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Gender-dependent disease severity in autosomal polycystic kidney disease of rats

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Gender-dependent disease severity in autosomal polycystic kidney disease of rats. The impact of gender on the course of chronic renal failure in polycystic kidney disease (PKD) has been under discussion for years. Recently an animal model of autosomal dominant PKD in the rat became available allowing this topic to be studied. The aim of this study was to evaluate disease severity according to gender, and the occurrence of anticipation and/or genetic imprinting. Male and female affected PKD rats were crossed with respective Wistar-Ottawa-Karlsburg (WOK) rats. From this P generation 26 affected F1 hybrids were obtained, which were then backcrossed with WOK rats, resulting in 275 backcrosses (BC generation). In BC rats the affected males had a significantly higher kidney weight, worse histology and poorer renal function than the females. In the male, but not the female rats of the BC generation, transmission from an affected F1 mother resulted in significantly higher kidney weight, worse histology and poorer renal function than when the gene was inherited through an affected father. Since at the same time body and kidney weight were higher in the respective unaffected males, the previous effect in the affected rats might be due to a growth factor transferred by the mother's milk. The sex of the P generation had no such impact on these parameters. Thus our data provide no evidence for disease anticipation and genetic imprinting (in the classical sense) in the PKD rats, and the assumption of a gender-dependent disease expressivity is favored.

In 1989 we demonstrated that end-stage renal failure appears earlier in men than in women with adult autosomal dominant polycystic kidney disease (PKD) (median age: 52.5 vs. 58.0 years) [1]. These findings were supported [1, 2] and reconfirmed recently [3] by data from the Australian and New Zealand Combined Dialysis and Transplant Registry. Also Gabow et al [4] reported that in patients having the PKD1 gene, male gender resulted in a more rapid loss of renal function. This gender-dependent acceleration of the disease process might be explained by gender-dependent disease expressivity.

In contrast, Bear et al [5] could not confirm this gender difference when analyzing patients having the PKD1 gene. They noted, however, that end-stage renal disease (ESRD) was related to the gender of the parent transmitting the disease. ESRD occurred earlier (50.5 vs. 64.8 years) in patients having inherited the disease from their mother than from their father. This observation would be consistent with a genetic imprinting effect, that is, the differential modification of genetic material depending on whether inheritance is from the male or female parent.

Recently Fick, Johnson and Gabow provided evidence for both genetic imprinting and disease anticipation, that is, an earlier onset and increased disease severity in successive generations [6]. Anticipation was noted, however, in only 53% of the informative families, if anticipation was defined as a 10-year-earlier onset of ESRD.

Thus there is both evidence for disease anticipation and genetic imprinting. Furthermore a sex-dependent disease expressivity cannot be ruled out. One approach to gain more insight into these features would be to analyze these aspects in a suitable animal model of PKD.

In 1989 Kaspareit-Rittinghausen et al reported on a rat model exhibiting autosomal dominant PKD [7, 8]. These rats develop histological lesions comparable to human PKD and progress to ESRD, though slower than described initially [9–15]. Progression can be accelerated by unilateral nephrectomy [16]. In the intial reports [17, 18] hypertension had been mentioned, a finding which subsequently could not be confirmed [19]. The most striking observation in this model, however, was that female rats exhibiting renal cysts did not develop renal failure up to the age of six months [15] or even 44 weeks [9]. This is clearly different from what had been observed in male animals, in which already at the age of two months increased serum urea concentrations occur [9, 15]. Thus a significant gender difference with respect to the development and progression of renal failure seems to exist in the PKD rat model.

These findings in humans and animals prompted us to examine in the PKD rat model the severity of the renal degeneration according to gender, and the presence of disease anticipation or genetic imprinting.

Animals and methods

Homozygous unaffected and heterozygous affected Han: SPRD rats exhibiting PKD were originally obtained from the Central Institute for Laboratory Animal Breeding in Hannover, Germany, and highly inbred WOK (Wistar-Ottawa-Karlsburg) rats from the

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Diabetes Research Center in Karlsburg, Germany. The PKD rats were transferred into our laboratory when inbred to the 11th generation, while the WOK rats were already inbred up to the 21st generation [10, 20]. The PKD rats were selectively inbred by us up to their 19th generation before starting this experiment. One affected male and one female PKD rat (P) was crossed with respective WOK rats in order to obtain (PKD × WOK) F1 hybrids from which 18 male and 8 female F1 hybrids were backcrossed onto their respective WOK mate. These [(PKD × WOK)(F1 × WOK)] first backcross hybrids (BC) were then studied.

All animals had free access to tap water and standard rat chow containing 19% protein (Altromin 1934®, Lage, Germany). All rats were sacrificed when the BC offspring had reached an age of two months. Body weight was taken, and under anesthesia (ketamine and diazepam) the animals were bled from the aorta, the kidneys were removed and fixed in formaldehyde after wet wt had been obtained. From the blood samples serum urea concentration was determined by standard laboratory methods.

The carrier status of each animal was established by histology. The extent of the cystic formation was assessed by using a scoring system:

Grade 1. Occasionally small and/or medium-sized cysts (diameter up to that of 2 glomeruli), cysts only in a few visual fields;

Grade 2. Few small and/or up to 5 medium-sized cysts per visual field, rarely large cysts (diameter larger than 2 glomeruli), cysts not in every visual field;

Grade 3. Several small and/or up to 10 medium-sized cysts (diameter up to that of 2 glomeruli) per visual field, few large cysts (diameter larger than 2 glomeruli), cysts in every visual field;

Grade 4. A large number of small and/or medium-sized and/or at least 2 large cysts per visual field, cysts in every visual field, occurrence of 'network-like structures' consisting of large connected cysts.

The histological examination and the scoring was performed by two independent observers in a blinded fashion. In all cases agreement on the diagnosis and the histological grading was obtained.

For data evaluation the SAS system was used. The following procedures were applied: PROC FREQ (Fisher's exact test for 2 × 2 or larger tables) [21], PROC GCHART (graphical presentation of data) [22], PROC TTEST (*t*-test) [23] and PROC UNIVARIATE (mean and SD) [24]. All data are given as $\bar{x} \pm$ SD.

Results

From 26 pairs of F1 animals 292 BC rats were obtained, of which 17 died during the first six weeks of life (range 0 to 2/litter). Thus 275 were entered into final evaluation. The overall effective litter size was 10.6 ± 2.8 animals. In the 275 animals the mode of inheritance was autosomal dominant, as the number of rats expected to be PKD positive or negative and the actual figures did not differ (PKD: positive $N = 151$ and negative $N = 124$; Fisher's exact test, $P = 0.143$). The overall gender distribution (males $N = 133$, females $N = 142$; Fisher's exact test, $P = 0.382$) and the distribution of affected and unaffected animals within both sexes (males: affected $N = 74$ and unaffected $N = 59$; females: affected $N = 77$ and unaffected $N = 65$; Fisher's exact test, $P = 0.455$) was not skewed.

Gender of the F1 animal transmitting the PKD gene had a significant impact on body wt (Table 1) and kidney mass (wet wt of the left and right kidneys; Table 1). In affected and unaffected male BC rats maternal gene transmission resulted in higher body wt and larger kidneys than in those rats in which paternal gene transmission had occurred. No such differences were noted in female offspring.

Table 1. Body weight and dney mass according carrier status and parental nd offspring gender

	F1 gene transmission						
	Maternal			Paternal			
BC offspring	*N*	$\bar{x}$	SD	*N*	$\bar{x}$	SD	*P*
Body weight *g*							
affected male	28	321	23	46	305	28	0.0135
unaffected male	19	339	24	40	308	26	0.0001
affected female	26	216	21	51	211	15	0.2198
unaffected female	22	210	13	43	208	14	0.4819
Kidney mass *g*							
affected male	28	5.4	0.9	46	4.7	0.9	0.0012
unaffected male	19	2.3	0.2	40	2.1	0.2	0.0015
affected female	26	2.3	0.4	51	2.3	0.3	0.2944
unaffected female	22	1.4	0.1	43	1.4	0.2	0.9049
Serum urea *mg/dl*							
affected male	28	53.1	10.9	46	46.6	7.0	0.0072
unaffected male	19	36.8	2.6	40	36.1	2.9	0.3547
affected female	26	36.9	5.2	51	38.6	4.7	0.1587
unaffected female	22	34.0	3.9	43	38.2	3.8	0.0001

N is number of animals.

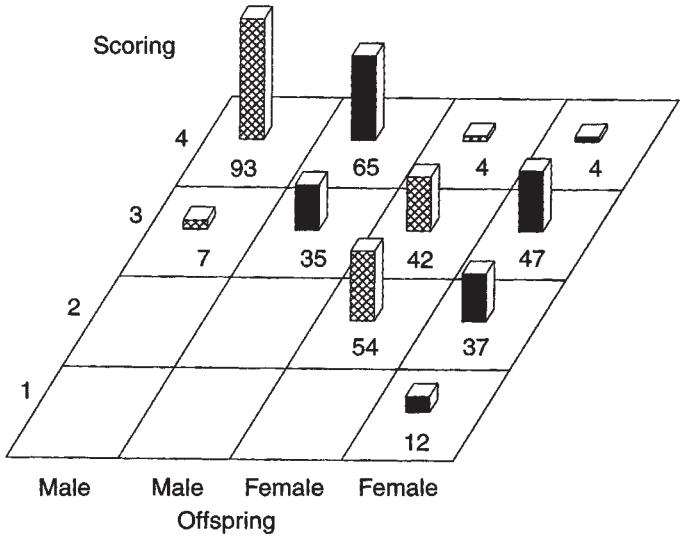

Fig. 1. *Percentage distribution of disease severity according to parental gender (F1) and that of the offspring after histological scoring.* Symbols are: (▨) maternal gene transmission; (■) paternal gene transmission).

The observation of a higher kidney weight after maternal gene transmission suggests the occurrence of more severe histological lesions in the affected male rats. Significantly higher disease severity scores were found in affected male rats inheriting the PKD gene from their mother (Fig. 1; Fisher's exact test, $P = 0.006$). Such a finding could not be observed in affected female rats (Fisher's exact test, $P = 0.222$). Overall, affected female BC animals had smaller cysts in comparison to affected male BC animals (Fisher's exact test, $P < 0.001$).

Serum urea values in affected male BC offspring of affected F1

Table 2. Impact of gender of the P generation on kidney mass, body weight and serum urea concentration

				Kidney mass *g*			Body weight			Serum urea *mg/dl*		
P	F1	BC	*N*	$\bar{x}$ ± SD		*P*	$\bar{x}$ ± SD		*P*	$\bar{x}$ ± SD		*P*
Affected animals												
fe	fe	ma	11	5.7	0.64	0.107	331	14.8	0.046	56.0	5.7	0.187
ma	fe	ma	17	5.2	0.94		314	24.4		51.2	13.1	
fe	ma	ma	21	4.8	0.79	0.626	311	28.0	0.189	47.9	7.0	0.260
ma	ma	ma	25	4.7	1.07		300	28.1		45.5	7.1	
fe	fe	fe	14	2.4	0.43	0.195	219	23.8	0.395	36.1	5.2	0.500
ma	fe	fe	12	2.2	0.31		212	16.0		37.8	5.3	
fe	ma	fe	21	2.4	0.26	0.082	215	15.0	0.065	38.4	4.2	0.861
ma	ma	fe	30	2.3	0.34		207	14.8		38.7	5.1	
Unaffected animals												
fe	fe	ma	13	2.3	0.22	0.282	342	28.1	0.212	36.4	2.6	0.335
ma	fe	ma	6	2.2	0.14		331	8.1		37.7	2.7	
fe	ma	ma	17	2.1	0.24	081.8	310	32.6	0.579	36.2	3.4	0.737
ma	ma	ma	23	2.1	0.19		306	20.5		35.9	2.6	
fe	fe	fe	6	1.4	0.08	0.145	213	13.5	0.577	34.5	3.1	0.751
ma	fe	fe	16	1.4	0.09		209	13.5		33.9	4.3	
fe	ma	fe	24	1.4	0.19	0.079	208	15.1	0.477	39.1	3.9	0.086
ma	ma	fe	19	1.4	0.11		206	11.4		37.1	3.6	

Abbreviations are: P, F1 and BC are defined in Methods section; fe, female, ma, male.

mothers were significantly higher when compared with affected male BC offspring of affected F1 fathers (Table 1). With respect to serum urea unaffected BC female offspring revealed a significant difference according to parental gender, though the magnitude of the difference was small.

Furthermore we checked the data for disease anticipation taking the gender of the P generation into consideration. After dividing each of the groups analyzed in Table 1 according to gender of the P parent, no major significant difference between the different groups of animals could be observed (Table 2). A marginally significant difference occurred with respect to body wt between the groups, in which either a male or a female transmitted the gene via a female onto a male animal. Also, in the affected groups no difference could be detected with respect to the scoring of the renal histology (Fisher's exact test, *P* ranging from 0.505 to 0.836).

Discussion

Our data clearly demonstrate that there is a considerable gender difference between affected male and female rats with respect to their renal function, kidney mass (Table 1) and degree of histological renal involvement (Fig. 1). Furthermore, a considerable impact of parental gender (F1 generation) on functional and histological parameters was noted, with a maternal gene transmission resulting in a more pronounced disease severity (Fig. 1, Table 1). On the other hand, no such difference could be revealed for the impact of gender on the P generation (Table 2). Thus these data provide priliminary evidence for a genetic imprinting effect and gender-dependent disease expressivity, but not for disease anticipation.

In addition, we could successfully transfer the PKD gene from one rat strain to another, that is, from a Sprague-Dawley to WOK background, resulting in the same histological and functional changes as described previously. Finally the autosomal dominant type of inheritance and a normal gender distribution was confirmed.

In a recent review on genetic heterogeneity, anticipation, and imprinting in PKD, Zerres and Rudnik-Schöneborn [25] pointed out that the number of pediatric patients suffering from the recessive or dominant type of PKD is about the same in Germany (129 and 143, respectively). Already in 1993 Fick et al [26] and Zerres, Rudnik-Schöneborn and Deget [27] reported on the occurrence of autosomal dominant PKD *in utero* and in the neonatal period. Surprisingly, the parents transmitting the autosomal dominant type of PKD to the children with early onset most often show the typical age at onset of the disease. Furthermore, these authors noted the clustering of the early onset cases in the same families and the predominance of maternal gene transmission. This high variability in disease severity, not only among but also within families, together with the fact that up to one third of PKD patients have no family history, probably reflecting unsuspected disease in earlier generations, suggested the occurrence of anticipation. This hypothesis, first made by Gabow et al [4], has recently been confirmed by Fick et al [6], who noted a 10-year-earlier onset of ESRD in 50 out of 94 informative families (53%). A similar percentage of anticipation (45%) was published in an abstract by Torra et al [28].

Moreover, a gender effect seems to be operative in the transmission of early onset autosomal dominant PKD since both Fick et al [26] and Zerres et al [27] noted that the transmitting parent in the pairs with anticipation was more often the mother than the father. Similarly, Bear et al [5] reported that ESRD occurred earlier (50.5 vs. 64.8 years) in patients having inherited the disease from their mother than from their father.

This gender-dependent transmission of both the early onset and the anticipation in autosomal dominant PKD resembles what had already been found in other genetic diseases, where an epigenetic modification of the disease locus, known as genetic imprinting, has been demonstrated to play a role in changing the degree of severity or the appearance of the disease [29]. Neither genetic imprinting nor the possible interaction of the PKD gene with other modifying genes, not yet identified, can be excluded at this stage. An effect of changes in the gene itself, as it occurs with

heritable unstable DNA, might offer the best explanation for the above observations [30, 31].

Data collected in the PKD rat model can contribute to the understanding of genetic mechanism underlying the above observations. Although the number of BC rats in the present study is quite high (N = 275), the number of parent animals (P and F1 generation) is limited (N = 2 and N = 26, respectively). Thus despite a random selection of the P animals there could be a selection bias due to the small number of rats. Furthermore, the number of parent-offspring sets might be insufficient to confirm or exclude a possible role of anticipation. One has to keep in mind that all rats (P, F1 and BC rats) were sacrified when the BC generation had reached the age of two months. Therefore, a longitudinal comparison of the parameters under study cannot be performed.

Despite these caveats it can be concluded that, with respect to parental gender, heterozygous male rats inheriting the disease gene from the mother exhibit an earlier onset and/or more severe form of PKD (Fig. 1, Table 1). This suggests that the maternal effect could be due to the existence of a "growth factor" transmitted from the affected female parent (F1) to the BC offspring via the placenta or during suckling.

Recently Lakshmanan and Eysselein [32] noted a hereditary error in epidermal growth factor (EGF) prohormone metabolism in the PKD rat model. They found massive amounts of 66 kD EGF prohormone in cyst fluid, suggesting that EGF and/or its prohormone might function as a cystogen. As EGF is secreted in rat milk [33], is protected by milk-borne peptidases in the gut from destruction [34], and influences the body wt of pups [35] with its effect being androgen-dependent [36], most of the observed differences in our study could be explained by an aberrant metabolism of EGF or its prohormone. Whether the early effects of such a growth factor persist throughout life is unclear. On the other hand, such a factor would favor the hypothesis of a sex-dependent disease expressivity in PKD, while the assumption of genetic imprinting is not necessary to explain our findings.

In the fawn-hooded rat, another hereditary model of chronic renal failure, a similar gender difference with respect to the occurrence of focal segmental glomerular sclerosis, proteinuria and hypertension has been observed [37, 38]. Further gender differences in hereditary and non-hereditary renal diseases both in humans and in animals have been reviewed recently [39].

In conclusion, our data support the notion that there is a sex-dependent expressivity of the renal disease in the PKD rat, as in other animal models of renal failure. The occurrence of genetic imprinting is not supported by our data and disease anticipation could not be proven.

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