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The Phenotypic Characteristics of Adult Polycystic Kidney Disease Have Greater Impact on the Course of Progressive Disease than the Type of Mutation of the Polycystin 1 Gene

Fenotyp zwyrodnienia wielotorbielowatego nerek i rodzaj mutacji – wpływ na postęp choroby

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Abstract

Background. Autosomal dominant polycystic kidney disease (ADPKD) is among the most frequent inherited kidney disorders and appears, according to different statistics, with a frequency of 0.25–0.1% in the general population.
Objectives. The aim of the present study was to evaluate the impact of phenotypic differences among ADPKD patients on the progressive course of the disorder. In addition, an evaluation of mutations and polymorphisms in a fragment of the *PKD1* gene was performed and the potential relationship between the kind of mutation and the clinical picture of the disorder was investigated.
Material and Methods. Genetic analyses were performed in 134 patients, among whom were members of 23 families. Mutation and polymorphism screening was performed on fragments of the 3' and 5' *PKD1* gene regions which included exons 43 to 46 and exon 15. The detailed clinical examination encompassed 81 ADPKD patients (46 female and 35 male, mean age: 42.3 ± 13.1 years). For the determination of the severity of the clinical course of ADPKD, the index of clinical severity (ICS) (age at the clinical examination divided by eGRF and multiplied by 10) was used.
Results. The normotensive patients exhibited the lowest ICS values, very significantly lower compared with severely hypertensive patients ($p < 0.001$) and significantly lower versus moderately hypertensive patients ($p < 0.05$). There was also a statistically significant difference in ICS values between the moderately and severely hypertensive ($p < 0.02$). The drop in GFR was significantly faster in hypertensive patients than in normotensive individuals ($p < 0.01$), the difference between moderately and severely hypertensive patients also being statistically significant ($p < 0.02$). Additionally, patients in whom sonography detected cardiac abnormalities (left ventricular hypertrophy, disorders of contractility, aortic and mitral valve insufficiency) had significantly higher ICS values than those with normal heart examinations ($p < 0.01$). Patients with moderate and excessive kidney enlargement (14.6%) had significantly higher ICS values than the others ($p < 0.05$). Overall, 42 of the 134 analyzed patients (11.9%) showed genetic disorders. No correlation between genetic changes and clinical features of the disease was found.
Conclusions. The presence of hypertension and its severity as well as moderate and excessive kidney enlargement are associated with worse renal prognosis in ADPKD patients, whereas no connections between genetic abnormalities and clinical course of the disease were detected (**Adv Clin Exp Med 2008, 17, 2, 155–159**).

Key words: adult polycystic kidney disease, clinical course, hypertension, kidney size, mutation of polycystin 1 gene.

Streszczenie

Wprowadzenie. Autosomalna dominująca postać zwyrodnienia torbielowatego nerek jest jedną z najczęstszych chorób genetycznych dotyczącą prawie 0,1% populacji.

Cel pracy. Określenie różnic fenotypowych wśród chorych na zwyrodnienie torbielowate nerek, ocena występowania zmian mutacyjnych i polimorficznych w genie policystyny 1 oraz próba ustalenia ewentualnej korelacji między zmianami genetycznymi a rozwojem i ciężkością zaawansowania choroby.

Materiał i metody. Badania genetyczne przeprowadzono u 134 chorych, wśród których można wyodrębnić 23 rodziny. Badania dotyczyły fragmentów genu *PKD1* obejmujące egzony 43–46 oraz egzon 15. Szczegółowym badaniom klinicznym poddano 81 chorych z ADPKD (46 kobiet i 35 mężczyzn w wieku 42.3 ± 13,1 roku).W celu określenia ciężkości przebiegu wady posłużono się współczynnikiem ciężkości choroby (wyliczanym według wzoru: wiek podczas badania/eGFR × 10).

Wyniki. Osoby normotensyjne charakteryzowały się istotnie mniejszym współczynikiem ciężkości wady w porównaniu z osobami z nadciśnieniem ciężkim ($p < 0.001$) i umiarkowanym ($p < 0,05$). Między obiema grupami z nadciśnieniem wykazano również istotne różnice w wielkości współczynnika ciężkości wady ($p < 0.02$). Chorzy z nadciśnieniem mieli istotnie szybszy ubytek filtracji kłębuszkowej (wyższy współczynnik ciężkości wady) niż osoby normotensyjne ($p < 0.01$). Osoby, u których za pomocą badania USG serca stwierdzono nieprawidłowości miały istotnie wyższy współczynnik ciężkości przebiegu wady w porównaniu z osobami z prawidłowym echokardiogramem ($p < 0,01$). Pacjenci mający nerki o prawidłowej wielkości (ocena ultrasonograficzna) mieli istotnie niższy współczynnik ciężkości wady w porównaniu z pacjentami mającymi nerki umiarkowanie powiększone lub olbrzymie ($p < 0,05$). W grupie 134 badanych wykryto 42 nieprawidłowości genetyczne. Nie stwierdzono korelacji między zmianami genetycznymi a przebiegiem klinicznym choroby.

Wnioski. Obecność i ciężkość nadciśnienia tętniczego oraz stopień powiększenia nerek wiążą się z szybszą progresją wady; nie wykazano natomiast związku obrazu klinicznego z wykrytymi nieprawidłowościami genetycznymi (**Adv Clin Exp Med 2008, 17, 2, 155–159**).

Słowa kluczowe: zwyrodnienie wielotorbielowate nerek, przebieg kliniczny, nadciśnienie tętnicze, wielkość nerek, mutacje w genie policystyny 1.

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited kidney disorder, appearing with a frequency of 0.25–0.1% in the general population according to different statistics. ADPKD is one of major causes of end-stage renal disease (ESRD) and is recognized in 10–14% of patients treated by renal replacement therapy. The cause of the disease is very heterogeneous, which is reflected by the fact that patients acquire ESRD in a wide range of ages, between forty and seventy-five years [1–3]. Mutations of the polycystin 1 gene (*PKD1*) are responsible for 85% of ADPKD cases [4, 5]. The aim of the present study was to evaluate the impact of phenotypic differences between ADPKD patients on the progressive course of the disorder. In particular, the analysis considered the effect of such clinical factors as the degree of kidney enlargement and the severity of hypertension on the rate of decline in glomerular filtration rate (GFR). In addition, mutations and polymorphisms in a fragment of the *PKD1* gene were evaluated and the potential relationship between the kind of mutation and the clinical picture of the disorder was investigated.

Material and Methods

Genetic analyses were performed in 134 patients, among whom were members of 23 families. Two milliliters of peripheral blood was taken from the patients into vacuteiners filled with EDTA. The study obtained acceptance by the Bioethics Committee of Silesian Piasts University of Medicine in Wrocław and the individual consent of each patient. Mutation and polymorphism screening was performed on fragments of the 3' and 5' *PKD1* gene regions including exons 43 to 46 and exon 15. Genomic DNA was isolated by salt extraction with application of proteinase K. The gene fragments of interest were amplified by PCR and analyzed with heteroduplex, SSCP (single-stranded conformational polymorphism), and MSSCP (multitemperature single-stranded conformational polymorphism) methods. The ultimate determination was based on DNA sequences. The genetic analyses were performed at the Institute of Molecular Biology and Biotechnology, University of Poznań.

The detailed clinical examination encompassed 81 ADPKD patients (46 female and 35 male, mean age: 42.3 ± 13.1 years) in whom the eGFR value was calculated according to the abbreviated MDRD formula and the severity of hypertension was scored by the number of antihypertensive drugs taken. Kidney size was established during ultrasonographic examination and ultrasonography was also carried out to evaluate cardiac status. To determine the severity of the clinical course of ADPKD, the index of clinical severity (ICS), which is the age at the clinical examination divided by eGRF and multiplied by 10, was calculated.

Statistical analysis was performed using the STATISTICA v. 8.00 software package. The ANOVA test, Wilcoxon test, χ-squared test, Pearson's correlation, and Spearman's rank correlation were applied where appropriate.

Results

Hypertension was determined in 65 patients (80.25%), i.e. 88% of the men and 74% of the women, the difference being statistically significant ($p < 0.01$). The degree of hypertension was divided into two categories: moderate, i.e. patients who took one or two antihypertensive drugs, and severe, i.e. patients receiving at least three antihypertensive drugs. The data on the relationship between the degree of hypertension and ICS are presented in Table 1.

Table 1. Relationship between the degree of hypertension and ICS

Tabela 1. Zależność ciężkości nadciśnienia i wartości współczynnika ciężkości wody (ICS)

Degree of hypertension (Ciężkość nadciśnienia)	Number of patients (Liczba pacjentów)	Mean ICS (Średnia wartość ICS)
Moderate (Umiarkowana)	39	9.9
Severe (Ciężka)	26	12.3

The normotensive patients exhibited the lowest ICS values, very significantly lower compared with the severely hypertensive patients ($p < 0.001$) and significantly lower versus the moderately hypertensive patients ($p < 0.05$). There was also a statistically significant difference in the ICS values between the moderately and severely hypertensive patients ($p < 0.02$). A strong negative effect of hypertension on the clinical course of ADPKD was shown in the analysis of its association with the rate of GFR decline (Table 2).

The drop in GFR was significantly faster in hypertensive patients compared with normotensive individuals ($p < 0.01$). The speed of GFR decrease was also significantly affected by the category of hypertension, the difference between moderately and severely hypertensive patients being statistically significant ($p < 0.02$). The negative effect of hypertension on the clinical course of ADPKD was also visible in the analysis of the results of cardiac sonography. The patients in whom sonography detected abnormalities (left ventricular hypertrophy, disorders of contractility, aortic and mitral valve insufficiency) had significantly higher ICS values than persons with a normal heart examination ($p < 0.01$)

Table 2. Effect of hypertension on the rate of GFR decline represented by ICS values

Tabela 2. Wpływ nadciśnienia tętniczego na szybkość ubytku filtracji kłębuszkowej reprezentowanej przez wartości czynnika ciężkości wody (ICS)

Blood pressure (Ciśnienie krwi)	Number of patients (Liczba pacjentów)		Mean ICS (Średnia wartość ICS)
Normal (Prawidłowe)	16		5.6
Moderate hypertensive (Umiarkowane)	39	65	9.9
Severe hypertensive (Ciężkie)	26		12.3

According to the evaluation of kidney size (Table 3), the patients were divided into three categories: 1) normal size, 2) moderate enlargement, not crossing over the minor pelvis line, and 3) excessive enlargement, descending to the minor pelvis. Excessive enlargement was found in 12 patients (14.8%). The ICS values in the group of patients with moderate and excessive kidney enlargement were significantly higher than in the group with normal kidney size ($p < 0.05$).

Table 3. Relationship of kidney size and ICS

Tabela 3. Zależność wielkości nerek i wartości współczynnika ciężkości wody

Kidney size (Wielkość nerek)	Number of patients (Liczba pacjentów)	Mean ICS (Średnia wartość ICS)
Normal (Prawidłowa)	10	4.9
Moderate enlargement (Umiarkowane powiększenie)	59	10.3
Excessive enlargement (Nerki olbrzymie)	12	11.3

The results of the genetic investigations are presented in Table 4. In a total of 134 patients, 42 genetic changes were found, 11 of which were mutations, namely 4 in the 3' *PKD1* gene region and 7 in the 5' region. Seven mutations caused changes in the restriction site, one of which induced a substitution in the amino-acid sequence

of polycystin 1. One mutation caused truncated polycystin 1 due to the appearance of a premature STOP codon. The other 31 changes were polymorphisms detected in the 5' *PKD1* gene region. Fourteen polymorphisms led to differences in the amino-acid sequence. The other polymorphisms did not show such a character. Particular patients displayed few genetic changes. In the total of 16 patients in whom genetic changes were found, multiple changes were detected in 14. Overall, in the 134 analyzed patients, 11.9% showed genetic disorders. Genetic changes occurred in 5 of the 23 examined families (21.7%). No correlation between genetic changes and clinical features of the disease was found.

Table 4. Results of genetic investigations

Tabela 4. Wyniki badań genetycznych

Gene region (Rejon genu)	No. of mutations (Liczba mutacji)		No. of polymorphisms (Liczba polimorfizmów)	
3'	4	restriction site change	–	
(3' koniec)		3		
5'	7 1 truncated mutation	restriction site change	31	amino-acid change
(5' koniec)		4		14

Discussion

The results of the present study unequivocally demonstrate that the presence and severity of hypertension exerted a powerful impact on the rate of GFR deterioration in the ADPKD patients. This was reflected by the fact that the normotensive patients exhibited the lowest values of ICS, very significantly lower compared with the severely hypertensive patients ($p < 0.001$) and significantly lower versus the moderately hypertensive patients ($p < 0.05$). There was also a statistically significant difference in the ICS values between the categories of moderate and severe hypertension ($p < 0.02$). It is known that hypertension is a prevalent complication in patients with ADPKD. However, studies devoted to a detailed analysis of the effect of hypertension on the clinical course of ADPKD are surprisingly scant [6–8]. The data of the present study confirm that the appearance and severity of hypertension are associated with a more rapid deterioration of renal function. The negative effect of hypertension on the clinical course of ADPKD was also shown in the distinct type of analyses respecting the data from cardiac sonography. The patients in whom sonography detected abnormalities (left ventricular hypertrophy, disorders of contractility, aortic and mitral valve insufficiency) had significantly higher ICS values compared with those with normal heart examinations ($p < 0.01$). The results of this evaluation are in agreement with published data indicating left ventricular hypertrophy as a risk factor for worse renal prognosis in ADPKD.

The degree of kidney enlargement is the second clinical factor significantly augmenting GFR deterioration [9, 10]. This view was confirmed in the present study by the significantly higher ICS values in the patients with moderate and excessive kidney enlargement versus those with normal kidney sizes ($p < 0.05$).

Overall, 42 (11.9%) of the 134 analyzed patients showed genetic disorders. This frequency of genetic abnormalities, smaller than that previously published [11–14], may be connected with the limitations of the present investigation. The search for mutations was performed in a fragment of the *PKD1* gene and some mutations could have been missed. The other limitation is the limited number of screened patients. No correlation between genetic changes and clinical features of the disease was found. This shows that the genetic background of ADPKD is very complex, of a broader character than mutations of the *PKD1* gene and, consequently, the clinical phenotype is probably dependent on a modifying effect of multiple unknown genes.

The authors conclude that the appearance and severity of hypertension are very significantly associated with worse renal prognosis in ADPKD patients, moderate and excessive kidney enlargement is the second most significant clinical factor negatively affecting the speed of GFR deterioration in ADPKD patients, no correlation between genetic changes and clinical features of the disease was found.

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