ORIGINAL ARTICLE

Imatinib-induced ophthalmological side-effects in GIST patients are associated with the variations of *EGFR*, *SLC22A1*, *SLC22A5* and *ABCB1*

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Imatinib-induced ophthalmological side-effects, including conjunctiva hemorrhage and periorbital oedema, although very common and still remain relatively little understood. The present study investigated the effects of genetic polymorphisms of drug targets and membrane transporters on these side effects. We found that the minor allele of *EGFR* rs10258429 and *SLC22A1* rs683369 were significant risk determinants of conjunctival hemorrhage with OR of 7.061 (95%CI = 1.791–27.837, $P = 0.005$ for *EGFR* rs10258429 CT +TT vs CC), and 4.809 (95%CI = 1.267–18.431, $P = 0.021$ for *SLC22A1* rs683369 GG+CG vs CC). The minor allele of *SLC22A5* rs274558 and *ABCB1* rs2235040 were protective factors to periorbital oedema with OR of 0.313 (95%CI = 0.149–0.656, $P = 0.002$ for *SLC22A5* rs274558 AA+AG vs GG), and 0.253 (95%CI = 0.079–0.805, $P = 0.020$ for *ABCB1* rs2235040 CT vs CC). These results indicated that variants in *EGFR*, *SLC22A1*, *SLC22A5* and *ABCB1* influenced the incidence of Imatinib-induced ophthalmological toxicities, and polymorphism analyses in associated genes might be beneficial to optimize Imatinib treatment.

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INTRODUCTION

Imatinib, the first targeted agent, represents a promising treatment modality in patients affected by gastrointestinal stromal tumor (GIST) and chronic myeloid leukemia (CML).¹ Although targeted cancer therapies may be more effective than other anticancer treatments and less harmful to normal cells,² Imatinib has been found to be associated with widely-occurring side effects, such as oedema, skin rash and ocular side effects, among which ocular side effects are generally under-reported and not well studied.

Imatinib is a 2-phenyl amino pyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes.³ It occupies the TK active site, leading to a decrease in activity. Imatinib is quite selective for BCR-ABL (the Abelson proto-oncogene),⁴ it inhibits other targets such as KIT and PDGFR (platelet-derived growth factor receptor),⁵ and also to a lesser degree it acts on EGFR.⁶ Imatinib inhibits these tyrosine kinase enzymes of both cancer cells and non-cancer cells. Therefore, germline genetic polymorphisms of drug targets such as *KIT* and *PDGFRA/B* might influence side effects of Imatinib, such as ophthalmological toxicities.

Since Imatinib pharmacokinetics was correlated with the therapeutic responses,^{7–9} the germline DNA that dictates drug pharmacokinetics may indirectly determine efficacy and toxicity.^{10,11} Increasing body of evidence recognized that

influx- and efflux- transporters of ABC (adenosine triphosphate—binding cassette)^{12,13} and SLC (solute carrier) families with great affinity to Imatinib influence clinical responses.^{14–18} Patients with low expression or activity of OCT1 (encoded by *SLC22A1*) had a lower probability of achieving a cytogenetic or molecular remission to CML. Improved progression free survival and overall survival was also observed in patients with higher OCT1 expression.¹⁹ Variants in the *SLC22A5* gene (coding for OCTN2) were found correlated with major molecular response or prognosis to Imatinib.^{14,20} The expressions of *ABCB1* (MDR1, P-glycoprotein) and *ABCG2* (BCRP) are correlated with Imatinib responses in patients with CML.¹² Polymorphisms in related transporters may influence the exposure of target cells to the drug and consequently affect the response of Imatinib therapy. Until now, no investigation has been reported to explore the effect of SNPs in related transporters on the incidence of Imatinib-induced ophthalmological side-effects.

In this study, we comprehensively analyzed the SNPs in the influx- and efflux- transporters that potentially interacted with Imatinib and SNPs of *EGFR*, *PDGFRB* in 118 Chinese Gastrointestinal stromal tumor (GIST) patients treated with Imatinib. We also investigated the correlation of polymorphisms in these genes and the trough plasma concentrations with the development of ophthalmological side-effects.

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MATERIALS AND METHODS

Patients and study design

From 2014 to 2016, a total of 118 GIST patients in Sun Yat-Sen University Cancer Center in Guangzhou, China were enrolled in this study. Inclusion criteria were ≥ 18 years old with adequate hematological, renal and hepatic functions, histologically or molecular diagnosis confirmed GIST and Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2 . Exclusion criteria were uncontrolled systemic disease, poor compliance and receiving CYP3A4 or CYP3A5 inhibitor such as St John's Wort, cimetidine. This study was approved by the ethical committee of Sun Yat-Sen University Cancer Center. Written informed consent was obtained from all participating subjects.

All patients were treated with Imatinib at 400 mg daily for at least 3 months. Toxicity assessment: physical examination and routine laboratory tests (hematology and biochemistry assessments) were performed once a month by investigators. All adverse events were documented and graded according to NCI Common Terminology Criteria for Adverse Events v4.03.

Imatinib and NDI quantitation

Blood samples (3 ml each) were collected into EDTA polypropylene tubes, centrifuged at 1000 g for 10 min for plasma separation. The remaining samples were used for germline mutation detection. All the blood samples were frozen in -80°C refrigerator until analysis. Imatinib and NDI concentrations were determined using a validated liquid chromatography–tandem mass spectrometry assay. The lower limit of quantification was 10 ng/ml for both Imatinib and NDI.

KIT & PDGFRA mutation detection

Genomic DNAs were extracted from paraffin-embedded tumor specimens or fresh frozen tissue using TIANGEN TIANamp FFPE DNA Kit (DP311) or TIANamp Genomic DNA Kit (DP304), according to the manufacturer's instructions. DNA fragments aligned respectively with exons 9, 11, 13 and 17 of *KIT*, and exons 12 and 18 of *PDGFRA* were amplified by PCR using different primers.²¹ RR53A, TAKARA PCR Kit were used to amplification the target region with 1 μl of genomic DNA, and 10 $\mu\text{mol/l}$ of each primers in a final volume of 20 μl . The cycling conditions were 35 cycles of 98°C for 10 s, $X^{\circ}\text{C}$ for 30 s and 72°C for 30 s, final extension at 72°C for 3 min, and ended at 4°C (Supplementary Table 1).

Germline polymorphisms genotyping

Peripheral EDTA-whole blood of each patient before Imatinib treatment were collected. Genomic DNA was extracted by genome TIANGENTM TIANamp Blood DNA Kit (DP348). 33 SNPs, including SNPs in *ABCB1* (rs10256836, rs1045642, rs1128503, rs2032582, rs2235040), *ABCC2* (rs2273697, rs1717620), *ABCC4* (rs2274406, rs3765534, rs4148551), *ABCG2* (rs2231137, rs2231142, rs72554040), *SLC22A1* (rs2282143, rs628031,

rs683369), *SLC22A3* (rs501470, rs62440430), *SLC22A4* (rs272893), *SLC22A5* (rs144261584, rs274558), *SLCO1A2* (rs12816889, rs5484), *SLCO1B3* (rs2053098, rs3764006, rs3834935, rs4149117, rs4149158, rs7311358), *EGFR* (rs10258429, rs11977388), *PDGFRB* (rs17110944, rs246388) were genotyped using Agena Sequenom MassArray Analyzer 4 system (MALDI-TOF platform).

Statistical analysis

All statistical analyses of the results were performed using SPSS version 21.0 (IBM). The concentrations of Imatinib and NDI were not in normal distribution and Mann-Whitney U-test was used to evaluate the association between C_{min} and toxicity. Pearson χ^2 test were used to analyze the association between genetic polymorphisms and toxicity. With consideration of confounding factors listed in Table 1, such as gender, age, BSA, BMI, localization, mutation state and surgery, statistical significance have been reanalyzed by multivariate logistic regression. Haploview 4.2 (Broad Institute) was used to determine the deviation from the Hardy–Weinberg equilibrium. The power calculation was performed with G*power version 3.1.9.2 (Heinrich-Heine-Universität Düsseldorf). Statistical significance was assumed for P values < 0.05 .

RESULTS

Patients' characteristics

Association of ophthalmological side effects and patients characteristics and Imatinib/NDI level are presented in Table 1. In this analysis, conjunctival hemorrhage and periorbital oedema were observed but sight-threatening side effects affecting the optic nerve, macula and retina were not observed. 12 (10.2%) patients developed grade 1 conjunctival hemorrhage and 2 (1.7%) patients developed grade ≥ 2 conjunctival hemorrhage. In total 83 (70.3%) patients developed grade 1 periorbital oedema and 1 (0.8%) patients developed grade ≥ 2 periorbital oedema. Clinical characteristics had no effects on ophthalmological side effects (Table 1). Plasma levels of Imatinib and NDI had no correlation with the side effects (Figure 1).

Correlation between polymorphisms and ophthalmological side-effects

Genotype frequencies of all 33 tested SNPs are in Hardy–Weinberg equilibrium as shown in Supplementary Table 2.

As shown in Table 2, *ABCC2* rs717620 ($P=0.046$, Fisher's exact test), *ABCG2* rs72554040 ($P=0.035$, Recessive model), *SLC22A1* rs628031 ($P=0.044$, Fisher's exact test, Dominate model), *SLC22A1* rs683369 ($P=0.007$, Co-dominate model), *SLC22A3* rs501470

Table 1. Clinical characteristics had no effects on ophthalmological side effects in GIST patients

Characteristics	No. of patients	Conjunctival hemorrhage	Periorbital oedema
		P	P
Median age, years (median, [range])	55, [44–63]	0.213	0.301
Gender (Male/Female)	63/55	0.164	0.218
Weight, Kg (median, [range])	57, [38.5–83.0]	0.119	0.521
Height, m (median, [range])	1.64, [1.47–1.80]	0.536	0.489
Median BMI, (median, [range])	20.95, [19.50–23.98]	0.062	0.694
Median BSA, m2 (median, [range])	1.72, [1.61–1.81]	0.302	0.457
Surgery, n (Surgery/non-surgery)	78/40	0.152	0.644
Localization, n (Stomach/Intestines/Others)	87/13/18	0.94	0.368
Mutation, n (<i>KIT</i> / <i>PDGFRA</i> /Wild Type/Unreported)	63/0/18/37	0.167	0.123
Imatinib, (mean $\pm$ s.d., ng/mL)	1501.1 $\pm$ 646.8	0.766	0.299
NDI, (mean $\pm$ s.d., ng/mL)	221.7 $\pm$ 92.5	0.898	0.766
Conjunctival hemorrhage (Grade 0/1/2/3)	104/12/1/1		
Periorbital oedema (Grade 0/1/2/3)	34/83/1/0		

Abbreviation: NDI, N-desmethyl Imatinib. ($n=118$). Data are presented as M (median) with P25–P75 (Percentile: 25–75%), mean $\pm$ s.d. or amount.

Figure 1. The association of Imatinib/NDI levels with conjunctiva hemorrhage (a, b) and periorbital oedema (c, d).

($P=0.006$, Fisher's exact test), *SLC22A5* rs274558 ($P=0.043$, Fisher's exact test, Co-dominant model), *EGFR* rs10258429 ($P=0.002$, Recessive model), *EGFR* rs11977388 ($P=0.012$, Fisher's exact test, Recessive model) were associated with conjunctival hemorrhage (Grade 0 vs 1⁺). *ABCB1* rs1045642 ($P=0.006$, Co-dominant model), *ABCG2* rs2231142 ($P=0.005$, Fisher's exact test, Dominant model), *SLC22A5* rs144261584 ($P=0.009$), *SLCO1A2* rs5484 ($P=0.011$, Fisher's exact test), *SLCO1B3* rs3834935 ($P=0.028$, Fisher's exact test) were associated with periorbital oedema (Grade 0 vs 1⁺).

Multivariate analysis of SNPs with ophthalmological side effects taking into account potential confounding factors

Logistic regression analyses were performed to identify the association of risk or protective factors with ophthalmological side-effects (Table 3). Polymorphisms in *EGFR* and *SLC22A1* were verified to be associated with conjunctival hemorrhage and SNPs in *SLC22A5* and *ABCB1* were verified to be associated with periorbital oedema. The minor alleles of *EGFR* rs10258429 and *SLC22A1* rs683369 were risk factors to conjunctival hemorrhage. The analysis of conjunctival hemorrhage risk revealed the following: OR is 7.061 (95% CI = 1.791–27.837, $P=0.005$) for *EGFR* rs10258429 T allele (CT+TT vs CC), and 4.809 (95% CI = 1.267–18.431, $P=0.021$) for *SLC22A1* rs683369 G allele (GG+CG vs CC). In consideration of interaction between rs10258429 and rs683369, logistic regression analyses of rs10258429×rs683369 with conjunctival hemorrhage (OR = 3.046, 95% CI = 1.579–5.878, $P<0.001$). The minor alleles of *SLC22A5* rs274558 and *ABCB1* rs2235040 were protective factors to periorbital oedema. In regard to the risk to develop periorbital oedema, OR is 0.313 (95% CI = 0.149–0.656, $P=0.002$) for *SLC22A5* rs274558 A allele (AA+GA vs GG) and 0.253 (95% CI = 0.079–0.805, $P=0.020$) for *ABCB1* rs2235040 T allele (CT vs CC). In consideration of interaction between rs274558 and rs2235040, logistic regression analyses of rs274558×rs2235040 with conjunctival hemorrhage (OR = 0.411, 95% CI = 0.237–0.710, $P=0.001$).

DISCUSSION

In our study, 83 (71%) patients developed periorbital oedema, which is consistent with the results in two randomized controlled trials.^{22,23} Conjunctival hemorrhage was noted in 14 (11%) patients in the absence of marrow suppression or systemic bleeding tendency, which is consistent with the result in a cohort of 87 GIST patients.²⁴ In our study, we found, for the first time, that the ocular side effects of Imatinib are independent of plasma concentration and *KIT* or *PDGFRA* mutation status, but significantly correlated with the SNPs of *EGFR*, *SLC22A1*, *SLC22A5* and *ABCB1*, indicating that these variants might be markers for predicting the ocular toxicity in patients treated with Imatinib.

Epidermal growth factor receptor (*EGFR*) contained over 800 SNPs with minor allele frequency >1% including biological importance SNPs.²⁵ The identification of *EGFR* as an oncogene has led to the development of anticancer therapeutics directed against *EGFR* (called 'tyrosine kinase inhibitors', TKIs), including gefitinib, erlotinib, afatinib and brigatinib for lung cancer,^{26,27} and cetuximab for colon cancer,^{28,29} therefore, the impacts of *EGFR* gene polymorphisms on the cytotoxicity of TKIs, such as gefitinib, erlotinib and cetuximab, have been investigated broadly in patients affected with NSCLC^{30,31} and colon cancer.³⁰

However, no investigation has been reported of the influence of *EGFR* polymorphisms on Imatinib toxicity in patients with GIST. The present study found *EGFR* mutation was significantly correlated with Imatinib-induced conjunctival hemorrhage. It is known that *EGFR* is expressed in various ocular structures, including corneal, limbal and conjunctival epithelium.²⁴ And Imatinib selectively inhibits *EGFR* to certain extent.³² Through the inhibition of *EGFR*, Imatinib may decrease the epithelial proliferation, migration and wound healing or influence the blood-retinal barrier, leading to the occurrence of toxicity.^{33,34} Compared with C allele, rs10258429 T allele increased the risk of conjunctival hemorrhage significantly with OR of 7.061. This SNP is located in exon 15 and is a synonymous variation coding for amino acid of His. As far as we know, this SNP has not ever been

Table 2. The association of Candidate SNPs with ophthalmological side effects

Gene	SNP	Genotype	Conjunctival hemorrhage (Grade)				Periorbital oedema (Grade)			
			0	1	2+	P ^a	0	1	2+	P ^a
ABCB1	rs10256836	GG	77	9	1	NS	24	63	0	NS
		CG	27	3	1		10	20	1	
		CC	0	0	0		0	0	0	
	rs1045642	GG	32	4	1	NS	6	30	1	0.006 ^b
		GA	58	6	1		26	39	0	
		AA	14	1	0		2	13	0	
	rs1128503	AA	39	4	0	NS	10	33	0	NS
		AG	49	6	2		19	37	1	
		GG	7	0	0		1	6	0	
	rs2032582	AA	16	1	0	NS	7	10	0	NS
		CA	52	4	2		16	41	1	
		CC	15	1	0		5	11	0	
ABCC2	rs2235040	TC	11	4	0	NS	2	13	0	NS
		TT	1	0	0		0	1	0	
		TA	8	1	0		4	5	0	
	rs2273697	CC	83	8	1	NS	24	67	1	NS
		CT	20	3	1		10	14	0	
		TT	0	0	0		0	0	0	
	rs717620	GG	87	9	2	NS	31	67	0	NS
		GA	17	3	0		3	16	1	
		AA	0	0	0		0	0	0	
	rs2274406	CC	61	4	0	0.046 ^c	17	48	0	NS
		CT	30	5	1		10	25	1	
		TT	4	1	1		3	3	0	
ABCC4	rs3765534	TT	23	4	0	NS	7	19	1	NS
		TC	60	5	2		21	46	0	
		CC	20	3	0		6	17	0	
	rs4148551	CC	92	11	2	NS	29	75	1	NS
		CT	11	1	0		5	7	0	
		TT	1	0	0		0	1	0	
	rs2231142	CC	26	6	1	0.050 ^d	12	21	0	NS
		CT	57	4	1		14	48	0	
		TT	21	2	0		8	14	1	
	rs72554040	GG	39	2	1	NS	12	30	0	0.005 ^e
		GT	41	7	1		18	30	1	
		TT	14	1	0		0	15	0	
SLC22A1	rs2282143	GG	34	9	0	0.035 ^{d,c}	10	33	0	NS
		GA	57	2	1		19	40	1	
		AA	13	1	1		5	10	0	
	rs628031	CC	78	6	2	0.087 ^b	27	58	1	NS
		CT	24	6	0		7	23	0	
		TT	1	0	0		0	1	0	
	rs683369	GG	55	3	0	0.044 ^{e,c}	17	41	0	NS
		GA	35	5	1		10	30	1	
		AA	5	2	1		3	5	0	
	rs501470	CC	78	6	0	0.007 ^b	23	61	0	NS
		CG	25	6	2		10	22	1	
		GG	1	0	0		1	0	0	
SLC22A3	rs62440430	TT	49	5	1	0.006 ^c	14	40	1	NS
		TG	51	4	0		18	37	0	
		GG	3	3	1		1	6	0	
	rs144261584	AA	94	9	0	0.055 ^c	31	71	1	NS
		AG	6	1	2		2	7	0	
		GG	0	0	0		0	0	0	
	rs274558	CC	76	9	0	NS	19	65	1	0.009
		C.DEL	27	3	2		15	17	0	
		DEL	0	0	0		0	0	0	
	rs12816889	GG	47	3	0	0.043 ^{b,c}	11	39	0	0.062
		GA	44	8	2		16	37	1	
		AA	13	1	0		7	7	0	
SLCO1A2	rs5484	GG	59	8	2	NS	48	21	0	NS
		GT	36	2	0		9	28	1	
		TT	0	0	0		0	0	0	
	rs2053098	CC	94	11	2	NS	27	79	1	0.011 ^c
		CT	8	1	0		5	4	0	
		TT	2	0	0		2	0	0	
SLCO1B3	rs2053098	GG	58	5	1	NS	16	47	1	NS
		GA	36	6	1		15	28	0	

Table 2. (Continued)

Gene	SNP	Genotype	Conjunctival hemorrhage (Grade)				Periorbital oedema (Grade)			
			0	1	2+	P ^a	0	1	2+	P ^a
EGFR	rs3764006	AA	10	1	0	NS	3	8	0	NS
		AA	53	7	0		15	45	0	
		AG	40	4	2		14	31	1	
	rs3834935	GG	11	1	0	0.059^c	5	7	0	0.028 ^c
		DEL	52	2	1		11	44	0	
		DEL.A	45	8	1		22	31	1	
	rs4149117	AA	7	2	0	NS	1	8	0	NS
		GG	53	5	1		41	17	1	
		GT	32	4	1		27	10	0	
	rs7311358	TT	10	1	0	NS	8	3	0	NS
		AA	25	2	0		7	20	0	
		AG	60	7	2		20	48	1	
	rs10258429	GG	10	1	0	0.002 ^d	3	8	0	NS
		CC	88	7	1		25	70	1	
		CT	11	4	1		6	10	0	
	rs11977388	TT	0	1	0	0.012 ^{c,d}	0	1	0	NS
		TT	49	4	1		15	39	0	
		TC	46	3	0		14	34	1	
PDGFRB	rs17110944	CC	10	4	1	NS	4	11	0	NS
		TT	99	12	1		33	78	1	
		TA	4	0	1		1	4	0	
	rs246388	AA	1	0	0	NS	0	1	0	NS
		TT	81	10	2		25	67	1	
		TC	21	1	0		7	15	0	
		CC	1	1	0		1	1	0	

P values between 0.05 and 0.10 were regarded as marginally suggestive of an association (boldfaced), whereas $P \leq 0.05$ was considered statistically significant. NS means no significant different $P > 0.10$. ^aGrade 0 versus 1+. ^bCo-dominant model. ^cFisher's exact test. ^dRecessive model. ^eDominant model.

Table 3. Multivariate logistic regression analyses of ophthalmological side effects

	Variables	P	OR (95% CI)
Conjunctival hemorrhage	rs10258429 (CT+TT vs CC ^a)	0.005	7.061,(1.791–27.837)
	rs683369 (CG+GG vs CC ^a)	0.021	4.809,(1.262–18.318)
	Constant	< 0.001	0.041
Conjunctival hemorrhage ^b	rs10258429 × rs683369	< 0.001	3.046,(1.579–5.878)
	Constant	< 0.001	0.019
Periorbital oedema	rs274558 (GG ^a)	0.009	
	rs274558 (GA vs GG)	0.03	0.292, (0.097–0.886)
	rs274558 (AA vs GG)	0.003	0.100, (0.022–0.456)
	rs2235040 (CT vs CC ^a)	0.02	0.250, (0.078–0.806)
	Constant	< 0.001	7.829
	rs2235040 × rs274558	0.001	0.411, (0.237–0.710)
Periorbital oedema ^b	Constant	< 0.001	14.317

^aGG or CC indicates homozygous wild type. ^bIn consideration of interaction between SNPs.

investigated in any pharmacogenetic study related to Imatinib, making it mandatory to validate this observation in independent data sets.

Meanwhile, we also found in the present study Imatinib-induced ophthalmological toxicity to be correlated with the mutations in *SLC22A1*, *SLC22A5* and *ABCB1*.

SLC22A1 (Organic Cationic Transporter 1, OCT1) mediates the uptake of many organic cations from the blood into epithelial cells, and in 2004 Thomas *et al.*¹⁷ reported that *SLC22A1* was a critical transporters of Imatinib. Subsequently, multiple studies have been conducted addressing the interaction between *SLC22A1* and Imatinib and consequently, a number of mutations have been found to be involved in the pharmacokinetics and pharmacodynamics of many chemotherapy drugs.^{35,36} In our study, we, for the first time, found that for the SNP rs683369 G > C

(Leu160Phe), genotype GG+CG was significantly associated with Imatinib-induced conjunctiva hemorrhage. On the basis of Di Paolo A's research, the rs683369 G > C genotype had a significant effect on apparent drug clearance (CL/F), and patients with GG/CG genotype had a significantly lower CL/F value with respect to the CC individuals. Our results indicated that GG+CG genotypes may be correlated with increased cellular exposure and increase risk of toxicities of Imatinib.³⁷

OCTN2 (*SLC22A5*) is another member of the *SLC22* family of plasma membrane solute carrier proteins.³⁸ OCTN2 is expressed ubiquitously, with high expression in kidneys and lower expression in heart, skeletal muscles, and other tissues.³⁹ Mutations in this gene have been reported in a few patients with primary carnitine deficiency,^{40,41} most of whom presented early in life with a severe metabolic decompensation. However, few studies have

been conducted on the impact of the mutation in *SLC22A5* on therapeutic effects and toxicity of Imatinib, except that Angelini *et al.*¹⁴ reported the polymorphisms in *SLC22A5* to be associated with prolonged progression time in unresectable gastrointestinal stromal tumors treated with Imatinib therapy. In our study, we found that the minor allele of *SLC22A5* rs274558 (A807G, L269L) decreased the risk to periorbital oedema in GIST patients treated with Imatinib therapy. Patients with A allele (GA+AA) had significant lower risk to periorbital oedema compared with GG carriers. This SNP is located in the coding region of *SLC22A5* and is a synonymous mutation, leading to no change of amino acid (L269L). The A allele of rs274558 ranges in allele frequency from 29.3 to 75% depending on ethnicity.

ABCB1 (also known as multidrug resistance protein 1 (MDR1), P-glycoprotein 1, abbreviated as P-gp), a member of ABC transporters family, is expressed at major physiological barriers, such as intestinal epithelium, the canalicular membrane of liver cells, kidney proximal tubule epithelial cells, blood-brain barrier and blood-testis barrier,^{42,43} where it exhibits protective and excretory functions.⁴⁴ Mutations in *ABCB1* have been associated with changes in drug disposition, sensitivity and toxicity.⁴⁴ In our previous study, *ABCB1* polymorphism was found to be correlated with Gefitinib skin toxicity.⁴⁵ We, for the first time, examined the association of *ABCB1* rs2235040 CT genotype with periorbital oedema in GIST patients treated with Imatinib, finding it a valuable protective factor in predicting this toxicity.

Imatinib-induced ophthalmological toxicity was found to be plasma-concentration-independent, given the function assigned to the *SLC22A1*, *SLC22A5* and *ABCB1* genes in Imatinib disposition.^{16,18} We speculate that the mutation of these transporters could influence the uptake or efflux of Imatinib, alter the intracellular concentration of Imatinib and subsequently lead to toxic variability in patients.

Although hypotheses do exist that the ocular side effect associated with Imatinib is due to dermal dendrocytes found in the periocular soft tissue that express particular molecular targets of Imatinib, namely KIT and PDGFR, and through the inhibition of PDGFR, Imatinib may decrease the interstitial pressure and increase transcapillary transport.^{46,47} In our study, *PDGFRB* variations did not influence the incidence of ophthalmological side effects, which was consistent with Brück P's report that development of severe oedema during treatment with Imatinib is not associated with *PDGFRB* gene polymorphisms.⁴⁸

Missing mutation can cause loss of information and hence reduced power in association analyses, therefore, we check the pattern and frequency of missingness and found that the frequencies of missingness are lower than 10% for all the candidate SNPs except for rs2231142 and within the confines of our limited sample size the pattern of missingness is random. Through direct imputation based on the haplotype analysis using Haploview, the associations between this SNP and Imatinib-induced ophthalmologic toxicity are not significant, which is similar to the results for the original group without imputation (data not shown). These analyses suggest that the present results are not strongly affected by these SNP missingness, indicating that our study may not be biased by these omissions.

In our study, we only focused on the early toxicity but not the late toxicity, however, Postel-Vinay S, *et al.* reported that more than 50% of the severe toxicities induced by TKIs occurred after cycle 1 of clinical trial, and more than 50% of the patients presented with their worst-grade toxicity after cycle 1.^{49–51} In addition, as a large number of candidate polymorphic loci were evaluated and multiple analyses of each genetic polymorphism were performed there this is a possibility that multiple testing have led us to the detection of spurious associations. Therefore our findings are in need of further confirmation in independent larger data sets addressing longer durations.

CONCLUSIONS

These results suggest that genetic polymorphisms of *EGFR*, *SLC22A1*, *SLC22A5* and *ABCB1* may influence the Imatinib-induced ophthalmological toxicities in GIST patients. Further studies with larger sample size are warranted to validate the results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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