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Genetic variations of *CYP2D6*, *CYP3A4* and *CYP3A5* in systemic lupus erythematous pediatric patients

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Abstract: This study aimed to investigate genetic variations of *CYP2D6*, *CYP3A4* and *CYP3A5* in systemic lupus erythematous (SLE) pediatric patients. Methods: *CYP2D6*2*, **4*, **5*, **10*, **41* and *CYP3A4*22* and *CYP3A5*3* were determined with commercially available TaqMan assays in 75 SLE patients and 145 healthy study participants. Results: The *CYP2D6*2* allele was the most common variant alleles among SLE patients and the healthy participants. No homozygous mutation was detected for *CYP3A4*22* in both studied groups. No significant difference in the distribution of allele frequencies was observed between the two groups. Conclusion: The results of this study confirm that there was no difference in the frequency of genetic variations in *CYP2D6*, *CYP3A4* and *CYP3A5* between controls and pediatric patients with SLE. Nonetheless, more extensive investigations with a greater number of patients are required to validate and strengthen our findings.

keywords: Systemic lupus, *CYP2D6*, *CYP3A4*, *CYP3A5*, Polymorphism

1.Introduction

Systemic Lupus Erythematous (SLE) is a chronic immune-mediated systemic disease of heterogeneous clinical phenotypes with variations in disease severity, characterized by a tendency for flare [1]. The incidence of SLE is ten-fold higher rate in females than males in adults, but male patients typically have more severe manifestations and outcomes [2, 3]. When compared to adults, the female to male ratio in juveniles is about five times higher [4, 5]. One of the most important phase I drug enzymes contributing to the metabolization of a significant number of medicinal drugs is cytochrome P450 (*CYP450*) [6-8]. *CYP2D6* polymorphism has been linked to susceptibility to autoimmune diseases, including SLE [9].

To date, at least 149 allelic variants for the *CYP2D6* gene, 35 for the *CYP3A4* gene, and 9 for the *CYP3A5* gene (not including suballeles) have been described and are available online at PharmVar [10-12]. *CYP2D6*1* serves as the reference allele ‘’wildtype’’, and therefore any allele might be assigned by default as *CYP2D6*1* when no SNPs are identified. Despite the fact that *CYP2D6*2* has slightly decreased function owing to the presence of two missense SNPs, *2851C>T* (rs16947) and *4181G>C* (rs1135840), which cause amino acid changes, this difference is not substantial, consequently *CYP2D6*2* is regarded as a wild-type enzyme activity and is categorized as a normal function allele [13, 14]. The non-function *CYP2D6*4* allele is defined by the presence of the splicing defect variant *1847G>A* (rs3892097) and the *100C>T* (rs1065852) in all of its thirty sub-alleles that have been identified to date on the PharmVar Consortium, except for *CYP2D6*4.012* where the *1847G>A* (rs3892097) has been detected without *100C>T* (rs1065852) being present [15].

The *CYP2D6*10* is characterized by the presence of the *100C>T* (rs1065852) SNP and the absence of *1847G>A* (rs3892097), resulting in P34S substitution, which has been linked to decreased *CYP2D6* expression and catalytic activity [16, 17]. The reduced *CYP2D6*41* function allele is defined by the presence of *2989G>A* (rs1135840) in combination with *2851C>T* (rs16947) and *4181G>C* (rs1135840) [18].

*CYP3A4*22* is one of the most significant alleles associated with decreased clearance and, as a result, lower dose requirements for a variety of drugs. This allele is characterized by the presence of *15389C>T* (rs35599367), which results in an alternative splice in intron 6 and, consequently impacts mRNA expression and decreases *CYP3A4* enzyme activity [19]. The most frequent SNP *6981A>G* (rs776746) in intron 3 (*CYP3A5*1* has an *A* at this location) that characterizes the *CYP3A5*3* causes atypical RNA splicing, resulting in a premature stop codon and, as a result, truncation of the *CYP3A5* protein that lead to protein inactivity [20].

Hence, this study aimed to find associations between SLE autoimmune disease in pediatric patients and functional *CYP2D6*2, *4, *10* and **41, CYP3A4*22,* and *CYP3A5*3* polymorphisms.

2. Materials and methods

We followed up 75 patients with systemic lupus with a diagnosis of SLE according to the SELENA-SLEDAI criteria [21]. Another group of 145 healthy participants was included also in the research. Participants in the study were recruited at Mansoura University Children's Hospital in Egypt. The study was authorized by the University of Mansoura's Institutional Research Board.

2.1. Genotyping:

TaqMan assays (Applied Biosystems, CA, USA) were used to genotype DNA samples from SLE patients and 145 healthy volunteers for the following SNPs: rs35599367, rs16947, rs1065852, rs3892097, rs2837172 and rs776746. PCR conditions were conditioned as previously described [22].

2.2.Statistical Analysis:

Data expression of qualitative data was reported as absolute frequency (N) and relative frequency (%, percentage) while quantitative data was reported as mean ± standard deviation (SD) if normally distributed or median and interquartile range (IQR) if not normally distributed. The Chi-square test was utilized to analyze the difference between allele frequencies of SLE patients and the healthy cohort. A p-value of < 0.05 was used to represent statistical significance.

3. Results and Discussion

The majority of patients were females [N=63 (84%)]. The demographic characteristics of these SLE patients are shown in (**Table 1)**. Fever, oral ulcers, and arthritis were among the symptoms that occurred 20-40% of the time. While vasculitis, neurological symptoms, serositis, and visual problems were among the manifestations with a frequency of less than 20%, malar rash, photosensitivity, and alopecia were more than 40% of the time **(Table 2)**.

Table (1): Demographic characteristics of SLE patients

Qualitative	**N**	**(%)**
Sex (Male/Female)	12 / 63	(16) / (84)
Positive consanguinity	17	(22.7)
Positive family history of autoimmune diseases	15	(20)
Quantitative	Median	$25^{th} – 75^{th}$ percentile
Age (years)	15	12 – 16
Duration SLE (years)	3	1 – 4
Quantitative	Mean	Standard deviation
BMI (kg/m^2)*	23.05	4.4

*BMI, Body mass index. Data presented as N (%) for qualitative data and mean ± SD or median (25^{th} percentile-75^{th} percentile) for quantitative data.

Table (2): Clinical manifestations of SLE patients

Clinical manifestations	**SLE patients**	**%**
Neurologic disorder	7	9.3
Visual disturbances	2	2.7
Vasculitis	7	9.3
Arthritis	30	40
Renal disorder	54	72
Malar rash	46	61.3
Photosensitivity	33	44
Non-scarring alopecia	35	46.6
Oropharyngeal ulcers	21	28
Serositis (pleurisy, pericarditis or myositis)	8	10.6
Fever	25	33.3

Previous studies of SLE disease in juveniles have shown a marked prevalence in females, which was also observed in this study, with a female to male ratio of 5.25 to 1 [4, 5]. *CYP2D6* has been thoroughly investigated and proven to have a role in the metabolism ofat least 20–25% of all drug metabolism of all clinically prescribed drugs, including analgesics, antidepressants, anti-arrhythmics, and tamoxifen [7, 8, 23].

CYP3A4 and *CYP3A5* have also been identified as significant enzymes in the metabolism of a variety of therapeutically important medications [6, 23-25]. The current study demonstrates the genetic variations of *CYP2D6*2, 4, *10, *41, CYP3A4*22, and CYP3A5**3 polymorphisms in SLE pediatric patients.

The *CYP2D6*2* allele was the most common variant alleles among SLE patients. There was no identification of a single participant of homozygous mutation for *CYP3A4*22* in both studied groups. The high frequency of the *CYP3A5*3* allelic variant (*6986A>G*) was comparable to that seen in the healthy participants. No significant difference was observed between the observed frequencies of SLE patients and the 145 healthy control subjects (**Table 3)**. The results of this study confirm that there was no difference in the frequency of genetic variations in *CYP2D6*, *CYP3A4* and *CYP3A5* between controls and pediatric patients with SLE.

Matching findings were demonstrated by Kortunay, Bozkurt [26], which indicated that no difference in *CYP2D6* between SLE and control was observed. Moreover, in the study of Baranska, Rychlik-Sych [27], of genetic polymorphisms of *CYP2D6* in patients with systemic sclerosis, no effect of the *CYP2D6* gene mutations on the incidence of SLE was reported. In contrast to these findings, a meta-analysis study explored whether functional *CYP2D6* polymorphisms are associated with susceptibility to autoimmune diseases indicated that *CYP2D6*4* and **3* polymorphisms were associated with susceptibility to autoimmune diseases in Caucasians [27].

In conclusion, the current study determined that there was no difference in the frequency of genetic variations in *CYP2D6*, *CYP3A4* and *CYP3A5* between controls and pediatric patients with SLE. However, more comprehensive studies with larger number of patients are needed to confirm and strengthen the clinical utility of our findings.

Table (3): Comparison of *CYP2D6*, *CYP3A4* and *CYP3A5* variants frequencies in all studied SLE patients and healthy control subjects.

*Denotes the p-value for Chi-Square.

Star alleles with SNP	Variant	SNP	genotype	SLE patients	Control	P*
CYP2D6*2, *41	2851C>T	rs16947	(C/C)	15	33	0.9
			(C/T)	38	70	
			(T/T)	22	42	
CYP2D6*4	1847G>A	rs3892097	(G/G)	60	116	0.56
			(G/A)	15	29	
			(A/A)	0	0	
CYP2D6*4, *10	100C>T	rs1065852	(C/C)	0	1	0.72
			(C/T)	21	37	
			(T/T)	54	107	
CYP2D6*41	2989G>A	rs28371725	(G/G)	55	103	0.94
			(G/A)	19	40	
			(A/A)	1	2	
CYP3A4*22	15389C>T	rs35599367	(C/C)	73	140	0.55
			(C/T)	2	5	
			(T/T)	0	0	
CYP3A5*3	6986A>G	rs776746	(A/A)	2	3	0.30
			(A/G)	11	34	
			(G/G)	62	108	
			(A/G)	11	34	
			(G/G)	62	108	

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