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Research Article

IDENTIFICATION OF FUNCTIONAL SINGLE NUCLEOTIDE POLYMORPHISMS OF MULTIDRUG RESISTANCE GENE-1 AMONG NEPHROTIC SYNDROME CHILDREN IN SOUTH INDIA

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ABSTRACT

Objective: This study was conducted to determine the frequency of C3435T and G2677T/C single nucleotide polymorphisms of multi drug resistance gene -1 (MDR1) in nephrotic syndrome (NS) children in relation to healthy subjects. The role and association of these SNPs were also determined, whether response and/or resistance to steroid treatment in children with NS in south India.

Methods: Genomic DNA was isolated from 371 blood samples collected from children with NS and controls. Among 173 cases, categorized into steroid-resistant NS (SRNS) were 90 and steroid-sensitive NS (SSNS) were 83 and 198 blood samples were included as controls. All samples were subjected to DNA extraction, and polymerase chain reaction followed by restriction fragment length polymorphism for identification of C3435T and G2677T/C genomic variations.

Results: The frequencies of MDR-1 C3435T, CT, TT, and CC genotypes and SNP G2677T/C GG and G allele genotypes were observed in this study group. In SRNS, children showed significantly higher frequencies of MDR-1 C3435T, CT, TT, and TT+CC genotypes were observed than SRNS and controls. The allele frequencies of SRNS children showed CC - 4%, CT - 32% and TT - 12% and in SSNS children, CC - 10.98%, CT - 27.2% and TT - 13.9% were observed. Furthermore, increased frequencies of MDR-1 C3435T CT, TT, TT+CC genotypes, or T allele were observed in children aged <9 years old. There were no different genotype and allele frequency observed in G2677T/C genotypes NS children and controls.

Conclusion: Based on these data, we are suggesting that MDR-1 C3435T gene polymorphisms are risk factors of increased susceptibility, earlier onset of NS as well as leads to steroid resistance. Whereas SNP G2677T/C gene polymorphisms do not have significant role observed in this study population.

Keywords: Nephrotic syndrome, Steroid-sensitive nephrotic syndrome, Steroid-resistant nephrotic syndrome, Multidrug resistance gene-1, Polymerase chain reaction followed by restriction fragment length polymorphism.

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INTRODUCTION

Nephrotic syndrome (NS) is the most common primary glomerular diseases affecting children in worldwide. In NS patient's immune mechanisms, rather than primary structural defects of the filtration barrier, play a more prominent role in this syndrome. All the NS patients have similar biochemical abnormalities and clinical manifestations, variable grades of steroid responsiveness, and pattern of disease relapse [1]. The laboratory confirmation of NS is massive proteinuria of >40 mg/m^2/hrs or a urine protein/creatinine ratio >2.0 mg/mg and hypoalbuminemia of <2.5 g/dl [2,3]. In remission cases, the reduction in proteinuria is <4 mg/m^2/hrs or urine albumin dipstick of 0 to trace for 3 consecutive days. Relapse cases are defined as recurrence of massive proteinuria of >40 mg/m^2/hrs, urine protein/creatinine ratio >2.0 mg/mg, or urine albumin dipstick ≥2+ on 3 consecutive days. In steroid-sensitive NS, the patients enter remission in response to corticosteroid treatment alone are referred to as having steroid-sensitive NS (SSNS) whereas steroid-resistant NS (SRNS) referred to fail of remission after 8 weeks of corticosteroid treatment referred as SRNS [2,3]. Among these two variations, children with SRNS are the most difficult and therapeutic challenge. The reasons for the steroid resistance among NS could be a change in the histopathologic pattern of the kidney varied from a minimal change disease to mesangial nephropathy or focal segmental glomerulosclerosis (FSGS) [4]. A report of the International Study of Kidney Disease in Children (1981) demonstrated that the patients who were initial steroid responders and those who were not, 3% and 47.5% proved as FSGS, respectively [2]. Among nephrotic syndrome, FSGS cases shows more resistant to drug treatment than other forms. Incidence and response to drug treatment also varied from ethnicity. As well as NS likely that genetic and environmental risk factors play a significant role in explaining these ethnic differences among children [5].

Genetic variations play a major role in this disease; till date, there are many genes are identified among NS patients. Among these, multidrug resistance gene-1 (MDR-1) plays a vital role among NS patients as well as other diseases. The gene which encodes for a transmembrane P-glycoprotein (P-gp), with 170 kD belonging to the superfamily of ATP-binding cassette (ABC) transporters that play an important role in controlling drug uptake and excretion [6,7]. P-gp is essential for many cellular processes that require the transport of substrates across cell membranes [8]. In lymphocytes, the MDR-1 gene expression has been negatively correlated with the response to the drugs of prednisone, cyclophosphamide, and cyclosporine A among NS children [9]. The MDR-1 gene polymorphisms can alter the drug transport function of P-gp and are often associated with resistance to vast of the drugs, results treatment failure. MDR-1 polymorphisms at 1236, 2677, and 3435 positions significantly minimize P-gp functionality *in vitro*, the extent of which appears to be substrate dependent [10]. So far, around 50 single nucleotide

polymorphisms (SNPs) were identified in MDR-1 gene, among these more attention has been focused at position 3435 which is situated in exon 26 (C3435T). This is the only silent polymorphism identified that might influence P-gp expression in different human tissues in different races [11]. Based on the origin and genetic nature, Indians are grouped into (i) the Indo-Aryan present in North India and (ii) the Dravidian are predominantly in south India [12]. Both origins are genetically dissimilarities for centuries. MDR-1 gene polymorphisms of the South Indian population are significantly different from other populations [13]. Patients from Northern India showed that NS patients carrying homozygous mutations in G2677T/C are more prone to developing steroid resistance. The synergistic effect of the presence of mutant genotype of the G2677T/C and C3435T MDR-1 gene in different combinations may increase the risk of developing steroid-resistance in NS patients [14].

MDR-1 gene polymorphisms among NS children of South Indian origin, who fail to show a remission with steroids (steroid-resistant), those who fail to have a long remission (frequently relapsing), and those who cannot be weaned from steroids (steroid-dependent) [15]. Hence, a rescue therapy in difficult cases of infantile NS is needed to avoid unnecessary exposure of toxic drugs to children. Currently, an optimal combination of immunosuppressive agents to induce remission and to reduce the undesirable effects of high dose and prolonged administration of drugs is still a challenge [16,17].

Therefore, we aimed to study on MDR-1 gene polymorphisms among South Indian children who were confirmed with NS. Whether the MDR-1 gene polymorphisms induce NS and lead to SRNS. The study was designed to validate the SNP frequencies and compared with clinical data among SSNS and SRNS patients and control groups.

METHODS

Patients

This study was approved by the Institutional Ethical Committee of SRM Medical College Hospital and Research Centre (No. 347/IEC/2012), Chennai, India.

A total of 173 NS children were enrolled in this study after screened, diagnosed, and confirmed for NS. Among these, 98 were males and 75 were females in the age ranged from 1 to 15 (median age=6.94) years. For controls, 198 healthy children age ranged from 1 to 15 years (median age=7.2) were included in this study. All patients and controls were ethnically from South Indian origin. Informed consent was obtained from all children's parents before enrolled into this study. Demographical details were obtained and recorded individually from all study children (Table 1) for further analysis. For about 2 ml of blood samples were collected in anticoagulated tubes from all children. All blood samples were stored at −70°C for till further process. Biochemical parameters were analysis by a standard protocol for all the patients, and the results were recorded.

Primer design and SNP analysis

There are three functional SNPs were identified in MDR-1 gene in worldwide; among these, two major role-playing SNPs were selected for this study. Genotyping of C3435T (rs1045642) and C2677T (rs2032582) SNPs was carried out for all the study participants. Genomic DNA was isolated using the standard salting-out DNA extraction method from all the blood samples, DNA was subjected into polymerase chain reaction (PCR) protocol. Primers were designed based on tetra-primer amplification refractory mutation system PCR concept used in the following website tool (http://cedar.genetics.soton.ac.uk). The PCR products were subjected to restriction fragment length polymorphism with the use of restriction enzymes of BSrI for fragmentation of G2677T SNP and Sau3AI used for fragmentation of C3435T SNP. The enzymatically digested PCR products were subjected to electrophoresis, and the fragments were resolved by 3.5% agarose gel stained with ethidium bromide dye. After electrophoresis, the banding pattern was analyzed (Fig. 1).

The outcome of this study of MDR-1 haplotype frequencies was compared among SSNS and SRNS children of various clinical variables as well as a control group.

Statistical analysis

Data were analyzed by SPSS statistical software version 16.0. The odds ratio at 95% confidence interval facilitated the comparison of genotype frequency distribution between the SSNS, SRNS, and controls and $p<0.05$ was considered statistically significant.

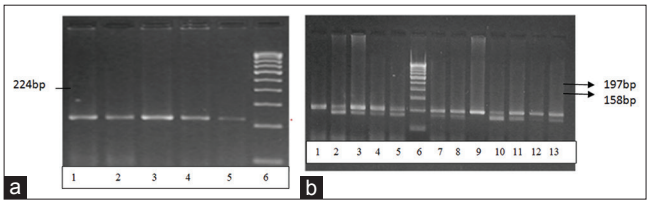

Fig. 1: Electrophoretic patterns for multidrug resistance gene-1 polymorphisms evaluated by polymerase chain reaction followed by restriction fragment length polymorphism-based assay, M (Marker, 100 bp ladder), and lane 6 markers: (a) 2677T>G: GG genotype lane 1-5. (b) C3435T>C: TT genotype (lane 1, 9), CT genotype (lane 2, 3, 4, 5, 7, 8, 10, 11, 12, 13)

Table 1: Demographic details about the study children and distribution of their allele frequencies

S. No.	Clinical data	#	%	CC	CT	TT	C allele	T allele
1	Abdominal pain	23	13.3	2	15	6	19	27
2	Abdominal pain and distension	13	7.5	2	6	5	10	16
3	Fever	12	6.9	1	9	2	11	13
4	Fever, Abl pain, urinary infection	6	3.5	1	3	2	5	7
5	Fever and infection	6	3.5	1	3	2	5	7
6	Dysuria	12	6.9	2	8	2	12	12
7	Nocturia and enuresis	6	3.5	0	3	3	3	9
8	OL and dysuria	9	5.2	1	7	1	9	9
9	OL and fever	10	5.7	1	7	2	9	11
11	OL and headache	2	1.2	0	0	2	0	4
12	OL, abdominal pain, and distension	15	8.7	4	7	4	15	15
13	OL	41	23.7	9	22	10	40	42
14	Polyuria and abdominal pain	1	0.6	1	0	0	2	0
15	Polyuria, abdominal Pain, and distension	7	4	1	6	0	8	6
16	Polyuria and enuresis	10	5.8	0	6	4	6	14
Total		173	100	26	102	45	154	192

OL: Oliguria

RESULTS

Demographic data

A total of 173 children with NS were included in this study, and 198 healthy children with age- and sex-matched were included as controls in this study. Among NS children, 98 (56.6%) were male children and 75 (43.4%) were female children, age ranged from 1 to 15 years in the median age of 6.94 years. The NS children were grouped into SRNS - 90 (52%) and SSNS - 83 (48%) in the mean age of onset was lower in SRNS (6.63 years) than SSNS (7.21 years). All study children were ethnically from South Indian origin.

Biochemical parameters

Serum was separated from blood samples for biochemical analysis. The biochemical parameters were done as per the protocol and results were recorded for further analysis. Proteinuria was the most common manifestation (100%) of all NS children. Biochemical parameters were studied and it found oliguria - 45%, abdominal pain - 20%, fever - 14% and polyuria - 11%; nocturia and dysuria were also reported in few cases. Biopsy was done and showed positive for 52 (30%) children of the total study population. The mean level of serum creatinine and albumin was slightly higher in SRNS (1.01 mg/dl and 2.37 mg/dl) than SSNS (0.73 mg/dl and 2.2 mg/dl) children. However, p-value of serum creatinine and serum protein was <0.05 on compare with other biochemical parameters (serum albumin and urea in urine p-value ≥0.05) (Table 2). Symptoms of either one/two or relevant to NS were observed 100% of patients. Family history was representing in 69.4% among the total study population with male-predominant (60.8%) children. Biopsy was done on 52 (30.1%) cases of the total study population. Of this, minimal change nephrotic syndrome (MCNS) shows a high frequency of 24.3% than FSGS (4.6%) and MCNS-hemolytic uremic syndrome was 2.1% (Table 3). On compare with NS children, the allele frequencies were little higher in NS children on compare with control children. When compared with T allele frequency, p-value was low as (p≤0.065) on compare with C allele frequencies (p<0.726).

Genomic data analysis

The genotypic frequencies of C3435T were 11.5% for CC, 55.8% for CT, and 32.7% were TT genotypes among NS children. In NS children, hematuria was reported in 3 (1.7%) cases and their genotype frequencies observed as 2 CT and 1TT. A total of 38 cases were administered second-line treatment of cytotoxic drug. Allele frequencies among biopsy-proven cases were T allele, i.e. 60.6% and 39.4% were C allele. Genotypic frequencies were observed G2677T/C, CT, TT, and TT+CC, whereas CT frequencies were high among children with family history (69.6%) than nonhistory (30.4%). In children with NS showed CT frequency (59.2%) was high when compared to control. On the contrary, the control group genotypic of TT (63.3%) frequencies was higher on compared to NS children (Table 4). Whereas comparing between SSNS and SRNS children, CT+TT frequencies and T allele frequencies were same, p≤0.032. Whereas TT genotype and C allele were higher in frequencies, p=0.864 (Table 5).

Table 2: Biochemical analysis of SSNS and SRNS of the study children, data analysis was done by Levene's test for equality of variances method

Biochemical parameters	Value range (mg/dl)	SRNS		SSNS		95% CI for mean difference	p-value
		Mean	Standard (X)	Mean	Standard (X)		
Serum creatinine (mg/dl)	0.2-5.1	1.01	0.955	0.723	0.316	0.49-0.76	0.008
Serum protein (mg/dl)	1.5-8.7	4.475	1.278	4.854	0.986	0.39-0.72	0.029
Serum albumin (mg/dl)	1-5	2.377	0.803	2.172	0.741	0.44-0.027	0.83
Proteinuria (mg/dl)	54-975	380.06	201.424	402.267	202.198	38.42-82.83	0.471

SSNS: Steroid-sensitive nephrotic syndrome, SRNS: Steroid-resistant nephrotic syndrome, CI: Confidence interval

Table 3: Comparison of C3435T genotype and allele frequencies among SSNS, SRNS, and remission, relapsed with control groups

SNP's	Genotype /allele	Male		Female		p-value	SSNS (%)	SRNS (%)	With family History	Without family	Remission (%)	Relapsed (%)
		NS cases	Control	NS Cases	Control							
MDR-1 C3435T	CC	14 (8.1)	25 (12.6)	12 (6.9)	20 (10)	0.06	7 (8.4)	19 (21.1)	17 (9.8)	9 (5)	15 (15.2)	11 (14.9)
	CT	58 (33.5)	65 (32.8)	44 (25.4)	33 (16.7)	0.07	55 (66.3)	47 (52.2)	71 (41)	31 (17.9)	62 (62.6)	40 (31.1)
	TT	26 (15)	30 (15.2)	19 (11)	25 (12.7)	0.72	21 (25.3)	24 (26.7)	32 (18.5)	13 (7.5)	22 (22.2)	23 (54.0)
	C allele	86 (24.9)	115 (29)	68 (19.6)	73 (18.4)	0.72	69 (19.9)	85 (24.6)	105 (30.4)	49 (14.2)	92 (26.6)	62 (17.9)
	t allele	110 (31.8)	125 (31.6)	82 (23.7)	83 (21)	0.06	97 (28)	95 (27.5)	135 (39)	57 (16.4)	106 (30.6)	86 (24.9)
MDR-1	GG	98 (56.6)	120 (60.6)	75 (43.4)	78 (39.4)	-	-	-	-	-	-	-
	G allele (%)	100	100	100	100		-	-	-	-	-	-

SSNS: Steroid sensitive nephrotic syndrome, SRNS: Steroid-resistant nephrotic syndrome

Table 4: Distribution of genotypes and alleles frequencies of MDR-1 gene polymorphisms of SNP C3435T among children with NS and controls

Genotypes/alleles	Groups	Controls		NS children	
		(n=198)	Odds ratio (95% CI)	(n=173)	Odds ratio (95% CI)
C3435T Genotypes	CC	45	2.059 (0.922-4.599)	52	0.805 (0.348-1.861)
	CT	98	0.696 (0.380-1.277)	102	1.403 (0.759-2.592)
	TT	55	0.884 (0.454-1.722)	45	0.757 (0.382-1.501)
	CT+TT	153	0.486 (0.217-1.084)	147	1.243 (0.537-2.874)
	C	188	1.131 (0.581-2.204)	154	1.321 (0.666-2.619)
Alleles	TS	208	0.486 (0.217-1.084)	192	1.243 (0.537-2.874)

MDR-1: Multidrug resistance gene-1, CI: confidence interval, NS: Nephrotic syndrome, SNP: Single nucleotide polymorphism

Table 5: Genotypic frequency comparison among SSNS and SRNS children

Genotypes/alleles	Groups	SSNS n=83 (%)	SRNS n=90 (%)	p-value	Odds ratio (95% CI)
C3435T Genotypes	CC	7 (8.4)	19 (21.1)	0.032	2.905 (1.152-7.327)
	CT	55 (66.3)	47 (52.2)	0.065	0.556 (0.301-1.029)
	TT	21 (25.3)	24 (26.7)	0.864	1.074 (0.544-2.120)
	CT + TT	76 (91.6)	71 (78.9)	0.032	0.344 (0.136-0.868)
	C	62 (74.7)	66 (73.3)	0.864	0.931 (0.472-1.840)
Alleles	T	76 (91.6)	71 (78.9)	0.032	0.344 (0.136-0.868)

SSNS: Steroid-sensitive nephrotic syndrome, SRNS: Steroid-resistant nephrotic syndrome, CI: confidence interval

DISCUSSION

NS is very common in the form of glomerular disease in childhood. Children with SRNS cause most difficult therapeutic challenge to clinicians. Studies shown glucocorticoid remains the mainstay of childhood NS treatment. There are many factors induced to modulate NS response to drug intervention such as the expression of P-gp, a product of MDR-1 [18,19]. In the kidney, the P-gp is expressed in the brush border membrane of proximal tubular epithelial cells [20]. Lung Resistance Protein (LRP) is a drug resistance protein which is highly expressed in tumors and drug resistance cell lines, and it does not belong to the ABC transporter family also have a significant role [21].

In this study, we investigated two SNPs, G2677T/C (rs2032582) in exon 21 and C3435T (rs1045642) in exon 26 which were more frequently reported worldwide and also seems to influence P-gp function [22,23]. Mutant genotypes of SNPs G2677T/C and C3435T were significantly higher in children with NS compared to healthy participants [24,25]. We also found that the SNP C3435T variations were high among patients on compare with healthy children. In this study, distribution of C3435T, CC, CT, and TT genotype frequency is close to that in the Asians such as Japanese, Chinese, and Indian population [26]. In this study, the genotypic distribution of CT, TT, and CC was relatively high on compare with controls were as are showed C3435T, CT, CC, and TT genotype (Figs. 1a and b). The genomic frequencies among patient's allele frequencies of CT were showed high frequencies than other alleles. The non-synonymous SNP (2677T>G/C) has also been found to be associated with altered expression, activity, and the substrate specificity of P-gp [27,28]. We also found that the C3435T, CC, CT, and TT allele frequencies had induced SRNS in children on compared with SSNS and control groups. Genomic frequencies of CC=4%, CT=32%, and TT=12% were found among SRNS, but in steroid responsive, were CC=10.98%, CT=27.2%, and TT=13.9% frequencies slight variations. In these genotypes of C3435T, CC genotype showed the high-level frequencies among patients on compared with SSNS and control groups. Whereas in the genotype frequencies data, the frequency was observed in patients were CC=15%, CT=59%, and TT=26% and controls were CC=26%, CT=48.5%, TT=25.5% observed, respectively. The silent 3435T>C polymorphism may have some effects on DNA structure, RNA stability, and P-gp function, or it is in linkage disequilibrium with other functional MDR-1 polymorphisms [29]. Our data showed that there was no such significant role of SNP G2677T/C among these study children. C3435T gene polymorphism significantly differs among populations and ethnicity. In this study, we found that the MDR-1 SNP C3435T polymorphism plays a significant role among NS children. In this study, the SNP G2677 T/C polymorphism did not show genomic variations and no role at all.

CONCLUSION

In this study, we selected two SNPs of G2677T/C and C3435T polymorphisms in MDR-1 gene which were more frequently reported in the world of childhood NS. Among these two SNPs, C3435T causes NS and leads to SRNS in children. However, in this study group, SNP G2677T/C did not show any variation. In the future, the more number of samples size and different groups of study population can reveal the right situation of the MDR-1 C3435T gene polymorphisms.

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REFERENCES

1. Ahmad H, Tejani A. Predictive value of repeat renal biopsies in children with nephrotic syndrome. Nephron 2000;84(4):342-6.
2. The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. A report of the International Study of Kidney Disease in Children. J Pediatr 1981;98(4):561-4.
3. Niaudet P. Steroid-resistant idiopathic nephrotic syndrome in children. In: Avner ED, Harmon WE, Niaudet P, editors. Pediatric Nephrology. Philadelphia, PA: Lippincott Williams & Wilkins; 2004.
4. Klimecki WT, Futscher BW, Grogan TM, Dalton WS. P-glycoprotein expression and function in circulating blood cells from normal volunteers. Blood 1994;83(9):2451-8.
5. Chanchlani R, Parekh RS. Ethnic differences in childhood nephrotic syndrome. Front Pediatr 2016;4:39.
6. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 2001;11(7):1156-66.
7. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 1993;62:385-427.
8. Lespine A, Ménez C, Bourguinat C, Prichard RK. P-glycoproteins and other multidrug resistance transporters in the pharmacology of anthelmintics: Prospects for reversing transport-dependent anthelmintic resistance. Int J Parasitol Drugs Drug Resist 2011;2:58-75.
9. Lin JH. Drug-drug interaction mediated by inhibition and induction of P-glycoprotein. Adv Drug Deliv Rev 2003;55(1):53-81.
10. Salama NN, Yang Z, Bui T, Ho RJ. MDR1 haplotypes significantly minimize intracellular uptake and transcellular P-gp substrate transport in recombinant LLC-PK1 cells. J Pharm Sci 2006;95(10):2293-308.
11. Li YH, Wang YH, Li Y, Yang L. MDR1 gene polymorphisms and clinical relevance. Acta Genet Sin 2006;33(2):93-104.
12. Umamaheswaran G, Krishna Kumar D, Kayathiri D, Rajan S, Shewade DG, Dkhar SA, *et al*. Inter and intra-ethnic differences in the distribution of the molecular variants of TPMT, UGT1A1 and MDR1 genes in the South Indian population. Mol Biol Rep 2012;39(5):6343-51.
13. Youssef DM, Attia TA, El-Shal AS, Abduelometty FA. Multi-drug resistance-1 gene polymorphisms in nephrotic syndrome: Impact on susceptibility and response to steroids. Gene 2013;530(2):201-7.
14. Emeneau MB. India as a lingustic area**.** Language 1956;32(1):3-16.
15. Anochie I, Eke F, Okpere A. Childhood nephrotic syndrome: Change in pattern and response to steroids. J Natl Med Assoc 2006;98(12):1977-81.
16. Coppo R. Non-steroidal and non-cytotoxic therapies for nephrotic syndrome. Nephrol Dial Transplant 2008;23(6):1793-6.
17. Moustafa BH, Tolba OA. Immunosuppressive therapy in children with steroid-resistant, frequently-relapsing, and steroid-dependent idiopathic nephrotic syndrome: A single center experience. Electron Physician 2016;8(2):2039-47.
18. Peña A, Bravo J, Melgosa M, Fernandez C, Meseguer C, Espinosa L, *et al.* Steroid-resistant nephrotic syndrome: Long-term evolution after sequential therapy. Pediatr Nephrol 2007;22(11):1875-80.
19. Cizmarikova M, Podracka L, Klimcakova L, Habalova V, Boor A, Mojzis J, *et al.* MDR1 polymorphisms and idiopathic nephrotic syndrome in Slovak children: Preliminary results. Med Sci Monit

2015;21:59-68.
20. Kim HJ, Kim HK, Kwon JT, Lee SH, El Park S, Gil HW, *et al.* Effect of MDR1 gene polymorphisms on mortality in paraquat intoxicated patients. Sci Rep 2016;6:31765.
21. Sreenivasan S, Alameen M, Krishnakumar S, Vetrivel U. Computational docking studies reveal the most potential inhibitory binding mode of demethoxylated and reduced furcumin compounds on lung resistance protein. Int J Pharm Pharm Sci 2014;6(5):234-8.
22. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci U S A 1987;84(21):7735-8.
23. Xue Y, Zhao ZQ, Hong D, Zhang HJ, Chen HX, Fan SW. MDR1 gene polymorphisms are associated with glucocorticoid-induced avascular necrosis of the femoral head in a Chinese population. Genet Test Mol Biomarkers 2014;18(3):196-201.
24. Alzoubi KH, Khabour OF, Al-Azzam SI, Mayyas F, Mhaidat NM. The role of multidrug resistance-1 (MDR1) variants in response to atorvastatin among Jordanians. Cytotechnology 2015;67(2):267-74.
25. Omar MS, Crowe A, Hughes J. Distribution of the single nucleotide polymorphism C3435T of MDR1 gene among people in Western Australia, Australia. Int J Pharm Pharm Sci 2013;5(4):470-3.
26. Jafar T, Prasad N, Agarwal V, Mahdi A, Gupta A, Sharma RK, *et al.* MDR-1 gene polymorphisms in steroid-responsive versus steroid-resistant nephrotic syndrome in children. Nephrol Dial Transplant 2011;26(12):3968-74.
27. Farhat K, Waheed A, Hussain A, Iqbal J, Mansoor Q, Ismail M. Polymorphisms of the ABCB1 gene in the Pakistani population. J Coll Physicians Surg Pak 2015;25(7):482-5.
28. Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, *et al.* Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 2001;70(2):189-99.
29. Sakurai A, Onishi Y, Hirano H, Seigneuret M, Obanayama K, Kim G, *et al.* Quantitative structure-activity relationship analysis and molecular dynamics simulation to functionally validate nonsynonymous polymorphisms of human ABC transporter BCB1 (P- glycoprotein/ MDR1). Biochemistry 2007;46(26):7678-93.