL.

Patients and Methods: Two-stage pharmacogenetic analyses were performed to identify genetic determinants of MP-related neutropenia in Korean pediatric ALL patients. A total of 185 patients with ALL who were diagnosed and treated at the Seoul National University Children's Hospital were included. First, targeted sequencing was carried out using a novel gene panel of 147 pharmacogenetics related genes and 8 SNPs in 44 patients who received less than 25% of initial dose during maintenance therapy due to MP intolerance. Next, significant genes identified in the first analysis were re-sequenced in a total of 185 patients. The relationship between mutation of each gene identified through sequencing and toxicity or dose of MP were analyzed.

Results: As a result of the first stage analysis, the 4 loci which were likely to be most relevant to the toxicity of MP (*NUDT15* rs116855232, *APEX1* rs2307486, *CYP1A1* rs4646422, *BAG3* rs78439745) were identified, and 24 variants in 13 genes potentially linked to MP adverse reactions including these 4 loci were selected as final candidates for subsequent validation in a cohort of 185 patients. The distribution of variant alleles were *NUDT15* rs116855232 (21.7%), *APEX1* rs2307486 (13.5%), *ABCC4* rs2274407 (34%), *ABCC4* rs3765534 (14.1%), *ABCC4* rs11568658 (23.2%), *CYP4F2* rs2108622 (51.9%), *CYP1A1* rs4646422 (29.1%), *SLCO1B1* rs11045879 (55.7%), *SLCOB1* rs4149056 (26.5%), *ITPA* rs1127354 (26%), *ITPA* rs7270101 (0%), *MTHFR* rs1801131 (60.5%), *MTHFR* rs1801133 (28.6%), *MTHFR* rs1901133 (50.8%), *GRIA1* rs4958351 (3.8%), *MOCOS* rs594445 (48.1%), *PACSIN2* rs2413739 (13.5%), *BAG3* rs78439745 (7%) and *TPMT* (* 1 / * 3C, 3.8%). Neutropenia (neutrophil<500/㎕) was observed in 121 patients (65.4%) during the administration of MP. When frequency of neutropenia during MP administration was crosstabulated with variant frequencies, the risk of neutropenia was 4.64 times

higher in patients with T allele in *ABCC4* rs3765534 (95% CI; 1.32 to 25.12, *P*=0.008). Among 121 patients with neutropenia, 15 patients had neutropenia within 30 days (early neutropenia) after starting MP, and the risk of early neutropenia was significantly higher in patients with G allele in *APEX1* rs2307486 (OR=3.44; 95% CI; 1.04 to 9.89, *P*=0.02). In addition, the cumulative incidence of early neutropenia was significantly higher in patients with variant forms of *APEX1* rs2307486 (AA:AG:GG=66.3%:73%:100%, *P*<0.001). The cumulative incidence of early neutropenia was also significantly increased in patients with *NUDT15* rs116855232 mutations (CC:CT:TT=65.0%:72.2%:100%, *P*<0.001). Comparing the average doses of MP used in the study, the T allele type of *NUDT15* rs116855232 was significantly associated with a lower tolerated dose of MP as 10.5, 19.4 and 28.7 mg/m^2/day for TT, CT, and CC genotypes (*P*<0.001). However, MP doses by *TPMT* mutations were not significantly different in this study.

Conclusion: In conclusion, this study revealed that genetic variation of *APEX1* increases the risk of early neutropenia

associated with MP, and the variant allele in *NUDT15* was related to MP intolerance. In Korean ALL patients, toxicity and tolerance of MP were more affected by *APEX1* and *NUDT15* than by *TPMT*, which has a lower variant allele frequency in Asian. Further validation will be needed to confirm the frequency and pharmacogenetic consequences of *APEX1* variants in other ethnic groups, including different Asian populations

Keywords : childhood acute lymphoblastic leukemia, 6-mercaptopurine, toxicity, pharmacogenetics

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Introduction

The long term survival rate of pediatric acute lymphoblastic leukemia (ALL) has been improved dramatically (1). Despite this improvement, treatment results are not satisfactory in some patients because of treatment failures in high risk group patients and severe treatment related toxicities still occur in about 20% of patients (2).

One of the explanations of treatment failures and drug toxicities is the pharmacogenetic effect. The germline polymorphisms in ALL patients can alter drug transporters, metabolizing enzymes, or drug targets and thus influence toxicity or efficacy of antileukemic agents (3). Thus, if the genetic determinants of inter-patient variability in pharmacokinetics were better defined, individualized therapy based on those factors could lead to better treatment outcomes.

Pharmacogenetic researches have been actively conducted in ALL, and mercaptopurine (MP) is the most actively studied drug in ALL pharmacogenetics. Mercaptopurine has a narrow therapeutic index with common dose-limiting toxicities in the hematopoietic tissues (4). Patients with ALL take MP daily

during the maintenance phase, which continues for 1–2 years. Toxicities related to MP were most likely to occur in the maintenance schedules if a patient had a susceptible genotypic trait for low metabolism so that treatment interruption is frequent. Because high–intra individual variability in thiopurine exposure can negatively affect ALL treatment outcome (5, 6), constant MP dose titration to maintain steady thiopurine exposure and minimize toxicity is important. However, constant MP dose titration is challenging in clinical practice.

Throughout previous studies, thiopurine methyltransferase (TPMT), which involves in the methylation of the metabolites of MP, is proved to be one of the most responsible genes in the pharmacogenetics of MP (7). Patients with nonfunctional variant allele of *TPMT* have lower TPMT enzyme activity so that 6–thioguanine nucleotide (6–TGN) is excessively accumulated and causes hematopoietic toxicities frequently. These results introduced the concept of individualized MP therapy according to *TPMT* genotype, and *TPMT*–guided MP dose adjustment was one of the first clinical implementation of pharmacogenetics.

However, the variant allele frequency of *TPMT* is relatively lower in Asian patients than in Western patients (8–10).

Determination of *TPMT* polymorphisms, therefore, has a limited clinical benefit to children with ALL in most Asian countries. In practice, some East Asian patients still showed sensitivity to MP dose intensity after adjusting for *TPMT* variants, suggesting the existence of other Asian-specific factors related to MP sensitivity.

Recently, a single nucleotide polymorphism (SNP) of nucleoside diphophate- linked moiety X-type motif 15 (*NUDT15*) has been shown to be associated with myelosuppression induced by MP or azathioprine, a prodrug of MP, in pediatric ALL and inflammatory bowel disease (4, 11-13). Although previous studies showed strong correlation between rs116855232 SNP and major 6-MP toxicity, thorough evaluation of rare clinical phenotypes and potential additive effects with other candidate genetic loci is still needed.

In this study, the genetic factors affecting the toxicity of MP were investigated in Korean children with ALL with targeted sequencing and computational prediction algorithm.

Patients and Methods

Patients and treatment

Of the patients who were diagnosed with ALL in Seoul National University Hospital (SNUH), 185 patients whose samples and informed consents were available were included. Bone marrow aspirates of peripheral blood samples at complete remission status from the patients were selected for sequencing. Patients were assigned to the standard-risk group if the age was 1 to 9 years at diagnosis, and the leukocyte count was less than 50×10^9/L, the others were assigned to the high-risk group. If a patient had one or more of the following: age younger than 1 year, hypodiploidy, presence of t(9;22), a leukocyte count of at least 200×10^9/L, or the 11q23 rearrangement, the patient proceeded to undergo hematopoietic stem cell transplantation when clinical remission was achieved and an appropriate donor was available.

In the standard-risk patients, the treatment protocol was modified from the Children's Cancer Group (CCG)-1891 (14) or 1952 protocol (15), and the protocols for high-risk patients were CCG-1882 (16) or 0601 protocol for high risk ALL

(Korean multicenter prospective regimen), which employed longer and stronger post induction intensification in patients with slow early response during induction.

In Korea, the planned dose of MP was reduced from 75 mg/m^2 to 50 mg/m^2 based on the previous experience, because many patients who had been given the same dose with the original CCG protocol had to delay or stop their chemotherapy due to moderate to severe toxicities during MP administration (17). Dose of MP during maintenance was adjusted to maintain a white blood cell (WBC) count of $2.0-3.5 \times 10^9$/L while neutrophil count over 500/$\mu\ell$, and hepatotoxicity related dose modification was done at the discretion of the treating physician. Doses of all drugs were not prospectively adjusted on the basis of patients' genotyping results.

Clinical data collection

The medical records of all the patients were retrospectively reviewed including complete blood cell counts, total bilirubin, serum liver enzyme levels, frequency and grades of hematologic and liver toxicity, and the first date of toxicity noticed during maintenance. Events were defined as any relapse, death, or secondary malignancies. The National Cancer

Institute Common Toxicity Criteria (NCI-CTC) version 4.03 was used to grade toxicities. Early neutropenia was defined as neutropenia which developed within 30 days after initiation of the maintenance phase, and otherwise defined as late neutropenia.

Because doses of MP or methotrexate (MTX) were reduced in response to toxicities during the maintenance treatment, toxicity was supposed to be in inverse proportion to the actual administered dosage of the antileukemic agents. Data of MP dose was collected on a cycle basis (12 weeks), and the dose of MP in the final maintenance cycle was supposed to be the maximum tolerated dose for each patient.

Primary targeted sequencing and analysis

Targeted sequencing was conducted with novel pharm-gene panel for screening of suspected genetic determinants of MP related toxicities. This panel was composed of 147 pharmacogenetics related genes and 8 SNPs known to be involved in the metabolism of various drugs, and the size of total targeted region was 464,798 base pairs (Table 1).

Table 1. Target regions of the gene panel.

(147 pharmacogenetics related genes and 8 SNPs)

Gene symbol	rsID	Chromosome	position	Exon ID	Start	End
ABCC6	rs2238472	16	16251599	ENSE00001769392	16251520	16251666
ACE	rs4343	17	61566031	ENSE00002349173	61566009	61566152
AGTR1	rs5182	3	148459395	ENSE00001907256	148459308	148459457
ANKK1	rs1800497	11	113270828	ENSE00001130275	113270763	113270912
APOB	rs1367117	2	21263900	ENSE00000932263	21263810	21263955
APOC3	rs5128	11	116703640	ENSE00001413859	116703574	116703723
APOE	rs7412	19	45412079	ENSE00001700162	45412089	45412238
ATIC	rs2372536	2	216190020	ENSE00001906603	216189964	216190052
ATP7A	rs2227291	X	77268502	ENSE00001628072	77268403	77268552
BDKRB2	rs1799722	14	96671139	ENSE00002276929	96671085	96671234
C22orf32,CYP2D6	rs5030655	22	42525085	ENSE00002338508	42525035	42525187
C9orf72	rs774359	9	27561049	ENSE00001367833	27560965	27561114
CBR1,SETD4	rs9024	21	37445313	ENSE00001734805	37445222	37445371
	rs20572	21	37444973	ENSE00001734805	37444922	37445071
CBR3	rs1056892	21	37518706	ENSE00001044176	37518613	37518762
	rs8133052	21	37507501	ENSE00001044175	37507413	37507562
CD3EAP,ERCC1	rs3212986	19	45912736	ENSE00002263283	45912678	45912825
CDA	rs60369023	1	20931474	ENSE00001041262	20931421	20931532
	rs2072671	1	20915701	ENSE00001465671	20915623	20915776
CHRNA4	rs1044396	20	61981134	ENSE00002112075	61981049	61981198
	rs2236196	20	61977556	ENSE00001452193	61977499	61977648
CHST3	rs4148950	10	73771706	ENSE00001459536	73771620	73771769
	rs1871450	10	73772014	ENSE00001459536	73771920	73772069

	rs12418	10	73773014	ENSE00001459536	73772920	73773069
	rs4148943	10	73769507	ENSE00001459536	73769420	73769569
	rs4148945	10	73769590	ENSE00001459536	73769520	73769669
	rs4148947	10	73770117	ENSE00001459536	73770020	73770169
	rs730720	10	73772762	ENSE00001459536	73772670	73772819
COMT	rs165599	22	19956781	ENSE00001297813	19956713	19956862
	rs4646316	22	19952132	ENSE00001554634	19952070	19952219
	rs4680	22	19951271	ENSE00001295143	19951220	19951369
CYBA	rs4673	16	88713236	ENSE00001113677	88713163	88713252
CYP4B1	rs4646487	1	47279175	ENSE00001690282	47279154	47279278
CYP4F2	rs2108622	19	15990431	ENSE00001608537	15990414	15990478
DRD3	rs6280	3	113890815	ENSE00001225059	113890735	113890874
EGFR	rs712829	7	55086755	ENSE00001633131	55086725	55086874
EPHX1,PARP1	rs2292566	1	226019653	ENSE00001836308	226000000	226000000
	rs1051740	1	226019633	ENSE00001836308		
	rs2234922	1	226026406	ENSE00000962130	226026355	226026525
ERBB2	rs1136201	17	37879588	ENSE00001121070	37879572	37879710
ERCC1	rs11615	19	45923653	ENSE00000749055	45923582	45923685
ERCC2	rs13181	19	45854919	ENSE00002225456	45854887	45854979
	rs1799793	19	45867259	ENSE00002244461	45867244	45867377
ESR2	rs4986938	14	64699816	ENSE00001564600	64699747	64699893
FCGR3A	rs396991	1	161514542	ENSE00002415838	161514491	161514668
FKBP5	rs3800373	6	35542476	ENSE00001193215	35542420	35542569
GGH	rs11545078	8	63938764	ENSE00000928431	63938717	63938855
GNB3	rs5443	12	6954875	ENSE00002278155	6954818	6954966
HMGCR	rs17238540	5	74655498	ENSE00002084757	74655443	74655592

	rs3846662	5	74651084	ENSE00002086875	74650993	74651142
HSPA1L	rs2227956	6	31778272	ENSE00001943225	31778236	31778385
HTR2C	rs518147	X	113818582	ENSE00001289925	113818551	113818700
KCNJ11	rs5219	11	17409572	ENSE00001366321	17409507	17409638
KIF6	rs20455	6	39325078	ENSE00001086070	39325053	39325150
LRP2	rs2075252	2	170010985	ENSE00002418879	170010970	170011113
MAML1	rs730012	5	179220638	ENSE00002065753		
MTHFR	rs1801131	1	11854476	ENSE00001225813	11854415	11854595
	rs1801133	1	11856378	ENSE00001225832	11856311	11856456
NOS3	rs1799983	7	150696111	ENSE00001088139	150696034	150696173
NPPA−AS1,NPPA	rs5065	1	11906068	ENSE00001470668	11905941	11906071
OPRM1	rs1799971	6	154360797	ENSE00001428473	154360696	154360845
PICK1	rs2076369	22	38463652	ENSE00001921127	38463577	38463727
PPARD	rs3734254	6	35395010	ENSE00001401034	35394935	35395085
	rs2016520	6	35378778	ENSE00002212717	35378764	35378914
PPP1R9A	rs854547	7	94923856	ENSE00001390261	94923781	94923931
RRM1	rs9937	11	4159457	ENSE00002166805	4159425	4159575
SLC10A2	rs2301159	13	103697728	ENSE00000854033	103697653	103697803
SLC19A1	rs1051266	21	46957794	ENSE00001872362	46957719	46957869
SLC22A16	rs714368	6	110778128	ENSE00001933561	110778053	110778203
SOD2	rs4880	6	160113872	ENSE00001643760	160113745	160113895
SPECC1L,ADORA2A,C22orf45	rs2298383	22	24825511	ENSE00002309813	24825436	24825586
SPG7	rs12960	16	89620328	ENSE00002304833	89620218	89620368
	rs2292954	16	89613123	ENSE00001434018	89613066	89613168
SULT1C4	rs1402467	2	108994808	ENSE00001926333	108994733	108994883
TLR3	rs3775291	4	187004074	ENSE00002051030	187003999	187004149

TMPRSS3,KCNJ6	rs2070995	21	39086965	ENSE00001033313	39086890	39087040
TP53	rs1042522	17	7579472	ENSE00002359670	7579397	7579547
TYMS,C18orf56	rs45445694	18	657646	ENSE00001753233	657604	657754
VDR	rs731236	12	48238757	ENSE00002372083	48238638	48238788
VKORC1	rs7294	16	31102321	ENSE00001820260	31102246	31102396
	rs9934438	16	31104878	ENSE00001921254	31104803	31104953
XPC	rs2228001	3	14187449	ENSE00001914924	14187374	14187524
XRCC1	rs25487	19	44055726	ENSE00000710568	44055719	44055869
ZBTB42	rs3803300	14	105269779	ENSE00001213415	105269704	105269854

Subject for targeted sequencing were selected among all patients, whose MP doses was reduced due to toxicities so that the final dose was less than 25% of initial planned doses. Total 44 patients were selected as a representative cohort for MP related prominent toxicity (Figure 1). DNA was extracted from normal blood cells in bone marrow aspirates or peripheral blood, which was collected in remission states.

Sequencing data of 44 subjects was generated by Ion Torrent Personal™(ThermoFisher Scientific,Waltham, MA, USA) (Figure 2). Sequence data in FastQ format were aligned to the hg19 version of the human genome (GRCh37) from UCSC (http://cole-trapnell lab.github.io/cufflinks/igenome_ table/index.html), transformed into SAM files and then converted into compressed BAM files by PICARD (http://picard.sourceforge.net/). Possible PCR duplicates were marked by PICARD and local realignment around indels was performed using the Genome Analysis Tool Kit v2.8-1 (GATK, https://www.broadinstitute.org/gatk/). The average depth of coverage was 415.71x ± 157.30.

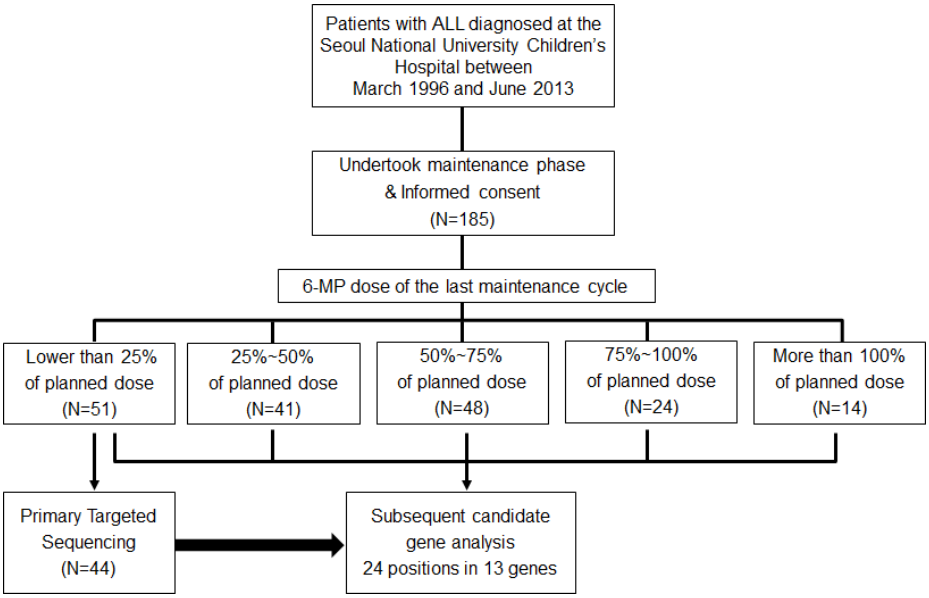


Figure 1. Total scheme of the study. Two-stage pharmacogenetic analyses were performed. A total of 185 patients with ALL who were diagnosed and treated at the Seoul National University Children's Hospital were included. First, targeted sequencing was carried out using a novel gene panel of 147 pharmacogenetics related genes and 8 SNPs in 44 patients who received less than 25% of initial dose (50 mg/m^2/day) during maintenance therapy due to MP intolerance. Next, significant genes identified in the first analysis were re-sequenced in a total of 185 patients.

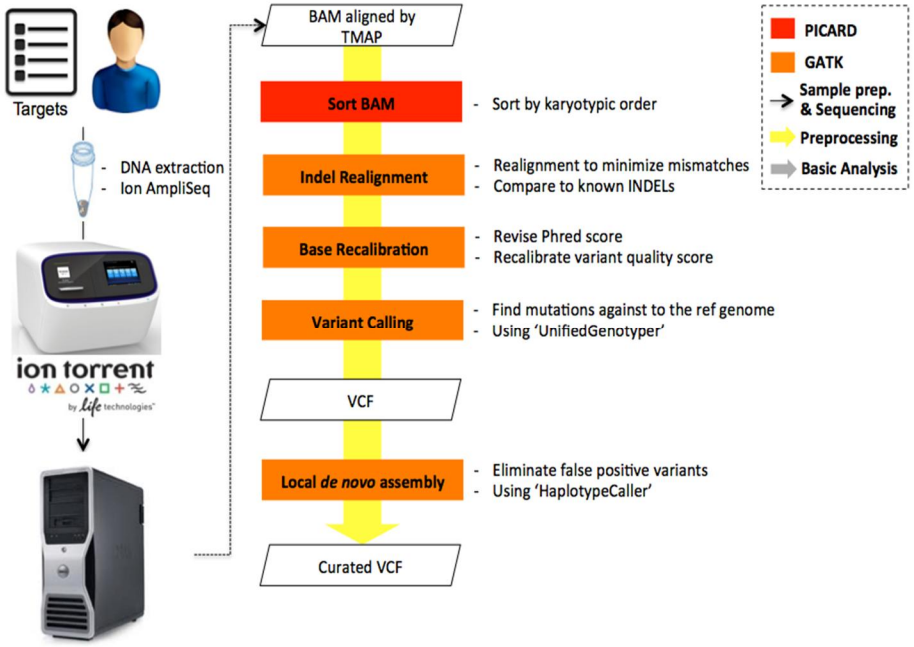


Figure 2. Primary targeted sequencing pipeline. Sequencing data was generated by Ion Torrent. Sequence data in FastQ format were aligned to the hg19 version of the human genome (GRCh37), transformed into SAM files and then converted into compressed BAM files by PICARD. Possible PCR duplicates were marked by PICARD and local realignment around indels was performed using the Genome Analysis Tool Kit v2.8-1.

Sequenced data of this cohort was compared with data of normal population and patients with other disease. As normal population data, 1,000 genome phase 3 data (N=2,504) and 22 Korea adults sequencing data was used, and data of 146 patients undergoing stem cell transplantation and 45 chronic obstructive pulmonary disease patients was also used as control data. Except for 1,000 genome data, other samples were sequenced by Ion Torrent with the pharm gene panel.

Subsequent validation with candidate gene analysis

Subsequent validation of suspected pharmacogenetics genes on the basis of primary sequencing results was conducted with candidate gene approach.

Candidate genes were also selected considering the previous clinical studies in which exhibited polymorphisms and encoded proteins of multiple genes involved in the pharmacokinetics or pharmacodynamics of antileukemic agents were described (Table 2).

Table 2. Candidate genes included in the subsequent analysis

Gene	rdID
NUDT15	rs116855232
APEX1	rs2307486
ABCC4	rs2274407
	rs3765534
	rs11568658
CYP4F2	rs2108622
CYP1A1	rs4646422
TPMT	rs144041067
	rs151149760
	rs75543815
	rs1800460
	rs1800462
	rs1142345
SLCO1B1	rs11045879
	rs4149056
ITPA	rs1127354
	rs7270101
MTHFR	rs1801131
	rs1801133
	rs1901133
GRIA1	rs4958351
MOCOS	rs594445
PACSIN2	rs2413739
BAG3	rs78439745

The validation analysis on 185 samples with an array-based high throughput method was conducted to simultaneously detect 22 positions in 13 genes using SNPtype assay (Fluidigm, San Francisco, CA, USA) (Table 3), and 2 positions in 2 genes using SNaPshot assay (Table 4).

The method of SNPtype assay was that the genomic DNA flanking the interested SNP was amplified with PCR reaction with STA primer set and Qiagen 2X Mutiplex PCR Master Mix (Qiagen) in 5 microliter reaction volume, containing 40ng of genomic DNA. The PCR reactions were carried out as follows : 15 min at 95℃ for 1 cycle, and 14 cycles on 95℃ for 15s, 60℃ for 4min. After amplification, the STA products were diluted 1:100 in DNA Suspension Buffer. 2.5 microliter of the diluted STA products were added to a Sample Pre-Mix containing 3microliter of 2X Fast Probe Master Mix, 0.3 microliter of the SNPtype 20X Sample Loading Reagent, 0.1 microliter of the SNPtype Reagent, and 0.036 microliter of the ROX. After the Assay Pre-Mix and the Sample Pre-Mix were loaded into the 192.24 Dynamic Array. SNPtype assay reaction was carried out as follows : 5 min at 95℃ for 1 cycle, 1 cycle on 95℃ for 15s, 64℃ for 45s, 72℃ for 15s , 1 cycle on 95℃ for 15s, 63℃ for 45s, 72℃ for 15s, 1 cycle on 95℃ for 15s, 62℃ for 45s, 72℃

for 15s, 1 cycle on 95℃ for 15s, 61℃ for 45s, 72℃ for 15s, 34 cycle on 95℃ for 15s, 60℃ for 45s, 72℃ for 15s, and 10s at 25℃ for 1 cycle. Analysis was carried out using Fluidigm SNP Genotyping Analysis software (version 4.0.1; Fluidigm).

The SNaPshot assay was performed according to the manufacturer's instructions (ABI PRISM SNaPShot Multiplex kit , Foster City, CA, USA). Analysis was carried out using Genemapper software (version 4.0; Applied Biosystems).

Table 3. Primer and probe sequences for the SNPtype assay

rsID	ASP1_SEQ	ASP2_SEQ	LSP_SEQ	STA_SEQ
rs11045879	AGGATCCAGGGTTAATATAACAGAATCAAG	AGGATCCAGGGTTAATATAACAGAATCAAA	GGGCATCTTGATGGCTTCCAA	TTGCAAGGTTTCAGGACACAG
rs1127354	GGTCGTTCAGATTCTAGGAGATAAGTTTA	GGTCGTTCAGATTCTAGGAGATAAGTTTC	GTCAATTTTCTGTGCCACCAAA	TGTTCTCTTTTCTCTTGGAACAGG
rs11568658	GGCCTGTGGTTGTCTTCCC	GGCCTGTGGTTGTCTTCCA	ACCTCTTTTATTTCAGGCACTTCGTCT	TGGACAGCAGATTGACTATCTGG
rs116855232	GGATCATAGCCTTGTTCTTTTAAACAACG	GGATCATAGCCTTGTTCTTTTAAACAACA	CCTCCCCTGGACCAGCTTT	CACCAGATGGTTCAGATCTTCTTTA
rs144041067	GCTAAACAAAAAAAGAAAAATTACTTACCATTTGC	AGCTAAACAAAAAAAGAAAAATTACTTACCATTTGT	CAAATATTGGCAAATTTGACATGATTTGGGAT	CCCAGAAAAAGTATAGTATACTAAAAAATTAAGACAG
rs151149760	CACCGAAATTCCTGGAACCAC	TCACCGAAATTCCTGGAACCAA	TGGATACAATTATTTACCCAAATCAAAACAAACCTT	ACTCAGAAGAACCAATCACCGA
rs1800460	CAAATTTGACATGATTTGGGATAGAGGAA	CAAATTTGACATGATTTGGGATAGAGGAG	ACTTACCATTTGCGATCACCTGGA	TAGGACAAATATTGGCAAATTTGACAT
rs1800462	CTGTGTCCCCGGTCTGG	TGTGTCCCCGGTCTGC	AAACATAATTTAAGTGTAAATGTATGATTTTATGCAGGTT	ACTGATTTCCACACCAACTACAC
rs1801131	GAGGAGCTGACCAGTGAAGA	AGGAGCTGACCAGTGAAGC	TCCGGTTTGGTTCTCCCGAG	CCAAGGAGGAGCTGCTGA
rs1801133	GCTGCGTGATGATGAAATCGG	AGCTGCGTGATGATGAAATCGA	AGGCTGACCTGAAGCACTTGA	TGTGTCAGCCTCAAAGAAAAGC
rs1901133	TTCCATTGGGTAGAAGAATATTTTTCAGC	CTTCCATTGGGTAGAAGAATATTTTTCAGT	GAATGCAAAATGAATGGCAAGCTAATTTTTAGA	CCCAGAATTACTGAGCATTGACT
rs2108622	ACCCATCACAACCCAGCTG	AACCCATCACAACCCAGCTA	GCACCTCAGGGTCCGGC	TGCCTCATCAGTGTTTTCGG
rs2307486	CAAACCTGCCACACTCAAGA	CAAACCTGCCACACTCAAGG	ATCCAGGCTCGAAGCCCAT	AAAACCTCACCCAGTGGC
rs2413739	CCTAAGTTTTATTTGAGGTCGGACG	GCCTAAGTTTTATTTGAGGTCGGACA	GCACTTGAAGGGAGAGCACAA	GATTCCATAGCAGCCCTTGG
rs3765534	CCTAAGTACCAGTTAAGATCTAGCTTCTC	CCTAAGTACCAGTTAAGATCTAGCTTCTT	CCCATGTTCCATAGGGCAAACA	CAATACACCAAATGATTAAACTTTACCTGAA
rs4149056	CACGAAGCATATTACCCATGAACG	CACGAAGCATATTACCCATGAACA	AAAGGAATCTGGGTCATACATGTGGAT	GTACTATGGGAGTCTCCCCTATTC
rs4646422	GCCCAATCAGAGGCCAGT	GCCCAATCAGAGGCCAGC	GGTCCCCAAAGGCCTGAAGA	GTTCTTTCCCAGGGTCAGC
rs4958351	CCTAGCAGTAGTTTCTAGATATCTAGTTTTCAT	CCTAGCAGTAGTTTCTAGATATCTAGTTTTCAC	GGTCCTCCCACACCCACTTT	AGCAGAAGCTATCCCTTAGCC
rs594445	CAACATTTTTTGGCCGTCCTTGTA	ACATTTTTTGGCCGTCCTTGTC	GCATTCCTTTGAGAGTTTGAACTTTGTTTG	GGAGAAAAAATTTCAAGCTGGTTGT
rs7270101	AAATTGACCGTATGTCTCTGTTTTGTTTTA	ATTGACCGTATGTCTCTGTTTTGTTTTC	ACAGGAAGACAGAGAAATCCAACCA	ATGCACTTTGGTGGCACA
rs75543815	GGATCATAAGAAAGAACACACAGGAGAA	GGATCATAAGAAAGAACACACAGGAGAT	AGATACAATGTTTTCCCTCCTGG	CCAACAACTTTACCTGGATGTTTAG
rs78439745	CCGTACCTGAGGCCTGTT	CCGTACCTGAGGCCTGTC	CCACTCCACTCCCCCTCG	TCTGCTGACGACTAACTTCCC

Table 4. Primer sequence and PCR condition for the SNaPshot assay

Gene	rsID	Strand	Primer sequence		Tm	Additive
TPMT	rs1142345	Forward	Forward Primer	TCCCTGATGTCATTCTTCA	60	–
			Reverse Primer	CTCCTCTCCAAAGGAGCTA		
			Genotyping Primer	TTGACTGTCTTTTTGAAAAGTTAT		
ABCC4	rs2274407	Reverse	Forward Primer	aattcatccacctcagctc	60	Betaine
			Reverse Primer	CCTCTGACACCCTCTCAAT		
			Genotyping Primer	cttctcagaatcttggaaatctcctt		

Statistical analysis

Statistical associations between categorical variables, such as the frequency of toxicities and SNPs were evaluated using Chi-square or Fisher's exact tests. Odds ratios (OR) and 95% confidence intervals (CI) associated with each SNP were determined using logistic regression assuming log-additive and dominant genetic models of inheritance. The comparison of genotype frequencies using a nonparametric method Cochran-Armitage trend test was also applied.

Event-free survival (EFS) and overall survival (OS) were estimated using Kaplan-Meier analysis, and the survival differences according to different genetic polymorphisms and prognostic variables were analyzed by log-rank test. Comparison of the administered dose according to each SNP group was analyzed by T-test or ANOVA for parametric data and Mann-Whitney U or Kruskal-Wallis test for nonparametric data.

Cumulative incidence curves were used to estimate the cumulative risk of the occurrence of neutropenia after the initiation of maintenance phase using the method of Fine and Gray (18), and comparison was performed by Gray's test (19). Competing risk estimation and Cochran-Armitage trend

test were conducted using R (R 3.2.0; http://www.r-project.org/), and other analysis was done by SPSS version 20.0 with statistical significance of $P < 0.05$.

Ethics statement

This study was approved by the Institutional Review Board of Seoul National University Hospital (H-0611-021-189). Informed written consents for blood sampling, collection, DNA analysis and review of their medical records were obtained.

Results

Patients and treatment

A total of 185 patients were analyzed (Table 5). There were 110 males and 75 females. The median follow up duration was 74.2 months (26.6~235.7 months). The median age at diagnosis was 4.9 years (1.1~17.3 years), and 45 patients were over 10 years old at diagnosis. Fifty-seven patients were assigned to the standard-risk group, and 1 and 56 patients were treated with modified CCG-1881 and 1952 protocols. One hundred twenty-eight patients were assigned to the high-risk group, and 2, 59, 20 and 47 patients were treated with the modified CCG 1882A, 1882B, 1882C and 0601 protocol for Korean multicenter study protocols, respectively.

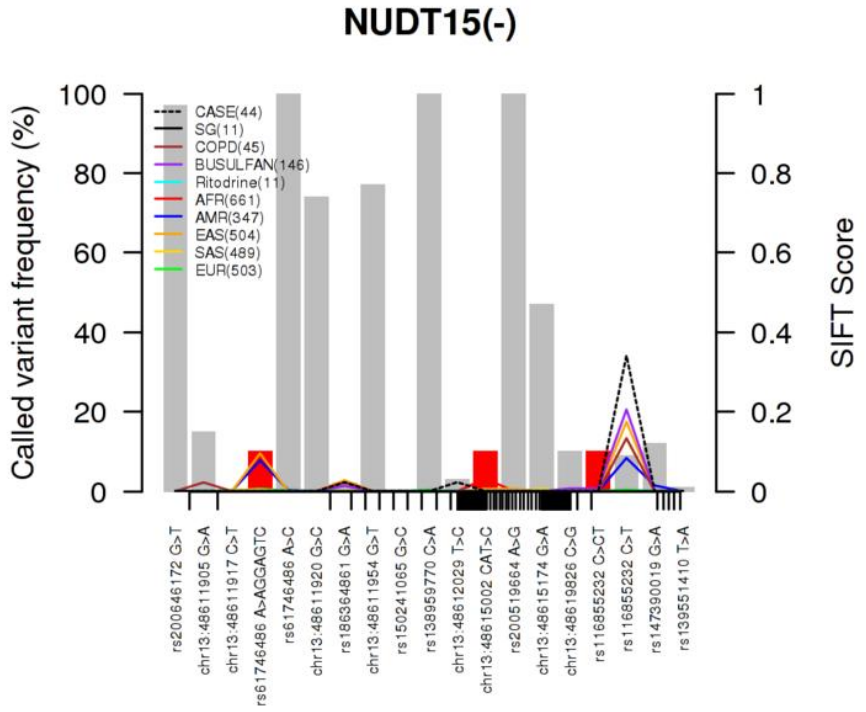
Table 5. Characteristics of all patients (N=185)

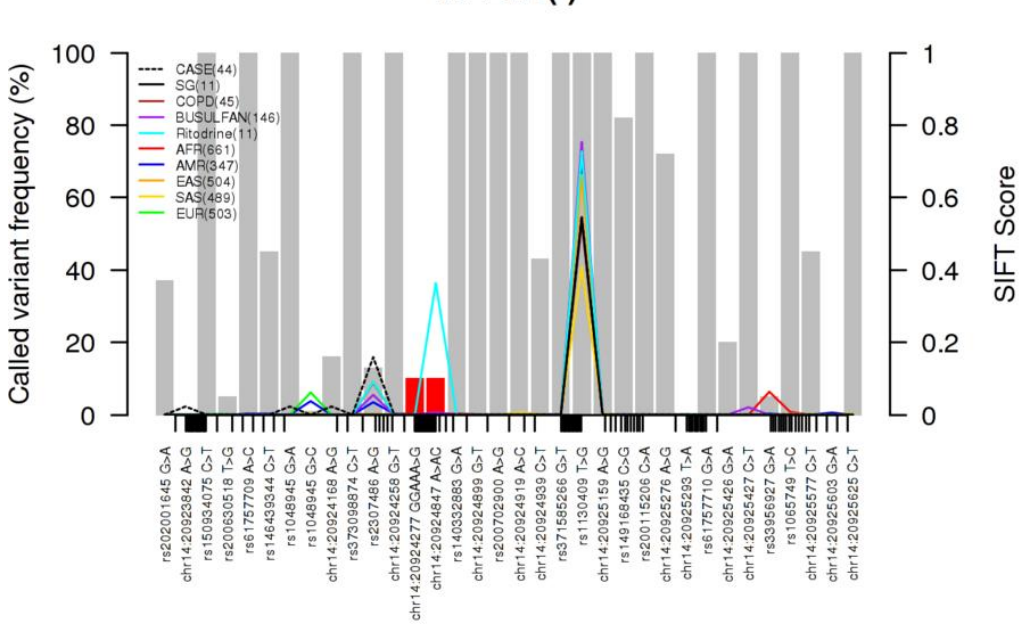
Characteristics	
Age (yr) at diagnosis, median (range)	4.9 (1.1−17.3)
1 y to less than 10 y	140
at least 10 y	45
Gender, No.	
Male	110
Female	75
Risk group, No.	
Standard−risk patients	57
modified CCG 1891	1
modified CCG 1952	56
High−risk patients	128
modified CCG 1882A	2
modified CCG 1882B	59
modified CCG 1882C	20
0601 protocol Korea	47

Results of primary targeted sequencing

Primary targeted sequencing was conducted in 44 patients. The median dose percent of last cycle MP in 44 patients was 17.9%. Sequencing data were analyzed with gene score based comparison between 44 subjects and other control group. Considering two tailed False Discovery Rate (FDR) and gene scores, 11 genes were selected for possible genetic determinants for MP intolerance. Furthermore, multiple SNPs within 11 genes were further explored in order to find out variants of which frequency are higher in Asian than other ethnic groups.

As a result, *NUDT15* rs116855232, *APEX1* rs2307486, *CYP1A1* rs4646422, and *BAG3* rs78439745 were selected as possible genetic factors for MP intolerance (Figure 3).


NUDT15(-)
Called variant frequency (%)
SIFT Score
CASE(44)
SG(11)
COPD(45)
BUSULFAN(146)
Ritodrine(11)
AFR(661)
AMR(347)
EAS(504)
SAS(489)
EUR(503)
rs200646172 G>T
chr13:48611905 G>A
chr13:48611917 C>T
rs61746486 A>AGGAGTC
rs61746486 A>C
chr13:48611920 G>C
rs186364861 G>A
chr13:48611954 G>T
rs150241065 G>C
rs138959770 C>A
chr13:48612029 T>C
chr13:48615002 CAT>C
rs200519664 A>G
chr13:48615174 G>A
chr13:48619826 C>G
rs116855232 C>CT
rs116855232 C>T
rs147390019 G>A
rs139551410 T>A


APEX1(-)
Called variant frequency (%)
SIFT Score
CASE(44)
SG(11)
COPD(45)
BUSULFAN(146)
Ritodrine(11)
AFR(661)
AMR(347)
EAS(504)
SAS(489)
EUR(503)
rs202001645 G>A
chr14:20923842 A>G
rs150934075 C>T
rs200630518 T>G
rs61757709 A>C
rs146439344 C>T
rs1048945 G>A
rs1048945 G>C
chr14:20924168 A>G
rs373098874 C>T
rs2307486 A>G
chr14:20924258 G>T
chr14:20924277 GGAAA>G
chr14:20924847 A>AC
rs1403328883 G>A
chr14:20924899 G>T
rs200702900 A>G
chr14:20924919 A>C
chr14:20924939 C>T
rs371585266 G>T
rs1130409 T>G
chr14:20925159 A>G
rs149168435 C>G
rs200115206 C>A
chr14:20925276 A>G
chr14:20925293 T>A
rs61757710 G>A
chr14:20925426 G>A
chr14:20925427 C>T
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rs1065749 T>C
chr14:20925577 C>T
chr14:20925603 G>A
chr14:20925625 C>T

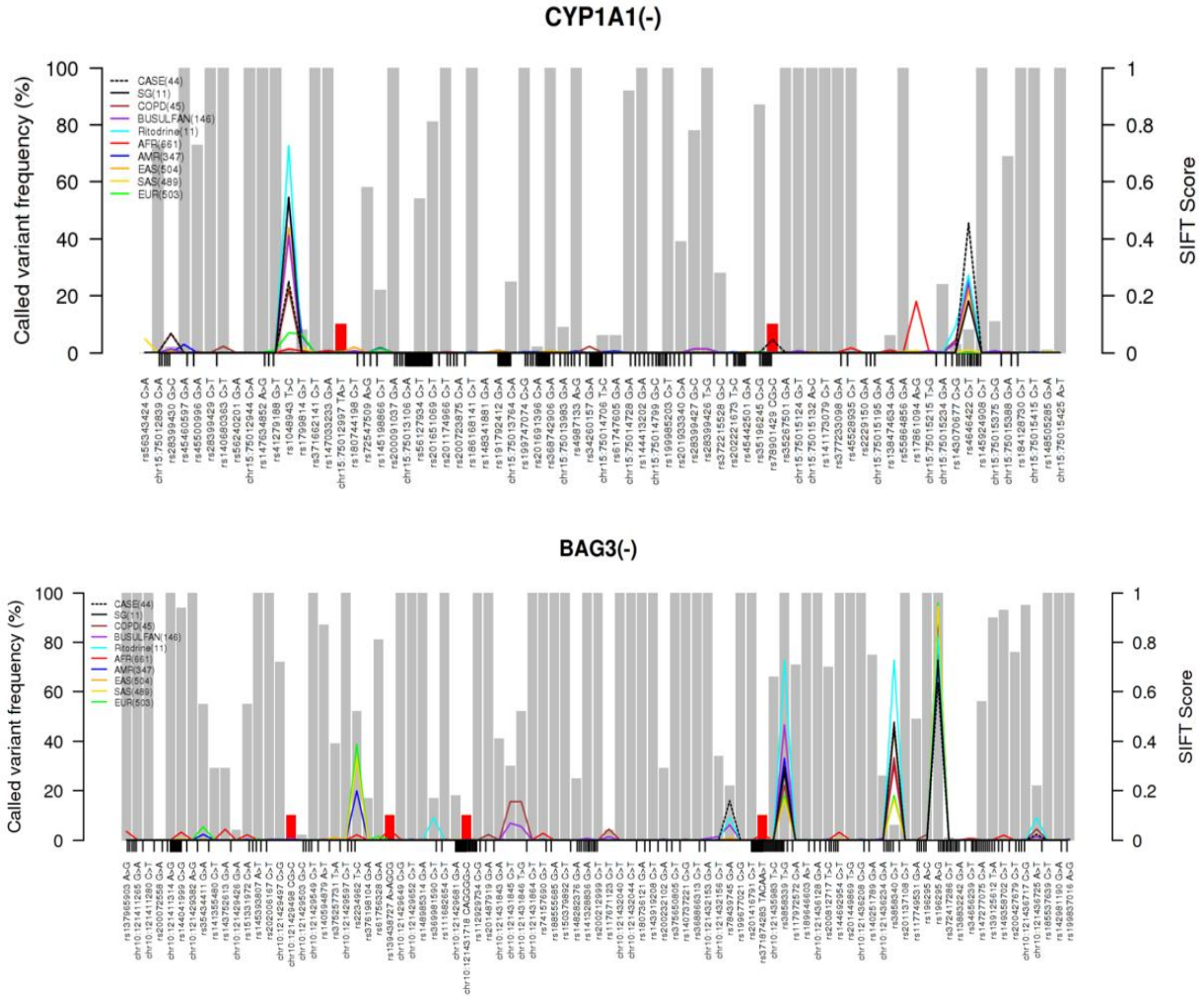

Figure 3. Plots for called variant frequencies and SIFT scores of each SNPs in *NUDT15, APEX1, CYP1A1,* and *BAG3*. The line plot is the called variant frequency of each variant locus and is shown for each race or cohort. The gray bar on the background of the graph is the SIFT score of each variant, and if the score is low, it is likely to be a variant that causes affecting mutation. In this study, variants with lower SIFT scores and higher variant frequency than other races were selected as meaningful variants.

Results of subsequent candidate gene analysis

Genotypes of the 24 candidate loci in 185 patients are summarized in Table 6. The distribution of patients with variant alleles were *NUDT15* rs116855232 (21.7%), *APEX1* rs2307486 (13.5%), *ABCC4* rs2274407 (34%), *ABCC4* rs3765534 (14.1%), *ABCC4* rs11568658 (23.2%), *CYP4F2* rs2108622 (51.9%), *CYP1A1* rs4646422 (29.1%), *SLCO1B1* rs11045879 (55.7%), *SLCOB1* rs4149056 (26.5%), *ITPA* rs1127354 (26%), *ITPA* rs7270101 (0%), *MTHFR* rs1801131 (60.5%), *MTHFR* rs1801133 (28.6%), *MTHFR* rs1901133 (50.8%), *GRIA1* rs4958351 (3.8%), *MOCOS* rs594445 (48.1%), *PACSIN2* rs2413739 (13.5%), and *BAG3* rs78439745 (7%). *TPMT* allele type was *1/*1 in most of the patients (96.2%), and variant allele was detected in only 7 patients (*1/*3C, 3.8%)

Table 6. The frequencies of candidate genetic loci (N=185)

Gene	rdID	Genotypes	No. of patients (%)
NUDT15	rs116855232	CC	131 (70.8)
		CT	36 (19.5)
		TT	4 (2.2)
APEX1	rs2307486	AA	146 (78.9)
		AG	24 (13)
		GG	1 (0.5)
ABCC4	rs2274407	GG	110 (59.5)
		GT	57 (30.8)
		TT	6 (3.2)
	rs3765534	CC	145 (78.4)
		CT	24 (13)
		TT	2 (1.1)
	rs11568658	CC	129 (69.7)
		CA	38 (20.5)
		AA	5 (2.7)
CYP4F2	rs2108622	CC	76 (41.1)
		CT	71 (38.4)
		TT	25 (13.5)
CYP1A1	rs4646422	CC	116 (62.7)
		CT	48 (25.9)
		TT	6 (3.2)
SLCO1B1	rs11045879	TT	68 (36.8)
		TC	71 (38.4)
		CC	32 (17.3)
	rs4149056	TT	122 (65.9)
		TC	46 (24.9)
		CC	3 (1.6)
ITPA	rs1127354	CC	123 (66.5)
		CA	44 (23.8)
		AA	4 (2.2)
	rs7270101	AA	185 (100)
MTHFR	rs1801131	GG	58 (31.4)
		GA	84 (45.4)
		AA	28 (15.1)
	rs1801133	TT	118 (63.8)

		TG	50 (27.0)
		GG	3 (1.6)
	rs1901133	GG	78 (42.2)
		GA	83 (44.9)
		AA	11 (5.9)
GRIA1	rs4958351	GG	166 (89.7)
		AG	7 (3.8)
MOCOS	rs594445	CC	84 (45.4)
		AC	76 (41.1)
		AA	13 (7)
PACSIN2	rs2413739	CC	145 (78.4)
		CT	25 (13.5)
BAG3	rs78439745	GG	160 (86.5)
		AC	13 (7)
TPMT	*1/*1		178 (96.2)
	*1/*3C		7 (3.8)

Neutropenia during maintenance & genotypes

Absolute neutrophil counts (ANC) under 500/㎕ developed in 121 patients during MP administration in the maintenance phase (65.4%) (Table 7). When first date of ANC under 500/㎕ after MP intake during maintenance was reviewed, early onset neutropenia (within 30 days) was detected in 15 patients (8.1%), and others was detected after 30 days after initiation of the maintenance phase (106 patients, 57.3%)

When frequency of neutropenia during MP administration was crosstabulated with variant frequencies, Grade 4 neutropenia (ANC<500/㎕)was significantly more frequent in the carriers of variant T allele in *ABCC4* rs3765534, and it was associated with 4.64 fold increase risk of severe neutropenia compared to homozygous wild-type patients (OR, 4.64; 95% CI;1.32~25.12, *P*=0.008) (Table 8).

Table 7. Neutropenia during maintenance phase in all patients

Toxicities during maintenance		No. of patients (%) (N=185)
Neutropenia	< 500/㎕	121 (65.4)
	Early (within 30 days)	15 (8.1)
	Late (after 30 days)	106 (57.3)

Table 8. *ABCC4* rs3765534 related to Grade 4 neutropenia (N=179)

Neutropenia (ANC<500 $\mu\ell$)	Allelic Frequency					Genotype (Dominant model)						CATT
Total	C	T	Chisq *P*	Fisher's *P*	OR (95% CI)	CC	CT	TT	Chisq *P*	Fisher's *P*	OR (95% CI)	*P*
YES	213	27	0.01	0.004	4.84 (1.44~25.48)	96	21	3	0.016	0.008	4.64 (1.32~25.12)	0.02
NO	115	3				56	3	0				
No *NUDT15* rs116855232 (N=140)												
YES	160	20	0.01	0.005	6.10 (1.43~54.9)	71	18	1	0.014	0.006	6.36 (1.43~58.73)	0.02
NO	98	2				48	2	0				

OR, odds ratio; CI, confidence internal; CATT, Cochran-Armitage trend test

Table 9. *APEX1* rs2307486 related to early onset Grade 4 neutropenia (N=178)

Early Grade 4 Neutropenia	Allelic Frequency					Genotype (Dominant model)						CATT
Total	A	G	Chisq *P*	Fisher's *P*	OR (95% CI)	AA	AG	GG	Chisq *P*	Fisher's *P*	OR (95% CI)	*P*
YES	24	6	0.03	0.02	3.44 (1.04~9.89)	10	4	1	0.09	0.06	3.18 (0.78~11.43)	0.003
NO	304	22				141	22	0				

OR, odds ratio; CI, confidence internal; CATT, Cochran-Armitage rend test

This statistical association was also present within patients without *NUDT15* rs1168552532 variant alleles (N=140), which was known as a potent risk factor for myelotoxicity of MP in previous studies, and the odd ratio was even higher as 6.36 (OR, 6.36; 95% CI;1.43~58.73, *P*=0.006) in these patients without *NUDT15* variants.

Although SNPs in *APEX1* rs2307486 were analyzed as a risk factor of lower tolerable MP dose according to the primary sequencing results, genetic polymorphisms *APEX1* rs2307486 showed no significant correlation with total frequencies of MP related neutropenia. However, considering onset of neutropenia, G allele in *APEX1* rs2307486 was highly related to the early onset Grade 4 neutropenia after the initiation of MP administration (OR, 3.44; 95 CI;1.04~9.89, *P*=0.02) in allele frequency based analysis (Table 9).

When multiple logistic regression analysis was performed on variants affecting Grade 4 neutropenia, the variant of *ABCC4* rs3765534 was found to increase the risk of neutropenia by 4.78 fold (Table 10). According to the cox regression analysis, the risk of Grade 4 neutropenia was significantly increased in patients with GG genotype of *APEX1* rs2307486 by 12.03 fold and in those with TT genotype in *NUDT15* rs116855232 by 11.42 fold (Table 11).

Table 10. Genetic variants affecting Grade 4 neutropenia (The result of multiple logistic regression analysis)

Variables	OR	95%CI	*P*
ABCC4 rs3765534	4.78	1.65-20.51	0.012
ABCC4 rs11568658	0.64	0.34-1.18	0.150
MTHFR rs1901133	1.57	0.91-2.79	0.111

OR, odds ratio; CI, confidence internal

Table 11. Genetic variants affecting Grade 4 neutropenia (The result of Cox-regression analysis)

Variables	HR	95%CI	*P*
APEX1 rs2307486			
AA genotypes (wild type)	1.00	-	-
AG genotypes	1.49	0.87-2.53	0.139
GG genotypes	12.03	1.43-101.20	0.022
NUDT15 rs116855232			
CC genotypes (wild type)	1.00	-	-
CT genotypes	1.25	0.78-2.01	0.358
TT genotypes	11.42	3.35-38.97	<0.001
ABCC4 rs3765534			
CC genotypes (wild type)	1.00	-	-
CT genotypes	1.72	1.05-2.80	0.031
TT genotypes	2.11	0.62-7.20	0.232

HR, hazard ratio; CI, confidence internal

Furthermore, cumulative incidence or proportion of patients experiencing Grade 4 neutropenia after MP initiation was significantly higher in patients with variants of *APEX1* rs2307486 so that AA, AG, GG genotypes were associated with the lowest, the intermediate, and the highest cumulative incidence during maintenance phase (P<0.001) (Figure 4).

One individual with homozygous variant (GG) of *APEX1* developed neutropenia lower than 500/$\mu\ell$ at the 21st day of maintenance as cumulative incidence of 100%. After about 2 years of maintenance treatment, the estimated cumulative incidence was 73% (95% CI 50.5~86.6%) in patients with AG genotype versus 66.3% (95% CI 57.9~73.5%) in patients with wild type (AA) in *APEX1* rs2307486.

In addition, *NUDT15* rs116855232 variants were also strongly associated with higher cumulative incidence of Grade 4 neutropenia that cumulative incidences were 100% in TT genotype, 72.2% (95% CI 53.9~84.3%) in CT genotype, and 65.0% (95% CI 56.2~72.4%) in CC genotype, respectively (P<0.001) (Figure 5). All three patients with homozygous variant genotype (TT) developed Grade 4 neutropenia at 19, 35, 42 days of 1st cycle of maintenance.

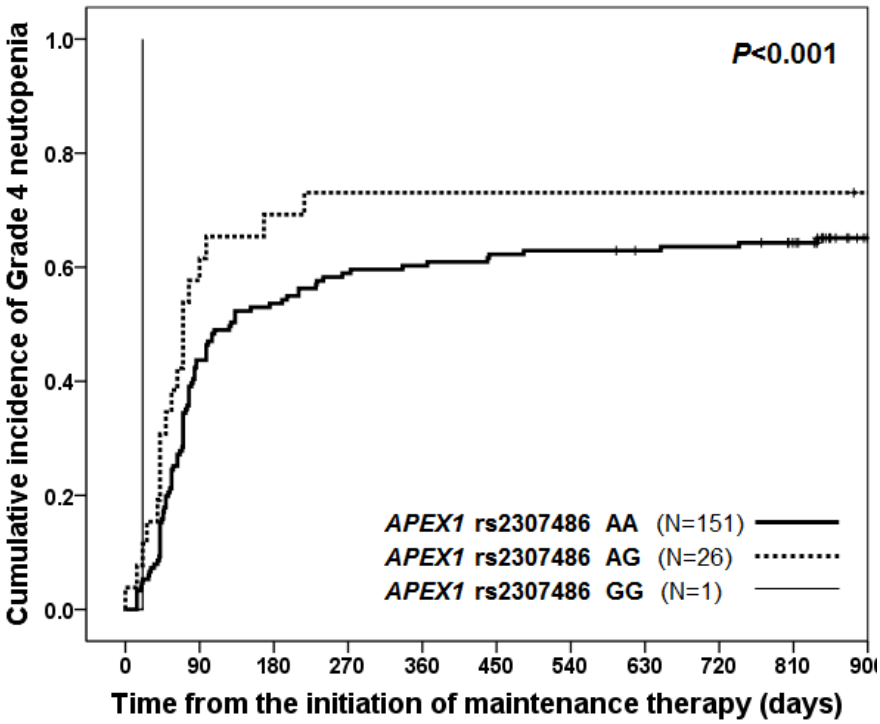

Figure 4. Estimated cumulative incidence of Grade 4 neutropenia after MP initiation according to genotypes of *APEX1* rs2307486. The cumulative incidence or proportion of patients experiencing early Grade 4 neutropenia after MP initiation was significantly higher in patients with variants of *APEX1* rs2307486. (AA:AG:GG=66.3%:73%:100%)

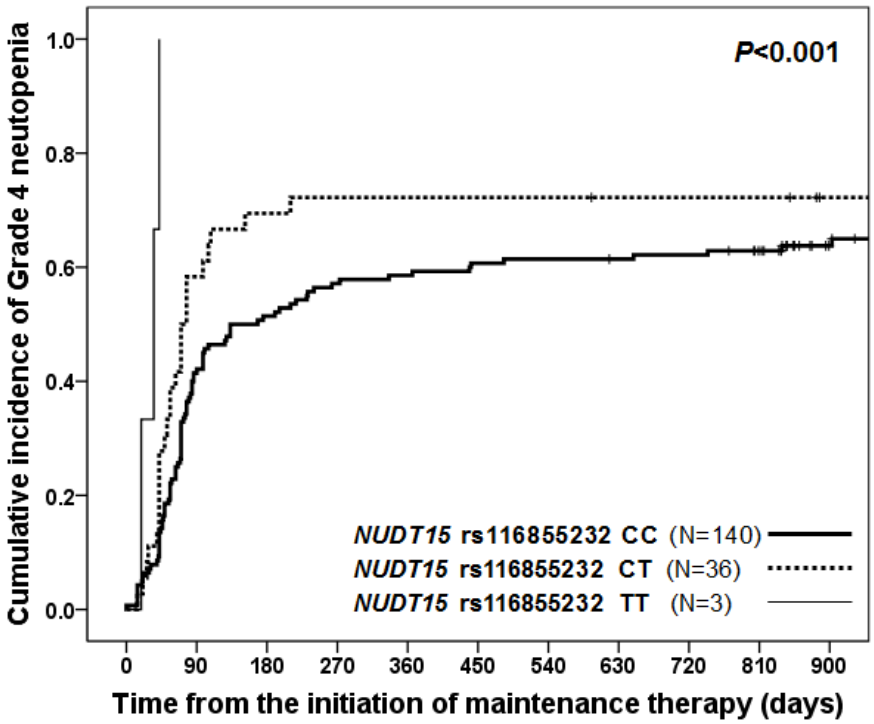

Figure 5. Estimated cumulative incidence of Grade 4 neutropenia after MP initiation according to genotypes of *NUDT15* rs116855232. The cumulative incidence or proportion of patients experiencing early Grade 4 neutropenia after MP initiation was significantly higher in patients with variants of *NUDT15* rs116855232. (CC:CT:TT =65.0%:72.2%:100%)

Primary targeted sequencing data of 44 patients was analyzed to find out further risk alleles for early onset neutropenia, SNPs of *GNB3*, which encodes Guanine Nucleotide Binding Protein (G Protein), Beta Polypeptide 3, were also related to the early onset MP related neutropenia. To evaluate the combined effects of both *APEX1* rs2307486 and *GNB3* rs2234757 variants, the frequency of early onset neutropenia in 44 patients was compared with different genotype combinations at these two loci. In these patients, there was a significant correlation between the burden of risk alleles and early onset MP related neutropenia, with individuals homozygous for either *APEX1* or *GNB3* risk variants showing almost 10 times higher risk than patients with wild type in both loci suggesting possible additive risk effects of *GNB3* variants (Table 12). This pattern of allele frequency differences was also replicated in general population and Asian ancestries from 1,000 genome data as control groups.

Table 12. Combined effects of *APEX1* and *GNB3* variants on early onset neutropenia during MP administration (N=40)

Control Group	Early Onset Neutropenia	Allelic Frequency				Genotype (Dominant model)					CATT
		*REF	§ ALT	Fisher's *P*	OR (95% CI)	*REF	†HET	‡ HOM	Fisher's *P*	OR (95% CI)	*P*
SNUCH, N=33	YES	6	8	<0.001	10.71 (2.48~51.19)	2	2	3	0.011	10.35 (1.33~133.67)	0.001
	NO	59	7			27	5	1			
1KP, N=2504	YES	6	8	<0.001	40.78 (12.25~144.27)	2	2	3	<0.001	38.28 (6.2~403.82)	<0.001
	NO	4850	158			2351	148	5			
EAS, N=504	YES	6	8	<0.001	14.77 (4.38~52.98)	2	2	3	0.002	12.77 (2.05~136.41)	<0.001
	NO	925	83			422	81	1			
EAS_CDX, N=93	YES	6	8	<0.001	13.79 (3.69~54.97)	2	2	3	0.004	11.60 (1.72~132.08)	<0.001
	NO	170	16			77	16	0			
EAS_CHB, N=103	YES	6	8	<0.001	9.92 (2.76~37.98)	2	2	3	0.013	8.03 (1.22~89.55)	<0.001
	NO	182	24			79	24	0			
EAS_CHS, N=105	YES	6	8	<0.001	14.75 (3.98~58.26)	2	2	3	0.003	12.5 (1.86~141.59)	<0.001
	NO	193	17			88	17	0			
EAS_JPT, N=104	YES	6	8	<0.001	21.03 (5.45~87.23)	2	2	3	0.001	20.03 (2.88~233.28)	<0.001
	NO	196	12			93	10	1			
EAS_KHV, N=99	YES	6	8	<0.001	16.99 (4.49~68.95)	2	2	3	0.002	14.55 (2.14~166.67)	<0.001
	NO	184	14			85	14	0			

*REF : *APEX1* rs2307486 wild type + *GNB3* rs2234757 wild type

†HET : *APEX1* rs2307486 heterogeneous variant ± *GNB3* rs2234757 heterogeneous variant

‡HOM : *APEX1* rs2307486 homogeneous variant ± *GNB3* rs2234757 homogeneous variant

§ALT : HET+HOM

OR, odds ratio; CI, confidence internal; CATT, Cochran-Armitage trend test; SNUCH, Seoul National University Children's Hospital; 1KP, data from 1000 genome database; EAS, data of East Asian from 1000 genome database; CDX, data of Chinese Dai in Xishuangbanna, China from 1000 genome database; CHB, data of Han Chinese in Bejing, China from 100 genome database; CHS, data of Southern Han Chinese from 1000 genome database; JPT, data of Japanese in Tokyo, Japan from 1000 genome database; KHV, data of Kinh in Ho Chi Minh City, Vietnam

Other toxicities during maintenance & genotypes

Among 185 patients, hyperbilirubinemia over 3.6 mg/dL (CTCAE Grade 3-4) was developed in 3 patients (1.6%), and liver enzyme (ALT or AST) elevation over 300 IU/L in 83 patients (44.9%) (Table 13). During maintenance, neutropenia fever occurred in 35 patients (18.9%), and fever without neutropenia in 113 patients (61.1%).

Assuming CTCAE Grade 3-4 hyperbilirubinemia and liver enzyme elevation over 300 IU/L as 'liver toxicity', the frequency of liver toxicity during MP administration was crosstabulated with the variant frequencies of analyzed genotypes. Among 22 genotypes, a mutation in solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) rs11045879 was associated with liver toxicity so that CC genotypes was associated with 2.86 fold increase risk compared to homozygous wild-type patients (OR, 2.86; 95% CI;1.45~5.73, P=0.001) (Table 14). This statistical risk correlation of rs11045879 was also present in patients without *NUDT15* rs1168552532 variant alleles (N=137).

Otherwise there was no notable correlation between other toxicities and the variant alleles of each candidate gene in this study.

Table 13. Toxicities during maintenance phase in all patients (N=185)

Toxicities during maintenance		No. of patients (%)
Hyperbilirubinemia Grade 3-4	> 3.6 mg/dL	3 (1.6)
ALT/AST elevation	> 300 IU/L	83 (44.9)
Neutropenic fever	Yes	35 (18.9)
Fever without neutropenia	Yes	113 (61.1)

ALT, alanine transaminase; AST, Aspartate transaminase

Table 14. *SLCO1B1* rs11045879 related to liver toxicity (N=178)

Gr 3-4 Liver Toxicity	Allelic Frequency					Genotype (Dominant model)						CATT
Total	T	C	Chisq *P*	Fisher's *P*	OR (95% CI)	TT	TC	CC	Chisq *P*	Fisher's *P*	OR (95% CI)	*P*
YES	129	103	0.01	0.009	1.87 (1.15~3.08)	36	57	23	0.002	0.001	2.86 (1.45~5.73)	0.01
NO	87	37				35	17	10				
No rs116855232 (*NUDT15*) (N=137)												
YES	98	86	0.07	0.07	1.67 (0.96~2.93)	24	50	18	0.007	0.007	2.94 (1.31~6.68)	0.06
No	59	31				23	13	9				

OR, odds ratio; CI, confidence internal; CATT, Cochran-Armitage trend test

Doses of mercaptopurine, methotrexate & genotypes

Because doses of MP and MTX were reduced in response to toxicities during maintenance, doses were gradually adjusted during 1st cycle of maintenance (12 weeks) in most of the patients. Most patients generally reached their tolerable doses of MP and MTX during 2nd cycle of maintenance, and the dose of MP and MTX in the last maintenance cycle was supposed to be the maximum tolerated dose for each patient to maintain a white blood cell count of 2.0−3.5 x 10^9/L without apparent toxicities.

Table 15 shows the distribution of average MP and MTX doses of 2nd and last maintenance cycles in all patients. Protocol based planned doses were 50 mg/m^2/day for MP, and 20 mg/m^2/week for MTX. Aside from 6 to 9 patients whose data was not available, median doses of MP at 2nd and last cycles in study population were 22.8 and 23.5 mg/m^2/day, and those of MTX were 10 and 13 mg/m^2/week. Only 38/178 (21.3%) and 64/176 (36.3%) patients tolerated more than 75% of planned MP and MTX doses at the last cycle, respectively.

Table 15. Doses of mercaptopurine and methotrexate of the 2nd and last maintenance cycles (N=185)

Mercaptopurine dose (mg/m^2/day)	2nd Cycle		Last cycle	
	No. of patients (%)	Median dose (mg/m^2/day)	No. of patients (%)	Median dose (mg/m^2/day)
MP < 12.5	38 (21.2)	7.8	51 (28.7)	7
12.5 ≤ MP < 25	58 (32.4)	17.9	41 (23.0)	17.4
25 ≤ MP < 37.5	37 (20.7)	27.8	48 (27.0)	31.3
37.5 ≤ MP < 50	41 (22.9)	41.7	24 (13.5)	42.7
MP ≥ 50	5 (2.8)	52.1	14 (7.9)	55.7
Data not available	6		7	
Total patients	179	22.8	178	23.5
Methotrexate dose (mg/m^2/week)	2nd Cycle		Last cycle	
	No. of patients (%)	Median dose (mg/m^2/day)	No. of patients (%)	Median dose (mg/m^2/day)
MTX < 5	38 (21.2)	3.3	29 (16.5)	7
5 ≤ MTX < 10	49 (27.4)	7.9	32 (18.2)	17.4
10 ≤ MTX < 15	42 (23.5)	11.8	51 (29.0)	31.3
15 ≤ MTX < 20	46 (25.7)	17.7	39 (22.2)	42.7
MTX ≥ 20	4 (2.2)	21.3	25 (14.2)	55.7
Data not available	6		9	
Total patients	179	10	176	13

In the analysis of 185 patients, nonsynonymous rs116855232 variant in *NUDT15* predisposed patients to significant MP dose reduction (Figure 6), most likely because of increased hematologic toxicities. Four patients were homozygous for the variant allele (TT genotype) at *NUDT15* rs116855232, and these patients were highly sensitive to MP, with a tolerated dose at 2^{nd} cycle of only 8.9 mg/m^2/day, compared with patients with CT genotype (N=36) or wild type CC genotype (N=131), who tolerated average dose of 15.6 and 27.4 mg/m^2/day, respectively (P<0.001). This effect of *NUDT15* variants were persisted during whole maintenance phase so that average doses during last maintenance cycle were 5, 17.6 and 26.9 mg/m^2/day for patients with TT, CT and CC genotypes, respectively (P<0.001).

Interestingly, this risk allele of rs116855232 was also highly associated with a lower dose of MTX (Figure 7). The average MTX doses during 2^{nd} maintenance therapy for patients with TT, CT, CC genotypes were 5.4, 7.8 and 11.7 mg/m^2/week, respectively (P=0.001). Also during last cycle, the tolerance for MTX in patients with the T allele, especially the TT genotype, was significantly lower than patients with CT or CC genotypes (TT:CT:CC = 1.7:10.6:13.1 mg/m^2/week, P<0.001).

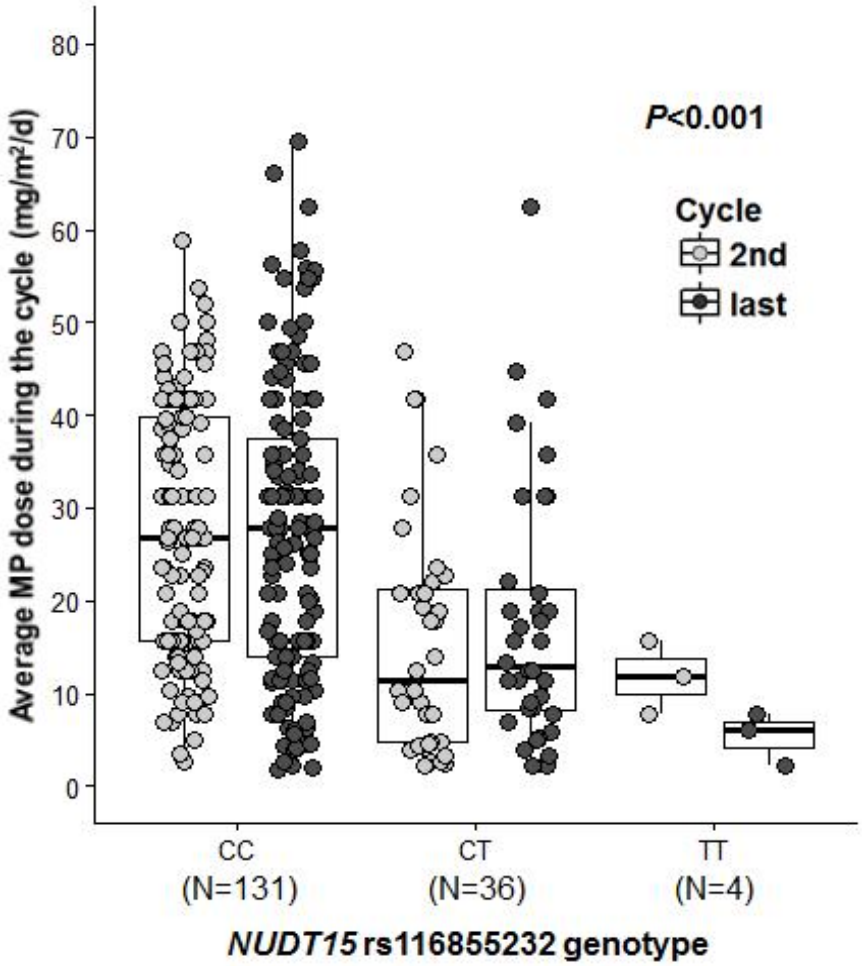

Figure 6. Average dose of mercaptopurine during the 2nd and last maintenance cycles according to *NUDT15* rs116855232 genotypes. Nonsynonymous rs116855232 variant in *NUDT15* predisposed patients to significant MP dose reduction. Tolerated doses at 2nd cycle were 8.9, 15.6, and 27.4 mg/m^2/day for patients with TT, CT, and CC genotypes (P<0.001). The average doses during last maintenance cycle were 5, 17.6 and 26.9 mg/m^2/day for patients with TT, CT and CC genotypes, respectively (P<0.001).

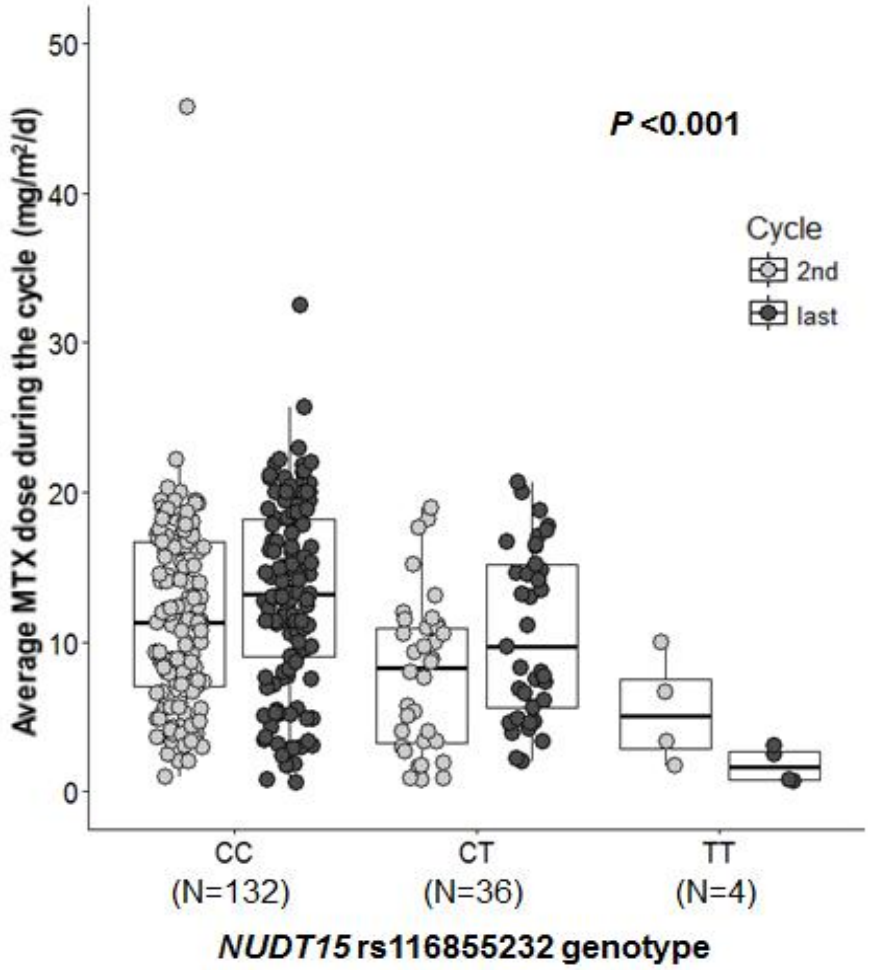

Figure 7. Average dose of methotrexate during the 2nd and last maintenance cycles according to *NUDT15* rs116855232 genotypes. The risk allele of rs116855232 in *NUDT15* associated with a lower dose of MTX. Tolerated doses at 2nd cycle were 5.4, 7.8, and 11.7 mg/m^2/day for patients with TT, CT, and CC genotypes (P=0.001). The average doses during last maintenance cycle were 1.7, 10.6 and 13/1 mg/m^2/day for patients with TT, CT and CC genotypes, respectively (P<0.001).

Survival analysis & genotypes

Among 185 patients reviewed, a total of 23 patients relapsed. The details of relapsed sites were as follows: bone marrow (N=4) and isolated central nervous system (N=4). Among those, 11 patients received allogeneic hematopoietic stem cell transplantation from unrelated donors (4 double units cord blood, 7 peripheral blood stem cell), and 6 patients from related donors (5 sibling, 1 haploidentical). No secondary malignancy was developed during observation period.

As a result of treatment, 13 patients died. The causes of death were disease progression in 6 patients, and treatment related mortality in 7 patients. Details of treatment related mortalities were septic shock (N=4), cytomegalovirus pneumonia (N=2), and acute respiratory distress syndrome (N=1).

Survival was analyzed with genotype variables in all patients. Only statistically significant result was that EFS was lower in the patient with minor alleles (A allele) in *ABCC4* rs11568658 (P=0.04) (Figure 8). EFS rate was 88.5% in wild type (CC genotype, N=138), 79.3% in CA variants (N=41), and 60% in AA variants (N=5).

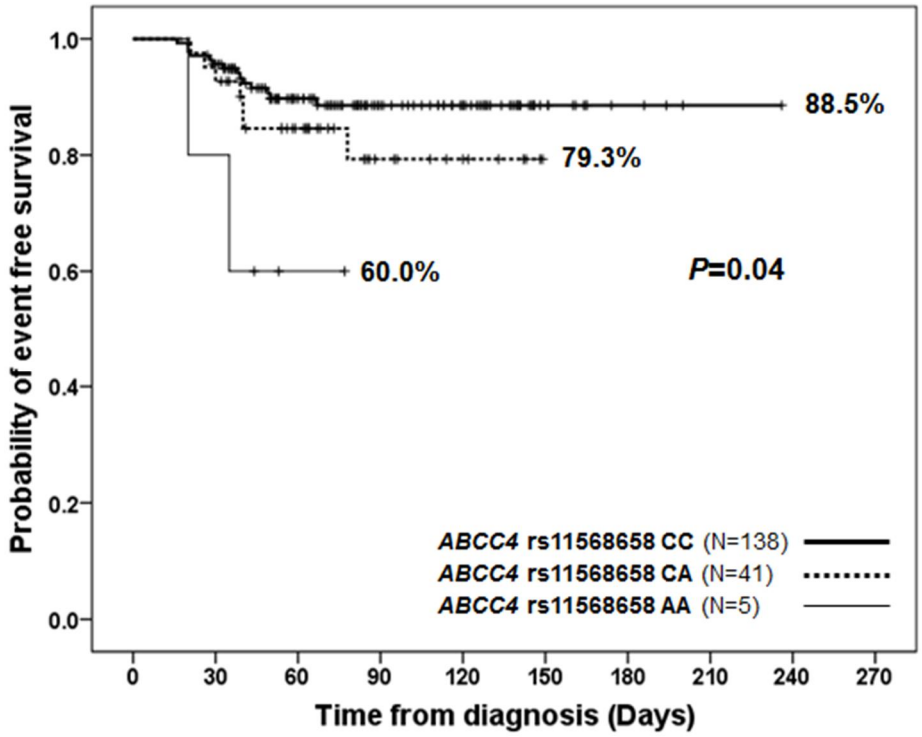

Figure 8. Evaluation of event-free survival (EFS) probabilities according to *ABCC4* rs11568658 genotype. EFS was lower in the patient with minor alleles (A allele) in *ABCC4* rs11568658 (*P*=0.04). EFS rate was 88.5% in wild type (CC genotype, N=138), 79.3% in CA variants (N=41), and 60% in AA variants (N=5).

Discussion

Maintenance therapy in ALL treatment is necessary to prevent or forestall relapse, and all patients who are not candidates for allogeneic stem cell transplantation receive maintenance chemotherapy for 2~2.5 years (20, 21). Daily mercaptopurine and weekly methotrexate with periodic vincristine, prednisone, and intrathecal therapy constitute the backbone of maintenance regimens. The importance of compliance with MP and constant dose titration was illustrated in a cohort study that demonstrated an association between decreased adherence rates and an increased risk of relapse (22).

Therefore, in order to appropriate dose optimization of MP, many researches were conducted about a variety of genetic factors associated with MP related toxicities (23). Of those, *TPMT* has been considered as a major locus in determining susceptibility to toxicity, and recently a nonsynonymous mutation in *NUDT15* was identified as a strong factor for myelotoxicity of MP during maintenance therapy for ALL (4, 12). However, still 30~40% of patients who could not tolerate full dose MP could not be explained with known genetic factors.

In this study, using the novel pharm-gene panel, targeted sequencing of patients who showed MP intolerance and staged candidate gene validation were conducted to find out genetic determintants of MP related adverse effects in Korean pediatric patients with ALL. This study showed that *APEX1* SNP rs2307486 conferred increased risk of MP related early onset neutropenia. *APEX1* rs2307486 was highly related to the early onset Grade 4 neutropenia after the initiation of MP administration, and cumulative incidence of Grade 4 neutropenia after MP initiation was significantly higher in patients with variants of *APEX1* rs2307486

Human apurinic/apyrimidinic endonuclease 1 (APEX1) is the major enzyme responsible for DNA base excision repair pathway (BER) (24). Its main role in the repair of damaged or mismatched nucleotides in DNA is to create a nick in the phosphodiester backbone of the apurinic/apyrimidinic (AP) site created when DNA glycosylase removes the damaged base (25). It is therefore not surprising that APEX1 has been implicated in sensitivities or resistance to a diverse range of chemotherapeutic agents. Indeed, past studies employing small molecule inhibitor strategies have revealed that APEX1

deficient cells exhibit hypersensitivity to a number of DNA-damaging agents, including alkylators and antimetabolites. In vivo study of McNeill DR et al, APEX1 deficient cells showed the most enhanced sensitivity to the antimetabolites so that apoptotic cell death was profoundly increased, supporting a role for mutant *APEX1* in thiopurine-induced cytotoxicity (24).

The variant in *APEX1* rs2307486 is missense mutation, and A to G change in *APEX1* rs2307486 makes a protein residue change from Isoleucine [Ile] to Valine [Val]. The allele frequencies of variant alleles in rs2307486 were nearly 0% in Europeans, 0.08% in Africans, and only 1.73% in Americans. However, G allele frequency in East Asian was 4.66% according to the 1000 genome results. Thus East Asians who have a higher frequency of *APEX1* variant alleles might be more susceptible to the influence related to these variants, while Caucasian or European population may not be easily related to these variants because of minor allele frequency difference between ethnic groups.

Although any relation between *APEX1* SNP rs2307486 and the total frequency of neutropenia was not found in this study cohort, the correlation was significantly strong within larger

East Asian data using 1000 genome database, thus influence of *APEX1* variants on the MP related neutropenia needs to be replicated in a larger population (Table 16).

NUDT15 encodes a nucleoside diphosphatase, and involves in degrading oxidized purine nucleoside triphosphates (eg, 8-oxo-dGTP) by dephosphorylation to prevent incorporation into DNA, thus protects cells from damage caused by their misincorporation into DNA (26). By removing the oxidative damaged guanine nucleotides, *NUDT15* is protecting cells to decrease DNA damage and avoid apoptosis (27). MP cytotoxicity is enhanced by its enzymatic conversion into thioguanine nucleotides (thio-GMP, thio-GDP, and thio-GTP), as well as deoxy thioguanosine phosphates (thio-dGMP, thio-dGDP, and thio-dGTP) (28). Afterwards, converted thio-dGTP is directly incorporated into DNA, which triggers DNA damage repair and eventually apoptosis. NUDT15 prefers other nucleotide substrates over 8-oxo-dGTP (29), structurally similar to 8-oxo-dGTP, thio-dGTP is a potential substrate for NUDT15, and thio-dGTP may be hydrolyzed to the inactive thio-dGMP or thio-dGDP by NUDT15 (4). Therefore, in patients with defective *NUDT15*, even normal dose of MP could

Table 16. Analysis of frequency of Grade 4 neutropenia according to genotypes of *APEX1* rs2307486

Control Group	Grade 4 Neutropenia	Allelic Frequency					Genotype (Dominant model)						CATT
		A	G	Chisq *P*	Fisher's *P*	OR (95% CI)	AA	AG	GG	Chisq *P*	Fisher's *P*	OR (95% CI)	*P*
EAS, N=504	YES	219	21	0.02	0.02	1.96 (1.09~3.42)	100	19	1	0.03	0.02	1.99 (1.07~3.61)	0.01
	NO	961	47				458	45	1				
EAS_CDX, N=93	YES	219	21	0.01	<0.001	4.35 (1.43~17.74)	100	19	1	0.01	<0.001	4.42 (1.41~18.47)	0.01
	NO	182	4				89	4	0				
EAS_CHB, N=103	YES	219	21	0.56	0.48	1.31 (0.62~2.88)	100	19	1	0.65	0.58	1.27 (0.57~2.89)	0.49
	NO	192	14				89	14	0				
EAS_CHS, N=105	YES	219	21	0.14	0.14	1.92 (0.84~4.67)	100	19	1	0.17	0.17	1.90 (0.80~4.78)	0.13
	NO	200	10				95	10	0				
EAS_JPT, N=104	YES	219	21	0.22	0.2	1.72 (0.77~4.05)	100	19	1	0.18	0.17	1.88 (0.79~4.73)	0.17
	NO	197	11				94	9	1				
EAS_KHV, N=99	YES	219	21	0.08	0.05	2.27 (0.94~6.08)	100	19	1	0.09	0.07	2.27 (0.90~6.26)	0.07
	NO	190	8				91	8	0				

OR, odds ratio; CI, confidence internal; CATT, Cochran-Armitage trend test; EAS, data of East Asian from 1000 genome database; CDX, data of Chinese Dai in Xishuangbanna, China from 1000 genome database; CHB, data of Han Chinese in Bejing, China from 100 genome database; CHS, data of Southern Han Chinese from 1000 genome database; JPT, data of Japanese in Tokyo, Japan from 1000 genome database; KHV, data of Kinh in Ho Chi Minh City, Vietnam from 1000 genome database

produce excessive accumulation of thio-dGTP and lead to extensive DNA damage, and cytotoxicity.

The variant in *NUDT15* rs116855232 is missense mutation, and C to T change in rs116855232 makes a protein residue change from Arginine [Arg] to Cysteine [Cys], and thought to be as a loss of function mutation. In fact, a recent GWAS in Korean patients with inflammatory bowel diseases also reported the same *NUDT15* variant associated with the susceptibility to thiopurine-related leucopenia, with each copy of the T allele conferring a remarkable 35.6 fold increase in toxicity risk (11). *APEX1* and *NUDT15* variants were significantly associated with MP induced early neutropenia in this study, however, the odds ratio of those SNPs were far lower than the study of Yang et al, as 3.44 fold increased risk for *APEX1* rs2307486 and risk of *NUDT15* was only validated in cumulative incidence analysis as in previous studies (12).

These risk difference seems to be due to the different dosage and modification guidelines between. Thiopurines are effective for both active disease and maintaining remission in inflammatory bower disease. Tailoring or optimization of thiopurine therapy can occur before or during treatment as in

ALL. Suggested starting dose of in inflammatory bowel disease is 1~1.5 mg/kg/day, and which is almost comparable to 50 mg/m^2/day in pediatric ALL (30). However, targets for tailoring dose and criteria for myelotoxicity are different between two disease. Target WBC counts for MP tailoring is generally 2.0–3.5 x 10^9/L in ALL, which is slightly subnormal range, for maintaining moderate myelosuppressive effect regarding antileukemic actions, whereas WBC lower than 3.0 x 10^9/L is regarded as leucopenia and myelotoxicity in inflammatory bowel disease, thus dose should be reduced in that case (31). For this reason, patients who showed dose-limiting myelotoxicity in inflammatory bowel disease would be selected more than ALL, which gives *NUDT15* variants a remarkable odds ratio for leucopenia.

Another interesting finding of this study was correlation between *NUDT15* rs116855232 variants and average dose of MTX. Chiengthong et al. and Yang et al. reported that *NUDT15* rs116855232 variants were related with lower median cumulative MP dose (4, 13) as in our study. Usually in ALL maintenance, doses of MP and MTX are alternately adjusted in response to adverse effects, therefore patients who showed

frequent myelosuppression would receive lowering doses of both MP and MTX. Therefore, the correlation between the dose percent of MP and MTX in each patient would be a linear relationship (17). In this regards, patients who had *NUDT15* variants showed early and frequent myelotoxicity so that they had reduced doses of MP and MTX at the same time.

The genetic variant in *TPMT* was not a significant factor for MP related toxicities in this study. It was confirmed by the previous studies that the variant allele frequencies of *TPMT* in Asian is lower than in other ethnic groups. Among the variant alleles in Caucasian, the allele frequency is the highest in *3A as 3.54%, and *3C is the next as the frequency of 0.42% (32). However, even *3C allele, which has the highest allele frequency among variant alleles in Asian, has been confirmed only in 0.9~1.8% of Asian population (33-35). In addition, there are about 0.33% of variant homozygotes in Caucasian, however, those are rarely observed in Asian. Therefore, determination of *TPMT* polymorphisms has a little clinical implication in children with ALL in most Asian countries.

The *1/*3C diplotype was the only variant heterozygote in this study and known as having intermediate TPMT activity so that

the starting dose for MP is recommended with 30~70% reduced dose compared to the full dose (32). This explains that there was no significant finding according to *TPMT* genotypes in this study, because 50 mg/m^2/day of MP as a starting dose was already at the same dose level recommended by the Clinical Pharmacogenetics Implementation Consortium guideline (32).

In this study, there were only 5 patients who had variant heterozygous for *TPMT*, and the average MP doses for the patients with *TPMT* *1/*1 and those with *1/*3C were not statistically different (Figure 9).

ATP-binding cassette sub-family C member 4 (ABCC4), also known as the multidrug resistance protein 4 (MRP4) belongs to the ATP-binding cassette transploter surperfamily (36). MRP4 is primarily involved in the efflux of nucleoside derivatives and has a role in the determination of drug sensitivity. It has the ability to transport anticancer drugs such as thiopurines and MTX, and is expressed in various blood cells. Cells expressing MRP4 are resistant to thiopurine-induced myelotoxicity because of their ability to export 6-thioguanine nucleotides

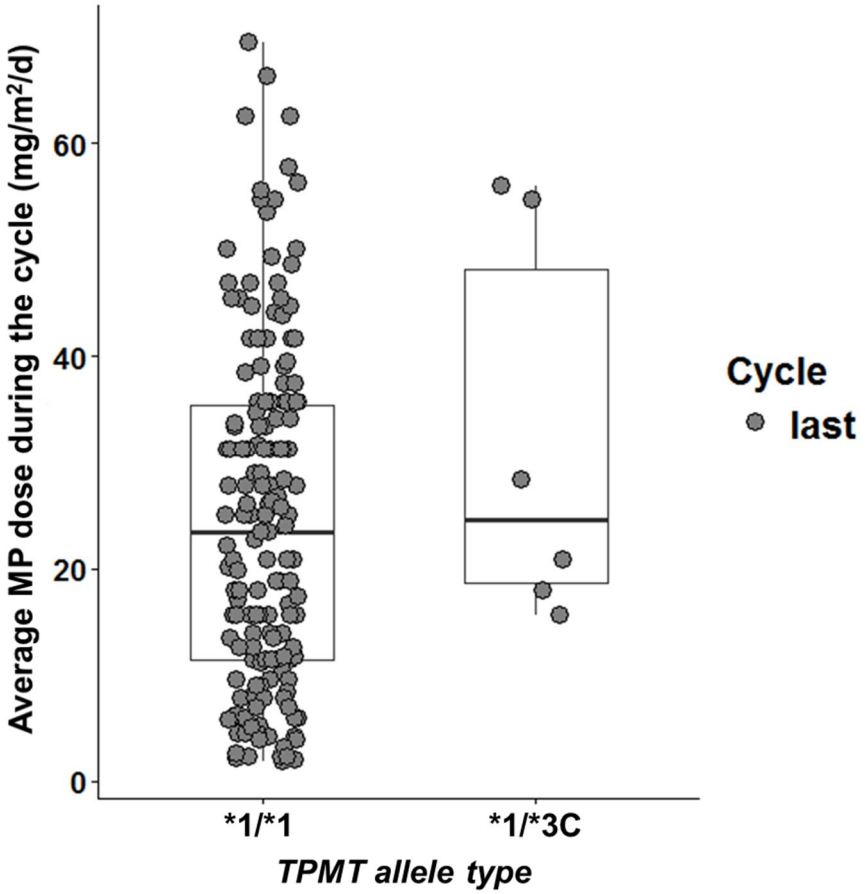

Figure 9. Average doses of mercaptopurine during last maintenance cycle according to *TPMT* genotypes. Average dose was 24.1 mg/m^2/day for *TPMT* ∗1/∗1, and 32.25 mg/m^2/day for *TPMT* ∗1/∗3 (*P*=0.41).

from the cells (37). Thus, decreased MRP4 expression on the cell surface would hinder efflux of the MP active metabolites so that patients with MRP4 mutation showed increased sensitivity for MP.

Tanaka Y et al. reported that average MP dose for patients with homozygous variant allele on *ABCC4* was significantly lower than that for patients with non-homozygous variant allele during maintenance therapy in Japanese ALL patients (36). In this study, *ABCC4* rs3765534 variants were related with Grade 4 neutropenia during maintenance without showing a significant dose reduction in MP, and *ABCC4* rs11568658 variants were related with lower EFS rates. However, these results could be caused by different sensitivities to many chemotherapeutic agents other than MP, and further studies are needed to elucidate the effects of *ABCC4* variants on MP related neutropenia.

Solute carrier organic anion transporter family member 1B1 (SLCO1B1) is an organic anion transporter that is known to transport methotrexate (38). Minor alleles for SNPs in the *SLCO1B1* gene are associated with both increased and

decreased transporter function, and known to influenza MTX clearance including rs11045879 (38). Trevino et al. studied 640 children with ALL who received high dose MTX and reported C allele at *SLCO1B1* rs11045879 was associated with low MTX clearance in a genome-wide association study (39). Although there has been no report that variants in *SLCO1B1* could influence the clearance of oral MTX during maintenance leading to clinical toxicity, statistical correlation between liver toxicities and *SLCO1B1* rs11045879 in this study is notable, and it deserves a further study in the future.

Throughout this study, a novel genetic risk factor for MP induced early onset neutropenia was found using an extensive pharm-gene panel based sequencing. Although *APEX1* is not directly related to MP metabolism, APEX1 and NUDT15 both participate in cell protection process from DNA damages or misincorporation, which can affect cellular drug sensitivities and toxicities.

This study has limitations, including the limited number of loci validated, a modest number of patients included, and absence of serum levels of drugs or metabolites. Further validation is

needed to confirm the frequency and pharmacogenetic consequence of this *APEX1* variant in other Asian populations, also in other ethnic groups.

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국 문 초 록

소아 급성림프모구백혈병의 치료 성적은 괄목할만한 발전을 이루어 왔으나, 아직도 약제 내성이나 재발이 해결해야 할 문제로 남아 있다. 유전적 다형성은 항 백혈병 제제의 효과와 독성에 영향을 주는 중요한 요인으로 알려져 왔으며, 약물유전체학은 약제 내성이나 재발을 줄이는 데 기여할 수 있을 것으로 기대되어 다양한 연구가 이루어지고 있다. 특히 소아 급성림프모구백혈병의 치료 중 1~2 년간 매일 복용 하는 mercaptopurine 의 약물유전체에 대한 연구가 활발하게 이루어져 왔으며, 고전적으로 *TPMT* 의 변이가 있는 환자에서 mercaptopurine 의 독성을 높은 것으로 알려져 왔다. 최근 아시아인에서 빈도가 많은 *NUDT15* 의 변이가 mercaptopurine 의 독성 및 intolerance 에 영향을 주는 강력한 유전 인자임이 확인되었으며, 본 연구에서는 한국인 소아 급성림프모구백혈병에서 *NUDT15* 를 포함하여 mercaptopurine 의 독성에 영향을 주는 유전 인자를 찾고자 하였다.

서울대학교 어린이병원에서 진단과 치료를 받은 185 명의 급성림프모구백혈병 환자가 대상이 되었으며, 완전 관해 시의 DNA 를 추출하였다. 먼저 유지요법 중에 mercaptopurine 의 독성으로 인하여 초기 용량 (50 mg/m^2/day)의 25% 이하를 투여 받았던 44 명의 환자군을 대상으로 유전자 패널을 이용한 targeted

sequencing 을 시행하였다. 본 유전자 패널을 통하여 44 명에서 총 147 개의 약물유전체 관련 유전자 및 8 개의 단일염기다형성 부위를 시퀀싱 하였으며, 이 결과를 분석하여 mercaptopurine 의 독성과 가장 관련성이 높을 것으로 보이는 4 개의 유전자 자리(locus)를 확인하였다 (*NUDT15* rs116855232, *APEX1* rs2307486, *CYP1A1* rs4646422, *BAG3* rs78439745). 이후 전체 185 명의 환자를 대상으로 1 차 분석에서 확인된 4 개의 유전자 자리를 포함한 총 24 개의 약물유전체 연관 유전자 자리를 시퀀싱 하였다. 시퀀싱을 통하여 확인된 각 유전자의 변이와 mercaptopurine 의 독성 및 용량과의 관련성을 분석하였다.

분석 결과, 185 명의 환자에서 *NUDT15* rs116855232 (21.7%), *APEX1* rs2307486 (13.5%), *ABCC4* rs2274407 (34%), *ABCC4* rs3765534 (14.1%), *ABCC4* rs11568658 (23.2%), *CYP4F2* rs2108622 (51.9%), *CYP1A1* rs4646422 (29.1%), *SLCO1B1* rs11045879 (55.7%), *SLCOB1* rs4149056 (26.5%), *ITPA* rs1127354 (26%), *ITPA* rs7270101 (0%), *MTHFR* rs1801131 (60.5%), *MTHFR* rs1801133 (28.6%), *MTHFR* rs1901133 (50.8%), *GRIA1* rs4958351 (3.8%), *MOCOS* rs594445 (48.1%), *PACSIN2* rs2413739 (13.5%), *BAG3* rs78439745 (7%), 그리고 *TPMT* (*1/*3C, 3.8%)와 같은 변이형 분포를 보였다. Mercaptopurine 의 투여 중에 호중구 감소증 (호중구<500/㎕)은

총 121 명 (65.4%)의 환자에서 관찰되었으며, *ABCC4* rs3765534 의 대립유전자 T 형이 있는 환자에서 호중구 감소증의 위험이 4.64 배 높았다 (95% 신뢰구간;1.32~25.12, P=0.008). 호중구 감소증을 보인 121 명의 환자 중에서 15 명의 환자는 mercaptopurine 복용 30 일 이내에 호중구 감소증 (조기 호중구 감소증)을 보였으며, *APEX1* rs2307486 의 대립유전자 G 형을 가진 환자에서 조기 호중구 감소증의 위험이 유의하게 높았다 (오즈비, 3.44; 95% 신뢰구간;1.04~9.89, P=0.02). 또한 조기 호중구 감소증 발생의 누적 발생률을 비교하였을 때에 *APEX1* rs2307486 의 변이형 (AG, GG)을 가진 환자에서 누적 발생률이 유의하게 높았다 (AA:AG:GG=66.3%:73.1%:100%, P<0.001). *NUDT15* rs116855232 의 변이형 (CT, TT)을 가진 경우에도 조기 호중구 감소증 발생의 누적 발생률이 의미 있게 증가하였으며 (CC:CT:TT=65.0%:72.2%:100%, P<0.001), 유지요법 중 mercaptopurine 의 평균 용량을 비교하였을 때, TT, CT, CC 형 환자에서 각각 10.5, 19.4 그리고 28.7 mg/m^2/day 으로 대립유전자 T 형이 낮은 mercaptopurine 내성용량 (tolerated dose)과 유의한 연관이 있었다 (P<0.001). 그러나 고전적으로 서구에서 mercaptopurine 의 독성 및 내성용량에 영향을 주는 것으로 알려진 *TPMT* 의 경우, 본 연구에서는 의미 있는 차이를 보이지 않았다.

결론적으로, 이번 연구를 통하여 *APEX1* 의 유전적 변이가 mercaptopurine 와 연관된 조기 호중구 감소증의 위험을 높인다는 것을 밝혀 내었으며, mercaptopurine 의 독성 및 내성용량에는 국내에서 변이형 빈도가 많지 않은 *TPMT* 의 역할보다는 *NUDT15* 등 아시아인에서 변이형의 빈도가 많은 유전 인자가 중요함을 확인하였다. 향후 보다 많은 환자 및 인종 군에서 *APEX1* 의 빈도 및 mercaptopurine 독성에 대한 영향을 확인하는 연구가 진행되어야 할 것이다.

주요어 : 소아 급성림프모구백혈병, 6-mercaptopurine, 독성, 약물유전학

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