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Research Article

Frequency of polymorphic variants in corticotropin releasing hormone receptor 1, glucocorticoid induced 1 and Fc fragment of IgE receptor II genes in healthy and asthmatic Tamilian population

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ABSTRACT

Background: Asthma is a chronic airway inflammatory disease characterized by increased hyper-responsiveness and recurrent episodes of reversible obstructions. Asthma pharmacogenomic studies report significant association of single nucleotide polymorphisms (SNPs) in genes corticotropin releasing hormone receptor 1 (CRHR1), Fc fragment of IgE receptor II (FCER2) and glucocorticoid induced 1 (GLCCI1) with inhaled corticosteroid (ICS) response. The present study was aimed to establish the allelic and genotypic frequencies of polymorphisms rs242941, rs28364072 & rs37972 in CRHR1, FCER2 and GLCCI1 genes, respectively in Tamilian healthy population and asthma patients and to compare with established frequencies of global populations.

Methods: The study groups consisted of healthy volunteers and persistent asthma patients who were drug naïve or without ICS treatment in the last ≥ 2 months, attending JIPMER hospital (n=111 and 78, respectively). SNP genotyping was done using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) and real time-PCR methods.

Results: Allelic and genotypic frequencies for all the studied variants found to be in hardy-weinberg equilibrium with minor allele frequencies (MAF) of rs 242941, rs 28364072 and rs 37972 at 0.51, 0.33 and 0.38, respectively, in healthy population. No significant difference in gene frequencies was obtained between healthy control and asthma patient groups. Significant difference in allele frequencies was observed between Tamilian healthy and specific global populations. West African frequency was found to be significantly different for all 3 SNPs (p<0.0001).

Conclusions: MAF of rs 242941, rs 28364072 and rs 37972 were 0.51, 0.33 and 0.38, respectively in Tamilian population which were significantly different from various global populations. The frequency distribution found helps to further with ICS response association studies in larger cohorts of asthma patients.

Keywords: Asthma, Tamilian, CRHR1, FCER2, GLCCI1

INTRODUCTION

As per global initiative for asthma (GINA) guidelines 2015, bronchial asthma is defined as a heterogeneous disease characterized by chronic airway inflammation, varying respiratory symptoms (wheeze, cough, shortness of breath and chest tightness) along with variable expiratory airflow limitation and episodes of exacerbations. It is a common respiratory disease affecting 1-18% globally and also a major causative of

poor quality of life, world-wide. Asthma phenotype being heterogeneous with varied underlying mechanisms, till date no strong association was reported between specific pathophysiological processes behind its clinical pattern and treatment response.[1]

In recent years, there is an increasing trend in asthma disease burden among Asian adults which was previously reported to be lower in Asian than Europeans. Thus confirms the expected increase in incidence of adult asthma in near future among Asians. Current adult asthma prevalence in India ranges from 2.4% to 3.5%.[2] As per World Health Survey (2002-03), on region and country-specific estimates of global adult asthma prevalence defined as doctor diagnosed asthma; clinical asthma and only with wheezing symptoms, the prevalence in India was reported as 3.16, 3.30 and 9.63 percentages, respectively.[3]

Currently, more than 150 drugs approved by the U.S-FDA already carry pharmacogenomic labels insisting its growing importance.[4] Inhaled corticosteroids (ICS) are prescribed to uncontrolled asthmatics, with the use of short-acting bronchodilators alone. Though ICS are the most commonly used asthma controller therapy, response varies widely between individuals. Despite maximal beneficial response from clinical observations, inter-individual variability in treatment response rate is significantly large.[5] Corticosteroids mediate immunological actions in treatment of inflammatory diseases but associated with significant lack of response and side effects. There are association studies reporting genetic contribution to ICS response variation in asthma via longitudinal change in lung function, from candidate genes crucial to biologic actions of corticosteroids. Significant inter patient variability in response to ICS and it's associations with pharmacogenomic variability are well reported in past decade.

CRHR1 gene (corticotropin releasing hormone receptor 1; chromosomal locus: 17q21.31; NCBI Gene ID: 1394) with 14 exons, encodes a G- protein coupled receptor, which binds to corticotropin releasing hormone (CRH), the major regulators of hypothalamic-pituitary-adrenal pathway, also involves in immune and stress response via activation of signal transduction pathways.[6] CRHR1 being the predominant CRH receptor in pituitary mediates release of adrenocorticotropic hormone (ACTH) and catecholaminergic response to CRH. Consistent association between specific polymorphisms in CRHR1 and enhanced ICS response was studied in three different populations where individuals homozygous for variant allele 'T' showed 2-4 fold increased response than wild type.[7]

Effect of 17th chromosomal inversion status with or without long-range LD on ICS response was also shown in ACRN population, as the chromosome 17 inversion polymorphism region encompasses the entire CRHR1 gene.[8] Similarly, in a recent North Indian study on

asthmatic children, genotypes GT/TT of the variant rs242941 (G>T) of CRHR1, were found significantly associated with better systemic corticosteroid response during acute exacerbations.[9] In contrast, a study on American children showed association between presence of minor alleles and poor response.[10] In a study on contribution of HPA axis-related SNPs to individual stress response during regular ICS treatment in asthmatic children, cortisol response after treatment was found to be delayed among TT variants.[11] In LOCCS (leukotriene modifier or corticosteroid or corticosteroid-salmeterol) trial, ~169 SNPs in 26 candidate genes were studied in asthma patients (n=189) randomized to 16 weeks of ICS. Five markers in CRHR1 (rs 242941, rs 739645, rs 1876831, rs 1876829 and rs 1876828) showed significant association with change in % predicted of Forced Expiratory Volume in 1second (ΔFEV1%pred), wherein rs242941 was associated with decreased FEV1%pred.[12] Moreover, rs 242941 was also studied as a biomarker for susceptibility to major depressive disorder (MDD) and fluoxetine response variation among Americans, Mexican-Americans and Chinese.[13-17]

GLCCI1 gene (Glucocorticoid induced 1; Chromosomal locus: 7p21.3; NCBI Gene ID: 113263) with eight exons encodes a protein of unknown function which has induced expression by glucocorticoids. A functional variant, rs 37972, C>T (in complete linkage disequilibrium with rs37973) located on GLCCI1 at 1473 bp upstream to ATG start codon, associated with substantial decrements in ICS response among asthmatics was initially reported through GWAS.[18] Moreover, these findings were replicative indicating reduced lung function despite ICS, among subjects with variant allele. Moreover, homozygous mutants 'TT' exhibited significantly higher poor response, compared to heterozygotes or wild-types.[19] No association of rs 37972 with altered ICS efficacy (estimates from exacerbations, hospital visits, oral steroids and asthma control questionnaire) in north European asthmatic children and young adults was also reported.[20] Though steroids are used as the first-line therapy for pediatric nephrotic syndrome, no significant association of rs37972 was found with treatment response variability.[21]

FCER2 gene (Fc fragment of IgE, low affinity II, receptor for (CD23); chromosomal locus: 19 p13.3; NCBI Gene ID: 2208) with 12 exons encodes a B-cell specific antigen which is a low-affinity receptor for IgE, which has its essential role in regulation of B cells and IgE production.[22] Association of polymorphic variant rs 28364072, A >G in FCER2 with increased exacerbations and serum IgE levels in asthma, despite ICS use was earlier reported.[23] Its association with chronic symptoms among steroid treated asthmatic children was also reported, despite recent reports on no association with asthma susceptibility among North Indians.[24,25]

With this background, the present study was undertaken. The primary objectives are to identify the allele and

genotype frequencies of CRHR1 (rs 242941, G >T), GLCCI1 (rs 37972, C>T) and FCER2 (rs28364072, A>G) variants in Tamilian healthy controls and mild to moderate persistent asthma patients. Secondary objectives are to compare the identified gene frequencies between study populations and also to with that of global population.

METHODS

Study population

The present study consists of two groups: Group I comprising of healthy volunteers as participants (n=111) and group II was bronchial asthma patients (n= 78). Unrelated healthy volunteers of Tamilian origin (residing in Tamilnadu or Pondicherry for the past 3 generations and with Tamil language as their mother tongue) and aged 18-50 years of either gender were recruited in the study group I - healthy control (HC). Mild-to-moderate persistent asthma patients attending JIPMER hospital, of Tamilian origin as defined above, aged 18-50 years of either gender who were treatment naive or without ICS treatment in last two months or more were recruited in the study group II - asthma patients (AP). Pregnant and lactating women, chronic smokers (i.e., >20 pk years; 1 Pk year =1 pack/day for 1 year), those under leukotriene, Anti-IgE and other steroid based medications, with COPD reported through <12% FEV1, concurrent upper or lower respiratory tract infections; TB positive were excluded.

Sample collection and DNA extraction

Institute ethics committee approval was obtained prior to start of the study. The purpose of study was explained and written informed consents were obtained. From recruited participants, 5 ml of venous blood was collected. Patient demographic details and anthropometric measurements were documented. Genomic DNA was extracted by standard phenol-chloroform method and stored suspended in Tris-EDTA buffer at -80 °C for future use.[26]

CRHR1 (rs 242941: G>T) genotyping

SNP Genotyping for the variant G>T (rs 242941) of CRHR1 (corticotrophin releasing hormone receptor 1) was done using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method, developed and validated earlier.[27] Primer sequences used are as follows: CRHR1 Forward Primer: 5'-GACACTTCAGGAGGGGAGGGTGGATATG-3'; CRHR1 reverse primer: 5'-CTGAGTCCAGCAGAGAAAGGGAGCCAAT-3'. Complementarity of primer sequences to the target site was confirmed before use through Insilico-PCR method. PCR conditions standardized in our laboratory (step 1: initial denaturation at 95.0°C for 120 s; step 2: 40 cycles of denaturation at 95.0°C for 60 s, Annealing at 68.0°C for 45s, extension at 72.0°C for 60s; step 3: Final extension at 72.0°C for 120 s) were followed using a thermocycler (Applied Biosystems®, U.S.A). The PCR product size was 381 bp. Restriction digestion of the PCR product was carried out through overnight incubation at 37oc with AciI restriction enzyme (New England Biolabs, U.S.A; Restriction site: 5'...C'CGC...3' and 3'...GGG'G...5') using 20 µl reaction mixture (10µl PCR product, 7µl MilliQ, 2.5µl buffer and 0.5µl RE). Digested product size was identified using 2% agarose gel electrophoresis (1X TBE; 180V; 45 minutes). Allelic discrimination was done through differentiation of product SIZES using gel-documentation system, as depicted in Figure 1. The results are validated with replicative analysis of 10% of the samples, in a random manner.
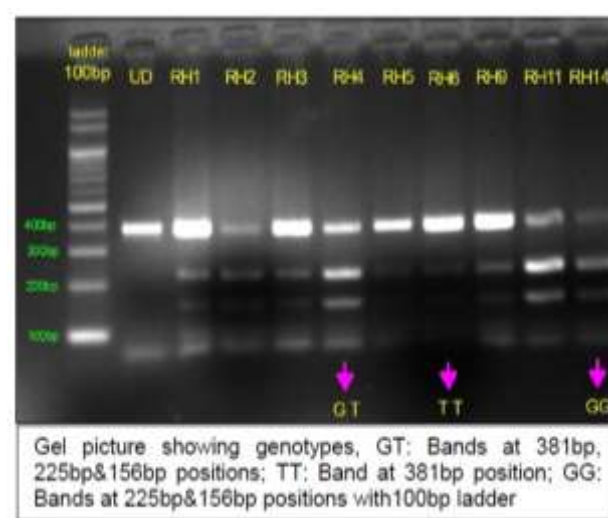

Gel picture showing genotypes, GT: Bands at 381bp, 225bp&156bp positions; TT: Band at 381bp position; GG: Bands at 225bp&156bp positions with100bp ladder

Figure 1: Banding pattern of rs242941 genotyping.

GLCCI1 (rs37972: C>T) and FCER2 (rs 28364072: A>G) genotyping:

SNP genotyping for the variants of GLCCI1 (rs37972: C>T) & FCER2 (rs28364072: A>G) was done through Real Time PCR using Taqman SNP Genotyping assays (Applied Biosystems®, U.S.A), as per manufacturer's recommendations. Allelic discrimination via Absolute Amplification and Quantification was carried out (ABI7300; Applied Biosystems®) with SDS software (ver.1.4). 10µl PCR reaction mixture containing 5µl of universal PCR master mix, 0.25µl of custom TaqMan assay, 2.25µl of autoclaved Milli Q water and 2.5µl of template DNA was prepared for both SNPs studied. Assay was performed using 96 well micro-titre plates loaded with template DNA samples and two NTC (non-template control). The plates were properly sealed with optical adhesive films before run. The reaction was done in 3 steps - pre-read for about 1 minute, followed by amplification for about 1:48 hours and finally post read for 1 minute. The results are obtained after post read run. Allele and genotype count was obtained by direct gene count method and their frequency was calculated. The

results are validated with replicative analysis of 10% of the samples, in a random manner.

Statistical analysis

Genotypic and allelic frequency distribution was checked for hardy-weinberg equilibrium using Chi-Square goodness of fit test. Fisher's exact test with Yate's continuity correction was used to analyse difference in allele frequencies between study groups. Frequencies were compared using Chi-square test for independence at 2 degrees of freedom. Allelic frequencies from the healthy control group of the present study were compared with that of other global populations using Fisher's exact test with Yate's continuity correction. All statistical analyses were done using Graphpad Instat 3 software and p<0.05 was considered significant.

RESULTS

Allele and genotype frequencies

77 female and 34 male healthy volunteers of Tamilian origin were recruited in the study group I-HC (n=111; Mean age±SD: 45.95±10.54 years). Similarly, in group II-AP, 58 female and 20 male mild-to-moderate persistent asthma patients (n=78; Mean age±SD: 37.21±10.2 years) were recruited as per inclusion and exclusion criteria, following GINA guidelines 2014 for interpretation of pulmonary function test parameters. DNA samples isolated from study participants were genotyped for the three genetic variants of interest. All three SNPs were found to be in hardy-weinberg equilibrium (HWE) at different significance levels in both the study groups.

Observed genotypic and allelic frequencies of the variants in study population are summarized in Tables 1 and 2.

CC, CT and TT genotypes of GLCCI1 rs 37972, C>T variant were observed at a frequency of 28%, 42.3% and 29.7% in HC group, whereas as 32.05%, 39.74% and 28.21% in AP group (Table 1), with minor allele frequency (MAF) or 'T' allele frequency at 51% and 48% in HC and AP groups (Table 2), respectively. Genotype frequencies were found to be at HWE (p >0.05). No significant difference was observed for genotype and allele frequencies of rs 37972 between the two study groups.

AA, AG and GG genotypes of FCER2 rs 28364072, A>G variant were observed at a frequency of 46%, 41.4% & 12.6% in HC group, whereas as 53.85%, 35.9% & 10.25% in AP group, with MAF ('G') at 33% and 28% in HC and AP groups, respectively (Table 1, Table 2). Genotype frequencies were found to be at HWE (p >0.05). No significant difference was observed for genotype and allele frequencies of rs 28364072 between the two study groups.

GG, GT and TT genotypes of CRHR1 rs 242941, G >T variant were observed at a frequency of 35.14%, 53.15% and 11.71% in HC group, whereas as 38.18%, 41.82% and 20% in AP group, with MAF ('T') at 38% and 41% in HC and AP groups, respectively. Genotype frequencies were found to be at HWE (p >0.05). No significant difference was observed for genotype and allele frequencies of rs242941 between the two study groups. (Table 1, 2).

Table 1: Comparison of genotype frequencies in Tamilian population.

Genotype frequencies of *GLCCI1* rs 37972					
Genotype	HC (N=111)		AP (N=78)		HC Vs AP	
	n (%)	HWE	n (%)	HWE	X^2 value	P value
		(P value)		(P value)		
CC	31 (28.0)		25 (32.05)			
CT	47 (42.3)	0.11	31 (39.74)	0.072	0.37	0.83
TT	33 (29.7)		22 (28.21)			
Genotype frequencies of *FCER2* rs 28364072					
Genotype	HC (N=111)		AP (N=78)		HC Vs AP	
	n (%)	HWE	n (%)	HWE	X^2	P value
		(P value)		(P value)	value	
AA	51 (46.0)		42(53.85)			
AG	46 (41.4)	0.48	28 (35.9)	0.32	1.16	0.56
GG	14 (12.6)		8(10.25)			
(C) Genotype Frequencies of *CRHR1* rs242941					
Genotype	HC (N=111)		AP (N=55)		HC Vs AP	
	n (%)	HWE	n (%)	HWE	X^2	P value
		(P value)		(P value)	value	
GG	39 (35.14)		21 (38.18))			
GT	59 (53.15)	0.19	23(41.82)	0.32	2.79	0.25
TT	13 (11.71)		11 (20.0)			

HC: Healthy Controls; AP: Asthma Patients; HWE: Hardy-Weinberg Equilibrium; X^2- Chi square

Table 2: Comparison of allele frequencies in Tamilian population.

Allele frequencies of GLCCI1 rs37972					
ALLELE	HC (N=222)		AP (N=156)		P value	OR (95% CI)
	n (%)		n (%)			
C	109	(49)	81	(52)	0.603	0.8931(0.5928; 1.346)
T	113	(51)	75	(48)		
Allele frequencies of FCER2 rs28364072						
ALLELE	HC (N=222)		AP (N=156)		P value	OR (95% CI)
	n (%)		n (%)			
A	148	(67)	112	(72)	0.312	0.7857 (0.5026; 1.228)
G	74	(33)	44	(28)		
Genotype frequencies of CRHR1 rs242941						
ALLELE	HC (N=222)		AP (N=110)		P value	OR (95% CI)
	n (%)		n (%)			
G	137	(62)	65	(59)	0.720	1.1160 (0.6997; 1.779)
T	85	(38)	45	(41)		

N,n represents no. of alleles; Data analyzed using Fisher's exact test with Yate's continuity correction; p <0.05 considered as significant

Comparison with global population

Observed gene frequencies of the variants in the present study were compared to that of established frequencies from global populations which were retrieved from NCBI dbSNP (Build 147), as summarized in the Tables. 3, 4, 5. MAF of rs 37972 in the study population was found to be significantly different from that of West African, Japanese, European and minor populations such as Kenyan and Italian, pooled out from Hap Map submissions.

Table 3: Global frequency comparison of rs37972 C>T (global MAF: 'T'= 0.38).

Population (Region)	N	2N	'A' (n)	'a' (n)	MAF (%)	Diff. (p value)	OR	(95% CI)
SI (Tamilian present study)	111	222	109	113	51			
CEPH*(Multinational)	92	184	94	90	49	0.77	0.92	(0.62; 1.37)
YRI (West Africa)	59	118	97	21	17.8	<0.0001	0.21	(0.12; 0.36)
CEU (North America)	60	120	62	58	48.3	0.73	0.90	(0.58; 1.41)
CHB+JPT (East Asia)	60	120	71	49	40.8	0.09	0.67	(0.42; 1.04)
CEU	113	226	125	101	44.7	0.22	0.78	(0.54; 1.13)
HCB (East Asia)	43	86	49	37	43	0.25	0.73	(0.44; 1.20)
JPT	86	172	106	66	38.4	0.02	0.60	(0.40; 0.90)
YRI	110	220	188	32	14.5	< 0.0001	0.16	(0.10; 0.26)
ASW	47	94	67	27	28.7	0.0003	0.39	(0.23; 0.66)
CHB	41	82	50	32	39	0.07	0.62	(0.37; 1.03)
CHD	85	170	82	88	48.2	0.92	1.04	(0.69; 1.54)
GIH	88	176	78	98	44.3	0.36	1.21	(0.81; 1.80)
LWK	90	180	162	18	10	< 0.0001	0.11	(0.06; 0.19)
MEX	50	100	54	46	46	0.47	0.82	(0.51; 1.32)
TSI	87	174	109	65	37.4	0.008	0.58	(0.38;0.86)

'N,' sample size; 'n,' no. of alleles; Data analyzed using Fisher's exact test; p<0.05 considered significant. MAF: Minor Allele Frequency; SI: South Indian; ASW: African ancestry in Southwest USA; CHD: Chinese in metropolitan Denver, Colarado; *Centre d'Etude du Polymorphisme Human (CEPH) pedigrees (UTAH (93%), French (4%), and Venezuelan (3%) samples from Coriell Cell Repository). CHB: Han Chinese in Beijing, China; CEU: One of the phase 3 HapMap populations; GIH: Gujarati Indians in Houston, Texas; JPT: Japanese in Tokyo, Japan; LWK: Luhya in Webuye, Kenya; MEX: Mexican ancestry in Los Angles, California; NI: North Indian; TSI: Toscans in Italy; #WUSTL Genome Sequencing Center, Washington University in St. Louis Genome Sequencing Center. YRI: Yoruba in Ibadan, Nigeria.

Table 4: Global frequency comparison rs 28364072 A>G (global MAF: 'G'= 0.3978).

Population (Region)	N	2N	'A' (n)	'a' (n)	MAF (%)	Diff. (p-value)	OR (95% CI)
SI (Tamilian present study)	111	222	148	74	33		
YRI (West Africa)	59	118	50	68	42.4	<0.0001	2.72 (1.72; 4.31)
CEU(North America)	60	120	89	31	25.8	0.18	0.70 (0.42; 1.14)
CHB+JPT(East Asia)	60	120	78	42	35	0.81	1.08 (0.67; 1.72)
CSAgilent (Europe)	645	1290	920	370	28.7	0.17	0.80 (0.59; 1.09)
PHAT project	443	886	646	240	27.1	0.07	0.74 (0.54; 1.02)

'N,' sample size; 'n,' no. of alleles; Data analyzed using Fisher's exact test; p<0.05 considered significant. MAF: Minor Allele Frequency; SI: South Indian; CEU: One of the phase 3 HapMap populations; CHB: Han Chinese in Beijing, China; JPT: Japanese in Tokyo, Japan; YRI: Yoruba in Ibadan, Nigeria

Table 5: Global frequency comparison rs242941 G> T (global MAF: 'A'= 0.32).

Population (Region)	N	2N	'A' (n)	'a' (n)	MAF (%)	Diff. (p value)	OR (95% CI)
SI (Tamilian present study)	**111**	**222**	**137**	**85**	**38**		
YRI (West Africa)	59	118	39	79	33.1	<0.0001	3.27 (2.04; 5.22)
CEU (North America)	60	120	85	35	29.2	0.098	0.66 (0.41; 1.07)
CHB+JPT(East Asia)	60	120	105	15	12.5	<0.0001	0.23 (0.13; 0.42)
PHAT project	447	894	586	308	34.5	0.31	0.85 (0.63; 1.15)
NI (North Indian)[27]	100	200	149	51	25.5	0.01	0.55 (0.36; 0.84)

'N,' sample size; 'n,' no. of alleles; Data analyzed using Fisher's exact test; p<0.05 considered significant. MAF: Minor Allele Frequency; SI: South Indian; YRI: Yoruba in Ibadan, Nigeria. CEU: One of the phase 3 HapMap populations; CHB: Han Chinese in Beijing, China; JPT: Japanese in Tokyo, Japan; PHAT: Pharmacogenetics of Asthma Treatment; NI: North Indian.

MAF of rs 28364072, A >G of the present study population was found to be significantly different from that of West Africans only. MAF of rs 242941 in the present study was found to be significantly different from that of West African, East Asian and North Indian population. Frequencies of all the study variants were found to be significantly different from that of West African population, consistently.

DISCUSSION

All the three genetic polymorphisms were found to be in hardy weinberg equilibrium at acceptable significant levels. No difference in MAF is observed between healthy and patient group, suggesting that none of the study variants might be associated with disease and thus we may further proceed with their drug response association. The observed significant difference in MAF between study and global population very well suggests the unique pattern of gene flow in Dravidian ethnicity, which in may be a result of ancestral South Indian (ASI) origin and its admixture. Moreover, the frequencies of all the 3 study variants were found to be significantly different from that of West African population, consistently. This observation may be more evaluated if a natural selection based testing in pharmacogenomic diversity of Dravidian origin is carried out with further research. Steroids being a major class of drug prescribed for most of the chronic illness, development of a predictive tool with the help of

significantly associated genetic biomarkers, which may classify the responders and non-responders group helps understanding its variable response rates and minimizing its adverse events.

MAF of rs 37972 ('T') was 0.51 in present study, which is significantly higher compared to other genetic association studies. Our study findings on rs 37972 are consistent with a recent case-control study on Saudi population, where MAF ('T') were 0.30 and 0.34 in asthma patients and healthy controls, respectively. Though no disease association was observed in our study, MAF is found to be higher in south Indian (0.51) than Saudi population (0.34).[28]

There are similar reports stating higher frequency of 'T' allele, which are consistent with our study results. Reported MAF of rs 37972 ('T') from replicative 4 independent American populations (Non-Hispanic whites) was 0.4.[19] Even in large cohort studies on Netherland and Scotland based children, MAF ('T') of rs 37972 was found to be >0.4.[20] Moreover, reported MAFs of rs37972 ('T') and rs28364072 ('G') in Roman and Hungarian population were 0.55; 0.44 and 0.25; 0.25, respectively.[29]

MAF of rs 28364072 ('G') in the present study was 0.33, which seems similar to other study results. In a genetic association study on north Indian children with asthma susceptibility, MAF of rs 28364072 ('G') was 0.3 and

0.39 in cases and controls, respectively.[25] MAF of rs28364072 ('G') among Northern Europeans, Polish Caucasians, Japanese East Asians and US based mixed populations were 0.28, 0.26, 0.19 and 0.26, respectively.[23]

In a cohort study on ICS response association among US based asthmatic children of mixed ethnicity, MAF of rs 28364072 ('G') and rs 242941 ('T') among ICS non-responders (G= 0.34 and T= 0.41) were found to be significantly higher compared to that of responders (G= 0.23 and T= 0.26).[10] MAF of rs 242941 ('T') was found to be 0.3, as stated in the first reports on pharmacogenomic association with ICS response among American population of mixed ethnicity (Caucasian, African American, Hispanic and others), which is consistent with our study results, i.e. 'T' allele frequency was found as 0.38.[7] Reported MAF of rs 242941 ('T') among Caucasian, Greece, Korean and North Indian populations were 0.34, 0.28, 0.08 and 0.26, respectively.[11,12,27]

From above stated studies on genetic association with ICS response rate, it can be assumed that, carriers of rs 37972 ('T'), rs 28364072 ('G') and rs 242941 ('T') are associated with either decrement or no improvement in lung function in spite of treatment, thus poorly responding to ICS. From the present study findings we conclude that, minor allele frequencies of rs 242941, rs 28364072 and rs37972 were 0.51, 0.33 and 0.38, respectively in Tamilian population which were significantly different from various global populations. As increased number of variant allele carriers is observed in the population, there is chance phenomenon for increased percentage of non-responders to ICS among south Indian asthmatics which has to be explored in further research. Thus the found frequency distribution helps to further with drug response association studies in larger cohorts of asthma patients.

The present study is the first to report the frequencies of genetic variants associated with steroid response in a south Indian Tamilian population. Though only asthma patients were considered for frequency comparison, establishment of allele frequencies of the study variants has its importance even in other diseases requiring long term or short term steroid (Systemic, oral or inhalational) treatment regimen. The STREGA statement's (Strengthening the reporting of genetic association studies (STREGA) - an extension of STROBE statement) recommendations are adapted in reporting the present study.[30]

Genomic biomarker based research is highly determined by the ethnicity and ancestry of the population under investigation. Gene frequency varies across global populations and may or may not exhibit hardy weinberg equilibrium. Genotypic knowledge on the variants of interest in healthy individuals of a population helps to understand the gene flow and possible mutation rates. Moreover, presence of population stratification which may be a potential confounder in genetic association studies has to be identified at the start of any population based genetic study. Study exploring the source of pharmacogenomic diversity in a south Indian Tamilian population with Dravidian origin, as a whole may give a clear understanding for personalized steroid treatment regimen in the future.

With this study we conclude that, the minor allele frequencies of polymorphic variants in CRHR1, GLCCI1 and FCER2 genes associated with inhaled corticosteroid response in Tamilian healthy population, were found to be 0.51, 0.33 and 0.38 for rs 242941, rs 28364072 and rs 37972, respectively, which is significantly different from various global populations. No significant difference in gene frequencies was obtained between healthy control and asthma patient groups of Tamilian origin. West African gene frequency found to be significantly different for all 3 SNPs (p <0.0001). Thus, the found frequency distribution may help to further with drug response association studies in larger cohorts of asthma patients.

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