Comp Clin Pathol (2015) 24:423–426
DOI 10.1007/s00580-014-1919-5

ORIGINAL ARTICLE

Contribution of HLA-DR to polycystic kidney disease in a sample of Egyptian patients

Rasha Mohamad Hosny Shahin · Ahmed Mahmoud Ahmed Shouman

Received: 3 February 2014 / Accepted: 17 March 2014 / Published online: 28 March 2014

Abstract The aim of this study was to investigate whether certain DR alleles might contribute to the genetic susceptibility among adult polycystic kidney disease patients in Egypt. This case–control study involved human leukocyte antigen (HLA)-DR typing for 40 non-related Egyptian patients with autosomal dominant polycystic kidney disease. Patients were compared with a group of 50 healthy subjects. Human leukocyte antigen DRB1 typing was carried out on allele level (DRB1*01 — DRB1*16) using polymerase chain reaction-sequence-specific oligonucleotide probes (PCR-SSOP). No statistically significant association of the disease with HLA-DRB1 was observed. So, HLA-DRB1 does not contribute to polycystic kidney disease in Egyptian patients.

Keywords Polycystic kidney disease · Human leukocyte antigen · Egypt

Introduction

Polycystic kidney disease (PKD) usually refers to a genetic or inherited disease that is sometimes called "adult PKD" because it normally appears in adult life. A less common type of PKD occurs primarily in babies and children (Amaout 2011).

The adult type of PKD (also called autosomal dominant PKD or ADPKD) is a multisystemic and progressive disorder characterized by the formation and enlargement of cysts in the kidney and other organs (e.g., liver, pancreas, spleen). Clinical features usually begin in the third to fourth decade of life, but cysts may be detectable in childhood and in utero. Up to 50 % of patients with ADPKD require renal replacement therapy by 60 years of age (Wilson 2004).

Scientists have located two genes associated with ADPKD. The first was located in 1985 on chromosome 16 and labeled *PKD1*. *PKD2* was localized to chromosome 4 in 1993. A mutation in either of the genes can lead to cyst formation, but evidence suggests that disease development also requires other factors, in addition to the mutation in one of the PKD genes (Rossetti et al. 2007).

The mutation in PKD genes is associated with abnormalities in the proteins (polycystin-1 and polycystin-2). The polycystin complex localizes to primary cilia and may act as a mechanosensor essential for maintaining the differentiated state of epithelia lining tubules in the kidney and biliary tract (Harris and Torres 2009).

A striking feature of ADPKD is the variability of the phenotype. ADPKD is fully penetrant, meaning that virtually 100 % of individuals who inherit a mutated PKD gene will develop renal cysts that can be detected sonographically by the age of 30. However, the severity of the disease, the age of onset of ESRD, and the spectrum of extrarenal manifestations vary widely between affected individuals, even within the same family. ADPKD may be modified by genetic, environmental, and stochastic factors independent of the germline PKD mutations (Torres et al. 2011).

One of these plausible genetic factors is antigens of the major histocompatibility complex (MHC), which is known in human as human leukocyte antigen (HLA) system. These antigens are genetically controlled by genes on the short arm of chromosome 6, and their expression shows an extensive polymorphism (Aajil 2011).

R. M. H. Shahin (✉)
Department of Clinical Pathology, Kasr Al Ainy Hospital, Faculty of Medicine, Cairo University, Cairo 11431, Egypt
e-mail: rasha.shaheen@kasralainy.edu.eg

A. M. A. Shouman
Department of Urology, Kasr Al Ainy Hospital, Faculty of Medicine, Cairo University, Cairo, Egypt

Therefore, the present investigation came to shed light on the frequencies of HLA-DR in a sample of Egyptian patients with ADPKD.

Materials and methods

The present case–control study included 40 non-related ADPKD patients diagnosed by kidney imaging studies. Patients were attending the outpatient clinic or the inpatient wards of the department of urology, Faculty of Medicine, Cairo University. Fifty, age, sex- and ethnically matched healthy volunteers were included in the current study as a control group. Peripheral blood samples were obtained from all subjects after they were provided with a written informed consent. The study was approved by the hospital's ethical committee (according to the WMA Declaration of Helsinki).

Genotyping

Total genomic DNA of patients and healthy controls was extracted from about 2 mL anticoagulated fresh or frozen whole blood on EDTA using Qiagen extraction kit (catalog number 51104, USA).

Molecular typing of HLA-DRB1 alleles was carried out on allele level (DRB1*01 — DRB1*16) using polymerase chain reaction (PCR) followed by reverse hybridization with a panel of sequence-specific oligonucleotides probes (reverse SSOP). The alleles were allocated based on the hybridization pattern for various probes using the interpretation software provided with the kit (Yoon et al. 2007).

PCR amplification of the second exon of the HLA-DRB1 locus was done using the INNO-LiPA HLA-DRB1 Amp Plus (Innogenetics, Belgium).

Genomic DNA were diluted to a concentration between 0.01and 0.02 ug/ul in TE buffer. PCR reactions were carried out in a 50-μL reaction mixture containing 5 μl extracted DNA, 10 u1 of primer solution (containing biotinylated primers, $MgCl_2$, and 0.05 % NaN_3 as preservative), 10 ul of amplification buffer (containing all dNTPs and 0.05 % NaN_3 as preservative), 1 u1 LiPATaq DNA polymerase, and 24 u1 autoclaved distilled water. The PCR amplifications cycles were performed as follows: initial denaturation at 95 °C for 5 min followed by a 35-cycle profile using 20 s at 95 °C denaturation, 20-s annealing at 58°C, and 20-s extension at 72 °C. The last PCR cycle was followed by an additional 10 min at 72 °C to ensure that the final extension step was complete. Temperature cycling was achieved using a DNA thermal cycler (Perkin-Elmer 9600, Singapore).

Reverse hybridization of amplified DNA product using the INNO-LiPA HLA-DRB1 plus (Innogenetics, Belgium).

Amplified biotinylated DNA material is chemically denatured, and the separated strands are hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. This is followed by a stringent wash step to remove any mismatched amplified material. After the stringent wash, streptavidin conjugated with alkaline phosphatase is added and bound to any biotinylated hybrid previously formed. Incubation with a substrate solution containing a chromogen results in a purple/brown precipitate. The reaction is stopped by a wash step, and the reactivity pattern of the probes is recorded.

Identification of all probe numbers that are positive on the INNO-LiPA HLA-DRB1 plus strip and deduce the DRB1 type by using the INN0-LiPA HLA-DRB1 plus typing table or a version of the LiPA interpretation software (LiRAS™).

Statistical methodology

Data were statistically described in terms of mean±standard deviation (±SD) or frequencies (number of cases) and percentages when appropriate. For comparing categorical data, chi-square (χ^2) test was performed. All P values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Demographic data of patients and controls were shown in Table 1. The study group included 40 ADPKD patients and the control group comprised 50 subjects, who were matched for gender (25 males and 15 females versus 36 males and 14 females, P=0.338), age (44.5000±9.666 versus 41.0200± 8.977, P=0.081), and ethnicity.

The distribution of the HLA-DRB1 alleles within the patients and controls was shown in Table 2. No statistical significant difference could be detected between patients and controls as regards the distribution of HLA-DRB1 allele frequencies.

Although HLA-DRB1*01 was apparently elevated in patients (15 %) compared with controls (8 %), the difference was

Table 1 Demographic data of patients and controls

		Patients (N=40)	Controls (N=50)	P value
Age		44.5000±9.666	41.0200±8.977	0.081
Sex	Males	25 (62.5 %)	36 (72 %)	0.338
	Females	15 (37.5 %)	14 (28 %)	

P<0.05 is considered statistically significant

Table 2 Distribution of HLA-DRB1 alleles in patients and controls

HLA-DRB1	Patients (N=40)	Controls (N=50)	P value
DRB1*01	6 (15 %)	4 (8 %)	0.294
DRB1*03	9 (22.5 %)	11 (22 %)	0.955
DRB1*04	14 (35 %)	15 (30 %)	0.614
DRB1*07	10 (25 %)	12 (24 %)	0.913
DRB1*08	2 (5 %)	3 (6 %)	0.837
DRB1*09	0 (0 %)	0 (0 %)	
DRB1*10	3 (7.5 %)	2 (4 %)	0.471
DRB1*11	10 (25 %)	18 (36 %)	0.263
DRB1*12	2 (5 %)	1 (2 %)	0.431
DRB1*13	10 (25 %)	15 (30 %)	0.599
DRB1*14	2 (5 %)	1 (2 %)	0.431
DRB1*15	3 (7.5 %)	8 (16 %)	0.221
DRB1*16	0 (0 %)	2 (4 %)	0.201

P<0.05 is considered statistically significant

not statistically significant (P=0.294). DRB1*04 was more common among patients (35 %) than the control subjects (30 %); however, the frequency values were not statistically significant (P=0.614).

HLA-DRB1*11 was more expressed in controls (36 %) compared to patients (25 %), as was HLA-DRB1*15 (expressed in 16 % controls versus 7.5 % patients). However, the differences were not significant (P=0.263, 0.221, respectively).

The frequencies of DRB1*03, HLA-DRB*07, and HLA-DRB*08 were almost similar in both control and patient groups. HLA-DRB1* 09 allele was completely absent in patients and controls. HLA-DRB1* 16 allele was absent in patients but present in 4 % of controls, however, the difference is statistically insignificant (P=0.201).

Discussion

It is increasingly recognized that the phenotype of Mendelian disorders is influenced by modifier genes, i.e., inherited genetic variations distinct from the disease locus. Such modifier genes have been evidenced in cystic fibrosis, familial Mediterranean fever, or familial hypercholesterolemia, as well as in murine models of polycystic kidney disease (Devuyst et al. 2003).

An argument for modifier genes in ADPKD is provided by the excess variability in the age at ESRD observed among siblings or dizygotic twins versus genetically identical monozygotic twins. The identification of major modifier genes in ADPKD is expected to improve our current understanding of the relevant molecular pathways. Such knowledge is essential for future development of individualized patient risk prediction and mechanism-based therapeutics for ADPKD—a promise of molecular medicine- (Pei 2005).

The antigens and/or alleles of the major histocompatibility system (MHS) have shown positive association with different diseases including non-congenital kidney diseases. Good pastures syndrome is associated with HLA-DR2 (Rees et al. 1978), idiopathic membranous nephropathy with HLA-DR3 (Klouda et al. 1979), and IgA nephropathy with HLA-DR4 (Fauchet et al. 1980). Also, an association between HLA-B5 and APKD has been reported (Dausset and Hors 1975). This association could be used as a risk classification marker, and thereby, progression to much more severe diseases could be protected or diagnosed at an earlier time.

In our study, no statistical significant difference could be detected between patients and controls as regards the distribution of HLA-DRB1 allele frequencies.

Similarly, Spengler et al. (1989) in their study in German patients with ADPKD did not find significant difference between patients and controls as regards the distribution of HLA-DR. Tissue typing was performed in their study using the lymphocytotoxicity test.

Also, Dyer et al. (1982) in their study showed absence of linkage between ADPKD and the MHS.

In contrast, Xu (1990) indicated the association of PKD with HLA–A9, BW22, DR5 haplotype in four of the nine members (involving three generations) of the studied Chinese family. Typing was performed using lymphocytotoxicity test.

Karahan et al. (2009) found a statistically significant association of HLA-DRB1*08 and HLA-DRB1*14 with polycystic kidney disease in Turkish population. Typing was performed using polymerase chain reaction- sequence-specific primer (PCR-SSP).

These contradictory findings could be the result of different ethnicity. It is important to explore HLA genes in diverse ethnic groups in order to understand the genetics of the disease.

We conclude that adult polycystic kidney disease as a multifactorial and heterogeneous disease is not associated with one of the HLA class II DRB1 alleles in Egyptian patients. Further studies with larger sample size are needed to improve our understanding of the genetic basis of PKD so that new therapies to prevent PKD or limit its severity can be developed.

Conflicts of interest The authors have no financial or proprietary interest in any product mentioned in this paper.

Funding This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

The authors have no financial or proprietary interest in any product mentioned in this paper.

References

Aajil A (2011) The association between HLA-class I antigens and polycystic ovary syndrome in a sample of Iraqi patients. Iraqi Journal of Cancer and Medical Genetics 4(1):52–56

Amaout MA (2011) Cystic kidney diseases. In: Goldman L, Ausiello D (eds) Cecil medicine 24th ed. Elsevier, Philadelphia, Chap128

Dausset J, Hors J (1975) Some contributions of the HLA complex to the genetics of human diseases. Transplantation Review 22:44–74

Devuyst O, Persu A, Vo-Cong M-T (2003) Autosomal dominant polycystic kidney disease: modifier genes and endothelial dysfunction. Nephrol Dial Transplant 18(11):2211–2215

Dyer P, Watters E, Klouda P, Harris R, Mallick N (1982) Absence of linkage between adult polycystic kidney disease and the major histocompatibility system. Tissue Antigens 20:108–111

Fauchet R, Le Pogamp P, Genetet B, Chevet D, Gueguen M, Simon P, Ramee MP, Cartier F (1980) HLA-DR4 antigen and IgA nephropathy. Tissue Antigens 16:405–410

Harris PC, Torres VE (2009) Polycystic kidney disease. Annu Rev Med 60:321–337

Karahan GE, Seyhun Y, Oguz F, Kekik C, Onal E (2009) Impact of HLA on the underlying primary diseases in Turkish patients with end-stage renal disease. Renal failure 31:44–49

Klouda PT, Manos J, Acheson ES, Dyer PA, Goldby FS, Harris R, Lawler W, Mallick NP, Williams G (1979) Strong association between idiopathic membranous nephropathy and HLA-DRW3. Lancet ii:770–771

Pei Y (2005) Nature and nurture on phenotypic variability of autosomal dominant polycystic kidney disease. Kidney Int 67:1630–1631

Rees AJ, Peters DK, Compston DAS, Batchelor JR (1978) Strong association between HLA-DRw2 and antibody mediated Goodpastures Syndrome. Lancet i:966–5268

Rossetti S, Consugar MB, Chapman AB, Torres VE, Guay-Woodford LM, Grantham JJ, Bennett WM, Meyers CM, Walker DL, Bae K, Zhang QJ, Thompson PA, Miller JP, Harris PC, CRISP Consortium (2007) Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. J Am Soc Nephrol 18:2143–2160

Spengler J, Wegener S, Schüler H, Kraatz G (1989) HLA antigen frequencies in patients with autosomal dominant polycystic kidney degeneration. Z Urol Nephrol 82(12):691–694

Torres VE, Grantham JJ, Chapman AB, Mrug M, Bae KT, King BF Jr, Wetzel LH, Martin D, Lockhart ME, Bennett WM, Moxey-Mims M, Abebe KZ, Lin Y, Bost JE, Consortium for radiologic imaging studies of polycystic kidney disease (CRISP) (2011) Potentially Modifiable Factors Affecting the Progression of Autosomal Dominant Polycystic Kidney Disease. Clin J Am Soc Nephrol 6(3):640–647

Wilson PD (2004) Polycystic kidney disease. N Engl J Med 350(2):151–164

Xu L (1990) Association of polycystic kidney disease and an HLA haplotype within a family clan. Urol Res 18:239–240

Yoon SH, Oh HB, Kim HK, Hong SC, Oh YM, Lee DS, Lee SD (2007) Association of HLA class II genes with idiopathic pulmonary arterial hypertension in Koreans. Lung 185(3):145–149