Association of Variants in Candidate Pharmacogenes with Response to Mercaptopurine and Methotrexate in Pediatric Acute Lymphoblastic Leukemia: A Single-center Experience from Croatia

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Introduction

Interindividual variability in the efficacy and toxicity of 6-mercaptopurine (6-MP) and methotrexate (MTX) drugs remains a major challenge in the treatment of pediatric acute lymphoblastic leukemia (ALL). Germline variation in pharmacogenes involved in drug metabolism, transport, and folate pathways may contribute to this variability.
The aim of this study was to determine the association of variants in candidate pharmacogenes with response to mercaptopurine and methotrexate in pediatric ALL.

Methods

In this retrospective cohort study, 43 pediatric patients with ALL treated at a tertiary referral centre in Croatia according to ALL IC-BFM 2002 or 2009 protocols were genotyped for eleven variants in TPMT, ITPA, NUDT15, PACSIN2, MTHFR, TYMS, SLC19A1 and SLCO1B1 genes. Associations with average 6-MP dose during maintenance therapy, MTX pharmacokinetics during consolidation and MTX-related toxicity were analyzed. A polygenic risk score (PRS) was constructed to assess cumulative genetic risk of developing toxicity when administering these two drugs.

Conclusions

Our findings support a role for ITPA and NUDT15 variants in modulating 6-MP dose requirements, while single-variant associations with MTX pharmacokinetics and toxicity were limited and not supported. These results underscore the complexity of antimetabolite pharmacogenetics and suggest that integrative polygenic approaches may better capture clinically relevant pharmacogenetic risk.

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Results

Carriers of the ITPA rs1127354 variant required significantly lower average 6-MP doses compared with wild-type carriers. The NUDT15 rs61973267 variant was associated with higher tolerated 6-MP doses after exclusion of outliers. No significant associations were observed between individual variants and MTX clearance. A trend toward reduced risk of oral mucositis was observed in carriers of the SLCO1B1 rs4149056 variant. PRS analyses that included carriers of the TPMT rs1142345 and ITPA rs1127354 variants showed a borderline association with the requirement for 6-MP dose reduction.

Table 1. Genetic variation in folate pathway genes as predictive markers of oral mucositis, GIT toxicity and hepatotoxicity (Toxicity grade 0-1 versus 2-4). To assess the association of genetic variants with toxicity, logistic regression was used, and all probabilities were adjusted for MTX dose, age and gender.

Gene	Variant	dbSNP	Allele	MAF	Oral mucositis						Hepatotoxicity		
					OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
MTHFR	c.677 C>T	rs1801133	C/T	30%	1.28	0.26-6.49	0.75	1.85	0.24-17.83	0.55	0.42	0.09-1.67	0.23
TYMS	28 bp repeats	rs34743033	2R-4R	48%	1.46	0.24-12.48	0.69	1.53	0.14-37.53	0.74	0.75	0.14-3.81	0.72
TYMS	6bp indel	rs11280056	WT/DEL	28%	0.69	0.13-3.41	0.63	NA	NA	0.99	1.14	0.27-4.91	0.85
SLC19A1	c.80 G>A	rs1051266	G/A	40%	1.09	0.21-5.80	0.91	NA	NA	0.99	1.01	0.26-4.72	0.89
SLCO1B1	c.521 T>C	rs4149056	T/C	20%	**0.15**	**0.006-1.06**	**0.10**	0.54	0.02-4.22	0.60	2.46	0.58-10.98	0.22
PACSIN2	Intronic	rs2413739	C/T	33%	0.59	0.10-3.18	0.53	NA	NA	0.99	0.37	0.08-1.56	0.18

Varijante	Genotip	N	Medijana 6-MP doze [IQR]	MAF	*p* vrednost za prosečnu dozu 6-MP
ITPA **rs1127354**	C/C	31	0.69 [0.61-0.76]	0.14	**0.045**
	C/A	12	0.60 [0.51 -0.68]		
	A/A	0	-		
TPMT **rs1142345**	A/A	40	0.67 [0.60 -0.74]	0.035	0.80
	A/G	3	0.68 [0.63 -0.69]		
	G/G	0	-		
PACSIN2 **rs2413739**	C/C	17	0.70 [0.60 -0.73]	0.34	0.90
	C/T	23	0.66 [0.60 -0.74]		
	T/T	3	0.63 [0.62 -0.65]		
NUDT15 **rs45465203**	G/G	32	0.68 [0.60 -0.74]	0.14	0.98
	G/A	10	0.65 [0.60 -0.71]		
	A/A	1	0.64 [0.64 -0.64]		
NUDT15 **rs79687000**	C/C	38	0.66 [0.60 -0.74]	0.06	0.7
	C/T	5	0.69 [0.62 -0.72]		
	T/T	0	-		
NUDT15 **rs61973267**	G/G	39	0.66 [0.60 -0.72]	0.05	0.30
	G/A	4	0.75 [0.68 -0.79]		
	A/A	0	-		

Table 2.
The association of genetic variants in mercaptopurine related pharmacogenes and MP average dose in Croatian acute lymphoblastic leukemia (ALL) pediatric patients.

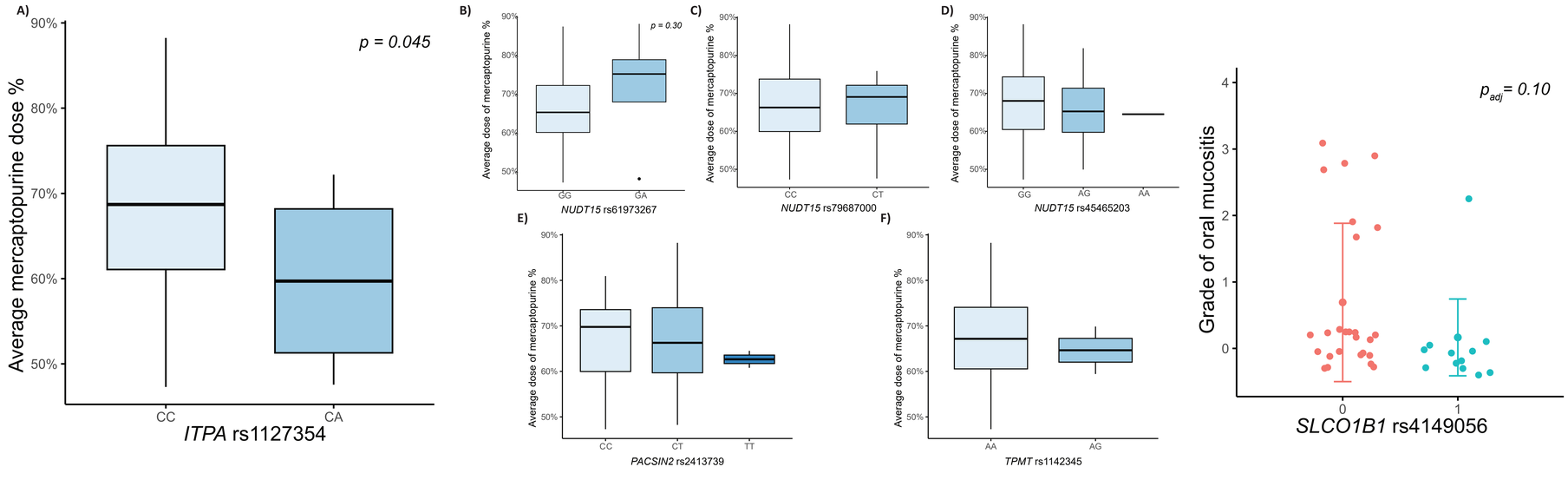

Figure 1: Correlation of the average dose of MP and genetic variants in pharmacogenes. A- rs1127354 (*ITPA*); B- rs61973267 (*NUDT15*); C- rs79687000 (*NUDT15*); D- rs45465203 (*NUDT15*); E- rs2413739 (*PACSIN2*); F- rs1142345 (*TPMT*)

Figure 2: Correlation of grade of oral mucositis and rs4149056 *SLCO1B1* TT and TC/CC genotype