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Original Article

GSTA1 gene polymorphisms are associated with cyclophosphamide effectiveness in lupus nephritis patients: A case-control study in Indonesia

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Abstract

Glutathione-S-transferase alpha-1 (*GSTA1*) is an enzyme with high conjugation activity against aldophosphamide, a metabolite of cyclophosphamide and promoter polymorphisms in *GSTA1* may influence the cyclophosphamide effectiveness. The aim of this study was to evaluate the effectiveness and side effects of cyclophosphamide in lupus nephritis patients, using *GSTA1* variants as predictors. A case-control study was conducted at Hasan Sadikin Hospital, Bandung, Indonesia, involving 100 lupus nephritis patients from February 2023 to January 2024. The PCR-Sanger sequencing was used to genotype five selected single nucleotide polymorphisms (SNPs) in the *GSTA1* promoter: -52 A>G, -69 T>C, -513 A>G, -567 G>T, and -631 G>T. The endpoint was assessed after six doses of cyclophosphamide by evaluating renal function, disease activity and side effects. Results indicated that six doses of intravenous cyclophosphamide treatment improved renal function and disease activity in the patients, as evidenced by significant changes in serum creatinine (0.79 vs 0.69 mg/dL), dipstick proteinuria (3.00 vs 1.50), creatinine clearance (98.50 vs 109.50 mL/min), and Modified Systemic Lupus Erythematosus Disease Activity Index 2000 (M-SLEDAI-2K) score (8.61 vs 6.95). The AG genotype at -513 A>G was associated with reduced cyclophosphamide effectiveness (odds ratio (OR): 0.19; 95%CI: 0.19–0.60; p=0.019). The GT genotype at -631 G>T independently increased the progression of anemia (OR: 2.41; 95%CI: 0.26–22.12; p=0.040). This study highlights that the presence of *GSTA1* variants affected cyclophosphamide effectiveness in lupus nephritis patients, with heterozygous polymorphisms at -513 (AA to AG) and -631 (TT to GT) predicting reduced effectiveness of cyclophosphamide by enhancing *GSTA1* promoter activity, while anemia further exacerbated lupus nephritis disease severity. *GSTA1* polymorphism was not associated with the presence of alopecia, amenorrhea, gastrointestinal disorders, and leukopenia during cyclophosphamide therapy.

Keywords: Promoter polymorphism, *GSTA1*, cyclophosphamide, effectiveness, lupus nephritis

Introduction

Cyclophosphamide, an alkylating agent, is a preferred treatment for lupus nephritis, primarily by suppressing T lymphocyte proliferation [1,2]. It is administered in six intravenous (IV) dosages

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for lupus nephritis induction therapy [3,4], with varying response rates across ethnic groups [5]. Asians have shown higher remission rates compared to Caucasians and Africans when treated with cyclophosphamide [6-12]. A meta-analysis found no significant difference in effectiveness between mycophenolate mofetil and cyclophosphamide for treating lupus nephritis when urine protein levels were ≥4 g/day (p=0.599) [13]. Mycophenolate mofetil, proven effective in randomized controlled trials even at lower doses, offers better tolerance than cyclophosphamide, which is associated with adverse effects such as pneumonia, gastrointestinal issues, and menstrual disturbances [14]. Cyclophosphamide's active metabolites, including aldophosphamide, 4-OH-cyclophosphamide, and phosphoramide mustard, can lead to organ damage, affecting heart function and causing myelosuppression and menstrual irregularities [15,16]. Despite these effects, cyclophosphamide lacks pharmacogenetic labeling as food and drug administration (FDA) biomarker [17].

Glutathione s-transferase alpha 1 (*GSTA1*) gene polymorphisms may affect the response to cyclophosphamide in lupus nephritis patients [8,11]. Previous studies on lupus nephritis populations from China and Egypt have identified different *GSTA1* genotypes affecting the effectiveness of cyclophosphamide [8,11]. In Egypt, the TT (-69) genotype was associated with non-remission and persistent proteinuria [8], whereas in China, heterozygous genotypes GA (-52) and TC (-69) were correlated with lower levels of cyclophosphamide active metabolites [11].

Prominent *GSTA1* promoter variants, including -52 A>G, -69 T>C, -513 A>G, -567 G>T, and -631 G>T, vary significantly across diverse human populations and form haplotypes through linkage disequilibrium [18-21]. Functional assays have demonstrated that single nucleotide polymorphisms (SNPs) at positions -52 (rs3957356) and -69 (rs3957357) play crucial roles in *GSTA1* haplotypes by interacting with SNPs at -513 (rs11964968) and -567 (rs4715332), while -631 (rs4715333) acts independently [21,22]. Individuals with specific *GSTA1* diplotypes, such as -52, -69, -567, -631 (ATGG/ATGG), have lower GSTA1 enzyme levels, whereas those with -52, -69, -513, -567, -631, -1142 (GCATGC/GCATGC) exhibit maximum promoter activity [18,20]. Given the geographic variability in *GSTA1* genotypes, the aim of this study was to evaluate how *GSTA1* variants, including diplotype sequences, predict cyclophosphamide efficacy and adverse effects. The present study's findings could inform precision medicine strategies for optimizing cyclophosphamide treatment in lupus nephritis.

Methods

Study design and setting

A case-control study involving lupus nephritis patients was conducted at Hasan Sadikin Hospital, Bandung, Indonesia, from February 2023 to January 2024. Outpatient rheumatology patients at Hasan Sadikin Hospital were enrolled in this study, with eligibility determined by their history of cyclophosphamide use, as documented in medical records. Data regarding lupus nephritis diagnosis, classification, associated symptoms, and laboratory findings were extracted from both manual and electronic medical records. Patients who met the inclusion criteria provided informed consent for genotyping during their routine follow-up visits. The evaluation of cyclophosphamide's effectiveness and side effects were assessed through physical examinations and laboratory data collected two weeks pre-treatment and post sixth cycle of cyclophosphamide treatment. Once the minimum required sample size was reached, patients were categorized into case and control groups according to specific definitional criteria (**Figure 1**).

Participants and criteria

Inclusion criteria comprised lupus nephritis patients aged over 18 years who had received six IV cyclophosphamide treatments according to the National Institutes of Health (NIH) or European Alliance of Associations for Rheumatology (EULAR) protocols [23,24]. Patients receiving the EULAR protocol were administered six doses of IV cyclophosphamide at a dose of 500 mg every two weeks, while patients following the NIH protocol received six doses of IV cyclophosphamide at a dosage of 0.5–1 g/m^2 administered monthly. Exclusion criteria included pregnancy, lactation, pregnancy planning, chronic kidney failure (CKD), mixed connective tissue disease (MCTD), systemic sclerosis (SSC), hepatitis, and neuro-psychiatric complications of systemic lupus

erythematosus (NPSLE). Patients who had used allopurinol, busulfan, chloramphenicol, or ciprofloxacin within the previous two months were also excluded due to potential interactions with cyclophosphamide metabolism.

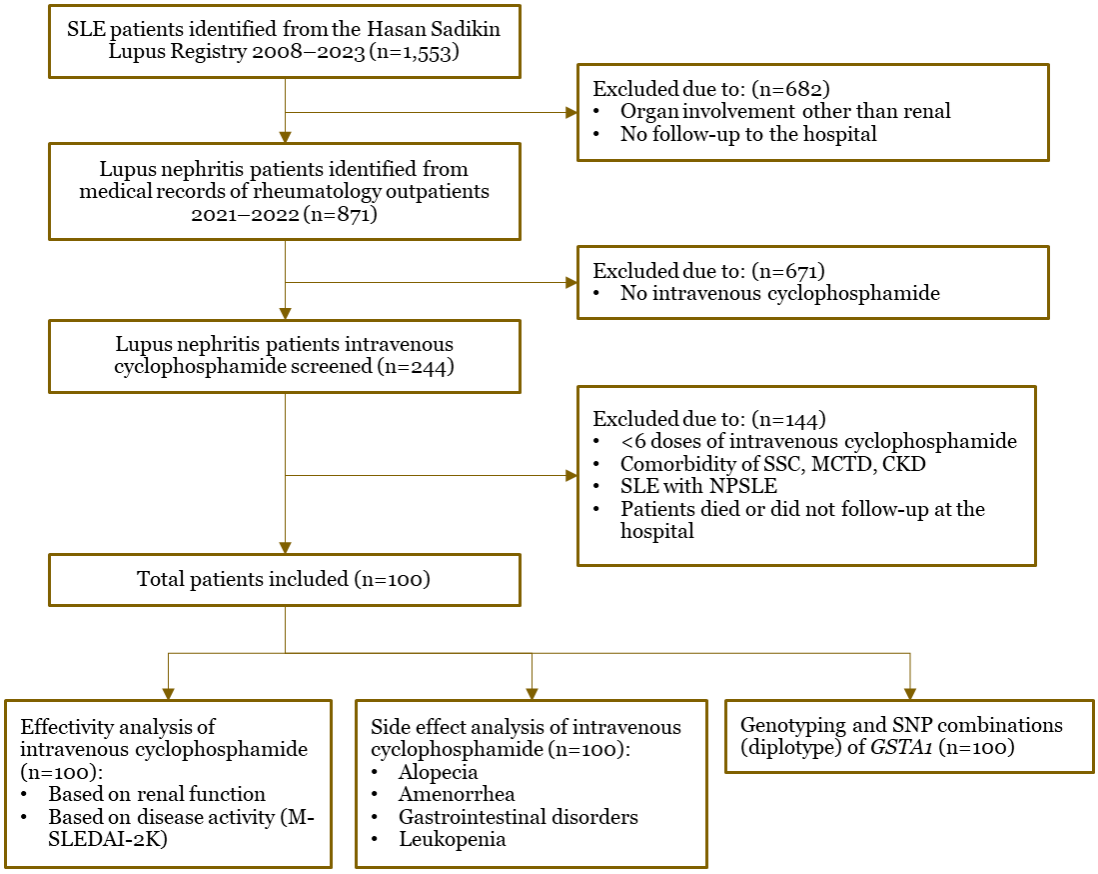

Figure 1. Study design and patient recruitment. This case-control study included adult lupus nephritis patients with six doses of cyclophosphamide to ensure homogeneity in the study sample. Dependent variables included the effectivity and side effects of cyclophosphamide, while independent variables were genotypes and diplotypes of the *GSTA1*. CKD: chronic kidney disease; MCTD: mixed connective tissue disease; NPSLE: neuro-psychiatric complications of systemic lupus erythematosus; SLE: systemic lupus erythematosus; SSC: systemic sclerosis

Sample and sampling method

Sample size calculation used the Cochrane formula, with a previous study involving 125 systemic lupus erythematosus (SLE) patients treated with cyclophosphamide [25]. The minimum sample inclusion of 96 cyclophosphamide-treated patients was deemed representative of the present study. To achieve 80% power and a 5% alpha error [8], a minimum sample size of 32 patients in both low and high-disease activity groups was required. Power analysis was conducted to assess the effectiveness of the primary outcome, where low disease activity and complete remission were categorized as case groups, while high disease activity and partial plus non-remission events were categorized as control groups. A power of 80% was calculated for each dependent variable to determine the number of representative subjects required for this study. In this retrospective case-control study, patient groups were not matched in equal numbers due to the requirement of genotype profiles of Indonesian patients as a preliminary investigation for *GSTA1*. To minimize bias from confounding factors, an initial stratification was conducted using a Chi-squared test to analyze low and high disease activity groups, followed by the identification of covariates for inclusion in the binary logistic regression analysis (regression adjustment). Both stratification and regression adjustment are recognized methodologies within propensity score measurement (PSM) [26,27]. Subsequently, propensity scores were calculated using multiple logistic regression, with the case group scores nearing 1 and the control group scores nearing 0. These scores were calculated based on the average of predicted probabilities in the multiple logistic regression, which indicated statistically significant differences. In this retrospective case-control

study, incidence density sampling was used to more accurately reflect the epidemiological conditions at the time of observation, with controls selected from the same population and during the same period as the cases.

Study procedures

Patients were diagnosed and treated based on the American College of Rheumatology (ACR) and the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline for the management of lupus nephritis [23,24]. Remission was assessed according to KDIGO 2023 [24,28,29], i.e. no proteinuria detected, creatinine clearance within normal range, and no edema and hypertension. The diagnosis of lupus nephritis in patients was based on signs of proteinuria and symptoms of SLE flare, along with the management of cyclophosphamide therapy. The duration from the initial diagnosis of SLE to the initiation of cyclophosphamide treatment was not constrained by a specific time frame. The lupus nephritis patients who were eligible to receive cyclophosphamide had nephritis class III, IV or V. Based on histological findings, class I was characterized by minimal mesangial lupus nephritis; class II with mesangial proliferative lupus nephritis; class III with focal lupus nephritis; class IV with diffuse lupus nephritis; and class V with membranous lupus nephritis [30,31]. Lupus nephritis disease activity was assessed using the Modified Systemic Lupus Erythematosus Disease Activity Index 2000 (M-SLEDAI-2K) [32]. Cyclophosphamide side effects such as alopecia, amenorrhea, gastrointestinal disorders, and leukopenia were evaluated according to the Common Terminology Criteria for Adverse Events version 5 (CTCAE v.5, 2017) [33].

DNA extraction

A volume of 0.5 mL of peripheral blood was collected from each patient using sterile ethylenediaminetetraacetic acid (EDTA) vacutainers during outpatient visits. The DNA of each patient was directly extracted from the fresh whole blood at the Molecular Genetics and Microbiology Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia, using Genomic DNA Mini Kit (Blood) (Geneaid, New Taipei City, Taiwan) following the manufacturer's instructions. The concentration and purity of the genomic DNA were assessed using a UV-Vis spectrophotometer at λ260/280 (Thermo Fisher Scientific Multiskan Go, USA). The DNA of each patient was stored at -20°C for further use as a template for polymerase chain reaction (PCR) amplification using Veriti Thermal Cycler, 96-well Fast (Thermo Fisher Scientific, Massachusetts, USA).

PCR-Sanger sequencing

A pair of primers (forward: 5'-GGAGGGTGTGAGGCAATGTA-3') and (reverse: 5'-CCCCCTACATGGTATAGGTGAA-3') was used to amplify the *GSTA1* fragment through PCR amplification. The PCR reaction mixture consisted of 1U of GoTaq Green Master Mix (Promega, USA), 150 ng of genomic DNA, 0.25 µM of each primer (Integrated DNA Technologies, Singapore), and nuclease-free water to a total volume of 50 µL (Promega, USA). The amplification protocol began with initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and elongation at 72°C for 30 seconds, with a final elongation step at 72°C for 5 minutes. PCR and gel electrophoresis of each amplicon was performed at Pharmacology and Pharmaceutical Biotechnology Laboratories, School of Pharmacy, Institut Teknologi Bandung, Bandung, Indonesia. The PCR products confirmed were subjected to Apical Scientific, Malaysia, for Sanger-sequencing. SNP genotypes were identified based on the *GSTA1* gene sequence from the GenBank database (AL590363.6). DNA sequences were analyzed using SnapGene software (Dotmatics, Illinois, USA) and Geneious Prime 2022 (Dotmatics, Auckland, New Zealand) for sequence alignment.

Determination of single nucleotide polymorphisms (SNPs) combination

Genotypes were assigned simultaneously from a single chromatogram of Sanger sequencing, allowing for the determination of five SNP genotypes of *GSTA1*. Each SNP genotype for *GSTA1* promoter (-52, -69, -513, -567, and -631) was confirmed to follow Hardy-Weinberg Equilibrium (HWE) (p>0.05). Allele frequency differences were assessed using the Chi-squared test for deviations from HWE [34]. The procedure for calculating the HWE involved the tabulation of

genotypes from five SNPs in the *GSTA1*, analyzed in relation to the binary disease activity group and remission group. SNPstat was utilized to determine the exact *p*-value for all subjects and within each subgroup. Subjects were considered to meet the HWE criteria when the *p*-value for the overall and each subgroup exceeds 0.05. The linkage disequilibrium value (r^2) in the Indonesian population was calculated to analyze interactions between the five SNPs, determined using SNPStats (Institut Català d'Oncologia, Barcelona, Spain) [34] with a strong correlation defined as $r^2>0.8$ [22]. Linkage disequilibrium data from the Indonesian population were also compared with the closest Chinese population (https://ldlink.nih.gov/). Based on previous study, three combinations were analyzed: first (-52, -69), second (-52, -69, -513), and third (-52, -69, -567) [21]. Diplotype frequencies for each combination were calculated as percentages.

Study variables

The dependent variables included disease activity as assessed by the M-SLEDAI-2K score, remission based on renal function/proteinuria, and the occurrence of side effects. The M-SLEDAI-2K scores for each patient were assessed two weeks pre and post cyclophosphamide treatment. Patients who were classified as having low disease activity demonstrated M-SLEDAI-2K scores ranging from 0 to 5, while those with high disease activity had scores between 6 and 15. Renal function was evaluated two weeks prior to the first cycle and post sixth cycle of cyclophosphamide therapy. Based on the renal function evaluation, patients were classified as in remission if they demonstrated a significant reduction in proteinuria and improvement in lupus nephritis manifestations, while those with persistent lupus nephritis manifestations and did not have a significant reduction in proteinuria were classified as being in non-remission. To assess the disease activity progression, the presence of anemia and non-infection cystitis were evaluated based on laboratory data two weeks post cyclophosphamide. Non-infection cystitis was defined as urine erythrocyte counts exceeding 5 cells/µL, while hemoglobin levels below 10 g/dL were classified in patients with anemia. Data on side effects were gathered from the manifestations observed during the administration of cyclophosphamide. This information was recorded in the medical records and validated during follow-up visits, along with the relevant laboratory results. The severity of these adverse effects was assessed according to the CTCAE v.5, 2017. Patients who experienced side effects were documented, and their occurrence rates were compiled. Detailed definition of remission, disease activity, and side effects of patients are presented in **Table S1**.

Statistical analysis

The Kolmogorov-Smirnov test was calculated to assess data normality distribution. Binary logistic regression assessed genotype and diplotype differences in response and side effects. The multiple logistic regression was adjusted by including covariates for age, lupus nephritis conditions, and cyclophosphamide protocol. Propensity scores from post-hoc multiple logistic regression analysis using probability density compared using one-way ANOVA. Poisson regression was used to analyze genotype and diplotype effects on proteinuria and M-SLEDAI-2K scores alongside covariates, with the first genotype or diplotype as the reference. The paired Student t-tests were used to assess the pre-post differences for quantitative data, while Chi-squared tests assessed associations. The associations between each characteristic in the low and high disease activity groups were evaluated using Chi-squared tests for qualitative data and one-way ANOVA for quantitative data. Two-way ANOVA illustrated interaction plots of genotype against M-SLEDAI-2K scores and hemoglobin levels post-cyclophosphamide. Minitab Statistical Software v.21 (Minitab LLC, Pennsylvania, USA) was employed for data analysis, with $p<0.05$ considered statistically significant.

Transcription factor prediction

Transcription factors were predicted using two *GSTA1* fragments (780 bp) containing variants and wild types. Using the JASPAR 2024 database (https://jaspar.elixir.no/), transcription factors were classified into classes based on binding positions, with a threshold of 80% for relative profile scores predicting a total of 1,518 transcription factor sites [35]. Position weight matrix (PWM) scores were used to compare variant and wild type binding scores at each position. Increased PWM scores indicated a higher likelihood of binding to the sequence motif, with transcription factor changes predicted based on SNPs positions within the *GSTA1* fragment.

Results

Characteristic of patients

A total of 100 lupus nephritis patients were involved in this study and were all successfully genotyped. The characteristics are presented in **Table 1**. Based on M-SLEDAI-2K scores, 40 had low disease activity (scores 0–5), while 60 had high disease activity (scores 6–15).

Table 1. Characteristics of lupus nephritis patients treated with cyclophosphamide

Characteristics	Low disease activity (n=40)	High disease activity (n=60)	*p*-value
Sex			0.669[a]
Male	3 (7.50)	6 (10.00)	
Female	37 (92.50)	54 (90.00)	
Age, mean±SD (years)	27.39±8.61	29.55±9.32	0.244[b]
Duration from SLE to lupus nephritis patients treated with cyclophosphamide, mean±SD (years)	3.58±4.78	2.57±3.55	0.227[b]
Protocol used			0.444[a]
EULAR	8 (20.00)	16 (26.67)	
NIH	32 (80.00)	44 (73.33)	
Ethnic groups			0.185[a]
Sundanese	38 (95.00)	53 (88.33)	
Javanese	2 (5.00)	5 (8.33)	
Balinese	0 (0.00)	1 (1.67)	
Batak	0 (0.00)	1 (1.67)	
Disease activity progression			0.126[a]
Without anemia	37 (92.50)	49 (81.67)	
Anemia	3 (7.50)	11 (18.33)	
Without non-infection cystitis	33 (82.50)	17 (28.33)	0.000[a**]
Non-infection cystitis	7 (17.50)	43 (71.67)	
Side effects			0.269[a]
Alopecia	23 (57.50)	41 (68.33)	
Without alopecia	17 (42.50)	19 (31.67)	
Amenorrhea	18 (45.00)	31 (51.67)	0.514[a]
Without amenorrhea	22 (55.00)	29 (48.33)	
Gastrointestinal disorders	7 (17.50)	11 (18.33)	0.915[a]
Without gastrointestinal disorders	33 (82.50)	49 (81.67)	
Leukopenia	5 (12.50)	8 (13.33)	0.903[a]
Without leukopenia	35 (87.50)	52 (86.67)	

EULAR: European Alliance of Associations for Rheumatology; NIH: National Institutes of Health; SLE: systemic lupus erythematosus
[a] Analyzed using Chi-squared test
[b] Analyzed using One-way ANOVA
[*] Statistically significant at $p<0.01$
[**] Statistically significant at $p<0.001$

The interval between the first diagnosis of SLE and the administration of cyclophosphamide varied, ranging from 0 to 22 years. All genotype frequencies adhered to Hardy-Weinberg equilibrium ($p>0.05$) (**Table S2**). The SNPs at positions -52 and -69 exhibited linkage disequilibrium (r^2)>0.8, specifically 0.87 (**Table S3**). Genotype and diplotype frequencies for each SNP (-52, -69, -513, -567, -631) were analyzed in the case (30% remission, 40% low disease activity) and control (70% non-remission, 60% high disease activity) groups (**Table S4**). Heterozygote proportions exceeded 25% for each SNP in the non-remission and high disease activity groups. GG (-52), CC (-69), AA (-513), and TT (-567) genotypes were significantly more prevalent in the non-remission group ($p<0.05$). Additionally, diplotypes GC/GC, GCA/GCA, and GCT/GCT differed significantly in frequency between remission and non-remission groups across the first to third combination distributions ($p<0.05$) (**Table S4**).

Improvement in renal function and disease activity

Following IV cyclophosphamide treatment, significant improvements were observed in 100 patients' serum creatinine (0.79 vs 0.69 mg/dL), dipstick proteinuria (3.00 vs 1.50), creatinine clearance (98.50 vs 109.50 mL/min), and M-SLEDAI-2K score (8.61 vs 6.95) (**Figure 2A**). Post-treatment, hemoglobin levels increased significantly (11.51 vs 11.98 g/dl; n=98), along with erythrocyte count (4.29 vs 4.44 × 10^3/µL; n=73), monocyte percentage (7.63 vs 10.00%; n=85),

and decreased urine erythrocyte count (14.07 vs 5.03 cells/µL; n=76) (p<0.05) (**Figure 2B).** Patients following the NIH protocol and aged 18–20 years showed the lowest levels of dipstick proteinuria and M-SLEDAI-2K post-cyclophosphamide, serving as covariates in multivariate analysis (**Figure 2C**), indicating that IV cyclophosphamide treatment significantly improved SLE activity post-treatment.

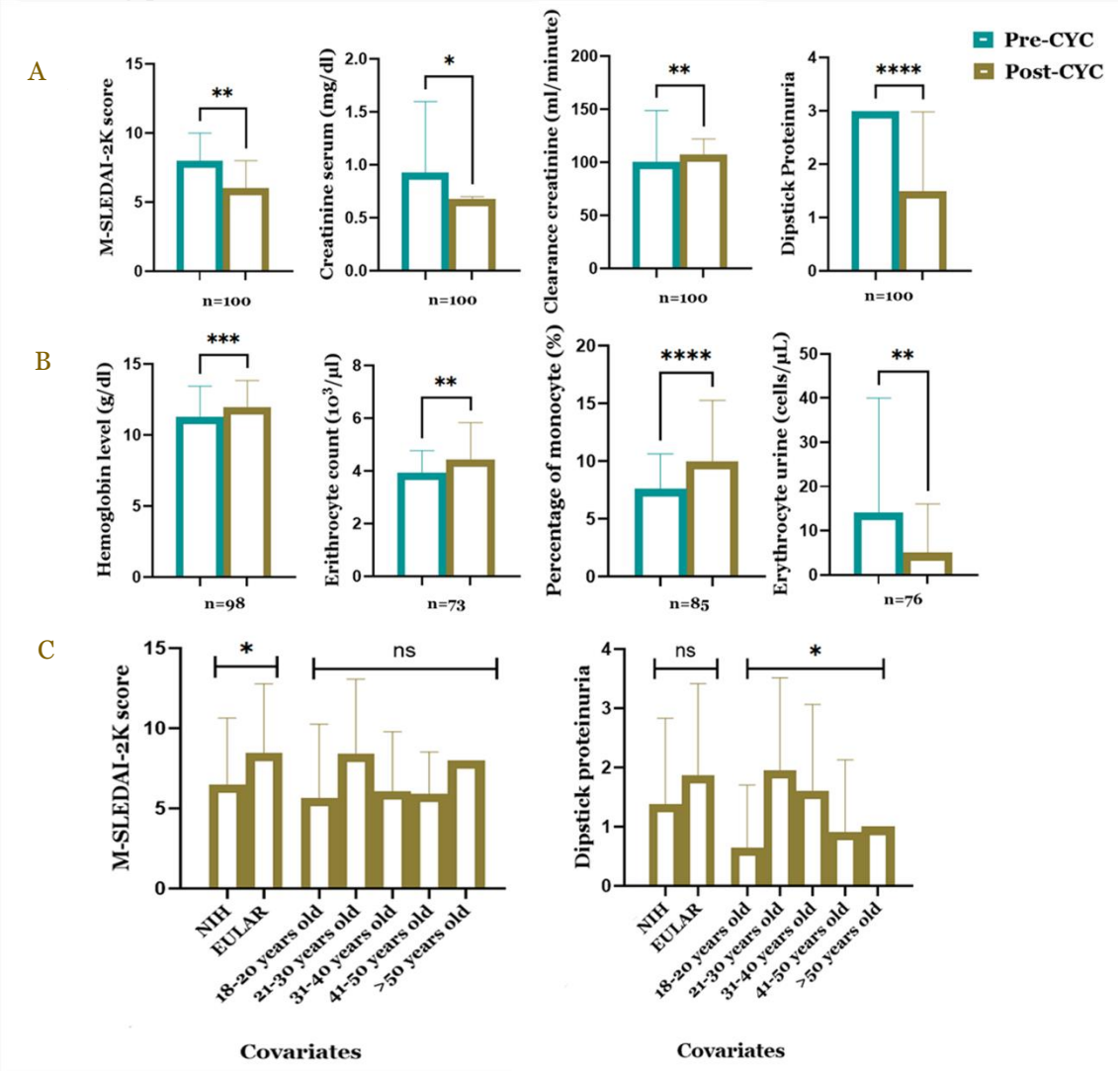

Figure 2. Evaluation of renal function and disease activity pre- and post-cyclophosphamide treatment. (A) Assessment of serum creatinine, creatinine clearance, dipstick proteinuria, and M-SLEDAI-2K scores. (B) Measurement of hemoglobin levels, erythrocyte counts, monocyte percentage, and urine erythrocyte counts. (C) Analysis of the impact of protocol IV cyclophosphamide and patient age on renal function and disease activity. ns: not significant. * Statistically significant at p<0.05; ** Statistically significant at p<0.01; *** Statistically significant at p<0.001; and **** Statistically significant at p<0.0001.

In this study, 100 Indonesian subjects were involved, with the majority (91%) being of Sundanese ethnicity. The Sundanese, Javanese, Balinese, and Batak ethnic groups were analyzed regarding proteinuria and M-SLEDAI-2K scores, which were not shown to have a significant effect (**Table S5**). The patients with non-infectious cystitis and anemia following cyclophosphamide treatment demonstrated higher scores in M-SLEDAI-2K compared to patients without anemia and non-infectious cystitis (**Table S6**).

Effect of *GSTA1* gene polymorphism on the effectiveness of intravenous (IV) cyclophosphamide

A homozygous polymorphism at position -513 (GG) correlated with minimal proteinuria (0.0) and a lower M-SLEDAI-2K score (3.0) post-cyclophosphamide (**Figure 3A**). Conversely,

genotype AG (-513) was associated with persistent proteinuria (1.93) and higher disease activity (9.22) (p<0.05), indicating reduced effectiveness of cyclophosphamide (**Table 2**). Receiver operating characteristic (ROC) analysis showed that detecting the -513 polymorphism could predict achieving low disease activity with an AUC of 0.7583 (sensitivity 72.50%, specificity 71.67%). Heterozygous polymorphisms at positions -52 (AG), -69 (TC), -513 (AG), and -567 (GT) consistently correlated with increased proteinuria and M-SLEDAI-2K scores, indicating worsened lupus nephritis outcomes. The second combination diplotype (-52, -69, -513) with ATA/GCG showed the highest post-cyclophosphamide proteinuria (2.15) and M-SLEDAI-2K score (9.60) (p<0.05), suggesting reduced cyclophosphamide efficacy.

Binary logistic regression (**Table 3**), revealed that AG (-513) and TT (-631) genotypes were associated with worsened disease activity (OR: 0.19 and 0.20, respectively), while AA (-52), TT (-69), and GG (-513) genotypes correlated with improvement (OR>1). Genotypes TT (-69) and GG (-513) were associated with higher odds of complete remission post-cyclophosphamide (OR>1) compared to CC and AA (**Table 4**).

Anemia and non-infectious cystitis were contributors to increased disease activity (**Table S6)**, suggesting potentially reduced cyclophosphamide efficacy (**Table 5**). The ATA/GCG diplotype in the second combination increased the likelihood of non-infectious cystitis (OR: 2.22) but did not reach significance. Approximately 14% of patients experienced low hemoglobin levels (<10 g/dL) post-cyclophosphamide. The GT (-631) genotype significantly increased the risk of anemia (OR: 2.41) compared to GG (p<0.05) (**Figure 3B**). ROC analysis for the -631 polymorphism in predicting anemia yielded an AUC of 0.8173 (sensitivity 78.57%, specificity 79.07%).

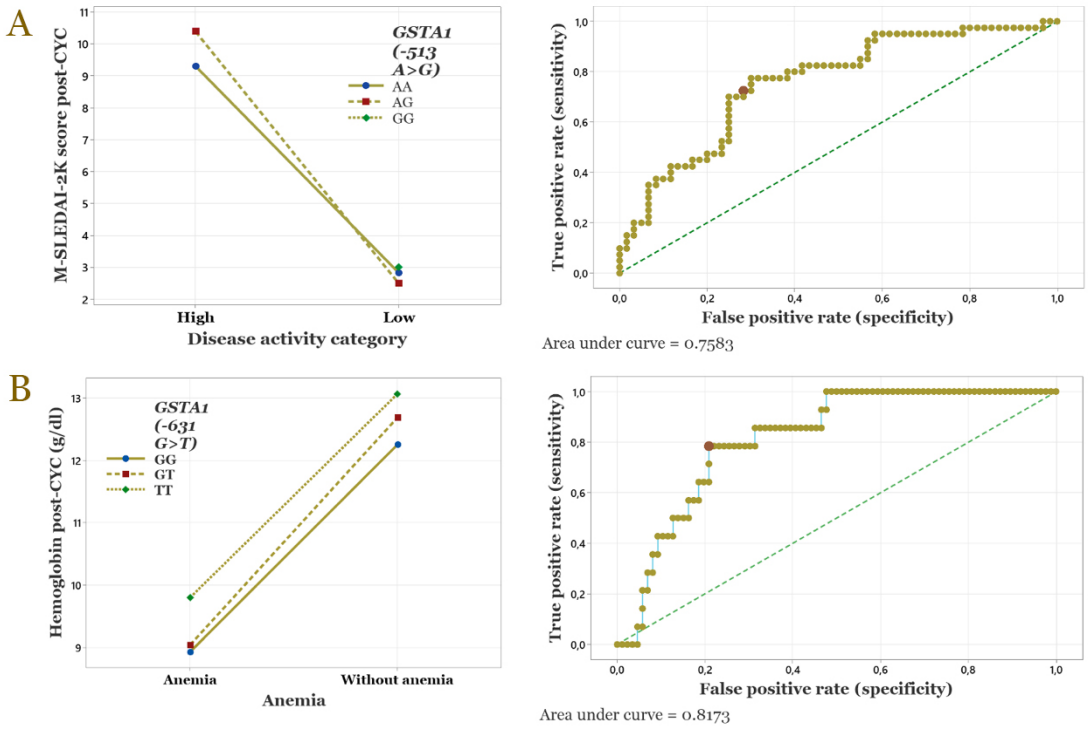

Figure 3. Effect of *GSTA1* polymorphisms on cyclophosphamide efficacy. (A) Interaction plot showing the *GSTA1* genotype at -513 versus M-SLEDAI-2K scores following cyclophosphamide treatment, alongside the receiver operating characteristic (ROC) curve of *GSTA1* (-513) as a predictor of achieving low disease activity. (B) Interaction plot depicting the *GSTA1* genotype at -631 versus hemoglobin levels, and ROC curve of *GSTA1* (-631) as a predictor of anemia in disease activity progression. Analysis was conducted using multiple binary logistic regression.

Table 2. Effect of genotypes and diplotypes on proteinuria and M-SLEDAI-2K scores following cyclophosphamide treatment

Predictors	Genotypes and diplotypes (n)	Dipstick proteinuria post-cyclophosphamide (mean±SD)	*p*-value [a]	M-SLEDAI-2K score post-cyclophosphamide (mean±SD)	*p*-value [a]
rs3957356 (-52 A>G)	GG (68) (*R*)	1.41±1.51	0.006**	6.52±4.05	<0.001**
	AG (28)	1.78±1.45		8.36±4.60	
	AA (4)	0.50±0.57		4.50±3.79	
rs3957357 (-69 T>C)	CC (69) (*R*)	1.45±1.51	0.009**	6.52±4.00	<0.001**
	TC (28)	1.71±1.47		8.18±4.80	
	TT (3)	0.67±0.57		5.33-4.16	
rs11964968 (-513 A>G)	AA (71) (*R*)	1.38±1.52	0.008**	6.19±4.10	<0.001**
	AG (27)	1.93±1.29		9.22±4.01	
	GG (2)	0.00		3.00±1.41	
rs4715332 (-567 G>T)	TT (64) (*R*)	1.39±1.53	0.007**	6.31±4.00	<0.001**
	GT (28)	1.75±1.43		8.14±4.66	
	GG (8)	1.50±1.31		7.88±4.49	
rs4715333 (-631 G>T)	GG (53) (*R*)	1.42±1.47	0.003**	6.72±3.99	<0.001**
	GT (39)	1.38±1.44		6.74±4.54	
	TT (8)	2.63±1.41		9.50±4.50	
First combination (-52, -69)	GC/GC (67) (*R*)	1.43±1.52	0.049*	6.43±4.03	<0.001**
	AT/GC (26)	1.77±1.48		8.27±4.77	
	AT/AT (3)	0.67±0.57		5.33±4.16	
	AC/GC (2)	2.00±1.41		9.50±0.71	
	AT/AC (1)	0.00		2.00	
	GT/GC (1)	2.00		12.00	
Second combination (-52, -69, -513)	GCA/GCA (66) (*R*)	1.44±1.53	0.082	6.38±4.03	<0.001**
	ATA/GCG (20)	2.15±1.35		9.60±4.15	
	ATA/GCA (5)	0.60±1.34		3.80±4.71	
	ATA/ATG (2)	1.00±0.00		7.00±4.24	
	ACA/GCG (2)	2.00±1.41		9.50±0.71	
	ATA/ACG (1)	0.00		2.00	
	ATG/ATG (1)	0.00		2.00	
	ATG/GCG (1)	0.00		4.00	
	GCA/GCG (1)	1.00		10.00	
	GTA/GCG (1)	2.00		12.00	
Third combination (-52, -69, -567)	GCT/GCT (63) (*R*)	1.41±1.53	0.084	6.32±4.03	<0.001**
	ATG/GCT (24)	1.79±1.47		8.13±4.80	
	GCG/GCG (4)	1.75±1.50		8.25-4.03	
	ATG/ATG (3)	0.67±0.57		5.33±4.16	
	ACG/GCT (2)	2.00±1.41		9.50±0.71	
	ATG/ACT (1)	0.00		2.00	
	ATG/GCG (1)	3.00		14.00	
	ATT/GCT (1)	0.00		6.00	
	GTG/GCT (1)	2.00		12.00	

[a] Analyzed using Poisson regression analysis with the greatest genotype as reference (*R*)
* Statistically significant at $p<0.05$
** Statistically significant at $p<0.01$
*** Statistically significant at $p<0.001$

Table 3. Effect of genotypes and diplotypes on achieving low disease activity analyzed through binary and multiple regression analysis

Predictors	Genotypes and diplotypes (n)	Low disease activity, OR (95%CI)	*p*-value [a]	Low disease activity, aOR (95%CI)	Adjusted *p*-value [b]
rs3957356 (-52 A>G)	GG (68) (*R*)	1.00	0.096	1.00	0.041*
	AG (28)	0.42 (0.38–38.40)		0.36 (0.12–1.04)	
	AA (4)	3.80 (0.38–38.40)		2.59 (0.23–28.29)	
rs3957357 (-69 T>C)	CC (69) (*R*)	1.00	0.267	1.00	0.072
	TC (28)	0.52 (0.20–1.34)		0.47 (0.17–1.30)	
	TT (3)	2.60 (0.22–30.05)		1.97 (0.16–24.73)	
rs11964968 (-513 A>G)	AA (71) (*R*)	1.00	0.019*	1.00	0.015*
	AG (27)	0.19 (0.19–0.60)		0.16 (0.04–0.55)	
	GG (2)	5.28 (1.65–16.85)		707,685.94 (0–1.79×10^{306})	
rs4715332 (-567 G>T)	TT (64) (*R*)	1.00	0.324	1.00	0.058
	GT (28)	0.48 (0.19–1.26)		0.39 (0.14–1.11)	
	GG (8)	0.72 (0.16–3.29)		0.61 (0.12–3.06)	
rs4715333 (-631 G>T)	GG (53) (*R*)	1.00	0.316	1.00	0.111
	GT (39)	1.09 (0.47–2.51)		1.09 (0.45–2.64)	
	TT (8)	0.20 (0.02–1.75)		0.28 (0.03–2.57)	
First combination (-52, -69)	GC/GC (67) (*R*)	1.00	0.668	1.00	0.217
	AT/GC (26)	0.45 (0.17–1.22)		0.40 (0.14–1.15)	
	AT/AT (3)	2.47 (0.21–28.53)		1.87 (0.15–23.68)	
	AC/GC (2)	0.00		0.00	
	AT/AC (1)	353,551.18 (0–9×10^{281})		150,272.52 (0–3.97×10^{281})	
	GT/GC (1)	0.00		0.00	
Second combination (-52, -69, -513)	GCA/GCA (66) (*R*)	1.00	0.434	1.00	0.291
	ATA/GCG (20)	0.13 (0.03–0.62)		0.11 (0.02–0.56)	
	ATA/GCA (5)	4.80 (0.51–45.28)		3.05 (0.29–32.16)	
	ATA/ATG (2)	1.20 (0.07–20.01)		0.00	
	ACA/GCG (2)	0.00		0.00	
	ATA/ACG (1)	343,995.74 (0–9×10^{281})		174,600.79 (0–4.62×10^{281})	
	ATG/ATG (1)	343,995.74 (0–9×10^{281})		133,138.98 (0–3.52×10^{281})	
	ATG/GCG (1)	343,995.74 (0–9×10^{281})		628,843.43 (0–1.38×10^{281})	
	GCA/GCG (1)	0.00		0.00	
	GTA/GCG (1)	0.00		0.00	
Third combination (-52, -69, -567)	GCT/GCT (63) (*R*)	1.00	0.925	1.00	0.509
	ATG/GCT (24)	0.48 (0.17–1.32)		0.42 (0.14–1.23)	
	GCG/GCG (4)	0.39 (0.03–3.96)		0.38 (0.03–4.48)	
	ATG/ATG (3)	2.34 (0.20–27.20)		1.74 (0.13–22.14)	
	ACG/GCT (2)	0.00		0.00	
	ATG/ACT (1)	8.2×10^{10} (0–~)		141,588.60 (0–3.75×10^{281})	
	ATG/GCG (1)	0.00		0.00	
	ATT/GCT (1)	0.00		0.00	
	GTG/GCT (1)	0.00		0.00	

R: reference group, the genotype and diplotype with the greatest frequency
[a] Analyzed using binary logistic regression
[b] OR: adjusted with the covariates
* Statistically significant at $p<0.05$

Table 4. Effect of genotypes and diplotypes on achieving remission, as assessed through binary and multiple regression analysis

Predictors	Genotypes and diplotypes (n)	Remission, OR (95%CI)	*p*-value [a]	Remission, aOR (95%CI)	Adjusted *p*-value [b]
rs3957356 (-52 A>G)	GG (68) (*R*)	1.00	0.757	1.00	0.224
	AG (28)	0.70 (0.26–1.88)		0.53 (0.18–1.53)	
	AA (4)	0.70 (0.07–7.09)		0.59 (0.05–6.50)	
rs3957357 (-69 T>C)	CC (69) (*R*)	1.00	0.793	1.00	0.239
	TC (28)	0.71 (0.26–1.92)		0.55 (0.19–1.59)	
	TT (3)	1.07 (0.09–12.42)		1.02 (0.07–13.11)	
rs11964968 (-513 A>G)	AA (71) (*R*)	1.00	0.291	1.00	0.112
	AG (27)	0.45 (0.15–1.32)		0.36 (0.11–1.12)	
	GG (2)	1.96 (0.12–32.70)		2.81 (0.11–70.43)	
rs4715332 (-567 G>T)	TT (64) (*R*)	1.00	0.218	1.00	0.087
	GT (28)	0.49 (0.17–1.37)		0.34 (0.11–1.04)	
	GG (8)	0.25 (0.03–2.20)		0.27 (0.03–2.44)	
rs4715333 (-631 G>T)	GG (53) (*R*)	1.00	0.325	1.00	0.240
	GT (39)	0.62 (0.25–1.54)		0.63 (0.24–1.61)	
	TT (8)	0.26 (0.03–2.24)		0.38 (0.04–3.56)	
First combination (-52, -69)	GC/GC (67) (*R*)	1.00	0.997	1.00	0.480
	AT/GC (26)	0.75 (0.28–2.05)		0.58 (0.19–1.70)	
	AT/AT (3)	1.02 (0.09–11.89)		0.93 (0.07–12.17)	
	AC/GC (2)	0.00		0.00	
	AT/AC (1)	0.00		0.00	
	GT/GC (1)	0.00		0.00	
Second combination (-52, -69, -513)	GCA/GCA (66)(*R*)	1.00	1.000	1.00	0.905
	ATA/GCG (20)	0.67 (0.21–2.07)		0.54 (0.16–1.81)	
	ATA/GCA (5)	1.33 (0.21–8.57)		0.73 (0.10–5.26)	
	ATA/ATG (2)	0.00		0.00	
	ACA/GCG (2)	0.00		0.00	
	ATA/ACG (1)	0.00		0.00	
	ATG/ATG (1)	8.21×10^{10} (0–~)		296,387.54 (0–7.84×10^{282})	
	ATG/GCG (1)	0.00		0.00	
	GCA/GCG (1)	0.00		0.00	
	GTA/GCG (1)	0.00		0.00	
Third combination (-52, -69, -567)	GCT/GCT (63)(*R*)	1.00	0.999	1.00	0.633
	ATG/GCT (24)	0.62 (0.21–1.79)		0.45 (0.14–1.41)	
	GCG/GCG (4)	0.00		0.00	
	ATG/ATG (3)	0.93 (0.08–10.86)		0.82 (0.06–10.88)	
	ACG/GCT (2)	0.00		0.00	
	ATG/ACT (1)	0.00		0.00	
	ATG/GCG (1)	0.00		0.00	
	ATT/GCT (1)	534,235.81 (0–1×10^{282})		1.83×10^{6} (0–4.85×10^{282})	
	GTG/GCT (1)	0.00		0.00	

R: reference group, the genotype and diplotype with the greatest frequency
[a] Analyzed using binary logistic regression.
[b] OR: adjusted with the covariates

Table 5. Effect of genotypes and diplotypes on anemia and non-infectious cystitis

Predictors	Genotypes and diplotypes (n)	Anemia, OR (95%CI)	*p*-value [a]	Non-infectious cystitis, OR (95%CI)	*p*-value [a]
rs3957356 (-52 A>G)	GG (68) (*R*)	1.00	1.000	1.00	0.300
	AG (28)	0.97 (0.27–3.38)		1.74 (0.71–4.26)	
	AA (4)	0.00		0.37 (0.04–3.79)	
rs3957357 (-69 T>C)	CC (69) (*R*)	1.00	1.000	1.00	0.380
	TC (28)	0.98 (0.28–3.44)		1.79 (0.73–4.36)	
	TT (3)	0.00		0.57 (0.05–6.67)	
rs11964968 (-513 A>G)	AA (71) (*R*)	1.00	0.860	1.00	0.290
	AG (27)	0.68 (0.17–2.66)		2.07 (0.83–5.14)	
	GG (2)	0.00		1.21 (0.07–20.26)	
rs4715332 (-567 G>T)	TT (64) (*R*)	1.00	0.990	1.00	0.670
	GT (28)	0.90 (0.26–3.15)		1.51 (0.62–3.69)	
	GG (8)	0.00		1.13 (0.26–4.93)	
rs4715333 (-631 G>T)	GG (53) (*R*)	1.00	0.040*	1.00	0.820
	GT (39)	2.41 (0.26–22.12)		0.86 (0.19–3.93)	
	TT (8)	0.42 (0.04–4.61)		1.12 (0.25–4.95)	
First combination (-52, -69)	GC/GC (67) (*R*)	1.00	1.000	1.00	0.840
	AT/GC (26)	1.03 (0.29–3.65)		1.60 (0.08–28.56)	
	AT/AT (3)	0.00		0.58 (0.05–6.71)	
	AC/GC (2)	0.00		0.00	
	AT/AC (1)	0.00		143,331.56 (0–3.8×10^{281})	
	GT/GC (1)	0.00		286,663.12 (0–7.63×10^{281})	
Second combination (-52, -69, -513)	GCA/GCA (66) (*R*)	1.00	1.000	1.00	0.980
	ATA/GCG (20)	0.98 (0.24–4.00)		2.22 (0.78–6.29)	
	ATA/GCA (5)	1.40 (0.14–13.85)		0.80 (0.12–5.10)	
	ATA/ATG (2)	0.00		1.20 (0.07–20.01)	
	ACA/GCG (2)	0.00		1.20 (0.07–20.01)	
	ATA/ACG (1)	0.00		0.00	
	ATG/ATG (1)	0.00		0.00	
	ATG/GCG (1)	0.00		343,995.7 (0–9.09×10^{281})	
	GCA/GCG (1)	0.00		343,995.7 (0–9.09×10^{281})	
	GTA/GCG (1)	0.00		343,995.7 (0–9.09×10^{281})	
Third combination (-52, -69, -567)	GCT/GCT (63) (*R*)	1.00	1.000	1.00	1.000
	ATG/GCT (24)	1.06 (0.29–3.77)		1.64 (0.63–4.24)	
	GCG/GCG (4)	0.00		1.17 (0.15–8.85)	
	ATG/ATG (3)	0.00		0.58 (0.05–6.80)	
	ACG/GCT (2)	0.00		1.17 (0.07–19.58)	
	ATG/ACT (1)	0.00		0.00	
	ATG/GCG (1)	0.00		336,087 (0–8.88×10^{281})	
	ATT/GCT (1)	0.00		336,087 (0–8.88×10^{281})	
	GTG/GCT (1)	0.00		336,087 (0–8.88×10^{281})	

R: reference group, the genotype and diplotype with the greatest frequency
[a] Analyzed using binary logistic regression

Effect of *GSTA1* gene polymorphism on the side effect of IV cyclophosphamide

Two weeks after cyclophosphamide treatment, patients without side effects returned to normal values on each parameter, whereas those with side effects showed deviations from normal. Among 49 female patients, amenorrhea was observed (**Table 1**), and binary regression analysis indicated that polymorphisms had no significant impact on cyclophosphamide side effects (**Table S7**). Regarding gastrointestinal side effects, a significantly higher incidence was noted among male patients compared to female patients (**Table S8**). Conversely, no significant differences were found between male and female patients with respect to the side effects of alopecia and leukopenia. The incidence of amenorrhea was elevated in patients with the diplotype third combination ATG/GCT (OR: 2.50), GCG/GCG (OR: 3.00), and the genotype GT (-567) (OR: 2.07).

Predicted transcription factor

A fragment of GSTA1's promoter region, including 5 sites of SNPs, was examined for the binding of potential transcription factors (**Figure 4** and **Table S9**). Changes in transcription factors were predicted to have a dual effect on *GSTA1* transcription, potentially both decreasing and increasing its expression.

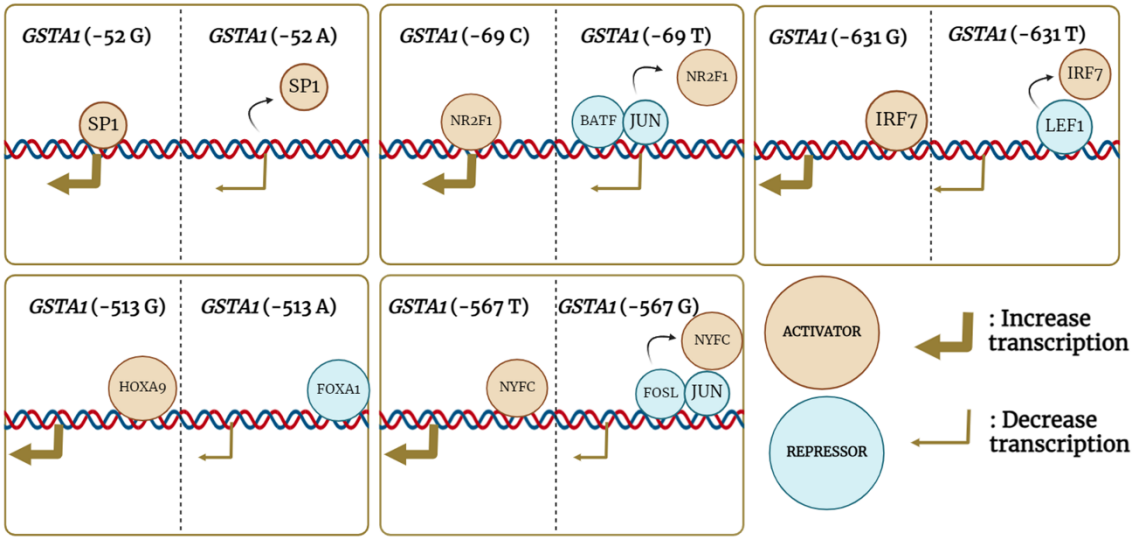

Figure 4. Illustration of predicted transcription factors for *GSTA1* polymorphisms at the -52, -69, -513, -567, and -631 promoter regions. Activators (depicted in chocolate) enhance transcription (bold arrow), while repressors (depicted in blue) diminish transcription (thin arrow) at the polymorphism-specific transcription factor binding sites.

Discussion

This case-control study examined the efficacy, side effects, and implications of *GSTA1* variants in cyclophosphamide treatment among lupus nephritis patients in Indonesia. The present study identified 5 SNPs in the -52 to -631 promoter region, including -513 A>G and -631 G>T variants relevant to lupus nephritis populations. Patients aged 25–35 years were critical due to SLE onset and therapy response dynamics [36]. Class I–II lupus nephritis patients typically exhibit milder symptoms, whereas class III, IV, and V lupus nephritis are more severe and often require treatment with cyclophosphamide, particularly for proliferative and membranous lupus nephritis [37]. The selection of either the EULAR or NIH protocols should be tailored to the individual patient, taking into account the severity of the disease, existing comorbidities, and the potential for adverse effects [38]. Patients seeking to preserve fertility or those with a higher susceptibility to infections may find greater advantages with the EULAR protocol; nonetheless, the outcomes related to achieving complete remission can differ among individuals [39,40]. The pharmacokinetics of cyclophosphamide (CYC) and its active metabolite, 4-hydroxycyclophosphamide, are affected by serum albumin levels and genetic polymorphisms in drug metabolism genes [41,42]. However, the NIH and EULAR protocols did not demonstrate a

significant impact on treatment outcomes in relation to the different CYC protocols [43,44]. Both protocols exhibited comparable efficacy and adverse effects, with no significant differences observed in blood levels or metabolism of CYC. Therefore, the choice between these protocols may be determined by other clinical factors rather than pharmacokinetic differences, allowing patients from both protocols to be included in this study.

Similar heterozygous polymorphisms at -52 and -69 were observed in Chinese lupus nephritis patients, affecting cyclophosphamide metabolism and efficacy [11]. Strong linkage disequilibrium (r^2) was found between SNPs at -52 and -69, influencing allele representation. The GC/GC diplotype frequency in the first combination (67%) differed from breast cancer patients in Pakistan (39.7%) [45], while the GCT/GCT frequency in the third combination contrasted with prostate cancer patients in Japan (73.7%) [46].

In the present study, post-cyclophosphamide evaluation demonstrated significant improvement in renal function and disease activity, though not all patients achieved complete remission or low disease activity. The present study highlighted the influence of protocol type and patient age on cyclophosphamide effectiveness. Due to limited 24-hour urine data, proteinuria dipstick was consistently used. Renal function was assessed using serum creatinine, clearance, and dipstick proteinuria, while disease activity was measured by M-SLEDAI-2K score. Effective cyclophosphamide induction therapy is crucial for predicting renal survival [47]. Lupus nephritis patients exhibited reduced blood monocyte counts pre-cyclophosphamide, potentially associated with kidney deposits and urine excretion [2,48,49]. Hematological conditions were prevalent in Indonesian SLE patients, with 73.5% showing hematological involvement [25]. Factors contributing to low erythrocyte counts in SLE include impaired kidney function and eryptosis [50,51].

Reduced promoter activity of *GSTA1* gene expression, associated with decreased enzyme levels and increased cell apoptosis [46], consistent with cyclophosphamide's mechanism of inhibiting lymphocyte proliferation in lupus nephritis [1,2]. Altered transcription factors at positions -52 (G to A) and -69 (C to T) reduced *GSTA1* gene expression in the liver and eliminated the active metabolite (4-OH-cyclophosphamide) [18,52,53]. In the present study, the high frequency of the GC/GC diplotype in the first combination potentially reduces cyclophosphamide effectiveness due to enhanced metabolite elimination. Heterozygous diplotypes (AT/GC) showed lower effectiveness (OR<1) compared to AT/AT and GC/GC, albeit not significantly. Promoter variations alter gene expression balance, impacting drug metabolism. Heterozygous polymorphisms (-52A, -69T, -567G/-52G, -69C, -567T) correlated with higher α-GST levels during sevoflurane administration, potentially reducing active metabolite concentrations via detoxification [54]. From the present study findings, the AT/AT diplotype in the first combination demonstrated greater effectiveness than GC/GC, suggesting reduced metabolite clearance and increased drug exposure.

Based on reporter assays in the present study, the GCG sequence in the second combination exhibited higher promoter activity compared to GCA and ATA, potentially reducing the active metabolite of cyclophosphamide and worsening lupus nephritis outcomes [21]. The GCG/GCG diplotype from the second combination was not observed in the present study. The change from A to G at position -513 increased *GSTA1* promoter activity, correlating with reduced cyclophosphamide effectiveness. The AG (-513) genotype significantly decreased cyclophosphamide efficacy. The GG genotype potentially increased promoter activity in sequences prior (-52 and -69), such as GC/GC and AT/GC, whereas ATG/ATG reduced promoter activity. The present study identified one patient with an ATG/GCG genotype, which reduced efficacy, and another with an ATG/ATG genotype, which showed increased efficacy. The second combination of SNPs (-52, -69, and -513) with the diplotype ATA/GCG demonstrated a significant association with decreased achievement of low disease activity (OR: 0.11; 95%CI: 0.02–0.56). This finding aligns with the results of the individual genotype analysis of AG (-513), which also indicated a decreased achievement of low disease activity (OR: 0.16; 95%CI: 0.04–0.55).

SP1 binding at the GC/GC site (-52 and -69) interacts with the transcription factor at -567, enhancing promoter activity [21]. The third combination ATG/ATG, associated with improved cyclophosphamide effectiveness, showed the lowest *GSTA1* gene expression in the present study. Additionally, the change from T to G at the -631 position increased *GSTA1* promoter activity. The

GT genotype at -631 reduced cyclophosphamide effectiveness and was associated with a higher risk of anemia compared to the TT genotype, which had the lowest promoter activity and improved cyclophosphamide effectiveness. Changes from G to C at position -1142 (rs58912740) affected function at -631 but not at -52 and -69. However, the present study did not analyze the genotype at position -1142. Based on a prior study [21], the SNP at position -513 was found to interact with the SNPs at positions -52 and -69, resulting in the second combination. However, the SNP at position -513 was not found to interact with the SNPs at positions -567 and -631. The lack of interaction between the SNPs at -513 and -631 was predicted due to opposing changes. The increase in promoter activity at position -513 was attributed to the transition from wild type to homozygous polymorphism (AA to GG), whereas at position -631, it was due to the transition from homozygous polymorphism to wild type (TT to GG).

Approximately 20–30% of cyclophosphamide remains unmetabolized in the liver, posing risks of multi-organ damage [16,55,56]. Cyclophosphamide-induced alopecia was prevalent among 64 patients in this study, attributed to cell division inhibition and apoptosis [57,58]. Preventive measures such as topical treatments have been recommended [59]. Amenorrhea affected 49 patients, a higher incidence compared to Chinese SLE patients treated with cyclophosphamide [14,60], causing physical and mental symptoms by increasing gonadotropins and decreasing estradiol [61]. Older SLE patients (>32 years) faced prolonged amenorrhea (>12 months) after brief cyclophosphamide IV therapy, necessitating alternative treatments [62]. Cyclophosphamide's inhibition of anti-Müllerian hormone in the endoplasmic reticulum was linked to ovarian function decline and premature insufficiency [63]. Increased GST activity on phosphoramide mustard in vivo mitigated ovotoxicity [64]. Polymorphism at position -567 (G to T) increased amenorrhea risk in breast cancer patients treated with cyclophosphamide [65]. The third combination, ATG/GCT, in the present study exhibited higher *GSTA1* expression than ATG/ATG, reducing ovarian toxicity without causing amenorrhea. Specific data on sex differences in cyclophosphamide side effects are lacking [66-68]. The limitation of this study was that infertility was not recorded in male patients.

Disease progression included increased non-infectious cystitis (50%) and anemia (14%) rates started 24–48 hours following IV cyclophosphamide and lasted 5–7 days or up to one month, managed with 2-mercaptethane sulfonate and hydration [69,70]. Cyclophosphamide suppressed erythropoiesis and hemoglobin synthesis, necessitating serum iron and ferritin assessments for anemia treatment [70,71].

SP1 enhances transcription by binding to the GC box, while NR2F1 initiates transcription via the PU box [72]. BATF::JUN negatively regulates AP-1/ATF transcription [73]. HOXA9 promotes B cell and lymphoid development [74], and FOXA1 binds to the ER element, reducing GST expression in the liver [75]. NFYC enhances transcription by binding to C/EBP sites with coactivators [76]. BATF::JUN and FOSL::JUN inhibit proliferation and induce cell death at the ATG (-52,-69,-567) [76]. IRF7, expressed in B cells, dendritic cells, and monocytes, induces IFN1 in the immune system [77]. Reduced binding of IRF7 to TT (-631) suggests lower anemia risk compared to GT and GG. The GT (-631) genotype likely increases GSTA1 gene expression while elevating IFN1 levels. FOXA1, NFYC, and IRF7 binding at this locus is crucial, given SP1's known interaction [78]. *GSTA1* expression occurs not only in the liver but also in renal, ovarian, fallopian tube, stomach, and intestinal tissues [79], influencing cyclophosphamide effectiveness and side effects based on target cell specificity.

The impact of *GSTA1* gene polymorphisms on promoter activity and gene expression varies by SNP position and combination [21]. Tissue-specific expression influences how GSTA1 affects cyclophosphamide effectiveness and side effects [18,52,53]. The present study confirmed significant associations between GSTA1 polymorphisms and cyclophosphamide effectiveness. The findings suggest that prior to use cyclophosphamide could be determined the presence of *GSTA1* polymorphism at positions -513 and -631. Based on the newest guideline for lupus nephritis management, if patients fail to reach the target effectiveness after cyclophosphamide treatment, alternative immunosuppressants such as mycophenolate mofetil (MMF) are recommended. Additionally, if remission is not attained, the administration of rituximab or belimumab may be considered [79].

Limitations of the present study include reliance on clinical lupus nephritis classification due to limited kidney biopsy data and retrospective medical records for laboratory measurements. Future research should focus on mechanistic insights into cyclophosphamide effectiveness and side effects mediated by *GSTA1* polymorphisms, requiring larger sample sizes to investigate amenorrhea, non-infectious cystitis and metabolite levels prospectively.

Conclusion

In lupus nephritis patients, *GSTA1* variants, particularly heterozygous polymorphisms at -513 (AA to AG) and -631 (TT to GT), significantly reduced cyclophosphamide effectiveness by enhancing *GSTA1* promoter activity and anemia exacerbated the disease severity in these patients. The alopecia, amenorrhea, gastrointestinal disorders, and leukopenia that occurred during cyclophosphamide use were not associated with *GSTA1* polymorphisms.

Ethics approval

The protocol of the present study was reviewed and approved by the Ethical Committee for Health Research, Hasan Sadikin Hospital, Bandung, Indonesia (Approval numbers: LB.02.01/X.6.5/37/2023 and DP.04/03/X.2.2.1/5757/2023), and adhered to the Declaration of Helsinki.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

All data generated or analyzed in this study are included in the published article and supplementary files (https://doi.org/10.6084/m9.figshare.26526781).

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