

# Gene frequency and haplotype analysis of HLA class I in patients with simple renal cysts

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## ABSTRACT

**Background:** The study of human leukocytes (HLA) alleles, and haplotype frequencies within populations provide an important source of information for anthropological investigation, organ and hematopoietic stem cell transplantation as well as disease association, certain diseases showed association with specific alleles specially those of known or suspected hereditary origin or immunological basis, whether simple renal cyst is congenital or acquired is still unclear and need to be investigated.

**Objectives:** To study the genetic aspect of simple renal cysts by detecting the gene frequency and the haplotype of HLA class I of patients with simple renal cysts, and to find the presence of these cysts in other family members.

**Method:** Thirty patients with simple renal cysts who were attending the outpatient clinic of urosurgery in the medical city were tested for HLA class I antigen using the microlymphocytotoxicity technique, in the period from February to June 2004 compared to 50 unrelated apparently healthy individuals. Gene frequency were calculated using square root formula ( $g=1-\sqrt{1-f}$ ), full history were taken including the family history.

**Results:** Certain gene frequencies were higher in the patients group than in the controls, yet not reached to a statistical significant level. No haplotype association with simple renal cysts was detected in this study; family history was detected in two patients which were proved by ultrasound examination.

**Conclusion:** Increasing the sample size may contribute to best results regarding gene frequency, haplotype and family study.

**Key words:** Gene frequency, Haplotype, Human Leukocyte Antigens.

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HLA, the human major histocompatibility complex (MHC), encompasses a series of tightly linked and highly polymorphic genes that function in histocompatibility and immune response<sup>1</sup>. The linkage of the HLA-A,-B,-C,-DR, and-DQ genes on the sixth chromosome create unique HLA haplotype, giving rise to potentially more than a billion genetically different individuals. Despite the vast number of potentially different haplotype, some combinations of HLA alleles have been conserved for extended period of time, exhibit linkage disequilibrium, and or are associated with disease susceptibility<sup>2</sup>.

Documentation and analysis of the role of the HLA complex in providing marker loci (or possible linked loci) for a variety of disease associated genes is of strong interest, since different human diseases especially those with a known or suspected hereditary factor or immunological basis may occur more frequently among individuals who carry particular HLA alleles<sup>3&4</sup>. Many renal disorders showed association with certain HLA loci, including cystic changes of the kidney<sup>5</sup>, however, simple renal cysts which are the most common type of cysts found in adult kidney had been agreed by many scientists that are not to be inherited<sup>6, 7&8</sup>, others suggested hereditary origin<sup>9</sup>.

Studying the gene frequency and haplotype analysis of patients having simple renal cysts may help in understanding the hereditary aspects of these cysts. Each chromosome having the HLA loci is referred to as

haplotype. Each individual has two haplotypes, inherits one paternal and one maternal haplotype from his parents in a simple mendelian autosomal inheritance and in allelic form. The term haplotype (from haploid genotype) was introduced by Ceppellini et al in 1967 to denote the combination of genes controlled by one chromosome and inherited in coupling<sup>10</sup>.

**Methods.** The current study was conducted in the period from February to June 2004 on the following groups; Thirty male and female patients with simple renal cysts attending the outpatient clinic of urosurgery in Baghdad medical City, compared with Fifty unrelated normal male and female control group, selected from healthy kidney donors attending kidney transplant unit in Baghdad Medical City. HLA typing to HLA class I was carried out using standard microlymphocytotoxicity technique<sup>11</sup>.

**Statistical analysis:** Gene frequencies were calculated using "square root formula"  $g=1-\sqrt{1-f}$  (12). P values of <0.05 were designated as statistical significant<sup>13</sup>.

**Results.** Familial history was detected in two patients which were proved by ultra sound examination, table 1. The gene frequency of HLA class I antigens in 30 patients with simple renal cysts was compared to those of 50 healthy unrelated individuals representing the control group as shown in table 2. The HLA Class I haplotypes was detected for these 30 patients with simple renal cysts as shown in table 3.

**Discussion:** Though the number of patients studied in this

work was very limited, yet familial history was quite prominent since two patients had family history of simple renal cysts. The first one is a male patient whose age was 37 years, his mother (68 years old) had cysts in both kidneys since 20 years, his brother (43 years old) had a cyst in one kidney since 5 years, both were proved by ultrasound

**Table 1:** Familial history of patients with Simple renal cysts.

Male patients with positive family history		No. of patients in the family	Female patients with positive family history		No. of patients in the family	Total Patients
No.	%		No.	%		
1	3.33	2	1	3.33	2	30

**Table 2:** The Gene frequency of HLA class I of Patients with simple renal cysts.

HLA A locus	30 individuals with simple renal cysts (patients)		50 healthy unrelated individuals (control)		P values
	Phenotype frequency	Gene frequency	Phenotype frequency	Gene frequency	
A1	0.233	0.125	0.28	0.152	>0.05
A2	0.233	0.125	0.28	0.152	>0.05
A3	0.066	0.034	0.10	0.052	>0.05
A11	0.033	0.017	0.10	0.052	>0.05
A23	0.033	0.017	0.06	0.031	>0.05
A24	0.066	0.034	0.10	0.052	>0.05
A25	0.033	0.017	0.02	0.011	>0.05
A26	0.066	0.034	0.02	0.011	>0.05
A28	0.166	0.087	0.12	0.062	>0.05
A29	0.066	0.034	0.02	0.011	>0.05
A30	0.066	0.034	0.02	0.011	>0.05
A33	0.066	0.034	0.06	0.031	>0.05
A34	0	0	0.02	0.02	>0.05
Blank	0.866	0.634	0.064	0.04	>0.05
HLA B locus					
	Phenotype frequency	Gene frequency	Phenotype frequency	Gene frequency	
B5	0	0	0.02	0.011	
B7	0.10	0.052	0.06	0.031	>0.05
B8	0.06	0.031	0.08	0.041	>0.05
B13	0	0	0.08	0.041	>0.05
B15	0.06	0.011	0.04	0.021	>0.05
B16	0	0	0.06	0.031	
B17	0.06	0.031	0.10	0.052	>0.05
B18	0.03	0.016	0.06	0.031	>0.05
B21	0.06	0.031	0	0	
B27	0.06	0.031	0.08	0.041	>0.05
B35	0.10	0.052	0.06	0.031	>0.05
B37	0.06	0.031	0	0	
B38	0.03	0.016	0.06	0.031	>0.05
B41	0	0	0.04	0.021	
B44	0	0	0.10	0.052	
B45	0	0	0.02	0.011	
B49	0	0	0.06	0.031	
B50	0.10	0.052	0.12	0.062	>0.05
B51	0.06	0.031	0.16	0.084	>0.05
B52	0	0	0.02	0.011	
B53	0.06	0.031	0.08	0.041	>0.05
B55	0.10	0.052	0	0	
B56	0.03	0.016	0.06	0.031	>0.05
B57	0.03	0.016	0.02	0.011	>0.05
B60	0.03	0.016	0.03	0.011	>0.05

B65	0.03	0.016	0	0	
B73	0	0	0.02	0.011	
HLA C locus					
C1	0.06	0.031	0.06	0.031	>0.05
C2	0.06	0.031	0.02	0.011	>0.05
C3	0	0	0.04	0.021	
C4	0.03	0.017	0.24	0.129	>0.05
C5	0	0	0	0	
C6	0	0	0.04	0.021	
C7	0.03	0.017	0.42	0.24	<0.05(S)
C8	0	0	0.052	0.052	

**Table 3:** The HLA Class I Haplotypes in 30 Patients with Simple Renal Cysts.

HLA class I Haplotypes frequencies	Female		Male	
	No.	%	No.	%
A1 A- B51 B6			1	3.33
A1 A- B35 B6			1	3.333
A1 A- B53 B6 C4			1	3.33
A1 A- B17 B4			1	3.33
A1 A- B50 B37			1	3.33
A1 A- B8 B6			1	3.33
A1 A- B38 B4 C2			1	3.33
A2 A- B17 B4			1	3.33
A2 A30 B35 B4			1	3.33
A2 A- B57 B4			1	3.33
A2 A3 B21 B6	1	3.33		
A2 A- BB65 B6	1	3.33		
A2 A- B50 B6	1	3.33		
A2 A- B15 B6	1	3.33		
A3 A- B18 B6			1	3.33
A11 A26 B53 B37			1	3.33
A23 A- B7 B6			1	3.33
A24 A28 B55 B4 C2			1	3.33
A24 A- B55 B6			1	3.33
A25 A- B37 B4			1	3.33
A26 A- B55 B35 C1			1	3.33
A28 A- B50 B6			1	3.33
A28 A- B7 B6			1	3.33
A28 A- B21 B6	1	3.33		
A28 A- B60 B6	1	3.33		
A29 A- B8 B6			1	3.33
A29 A- B15 B4			1	3.33
A30 A- B27 B4			1	3.33
A33 A- B7 B51 C1 C7			1	3.33
A33 A- B56 B6			1	3.33

examination. The second one was a female patient (70 years old), with two members of her family having simple renal cysts, her mother incidentally showed a cyst on US examination at the age of 80 years, her ante (80 years) had

a cyst since 10 years. This information encourage us to investigate the genetic aspect of this disease including gene frequency and haplotype analysis for HLA class I antigens, certain alleles showed genotype frequencies higher than control group (HLA -A26,A29,A30,B7,B35 and Cw2)but not reached to significant levels. Only Cw7 had been found to be significantly decreased which may be considered as a protective factor, Al Hamami (1996) studied the association of certain HLA alleles and nephritic syndrome in Iraqi children, the HLA A1 was significantly decreased with a phenotype frequency of 0.07 and gene frequency of 0.036<sup>14</sup>.

The study of haplotype frequencies within populations provides an important source of information for anthropological investigation, organ and hematopoietic stem cell transplantation purposes as well as disease association studies<sup>15</sup>, No haplotype association with simple renal cysts was detected in this study.

A study using molecular technique denoted that HLA-DRB1\*0701 is closely associated with the susceptibility to familial hepatitis B, and may be the susceptible or linkage gene. HLA-DRB1\*0401/0403/0405 and HLA-DRB1\*1301/1302, in inverse proportion to the susceptibility to familial hepatitis B, may be the resistant factor<sup>16</sup>.

The results of a study on genetic aspects of Behcet's disease indicates that HLA-A\*2601 is possibly associated with ocular BD, independent of HLA-B\*5101, indicating that HLA-A\*2601 is an additional susceptibility allele candidate of ocular BD in Japan<sup>17</sup>. However, another study done in Pakistan on patients with SLE showed a significant increase in certain alleles indicating positive association, a significant decrease in other alleles were considered as negative association<sup>18</sup>. A recent study on Psoriatic Arthritis, showed that susceptibility was associated with several class I alleles, B\*27:05:02 was positively associated with enthesitis, dactylitis and symmetric sacroiliitis, whereas B\*08:01:01-C\*07:01:01 were positively associated with joint fusion and deformities, asymmetrical sacroiliitis and dactylitis<sup>19</sup>. A Study done on Saudi Arabia population showed that HLA-B\*15,B\*18,B\*49 and DRB1\*03 were positively associated with end stage renal disease, the haplotype HLA-A\*01,DRB1\*13,HLA-A\*30-DRB1\*03 were positively associated as well<sup>20</sup>, Alksandrov and Skriabin studied the association of HLA in urolithiasis ,where they reveled specific distribution of HLA class I<sup>21</sup>, However, Hopkins and his colleagues found no significant difference in the proportion of HLA class I and class II in female patients with recurrent urinary tract infection<sup>22</sup>, Hiki and others found that HLA Dr 2 antigen was related to the occurrence of idiopathic membranous nephropathy<sup>23</sup>, meanwhile , other investigators announced that HLA A1,A26 and DR11 alleles are significantly elevated in patients with renal cell carcinoma<sup>24</sup>.

In conclusion, the HLA class I haplotype was investigated in this study for the 30 patient, each haplotype was detected only once ,the small sample size may be the reason for this result, these haplotypes may need to be investigated for larger number in addition to class II haplotypes.

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