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Research Article

Effects of MDR1 Gene Polymorphism on Efficacy and Hematotoxicity of Epirubicin-Based Regimen in Patients with Breast Cancer in Southwest China

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ARTICLE INFO

Article history:
Received: 15 March, 2021
Accepted: 19 March, 2021
Published: 30 March, 2021

Keywords:
MDR1 gene
polymorphism
breast cancer
Epirubicin
chemotherapy response
blood toxicity

ABSTRACT

Objective: To investigate the predictive value of multi-drug resistance gene (*MDR1*) polymorphism in the efficacy and hematological toxicity of chemotherapy regimen based on Epirubicin in patients with breast cancer in Southwest China.

Methods: Patients who received Epirubicin-based chemotherapy were included, and polymorphism of C1236T, G2677T/A and C3435T were detected by time-of-flight mass spectrometry (TOF-MS). The correlation between different genotypes and chemotherapy efficacy and blood toxicity were evaluated.

Results: A total of 102 patients were included, 44 of them were treated with neoadjuvant chemotherapy. There was no significant correlation between all genotypes and alleles of the three SNPs and the efficacy of neoadjuvant chemotherapy regimen containing Epirubicin in patients with breast cancer. There was a significant correlation between C3435T polymorphism and grade III-IV leukopenia in patients with breast cancer, the incidence of grade III-IV leukopenia in patients with C allele was significantly lower than that in patients with T allele.

Conclusion: T allele of C3435T polymorphism may be a risk factor for grade III-IV leukopenia in patients with breast cancer after chemotherapy.

Background

According to the latest statistical analysis of global cancer data, in 2018, the incidence of breast cancer is ranked first place as a deadly disease among female cancer patients [1]. Its incidence also ranks first in Chinese female population and exerts an increasing trend year by year [2, 3]. the number of new breast cancer cases and the number of deaths in China account for 12.2% and 9.6% per year [4]. Cytotoxic drug chemotherapy plays an important role in the treatment of breast cancer, in which the combination of Anthracycline and/or Taxanes is the first-line recommended [5].

Both anthracyclines and taxanes need to enter the tumor cells to act on the related targets and exert their anti-proliferation effects [6]. It has been confirmed that the transport of anthracyclines and taxanes is mediated by P-glycoprotein (P-gp), which is a transmembrane glycoprotein encoded by multi-drug resistance gene (*MDR1*) [7]. The gene polymorphism of MDR1 may affect the expression and functional activity of P-gp, thus affects the transport and even therapeutic effect of anthracyclines and taxanes, which is one of the important reasons for the occurrence of multi-drug resistance in tumor cells [8, 9]. At present, more than 50 single nucleotide polymorphisms (SNP) of *MDR1* gene have been found, and the mutation frequencies of these SNPs are different in different regions or populations [10, 11]. So, there are some

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http://dx.doi.org/10.31487/j.COR.2021.04.04

differences in the expression, activity and function of P-gp in different organisms [12]. Among them, C1236T, G2677T/A and C3435T have been proved to be closely related [13]. Therefore, the purpose of this study is to explore the correlation between the polymorphisms of C1236T, G2677T/A and C3435T and the efficacy and hematotoxicity of Epirubicin-based regimens in patients with breast cancer in Southwest China, which may provide predictive indicators for clinical treatment options for breast cancer patients and promote individualized treatment.

Materials and Methods

I Research Objects

To collect female patients with breast cancer diagnosed by pathology and immunohistochemistry from the Affiliated Hospital of Southwest Medical University. Inclusion criteria: no antitumor drug therapy in the first treatment or in the previous 6 months; Eastern Cooperative Oncology Group (ECOG) score of physical condition is 0-2; No abnormal blood routine, heart, liver and kidney function. Excluding patients with clinical diagnosis, unclear primary tumor or complicated with other tumors, pregnant or lactating patients.

II Determination of Chemotherapy Regimen, Efficacy and Hematotoxicity

All the patients in the study were given Epirubicin-based chemotherapy regimens, including EC, TEC or ET regimens (E: Epirubicin, T: docetaxel, C: cyclophosphamide). Doxorubicin 90mg/m^2, intravenous drip (iv drop); cyclophosphamide 600mg, iv drip; docetaxel 75mg, iv drip. All patients took 21 days as a chemotherapy cycle. After 2-4 cycles of chemotherapy, the short-term efficacy of chemotherapy was judged according to the solid tumor efficacy evaluation standard RECIST1.1 [5]. The combination of complete remission (CR) and partial remission (PR) was judged to be effective, and stable disease (SD) and progression disease (PD) were judged to be ineffective. The hematotoxicity of chemotherapy, including leukopenia, neutropenia, thrombocytopenia and anemia, was evaluated according to the criteria for evaluating the toxicity of anticancer drugs issued by the World Health Organization (WHO). The lowest value monitored during chemotherapy was used as the test result. The efficacy and hematotoxicity of preoperative neoadjuvant chemotherapy were evaluated, while those of postoperative adjuvant chemotherapy were only monitored.

III Extraction of DNA from Peripheral Venous Blood

Before chemotherapy, 4mL of peripheral fasting venous blood was collected with disodium ethylenediamine tetraacetate (EDTA-Na_2) purple anticoagulant tube. DNA was extracted by commercial Kit (Beijing BioTeKe Company). OD value was detected by NanoDrop2000 instrument, and 1.25% agarose gel electrophoresis was detected. DNA was stored in the refrigerator at-20℃ after passing quality test.

IV The Primer Design of *MDR1* Gene SNP

The primer design and synthesize of *MDR1* gene SNP was performed by Huada Genome Technology Co., Ltd. C1236T (rs1128503): forward-ACGTTGGATGTTT CTCACTCGTCCTGGTAG; reverse-ACGTTGGATGCACAGCCACTGTTTCCAA CC; G2677T/A (rs2032582): forward- ACGTTGGATGGCACTCATCGCCACTTAA TG; reverse- ACGTTGGATGACTGCTGCGGGTTCCTAAAG; C3435T (rs1045642): forward-ACGTTGGATGTTGCCTATGGAGACAACAGC; reverse-ACGTTGGATG AAGGCATGTATGTTGGCCTC.

V SNPs Determination of *MDR1* Gene

SNPs genotyping was performed by Agena Mass ARRAY system. Briefly, the target DNA fragment was amplified by polymerase chain reaction (PCR). The total mixture of PCR reaction was 5ul, containing reaction solution 4ul and DNA sample standardized to 20ng/ul. The specific reaction procedure was as follows: pre-denaturation at 94℃ for 5 min, annealing at 56℃ for 30s, extension at 72℃ for 1 min, cycle 45 times, complete extension at 72℃ for 3 min, and end the reaction. The amplification results were verified by 1.25% agarose gel electrophoresis. Then, under the action of shrimp alkaline phosphatase (SAP), the excess deoxyribonucleotide (dNTPs), was removed and the SAP reaction solution was increased by 2ul. The reaction procedure was set as follows: 37℃ 20 min/85℃ 5 min, and the reaction was finished. Then the single base extension reaction was carried out and the extension reaction solution was increased by 2ul. The specific reaction procedure was as follows: pre-denaturation at 94℃, denaturation at 94℃ for 5s, annealing at 52℃ for 5s, extension at 80℃ for 5s, cycle 45 times, complete extension at 72℃ for 3 minutes, and end the reaction. Finally, the product was purified by resin and detected by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

VI Statistical Analysis

Statistical analysis was carried out by SPSS 22.0 software. Fisher test and Fisher exact probability method were used to test the genetic balance between gene polymorphism and Hardy-Weinberg, and to analyse the relationship between *MDR1* gene polymorphism and basic clinical characteristics, pathological stage, molecular classification, chemotherapeutic efficacy and chemotoxicity. The relative risk of different genotypes and hematotoxicity of breast cancer chemotherapy was compared by binary logistic regression analysis, and the odds ratio (OR) and 95% confidence interval (CI) were calculated. $P < 0.05$ was considered to be statistically significant.

Results

I *MDR1* Gene Polymorphism Typing

A total of 102 patients with breast cancer were included in this study. The results of *MDR1* gene polymorphism detection showed that there were three genotypes of C1236T: CC, CT and TT, and the variation frequency of T alleles was 66.7%. Three genotypes of C1236T: GG, GT and TT, and the variation frequency of T allele was 53.9%. Three genotypes of C3435T: CC, CT and TT, and the variation frequency of T alleles was 45.6%. By χ^2 test, the genotypic distribution of the three SNPs loci conformed to the Hardy-Weinberg genetic balance ($P > 0.05$), as shown in (Table 1).

Table 1: C1236T, G2677T/A and C3435T polymorphisms and the results of Hardy-Weinberg genetic balance test.

	Cases	C1236T Genotype			Allele gene		$\chi2$	*P*
		CC	CT	TT	C	T		
Actual frequency	102	15	38	49	68	136	1.37	0.50
Theoretical frequency	102	11.3	45.4	45.3				
	Cases	G2677T/A Genotype			Allele gene		$\chi2$	*P*
		GG	GT	TT	G	T/A		
Actual frequency	102	28	38	36	94	110	3.16	0.21
Theoretical frequency	102	21.7	50.7	29.6				
	Cases	C3435T Genotype			Allele gene		$\chi2$	*P*
		CC	CT	TT	C	T		
Actual frequency	102	32	47	23	111	93	0.32	0.85
Theoretical frequency	102	30.2	50.6	21.2				

II Relationship between *MDR1* Gene Polymorphism and Clinicopathology

All the patients included in the study were Han women, ranging in age from 23 to 71-year-old, with an average age of 49-year-old. The genotypes of C1236T, G2677T/A and C3435T were compared with the baseline of clinicopathological characteristics by χ^2 test, including age, menstrual status, clinical stage, degree of tissue differentiation and molecular typing, as shown in (Table 2).

Table 2: Baseline clinical characteristics of patients with different *MDR1* genotypes.

	Cases	C1236T			*P*	G2677T/A			*P*	C3435T			*P*
	n	CC	CT	TT		GG	GT	TT		CC	CT	TT	
Age													
≤35	6	2	3	1	0.25	1	3	2	0.96	2	1	3	0.40
36~50	51	8	15	28		14	19	18		16	26	9	
＞50	45	5	20	20		13	16	16		14	20	11	
Menstrual state													
Premenopausal	48	7	17	24	0.93	12	18	18	0.85	17	22	9	0.59
Menopause	54	8	21	25		16	20	18		15	25	14	
Clinical staging													
I	12	2	1	9	0.13	2	7	3	0.45	4	6	2	0.37
II	39	8	15	16		12	15	12		11	15	13	
III	51	5	22	24		14	16	21		17	26	8	
Histological grading													
I	29	2	10	17	0.57	8	15	6	0.14	10	12	7	0.84
II	61	11	24	26		15	19	27		19	30	12	
III	12	2	4	6		5	4	3		3	5	4	
Molecular type													
triple-negative type	83	11	29	43	0.57	23	33	27	0.37	27	41	15	0.22
non-triple-negative type	16	3	7	6		4	4	8		4	6	6	

III The Relationship between *MDR1* Gene Polymorphism and the Efficacy of Chemotherapy

In this study, a total of 44 breast cancer patients received neoadjuvant chemotherapy. The evaluation results of chemotherapy efficacy were: CR: 2 cases, PR: 22 cases, SD: 15 cases and PD: 5 cases. The overall response rate (CR+PR) of chemotherapy was 54.5%. The results of correlation analysis between C1236T, G2677T/A and C3435T polymorphisms and chemotherapeutic efficacy showed that the genotypes or alleles of the three SNPs were not related to the chemotherapeutic efficacy of Epirubicin-based regimen in breast cancer patients (all $P > 0.05$), as shown in (Table 3).

Table 3: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy response in patients with breast cancer.

Genes	**Genotype**	**Cases**	**Effective（CR+PR）**	**Invalid（SD+PD）**	**Efficiency**	**$\chi2$**	**P value**
C1236T	CC	6	2	4	33.3%	1.57	0.46
	CT	17	9	8	52.9%		
	TT	21	13	8	61.9%		
	C	29	13	16	44.8%	1.65	0.26
	T	59	35	24	59.3%		

G2677T/A	GG	10	6	4	60.0%	0.58	0.75
	GT	19	11	8	57.9%		
	TT	15	7	8	46.7%		
	G	39	23	16	59.0%	0.55	0.52
	T	49	25	24	51.0%		
C3435T	CC	16	10	6	62.5%	2.37	0.31
	CT	21	12	9	57.1%		
	TT	7	2	5	28.6%		
	C	53	32	21	60.4%	1.83	0.20
	T	35	16	19	45.7%		

IV Relationship between *MDR1* Gene Polymorphism and Hematotoxicity

The results of blood toxicity evaluation of all 102 patients were as follows: the incidence of severe leukopenia: 18.6% (n=19); severe neutropenia: 35.3% (n=36); severe thrombocytopenia: 3.9% (n=4); severe anemia: 4.9% (n=5). The correlations between C1236T, G2677T/A and C3435T genotypes and alleles and blood toxicity were analysed. The results showed that C3435T polymorphism was associated with severe leukopenia. Further allele analysis showed that the incidence of severe leukopenia in patients with C allele was 12.6%, which was lower than that in patients with T allele, as shown in (Table 4). The C3435T polymorphism was associated with severe neutropenia, and the incidence of severe neutropenia in patients with C allele was significantly lower than that in patients with T allele, and the incidence of severe neutropenia in patients with C allele was significantly lower than that in patients with T allele. The genotypes or alleles of other SNPs were not associated with severe hematological toxicity, as shown in (Tables 5-7).

Table 4: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy-induced leukopenia.

Genes	**Genotype**	**Cases**	**Leukopenia**		χ^2	***P* value**
			0-II	**III-IV**		
C1236T	CC	15	11	4	1.40	0.50
	CT	38	30	8		
	TT	49	42	7		
	C	68	52	16	1.62	0.25
	T	136	114	22		
G2677T/A	GG	28	23	5	1.19	0.55
	GT	38	29	9		
	TT	36	31	5		
	G	94	75	19	0.29	0.59
	T	110	91	19		
C3435T	CC	32	29	3	5.85	0.05
	CT	47	39	8		
	TT	23	15	8		
	C	111	97	14	5.81	0.02*
	T	93	69	24		

Note: *P < 0.05

Table 5: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy-induced neutropenia.

Genes	**Genotype**	**Cases**	**Neutropenia**		χ^2	***P* value**
			0-II	**III-IV**		
C1236T	CC	15	10	5	4.10	0.13
	CT	38	20	18		
	TT	49	36	13		
	C	68	40	28	3.65	0.06
	T	136	112	44		
G2677T/A	GG	28	20	8	2.10	0.35
	GT	38	26	12		
	TT	36	20	16		
	G	94	66	28	2.32	0.14
	T	110	66	44		
C3435T	CC	32	27	5	8.83	0.01*

	CT	47	28	19		
	TT	23	11	12		
	C	111	82	29	8.91	0.003**
	T	93	50	43		

Note: *P < 0.05; **P < 0.01

Table 6: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy-induced thrombocytopenia.

Genes	**Genotype**	**Cases**	**Thrombocytopenia**		χ^2	***P* value**
			0-II	**III-IV**		
C1236T	CC	15	14	1	0.94	0.63
	CT	38	36	2		
	TT	49	48	1		
	C	68	64	4	1.04	0.45
	T	136	132	4		
G2677T/A	GG	28	28	0	2.86	0.24
	GT	38	35	3		
	TT	36	35	1		
	G	94	91	3	0.25	0.73
	T	110	105	5		
C3435T	CC	32	32	0	2.07	0.36
	CT	47	44	3		
	TT	23	22	1		
	C	111	108	3	0.96	0.47
	T	93	88	5		

Table 7: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy-induced anemia.

Genes	**Genotype**	**Cases**	**Anemia**		χ^2	***P* value**
			0-II	**III-IV**		
C1236T	CC	15	13	2	3.16	0.21
	CT	38	36	2		
	TT	49	48	1		
	C	68	62	6	3.37	0.09
	T	136	132	4		
G2677T/A	GG	28	27	1	1.19	0.55
	GT	38	35	3		
	TT	36	35	1		
	G	94	89	5	0.07	1.0
	T	110	105	5		
C3435T	CC	32	31	1	0.45	0.80
	CT	47	44	3		
	TT	23	22	1		
	C	111	106	5	0.08	1.0
	T	93	88	5		

With three SNPs as covariates, hematotoxicity as a dependent variable, binary logistic regression analysis of leukocytes and neutrophils was carried out. The results showed that there was a correlation between C3435T polymorphism and severe leukopenia (P < 0.05). Patients with CC genotype had a significantly lower risk of severe leukopenia than patients with other genotypes [OR=6.40, 95%CI (1.032-39.662)], as shown in (Table 8). However, the results did not show that C3435T polymorphism was a risk factor for severe neutropenia after chemotherapy (Table 9). The goodness-of-fit test results of binary logistic regression analysis showed that the calibration of the risk research model was high, and the accuracy of the prediction model was high.

Table 8: Binary Logistic analysis of C1236T, G2677T/A and C3435T polymorphisms and chemotherapy-induced leukopenia.

Genes	β	SE	Wald	*P*	OR	95%CI
C1236T	-0.546	0.692	0.623	0.43	0.579	0.149-2.247
G2677T/A	-1.085	0.85	1.629	0.202	0.338	0.064-1.788
C3435T	1.856	0.931	3.976	0.046	6.399	1.032-39.662

Note: Nagelkerke (pseudo) R^2=0.086; Goodness-of-fit test: χ^2=6.558 (*P*=0.087).

Table 9: Binary Logistic analysis of C1236T, G2677T/A and C3435T polymorphisms and chemotherapy-induced neutropenia.

Genes	β	SE	Wald	*P*	OR	95%CI
C1236T	-1.244	0.642	3.760	0.052	0.288	0.082-1.013
G2677T/A	-1.136	0.673	2.854	0.091	0.321	0.086-1.200
C3435T	0.139	0.685	0.041	0.840	1.149	0.300-4.398

Note: Nagelkerke (pseudo) R^2=0.134; Goodness-of-fit test: χ^2=3.377 (*P*=0.337).

Discussion

MDR1 gene, also known as *ABCB1* gene, is located on the long arm of human chromosome 7 q21.1 and encodes the P-gp [14-16]. P-gp can bind to ATP through a specific region and pump "harmful substances" out of the cell, which plays an important role in the absorption, distribution and excretion of its substrates including drugs [17]. Some studies have shown that *MDR1* gene-related SNPs polymorphism may affect the Area Under Curve (AUC) or clearance rate of docetaxel [18, 19]. At present, it has been found that there exits more than 50 SNPs in *MDR1* gene, of which exon 12 (C1236T), 21 (G2677T/A) and 26 (C3435T) have high mutation frequency [20-23]. Liu *et al.* found that breast cancer patients with T allele at C3435T locus had better chemotherapeutic efficacy than patients with C allele, but no significant difference was found in C1236T and G2677T/A polymorphism [24]. While in breast cancer patients in Saudi Arabia, Alsaif *et al.* thought that the CT/ TT type of *C1236T* gene had a low response rate to anthracycline and/or yew chemotherapy regimens [25].

Chang *et al.* obtained the opposite results in the study of paclitaxel monotherapy for breast cancer [26]. They found that in patient with CC type of C3435T gene, the disease control rate was significantly higher than that with mutant type, and the GG type of G2677T/A locus was related to paclitaxel resistance. In a Meta-analysis, Adela *et al.* considered that under the existing evidence, the above three SNPs did not have a significant correlation with the chemotherapy efficacy in patients with breast cancer, which did not support it as an effective predictor [27]. Considering that the gene polymorphism may affect different drugs, the inconsistency of these findings may be attributed to the different chemotherapy regimens. This study included breast cancer patients in southwest China as the study population and chooses Epirubicin-based chemotherapy regimen, which is different from other taxus-based regimens, and has a reference value for the clinical treatment in this area.

The effect of *MDR1* gene polymorphism on anthracycline and taxane drug transport may also lead to differences in drug toxicity. Tran *et al.* found that docetaxel-treated patients with T allele (C3435T) had a significantly higher frequency of leukopenia than those with C allele [28]. Meanwhile, Ji *et al.* found that T allele carriers may have a higher risk of neutropenia [29]. However, the study of Chang *et al.* failed to detect a significant correlation between *MDR1* gene polymorphism and the toxicity of paclitaxel chemotherapy [30]. All the above results of this study showed that C3435T polymorphism was a risk factor for severe leukopenia in breast cancer patients treated with Epirubicin. Meanwhile, the judgment of blood toxicity in this study is based on the lowest monitoring value during the study period, and the impact of doctors' immediate treatment of adverse reactions on the results cannot be ruled out.

Since multiple SNPs of *MDR1* gene may jointly affect the function and activity of P-gp, multivariate analysis may be more convincing than independent studies of single SNPs. Wang *et al.* used multiple SNPs combination analysis to analyse the combination of pairwise haploid and three haploids [31]. The results showed that the effective rate of chemotherapy in 3435T-2677T-1236T haplotype carriers was lower than that in other haplotype carriers. In addition, Ji *et al.* analysed the combined effect of *GSTP1* gene and *MDR1* gene on the prognosis of chemotherapy in Chinese breast cancer patients, suggesting the predictive value of these genes in tumor therapy [32]. In this study, after excluding the influence of three SNPs factors in binary Logistic regression analysis, C3435T polymorphism cannot act as a risk factor for severe neutropenia.

Conclusion

To sum up, this study suggests that C3435T polymorphism may be a risk factor for grade III-IV leukopenia in breast cancer patients treated with Epirubicin regimen in Southwest China. The incidence of grade III-IV leukopenia in patients with T allele was significantly higher than that those with C allele after chemotherapy. So, breast cancer patients with T allele should be strengthened to actively prevent the occurrence of leukopenia in clinical monitoring. The above results need to be further confirmed by large sample, multicenter clinical studies.

Acknowledgement

We sincerely thank the Professor Bin Wu and Dr. Mingquan Huang (Department of Pharmacy, The Affiliated Hospital of Southwest Medical University, Luzhou, China) to diagnosed the breast cancer cases, it' s our pleasure to acknowledge their contributions.

Author Contributions

Q.W., Y.H., and X.Z. designed this research and wrote the manuscript; Q.F. and W. Z. collected blood samples; Z.W., C.X., M.L., C.Z., Y.L. and J.Z. performed data collection and pre-processing of data.

Funding

This study was supported by research funding from the Science and Technology Planning Project of Sichuan Province (No.2019YFS0180), Science and Technology Program of Luzhou (No.2018-JYJ-41, 2015LZCYD-SO2-9/11), the Foundation for Young Scholars of Southwest Medical University (2019-ZQN-125, 2019-ZQN-151, 2018-ZQN-098), the Foundation of southwest medical university (2017-ZRZD-003).

Availability of Data and Materials

All data generated or analysed in this study are included in this published article.

Ethics Approval and Consent to Participate

All of the patient samples used in this study was approved by the ethics committee of The Affiliated Hospital of Southwest Medical University (Luzhou city, Sichuan Province, China) before we start the experiment.

Consent for Publication

Not applicable.

Conflicts of Interest

None.

Abbreviations

***MDR1* gene:** Multi-Drug Resistance Gene 1
P-gp: P-glycoprotein
SNP: Single Nucleotide Polymorphisms
ECOG: Eastern Cooperative Oncology Group
EC: Epirubicin + Cyclophosphamide
TEC: Docetaxel+ Epirubicin+ Cyclophosphamide
ET: Epirubicin+ Docetaxel
RECIST: Response Evaluation Criteria in Solid Tumors
CR: Complete Remission
PR: Partial Remission
SD: Stable Disease
PD: Progression Disease
WHO: World Health Organization
EDTA-Na2: Ethylenediaminetetraacetic Acid Disodium Salt
PCR: Polymerase Chain Reaction
SAP: Shrimp Alkaline Phosphatase
dNTPs: Deoxyribonucleotide
MALDI-TOFMS: Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry
OR: The Odds Ratio
CI: Confidence Interval
AUC: Area Under Curve
GSTP1: Glutathione S-Transferase P1

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