Rat models of autosomal dominant polycystic kidney disease

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Abstract. Several rat models of polycystic kidney disease (PKD) have been published. The only rat model of autosomal dominant polycystic kidney disease currently used is the so-called Hannover rat (Han:SPRD cy/+). This model is characterized by a slow progression of uraemia, proteinuria and hyperlipidaemia. Histological changes clearly resemble those seen in human PKD. The localization of Na⁺/K⁺-ATPase correlating with the phenotype of the cysts—basal in moderately expanded and apical in highly expanded cysts—suggests that the mislocation of the Na⁺/K⁺-ATPase is involved in the mechanism of cyst expansion rather than formation, and a consequence of cell dedifferentiation rather than an initial event. Of note is a considerable gender difference in disease severity. Disease anticipation or genetic imprinting does not occur. In addition to gender, a number of interventions influence the progression rate: acceleration is noted after unilateral nephrectomy, the induction of acidosis, chloride feeding or an increased protein intake; slowing down of the course occurs after the induction of alkalosis and castration, and after treatment with lovastatin and methylprednisolone. Thus the Han:SPRD cy/+ rat represents the only well-documented rat model of autosomal dominant PKD resembling a number of features of the human disease.

Key words: genetics; histology; polycystic kidney disease; rat models; uraemia

Introduction

In contrast to mouse models, genetically determined rat models of autosomal dominant [1,2] or recessive [3] polycystic kidney disease (PKD) have only rarely been described, although PKD is a frequent, spontaneously occurring disease in all species.

Lozzo et al. described some cyst formation in a mutant strain of the so-called Gunn rat, a mutant of the Wistar strain [1]. The major feature of this strain, however, was the occurrence of hydronephrosis (85%). Cysts were noted in only 5% of all rats. A genetic analysis suggested that all these renal lesions were inherited as an autosomal dominant trait. As a cyst formation was only observed in 5% of all animals and as no features of uraemia were described in these rats, it seems to be questionable whether this model has anything to do with autosomal dominant PKD.

In 1973 Soloman reported on a rat strain developing renal cysts [2]. In his strain the number of cysts increased with increasing age. Unfortunately the observation period was only 52 days, a short period in the lifespan of a rat, and the number of animals analysed was only 21. Furthermore, neither the genetic transmission of the disease nor the development of uraemia was established.

None of these models seems to be suitable to resemble human autosomal dominant PKD. Furthermore, no papers presenting additional details on these models have been published.

The aim of this paper is to summarize our knowledge on a recently described rat model of autosomal dominant PKD, the so-called Hannover rat (Han: SPRD cy/+). In addition, we will present new data on the localization of Na⁺/K⁺-ATPase in the renal epithelial cells of these rats.

The Hannover rat model of autosomal dominant PKD

In 1989 Kaspareit-Rittinghausen et al. [4–6] noted that in their Sprague-Dawley colony a spontaneous
mutation resulting in PKD had occurred. Beside the histological features of PKD, death due to uraemia and enlarged parathyroid glands were found. Furthermore, they observed that only male rats developed uraemia, while females were healthy despite exhibiting histological features of PKD. Genetic analysis suggested that there was an autosomal dominant transmission of the disease. In due course they also noted that their rats became hypertensive [7,8].

In 1990 we obtained 60 animals of the 11th generation of this rat strain to start a new breeding colony [9]. At that time the only feature we could reproduce was the occurrence of cysts in the kidneys and an autosomal dominant pattern of inheritance. In our rats aged <12 months no progressive renal disease or hypertension could be noted [10]. This observation was later on confirmed by Cowley et al. [11].

Heterozygous rats develop slowly progressive cystic disease (Figure 1) with interstitial fibrosis and thickened basement membranes [11-14]. About 75% of the cysts are derived from the proximal tubule. The cystic transformation starts with a sharp onset of basement membrane alterations and a loss of epithelial differentiation restricted to small focal areas, where there was also noted an increased message for α1 (IV) collagen and laminin B1 mRNA. Fibroblasts underlying the affected tubular portions are involved in matrix overexpression, resulting in the subepithelial accumulation of collagen IV and laminin. As shown by in situ hybridization, renin production is reduced. The expression of c-myc is elevated, suggesting abnormal regulation of cell proliferation [1].

Epithelial cells, including normal renal tubules cells, are polarized in terms of the distribution of cell adhesion molecules, ion channels, co-transporters and pumps. These molecules are restricted either to the apical (ultrafiltrate) or basolateral (blood supply) membrane domain, which results in the vectorial transepithelial transport of ions and solutes. A common explanation for the fluid accumulation in the cavity of renal cysts in humans is the fact that the Na+/K+-ATPase pump is mislocated to the apical membrane domain in the human cystic epithelium [15], resulting in a decrease in fluid reabsorption.

This mislocation of Na+/K+-ATPase has been reported not only in humans but also in several animal models of PKD (Table 1). In the cpk mouse, Avner demonstrated that the activity of Na+/K+-ATPase increased in the basolateral membrane of proximal tubule cysts while mislocated to the apical domain in collecting tubule cysts, coinciding with the immature phenotype of these tubules [16]. Apical mislocation of Na+/K+-ATPase had also been demonstrated in the glucocorticoid-induced PKD mouse, in the pcy mouse and in the diphenylthiazole-induced (DPT) PKD rat [17], while cysts derived from the MCDK cell line present a basolateral located Na+/K+-ATPase [15].

When initially analysing the location of Na+/K+-ATPase in the Han:SPRD cy/+ rat by using fluorescence microscopy no mislocation of Na+/K+-

Fig. 1. Light microscopy of kidney in Han:SPRD cy/+ rats exhibiting cystic degeneration preferentially located in the inner part of the renal cortex (H&E, magnification x80).
Table 1. Localization of Na+/K+-ATPase in PKD

<table>
<thead>
<tr>
<th>Model</th>
<th>Na+/K+-ATPase</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cpk mouse</td>
<td>apical in collecting tubule cysts, basolateral in proximal tubule cysts</td>
<td>[15]</td>
</tr>
<tr>
<td>Glucocorticoid induced (mouse) pcy mouse, DPT induced (rat)</td>
<td>apical</td>
<td>[16]</td>
</tr>
<tr>
<td>MDCK cell line cysts</td>
<td>basolateral</td>
<td>[17]</td>
</tr>
<tr>
<td>Phenol II induced (rats)</td>
<td>focal loss from basolateral cytoplasm, rarely apical</td>
<td>[18]</td>
</tr>
<tr>
<td>Han:SPRD cy/+</td>
<td>moving from basolateral to apical depending on cyst size</td>
<td>[own data]</td>
</tr>
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</table>

ATPase could be detected, but a weaker staining was evident [12]. Recently the re-evaluation of the location of Na+/K⁺-ATPase has become possible by using confocal microscopy, a more sensitive approach to detect minor changes in location. In moderately expanded cysts lined with a largely intact proximal tubular epithelium containing apparently differentiated cells the Na+/K⁺-ATPase was located in the basolateral...
membrane domain, but also in the more basal half of the cytoplasm (Figure 2a). In highly expanded cysts lined by flattened cells—apparently dedifferentiated and forming a less organized epithelium—the Na\(^+\)/K\(^-\)-ATPase was mislocated and restricted to the apical domain (Figure 2b). The localization of Na\(^+\)/K\(^-\)-ATPase correlating with the phenotype of the cysts suggests that the mislocation of the Na\(^+\)/K\(^-\)-ATPase is involved in the mechanism of cyst expansion rather than formation, and represents a consequence of cell dedifferentiation rather than an initial event.

Earlier reports [4–6] have suggested a rapid course of the disease, a finding which we could not reproduce. In our male heterozygous animals death due to uraemia occurs at the age of 17 months (median, \(n = 35\)) [20], while in heterozygous females no death had been observed up to the age of 14 months. The latter finding is in agreement with data from Cowley et al. [11]. We meanwhile learned by chance that death due to uraemia also occurs in female rats, but significantly later (age >17 months) [21]. Thus this gender dimorphism, also noted with respect to histology [11,12,22], seems to be more pronounced than in humans [23,24].

The disease process can be accelerated by unilateral nephrectomy (death due to uraemia occurring at a median age of 11.6 months [20]; significantly increased serum urea at day 150 [25]), chloride and protein feeding [26], and inducing acidosis [27].

The long-term analysis of body weight, biochemical parameters and blood pressure revealed that male heterozygous animals exhibit a normal growth rate when compared with healthy homozygous unaffected rats [251. The increase in proteinuria, serum creatinine and urea values (616 ± 240 mg/dl) and also increased serum creatinine (2.4 ± 1.1 mg/dl). In addition, kidney weight is dramatically increased (4.3 ± 1.3 g/kidney). These kidneys exhibit dramatic cystic changes [11,12].

Only recently have extrarenal manifestations of the cystic disease been detected, i.e. liver and pancreatic cysts [21]. It is apparent that these changes need time to develop, as they were found only in heterozygous female rats aged >17 months.

Recently Fick and Gabow claimed that disease anticipation occurs in PKD patients [29]. In our rat strain we checked the occurrence of disease anticipation, genetic imprinting and gender-dependent disease severity [30]. Male and female affected PKD rats were crossed with respective Wistar–Ottawa–Karlsburg (WOK) rats, a highly inbred strain. From this P generation 26 affected F\(_1\) 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 greater kidney weight, worse histology and poorer renal function than the females. These findings again point to a gender difference in disease severity.

In the male but not the female rats of the BC generation, transmission from an affected F\(_1\) mother resulted in significantly increased kidney weight (Figure 3), worse histology (Figure 4) and poorer renal function than when the gene was inherited through an affected father [30]. Such an effect was not noted in female offspring. As at the same time body and kidney weight were greater in the respective unaffected males (Figure 5), the previous effect in the affected rats might be due to a growth factor transferred by the mother's milk.

![Graph showing body weight (g) for maternal and paternal offspring](image)

**Table 2. Resemblance of human PKD by the Han:SPRD cy/+ model**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Han:SPRD cy/+</th>
<th>humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>autosomal dominant</td>
<td>autosomal dominant</td>
</tr>
<tr>
<td>Gene locus</td>
<td>?</td>
<td>16 and 4</td>
</tr>
<tr>
<td>Anticipation</td>
<td>–</td>
<td>?</td>
</tr>
<tr>
<td>Genetic imprinting</td>
<td>–</td>
<td>?</td>
</tr>
<tr>
<td>Gender difference</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Uraemia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Progression of uraemia</td>
<td>slow</td>
<td>dependent on influencing factors</td>
</tr>
<tr>
<td>Hypertension</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glomerular cysts</td>
<td>+?</td>
<td>+</td>
</tr>
<tr>
<td>Matrix alterations</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Laminin</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Na+/K+-ATPase</td>
<td>basal/apical</td>
<td>apical</td>
</tr>
<tr>
<td>Extrarenal manifestations</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
This assumption seems to be valid as the weight at birth does not depend on maternal or paternal gene transmission (N. Gretz, unpublished data). A good candidate for such a growth factor would be epidermal growth factor (EGF). Recently Lakshmanan and Eysselle [31] pointed out that in the Han:SPRD a hereditary error in EGF prohormone metabolism occurs. EGF is secreted in the mother’s milk and is absorbed by the pup without being digested. The difference between the male and female offspring could be explained by the effect of testosterone on action of EGF.

The gender of the P generation had no such impact on any of the analysed parameters. Thus our data provide no evidence for disease anticipation and genetic imprinting (in the classical sense) in the PKD rats, but the assumption of a gender-dependent disease expressivity is favoured.

Animal models of diseases are only as good as the resemblance to their human counterpart. Table 2 lists the different features discussed in this review. Taking all these features together, it is quite striking how closely the Han:SPRD cy/+ model resembles human autosomal dominant PKD. The major difference seems to be the renin production and release [12,25,32]. One has, however, to keep in mind that all the animal data were sampled early on in the rat’s life. Hypertension seems also to be of no relevance in this rat model. Again, all the measurements are taken early on in life, and sodium- and chloride loading are not constant features in animals but are probably frequent in humans. Besides these potential differences, a number of equivalences can be found. They make the model an interesting tool in analysing the pathophysiological events and evaluating therapeutic interventions.

Taking the gender difference into account, Zeier et al. [25] tested the impact of castration on progression. When performing orchidectomy at day ~60-70 a significant slowing of progression occurred, although the serum urea concentrations were still greater than usually seen in female rats.

In the paper by Zeier et al. [25] the impact of enalapril treatment on progression was analysed. As expected from histological findings indicating low mRNA for renin in the kidney, no beneficial effect of enalapril treatment on progression could be noted. Furthermore, these authors confirmed previous findings that unilateral nephrectomy accelerates progression in this model.

Torres et al. [27] demonstrated that inducing acidosis increased cyst growth and decreased creatinine clearance in female animals considerably, but conversely that bicarbonate treatment resulted in an increased creatinine clearance and a decreased kidney/body weight ratio. Thus, treating acidosis might have a considerable impact on progression.

Gile et al. [22] tested the effect of lovastatin on the development of PKD. Their hypothesis was that rins-proteins are important in the control of renal cell proliferation in PKD. Farnesyl pyrophosphate, an intermediate in the conversion of acetyl-CoA to cholesterol, is required for the activation of ras guanosine triphosphate (GTP)-binding proteins that are important in cell proliferation. Lovastatin is known to reduce farnesyl pyrophosphate production. Animals were treated from age 4 to 10 weeks. In male rats lovastatin significantly decreased the kidney/body weight ratio and serum urea nitrogen. The corresponding changes in females were insignificant. One might speculate that lovastatin did not have the expected effect on cell proliferation, but could have had an effect on hormone production of the adrenal gland. Thus, testosterone-mediated effects could have been reduced. Furthermore, it is of note that in the rat lovastatin does not reduce serum cholesterol concentrations. Unfortunately, cholesterol was not determined in this study, thus another effect of lovastatin than the expected one might explain the differences.

Gattone et al. [33] treated male and female rats for 42 days with methylprednisolone. The hypothesis was that interstitial inflammation and fibrosis, a common feature of PKD, could be reduced. Methylprednisolone administered to male rats resulted in decreased serum urea nitrogen concentrations, reduced kidney/body weight ratio and less severe interstitial fibrosis. No effect was noted in female rats.

In summary, the Han:SPRD cy/+ model represents a slowly progressive model of autosomal dominant PKD. Despite the fact that location and product of the PKD gene in this model is unknown, the model seems to be an interesting tool for studying pathophysiological events and evaluating therapeutic interventions.

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