Original Article

Interethnic Differences in Single and Haplotype Structures of Folylpolyglutamate Synthase and Gamma-glutamyl Hydrolase Variants and Their Influence on Disease Susceptibility to Acute Lymphoblastic Leukemia in the Indian Population: An Exploratory Study

Abstract

Aim: We aim to establish the genotype and haplotype frequencies of *folylpolyglutamate synthase* (*FPGS* rs10106 and rs1544105) and *gamma-glutamyl hydrolase* (*GGH* rs3758149 and rs11545078) variants in the South Indian population (SI) and to study the association of these variants with susceptibility to acute lymphoblastic leukemia (ALL). We also aim to compare the genotype and haplotype frequencies of studied variants with those of superpopulations from the 1000 Genomes Project collected in phase-3 and other published studies in the literature. **Materials and Methods:** A total of 220 unrelated healthy volunteers and 151 patients with ALL of both sexes were recruited for the study. Extracted DNA was subjected to genotyping by allelic discrimination using quantitative real-time-polymerase chain reaction. Genotype details of the studied variants in other ethnicities were obtained from 1000 genomes project Phase 3 data. Haploview software was used to construct haplotypes. **Results:** In our study, the frequencies of *FPGS* rs1006'G' and rs1544105'A' alleles were found to be 37% and 37.2%, respectively, and the frequencies of *GGH* rs3758149'T' and *GGH* rs11545078'T' alleles were found to be 29.8% and 16.7%, respectively. Among the studied variants, *FPGS* rs1544105'AA' genotype carriers were found to be susceptible to the risk of ALL (odds ratio: 2.16; 95% confidence interval [CI]: 1.15–4.07; $P = 0.02$). Haplotype structures of *FPGS* and *GGH* variants in SI population were significantly different from other ethnicities ($P < 0.05$), except the South Asian superpopulation. **Conclusion:** *FPGS* rs1544105'AA' genotype was found to influence the risk for ALL. Intra and interethnic differences exist in the distribution of studied variants. Therefore, the impact of each variant on the susceptibility and outcome of diseases may differ between populations.

Keywords: *Antifolates, folate, folylpolyglutamate synthase, gamma-glutamyl hydrolase, haplotypes, polymorphism*

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Introduction

Acute lymphoblastic leukemia (ALL) is a hematologic malignancy characterized by the production of immature leukocytes. The estimated number of new cases of ALL in the United States in 2018 is 5960 and there are 1470 predicted deaths from ALL this year.[1] In India, the lymphoid leukemia cases are expected to be 18,449 by the year 2020.[2] Both genetic and nongenetic factors play a role in ALL; however, despite many studies, the etiology of ALL is still poorly understood. Folate deficiency has been associated with the increased risk of some cancers,[3,4] and lower folate levels were found to be associated with ALL in the Indian population.[5] Folates

and antifolates are small molecules that are metabolized intracellularly into their more potent polyglutamate derivatives. *Folylpolyglutamate synthase* (*FPGS*) and *gamma-glutamyl hydrolase* (*GGH*) are genes located on chromosomes 9 and 8, respectively, that are essential for the intracellular accumulation of folate and antifolate polyglutamates.[6] Mutations in *FPGS* and *GGH* genes might affect the activity of these enzymes, altering intracellular levels of polyglutamates [Table 1].[7-9] Variants in *FPGS* and *GGH* are also relevant in the context of the efficacy and safety of antifolate-based therapy.[10-12] Genetic variants associated with disease among the

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Table 1: Genetic variants investigated in the study						
Gene	rsid	Nucleotide change	Type of variant	Chromosome number: position	Effect on enzyme	Consequences on folate and MTX levels
FPGS	rs1544105	2572 G>A	Intron	9:127800446	Decreased transcripts	Decreased[7]
	rs10106	1944 A>G	3'UTR	9:127813796	Not known	-
GGH	rs3758149	-401 C>T	5' UTR	8:63039169	Increased expression	Decreased[8]
	rs11545078	452 C>T	Missense	8:63026205	Decreased activity	Increased[9]

UTR – Untranslated region; MTX – Methotrexate; *FPGS* – Folylpolyglutamate synthase; GGH – Gamma-glutamyl hydrolase

populations of other countries may not be associated with those in India[13,14] because Indians are genetically diverse and may differ from other populations.[15-17] To date, very few studies are available regarding the influence of *FPGS* and *GGH* variants and their haplotypes on the risk of ALL in the global populace. Therefore, we aimed to establish the frequency of *FPGS* and *GGH* variants in healthy volunteers to provide a normative frequency which can be used to compare with those of patients with cancer risk.

Materials and Methods

The present study was approved by the JIPMER Institute's Ethics Committee (IEC; Number: JIP/IEC/SC/2/2012/28). Written informed consent was obtained from all study participants, and in the case of children, consent was obtained from their legally accepted representatives.

Study population

A case–control study consisting of 220 unrelated healthy volunteers (controls) and 151 patients (cases) with ALL of either sex was conducted. All the participants were residing in South India for at least three consecutive generations and spoke one of the Dravidian languages (Tamil, Telugu, Malayalam, or Kannada) as their mother tongue. The mean ages (± standard deviation) of the cases and controls were 15.5 (±10.5) and 24.5 (±4.8) years, respectively. There were 99 (65.6%) males and 52 (34.4%) females in the patient group and 118 (53.6%) males and 102 (46.4%) females in the control group. We could not recruit age-matched healthy children due to difficulty in obtaining consent from patients' parents/legal guardians that resulted in the difference in mean age between cases and controls. Details of sample collection, DNA extraction, and quantification have been described previously.[18] *FPGS* (rs10106 and rs1544105) and *GGH* (rs11545078 and rs3758149) TaqMan assays were procured, to detect considered variants, from Applied Biosystems (Foster City, CA; USA). Genotyping was done by allelic discrimination using real-time polymerase chain reaction (Applied Biosystems-7300) according to the manufacturer's instructions. Genotyping was done in duplicates in 30% of the randomly selected samples and were found to be in 100% concordance. Genotype details of the studied SNPs in other ethnic populations were obtained from the 1000 Genomes Project, phase-3, which include five major populations: Africans (AFR), Americans (AMR), East Asians (EAS), Europeans (EUR), and South Asians (SAS). We have also considered subpopulations of SAS such as Gujarati Indians from Houston (GIH), Punjabis from Lahore, Pakistan (PJL), Bengalis from Bangladesh (BEB), Sri Lankan Tamils from the UK (STU), and Indian Telugu from the UK (ITU) for the comparison of genotype frequencies.

Construction of haplotypes and linkage disequilibrium

To construct haplotype blocks and to obtain their corresponding frequencies, the genotype data of two loci per gene (*FPGS*, rs10106 and rs1544105 and *GGH*, rs3758149 and rs11545078) were used. Details of the variants are mentioned in Table 1.

A total of 218 and 211 samples of healthy volunteers were used for haplotype analysis of *GGH* and *FPGS* variants, respectively.

Haploview software v4.2 (Broad, Cambridge, MA, USA)[19] was used to estimate the pairwise Linkage Disequilibrium (LD) pattern and haplotype frequencies. All markers/SNPs with minor allele frequencies <0.05 were excluded, and the minimum haplotype frequency was set at 1%. Strong LD between a pair of markers is indicated by D' values from 0.7 to 1, whereas moderate LD is indicated by D' values from 0.2 to 0.7 and D' values from 0 to 0.2 indicate no linkage disequilibrium.

Statistical analysis

The observed genotype frequencies were tested for Hardy–Weinberg equilibrium (HWE) using the Chi-squared test. Fisher's exact test was used to test the differences in genotypes between ALL patients and healthy volunteers (controls), and odds ratios with 95% confidence interval were obtained. Comparison between genotype and allele frequencies of South Indians (SIs) with the 1000 Genomes Project data was made using the Chi-squared test. GraphPad InStat 3 (GraphPad Software Inc., San Diego, CA, USA) and SPSS software (version 16, SPSS Inc.; Chicago, IL, USA) were used for statistical analysis. The threshold for statistical significance was set at $P < 0.05$.

Results

Comparison of genotype distribution of *FPGS* and *GGH* variants between patients with acute lymphoblastic leukemia and healthy individuals

The observed genotype frequencies of *FPGS* and *GGH* variants in healthy individuals and patients with ALL

were found to be in HWE ($P > 0.05$). Among the studied variants, *FPGS* rs1544105'AA' genotype carriers were found to be at risk of developing ALL [Table 2].

Comparison of haplotype structures of studied *FPGS* and *GGH* variants between cases and controls

Haplotype structures (HS) of *FPGS* and *GGH* variants were compared between cases and controls and were not found to be significantly different. There was however a trend observed with the *GGH* HS3 haplotype (carrying the variant allele 'T' of both GGH-401 and 452) towards the protection against risk of ALL [Table 3], but it was not statistically significant ($P = 0.06$).

Comparison of frequency of studied variants in South Indian population with data from the 1000 Genomes Project and other studies

Allele frequencies of *FPGS* and *GGH* variants in our healthy volunteers were compared with those of five superpopulations found in the 1000 Genomes Project phase

3-data and with other studies. Both *FPGS* rs10106'G' and rs1544105'A' alleles in the SI population (37%) were significantly lower when compared to AFR, AMR, EAS, PJL, and Thai populations,[20] but were similar to EUR and subpopulations of SAS (except PJL) [Table 4].[21-29]

The frequency of the *FPGS* rs10106'G' allele in the SI population was also lower when compared to Puerto Rican,[21] Dutch,[22] and Singapore Chinese populations,[29] whereas rs1544105'A' allele frequency was lower when compared to a Chinese population (65.9%).[11] There was also a significant difference in the distribution of genotype and allele frequencies of *GGH* variants (*GGH*-401 (rs3758149) and 452 C>T (rs11545078)) between the SI population and other ethnicities, except for subpopulations of SAS such as BEB, GIH, ITU, PJL, and STU [Table 4]. Frequency of the *GGH*-401'T' allele in the SI population (29.8%) was significantly lower when compared to West Indians (61%),[28] but it was similar to EUR, North Indian,[23] and Thai

Table 2: Distribution of genotypes and allele frequencies of *folylpolyglutamate synthase* (rs10106 and rs1544105) and *gamma-glutamyl hydrolase* (rs3758149 and rs11545078) polymorphisms in patients with acute lymphoblastic leukemia and normal healthy individuals

Genotypes and Alleles	Patients with ALL	Healthy volunteers	*P* value	OR (95% CI)
FPGS 1944 A>G (rs10106)	N=145; *n* (%)	N=212; *n* (%)		
AA	49 (33.8)	82 (38.7)		1.00 (reference)
AG	70 (48.3)	103 (48.57)	0.63	1.13 (0.71-1.81)
GG	26 (17.9)	27 (12.6)	0.18	1.61 (0.84-3.07)
A	168 (57.9)	267 (63)		1.00 (reference)
G	122 (42.1)	157 (37)	0.81	1.2 (0.91-1.67)
FPGS 2572 G>A (rs1544105)	N=149; *n* (%)	N=219; *n* (%)		
Genetic models				
Codominant model				
GG	44 (29.5)	83 (37.9)		1.00 (reference)
GA	74 (49.7)	109 (49.8)	0.34	1.16 (0.86-1.57)
AA	31 (20.8)	27 (12.3)	0.02*	2.16 (1.15-4.07)
Recessive model GG + GA versus AA	118 (79.2)	192 (87.7)		1.00 (reference)
	31 (20.8)	27 (12.3)	0.04*	1.40 (1.06-1.85)
G	162 (54.4)	275 (62.8)		1.00 (reference)
A	136 (45.6)	163 (37.2)	0.02*	1.41 (1.05-1.91)
GGH -401 C>T (rs3758149)	N=151; *n* (%)	N=220; *n* (%)		
CC	74 (49.0)	108 (49.1)		
CT	67 (44.4)	93 (42.3)	0.82	1.00 (0.79-1.32)
TT	10 (6.6)	19 (8.6)	0.68	0.84 (0.49-1.44)
C	215 (71.2)	309 (70.2)		1.00 (reference)
T	87 (28.8)	131 (29.8)	0.80	0.97 (0.80-1.17)
GGH 452 C>T (rs11545078)	N=151; *n* (%)	N=218; *n* (%)		
CC	116 (76.8)	151 (69.3)		1.00 (reference)
CT	34 (22.5)	61 (28)	0.41	0.39 (0.06-2.50)
TT	1 (0.7)	6 (2.7)	0.24	0.32 (0.05-2.03)
C	266 (88.0)	363 (83.25)		1.00 (reference)
T	36 (12)	73 (16.74)	0.07	0.78 (0.58-1.03)

*$P<0.05$. OR – Odds ratio; CI – Confidence interval; N – Total number of patients considered to study the respective genetic variant; *n* (%) – Number of patients possessing respective particular genotype; ALL – Acute lymphoblastic leukemia

Table 3: Frequency of haplotype structures of *folylpolyglutamate synthase* and *gamma-glutamyl hydrolase* variants in patients with acute lymphoblastic leukemia and healthy volunteers

HS	rs1544105 G>A Allele 1	rs10106 A>G Allele 2	Cases (N=145)	Controls (N=211)	P value
FPGS					
HS1	A	G	41.4	35.8	0.13
HS2	G	A	54.0	61.6	0.07
HS3	G	G	-	-	0.51
HS4	A	A	3.10	1.40	0.12

HS	rs11545078 C>T	rs3758149 C>T	Cases (N=151)	Controls (N=218)	P value
GGH					
HS1	C	C	70.8	69.7	0.73
HS2	C	T	17.3	13.6	0.17
HS3	T	T	11.5	16.5	0.06
HS4	T	C	-	-	-

$P < 0.05$. Chi-square test was used. HS – Haplotype structures; – – Frequency is either absent or <1%; FPGS – Folylpolyglutamate synthase; GGH – Gamma-glutamyl hydrolase; ALL –Acute lymphoblastic leukemia

Table 4: Frequency of *folylpolyglutamate synthase* and *gamma-glutamyl hydrolase* variants in South Indians, major populations of 1000 Genome Project phase-3, and other ethnic groups

Population	FPGS 1944 A>G (rs10106)				FPGS 2572 G>A (rs1544105)				GGH -401 C>T (rs3758149)				GGH 452 C>T (rs11545078)			
	N	OFH	A	G	N	OFH	G	A	N	OFH	C	T	N	OFH	C	T
SI (present study)	212	49.0	63.0	37.0	219	49.8	62.8	37.2	220	42.3	70.2	29.8	218	28	83.25	16.7
AFR	661	49.2	49.5	50.5*	661	46.1	38.0	62.0*	661	28.0	83.3	16.7*	661	10.0	94.4	5.60*
AMR	347	49.9	53.2	46.8*	347	49.6	51.9	48.1*	347	34.6	77.2	22.8*	347	8.10	96.0	4.00*
EAS	504	42.5	31.0	69.0*	504	42.9	31.0	69.0*	504	33.5	78.1	21.9*	504	16.3	91.3	8.70*
EUR	503	48.3	60.9	39.1	503	49.5	60.3	39.7	503	38.2	72.2	27.8	503	16.5	90.8*	9.20*
SAS subpopulation																
BEB	86.0	46.5	62.8	37.2	86.0	46.5	62.8	37.2	86.0	41.9	68.6	31.4	86.0	27.9	82.6	17.4
GIH	103	56.3	58.3	41.7	103	56.3	58.3	41.7	103	43.7	70.4	29.6	103	23.3	82.5	17.5
ITU	102	40.2	70.1	29.9	102	41.2	69.6	30.4	102	29.4	74.5	25.5	102	18.6	85.8	14.2
PJL	96.0	51.0	51.6	48.4*	96.0	51.0	50.5	49.5*	96.0	39.6	72.9	27.1	96.0	20.8	89.6	10.4
STU	102	49.0	58.8	41.2	102	46.1	58.3	41.7	102	44.1	70.1	29.9	102	27.5	85.3	14.7
Puerto Rican[21]	940	48.5	50.3	49.7*	-	-	-	-	966	37.7	73.5	26.5	899	53.4	72.6	27.4
Dutch[22]	360	-	57.2	42.8	-	-	-	-	-	-	-	-	360	16.5	91.3	8.70*
Chinese[11]	-	-	-	-	91.0	37.4	34.1	65.9*	91.0	29.7	80.8	19.2*	-	-	-	-
North Indian[23]	-	-	-	-	77.0	-	69.0	31.0	77.0	-	75.0	25.0	77.0	18.6	81.0	19.0
Thai[20]	95.0	32.0	28.0	72.0*	98.0	29.6	21.2	71.8*	100	39.0	76.5	23.5	-	-	-	-
Thai[10]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Japanese[24]	-	-	-	-	-	-	-	-	-	-	-	-	269	10.4	94.4	5.60*
Chinese[25]	-	-	-	-	-	-	-	-	-	-	-	-	132	12.1	90.9	9.10*
Chinese[26]	-	-	-	-	-	-	-	-	-	-	-	-	82.0	16.9	87.0	13.0*
Brazilian[27]	-	-	-	-	-	-	-	-	-	-	-	-	200	-	93.0	7.00*
Mexican[12]	-	-	-	-	-	-	-	-	140	21.4	85.7	14.3*	140	3.60	98.2	1.80*
West Indian[28]	-	-	-	-	-	-	-	-	144	49.0	39.0	61.0*	-	-	-	-
Singapore Chinese[29]	462	41.8	29.5	70.5*	-	-	-	-	472	32.2	79.0	21.0*	474	18.6	89.2	10.8*

The values are significant (P<0.05) when compared to those of South Indian population. OFH – Observed frequency of heterozygosity; N – Number of individuals in that particular group. '–' not studied. AFR – Africans; AMR – Americans; EAS – East Asians; EUR – Europeans; SAS– South Asians; BEB – Bengalis from Bangladesh; GIH – Gujarati Indians from Houston; ITU – Telugu from the UK; PJL – Punjabis from Lahore, Pakistan; STU – Sri Lankan Tamils from the UK; FPGS – Folylpolyglutamate synthase; GGH – Gamma-glutamyl hydrolase

populations.[10] In the present study, the frequency of *GGH*-452 'T' allele (16.7%) in SI individuals was significantly higher when compared to Dutch,[22] Japanese,[24] Chinese,[25,26] Brazilian,[27] and Mexican populations,[12] but similar to that found in Puerto Rican[21] and North Indian populations[23] [Table 4].

Comparison of frequency of studied haplotype structures in South Indian population with the 1000 Genomes Project data

FPGS rs10106 and rs1544105 allele frequencies were found to be in complete LD (D' =1) in BEB and PJL populations. A high LD pattern was observed between the considered *FPGS* variants in SI (0.94) and other populations. Similarly, *GGH* rs11545078 and rs3758149 (D' =0.97) alleles in SI, AFR, EUR, and GIH were also found to be in high LD. A complete linkage (D' =1) between *GGH* rs11545078 and rs3758149 alleles was observed in AMR, BEB, EAS, PJL, STU, and ITU populations. There was a difference in the distribution of HS of *FPGS* and *GGH* alleles between SI and other populations, except for the SAS population [Table 5].

Discussion

In the present study, the genotype frequencies of *FPGS* (rs10106 and rs1544105) and *GGH* (rs11545078 and rs3758149) variants have been established in SIs. Our study is the first to report that the rs1544105'A' allele confers a potential risk of susceptibility to ALL disease in Indians. We have also found a significant intra- and interethnic differences in the allelic distribution of studied *FPGS* and *GGH* variants.

All studied *FPGS* and *GGH* variants were found to be in HWE, indicating the absence of inbreeding or population stratification.[30] The frequencies of *FPGS* rs10106'G' and rs1544105'A' alleles were found to be 37% and 37.2%, respectively, whereas the frequencies of *GGH*-401'T' and *GGH*452'T' alleles were observed to be 29.8% and 16.7%, respectively, in our study population. We observed the frequency of *FPGS* rs1544105'A' allele to be higher (45.6%) in patients with ALL compared to healthy volunteers (37.2%), making it a potential susceptibility factor for the development of ALL. *FPGS* rs1544105 G>A was predicted to modulate the affinity of the cyclic adenosine monophosphate response element-binding protein (CREB) transcription factor. CREB is reported to be overexpressed in childhood ALL and plays an important role in leukemogenesis.[31] The molecular mechanisms involved in the role of CREB in the pathogenesis of ALL ought to be explored in the future. The *FPGS* rs1544105'A' allele was associated with decreased FPGS mRNA levels compared to the 'G' allele in a Chinese population.[7] This might have led to decreased intracellular folate polyglutamates. Folate deficiency can increase the risk of cancer through altered methylation and uracil misincorporation during DNA synthesis. However, the differences in intracellular folate concentrations between 'A' allele carriers and 'G' allele carriers in the future should be measured to validate the above findings. Our study results are similar to Huang *et al.*,[32] but contradictory to the report by Piwkham *et al.* where 'AG' genotypes of *FPGS* rs1544105 and rs10106 were found to be associated with the risk of ALL in the Thai population.[20]

			Frequency in SI (%) (*n*=211)	Frequency in AFR (%) (*n*=661)	Frequency in AMR (%) (*n*=347)	Frequency in EAS (%) (*n*=504)	Frequency in EUR (%) (*n*=503)	Frequency in BEB (%) (*n*=86)	Frequency in GIH (%) (*n*=103)	Frequency in ITU (%) (*n*=102)	Frequency in PJL (%) (*n*=96)	Frequency in STU (%) (*n*=102)
HS	Allele 1	Allele 2										
FPGS	rs1544105 G>A	rs10106 A>G										
HS1	A	G	35.8	48.6*	45.6*	68*	38.1	37.2	41.3	29.4	48.4*	40.7
HS2	G	A	61.6	36.2*	50.7*	30*	59.3	62.8	57.8	69.1	50.5*	57.8
HS3	G	G	1.20	1.80	1.20	1.00	1.00	-	-	-	-	-
HS4	A	A	1.40	13.3*	2.50	1.00	1.60	-	-	1.00	1.00	1.00
GGH	rs11545078 C>T	rs3758149 C>T										
HS1	C	C	69.7	83.0*	77.2*	78.1*	72.0	68.6	69.8	74.5	72.9	70.1
HS2	C	T	13.6	11.4	18.7*	13.2	18.7*	14.0	12.7	11.3	16.7	15.2
HS3	T	T	16.5	5.30*	4.00	8.70*	9.10*	17.4	16.9	14.2	10.4	14.7
HS4	T	C	3.00	3.00	-	-	-	-	5.00	-	-	-

Table 5: Comparison of haplotype frequencies of folylpolyglutamate synthase and gamma-glutamyl hydrolase variants in South Indian population with the superpopulations of 1000 Genomes Project phase-3

*The values are significant (*P*<0.05) when compared to those of South Indian population (SI). -: Indicate either absent or <1%. FPGS – Folylpolyglutamate synthase; GGH – Gamma-glutamyl hydrolase; AFR – Africans; AMR – Americans; EAS – East Asians; EUR – Europeans; SAS – South Asians; BEB – Bengalis from Bangladesh; GIH – Gujarati Indians from Houston; ITU – Telugu from the UK; PJL – Punjabis from Lahore, Pakistan; STU – Sri Lankan Tamils from the UK; HS – Haplotype structures

In the present study, the GGH452'T' allele was not significantly associated with the risk of ALL [Table 2], and our results are in accordance with the previous studies conducted on Mexican[12] and Chinese populations.[25]

Furthermore, in the present study, the *GGH*-401C>T polymorphism was also not associated with the susceptibility to ALL. Our study results are similar to the findings by Koomdee *et al.* in a Thai population,[10] but not in line with a study done on Mexican population where the -401'T' allele was associated with the risk of ALL (*P* = 0.001).[12]

These contradictory results could be due to differences in the frequency distribution of alleles (*GGH*-401'T' allele in SI [29.8%] vs. Thai population [23.5%], *P* = 0.2 and SI vs. Mexicans [14.3%], *P* < 0.05) [Table 5], and also the involvement of other enzymes in folate metabolism and differences in gene-environment interactions. Therefore, the effect of the *GGH*-401C>T polymorphism on the risk of ALL ought to be further studied along with other variants in the genes encoding folate-metabolizing enzymes. Comparison of HS of *GGH* and *FPGS* variants between healthy volunteers and patients with ALL did not show a significant difference [Table 2].

Observation of genotype distribution of studied variants in other ethnicities revealed that *FPGS* rs10106'A' allele frequency was highest in ITU (70.1%) followed by SI populations (63%), and the 'G' allele was found at a higher frequency in the Thai population (72%)[20] followed by EAS (69%). A significant difference existed in the distribution of *FPGS* variant alleles between SI and other populations from the 1000 Genomes Project, except Europeans and SAS [Table 5]. The allelic frequencies of *FPGS* variants in the SI population showed greatest similarity to genetically closer populations such as SAS, except the PJL population. BEB and GIH populations had a high occurrence of the *GGH*-401'T' allele (31.4%) and the *GGH*452'T' allele (17.5%), respectively. In a Mexican population, the frequencies of both *GGH*-401'C' (85.7%) and 452'C' (98.2%) alleles were found to be higher compared to the present study.[12] *GGH*-401'T' allele frequency in the SI population significantly differed from the frequency in a West Indian population.[28] The significant differences in *FPGS* variants between SI and PJL populations and *GGH* variants between SI and West Indians suggest that populations with similar geographical background may not be considered together because they may possess significant differences in their genetic loci.

Haplotype analysis revealed a high LD pattern between studied *FPGS* (rs10106 and rs1544105) variants (D' >0.95). *FPGS* HS2 was the most frequent haplotype in SI, followed by HS1, HS4, and HS3, in descending order. HS3 frequency was found to be <3% in all populations. The frequency of HS4 carrying variant allele of rs1544105'A' was high in AFR (13.3%) whereas it was either absent

or occurred at <3% frequency in other populations. Therefore, the influence of these polymorphisms on disease susceptibility and drug response might vary in AFR, relative to other populations. There was a significant difference in the distribution of the HS of *FPGS* alleles between SI and other populations. *GGH* rs11545078 and rs3758149 alleles were also found to be in strong LD in SI population. HS1 of *GGH* was the predominant haplotype in all populations, followed by HS3 and HS2 in SI, BEB, GIH, and ITU. HS2 is the second most frequent haplotype in AMR, EAS, EUR, PJL, and STU, followed by HS3. HS4 was found at very low frequencies in SI, AFR, and GIH populations and was absent in other populations. The HS3 haplotype (16.5%) of *GGH* carrying variant allele was higher in SI population compared to other populations, except BEB and GIH. Significant differences in HS between SI and other populations could be due to differences in allele frequencies, suggesting interethnic variations in the susceptibility to disease and response to treatment. Limitation of our study might be a lack of data on folate levels that could have strengthened our findings. Therefore, folate levels need to be measured at the time of disease diagnosis in the future. Other variants in the genes encoding folate-metabolizing enzymes also need to be explored, to find a reliable biomarker for susceptibility to ALL disease.

Clinical relevance of *FPGS* and *GGH* variants in acute lymphoblastic leukemia

FPGS and GGH enzymes are involved in both folate and antifolate metabolism. Therefore, changes in the activities of these enzymes due to genetic variants may the influence the levels of antifolates and there by affect the treatment response also. Patients with *FPGS* rs1544105'CC' genotype had lower relapse-free survival (*P* = 0.01) and event-free survival (*P* = 0.04), but did not develop MTX toxicity.[7] Higher FPGS activity was associated with accumulation of long-chain MTXPGs and better overall survival in patients with ALL.[33] The *GGH*452'TT+CT' genotype was associated with increased risk of hepatotoxicity and mucositis, but not hematological toxicity, in a Chinese population.[25] In European children, the *GGH*452'T' allele was associated with thrombocytopenia, but neither *GGH* polymorphisms nor haplotypes were associated with MTX response and survival.[34] *GGH* -401C>T and 'TT' genotype carriers were at increased risk of developing leukopenia and thrombocytopenia after high-dose methotrexate in a Thai population.[10] In a Mexican population, the *GGH*-401C>T polymorphism was found to increase the risk of relapse significantly whereas *GGH* 452C>T polymorphism did not affect ALL outcome.[12] In Chinese patients, a higher serum MTX concentration/dose ratio and a higher concentration of MTX above the therapeutic threshold (>40 μM) were observed in *GGH* rs3758149'CT' or 'TT' genotype carriers when compared to 'CC' genotype carriers after high-dose MTX therapy. However, *FPGS* polymorphism was not

associated with serum MTX levels.[11] The above-observed differences in clinical outcome of ALL between various ethnicities could partly be explained by the differences in the distribution of *GGH* and *FPGS* variant alleles. Therefore, the impact of each SNP on the susceptibility and outcome of diseases might vary among different populations.

Conclusion

In our study, the *FPGS* rs1544105'AA' genotype was found to be associated with the susceptibility to ALL in SI population. Genotype and haplotype distributions of *FPGS* (rs10106 and rs1544105) and *GGH* (rs3758149 and rs11545078) variants in the SI population significantly differed from those of other ethnicities. Our data emphasized that each ethnicity has unique allele frequencies of studied *FPGS* and *GGH* variants. Thus, knowledge of genotype frequency distribution within a population can be useful to tailor drug therapy by optimizing drug doses and identifying potential risk groups which may develop toxicity.

Compliance with ethical standards

All procedures performed in our study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflicts of interest

There are no conflicts of interest.

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