Advances in Genomics and Genetics

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ORIGINAL RESEARCH

Polycystic kidney disease gene in the Lewis polycystic kidney rat is mapped to chromosome 10q21–q26

This article was published in the following Dove Press journal:
Advances in Genomics and Genetics
9 August 2012
Number of times this article has been viewed

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Background: Polycystic kidney disease (PKD) is a life-threatening disorder that affects the kidneys of millions of people across the world. The disease is normally inherited, but it can also be acquired, and leads to development of many cysts in the renal nephrons. In this study, the aim was to characterize PKD in the Lewis polycystic kidney (LPK) rat, the newest model for human PKD.

Methods: Mating experiments were performed between male LPK rats with PKD and female Brown Norway and Wistar Kyoto rats without PKD to raise second filial (F2) and backcross 1 (BC1) progeny, respectively. Rats that developed PKD were identified. Histological examination of the kidneys and liver was performed. Liver tissue samples were collected from each rat and used to extract DNA. The extracted DNA was amplified, and mapping and linkage analyses were performed to identify the quantitative trait locus that controlled the disease phenotypes.

Results: It was established that the disease was controlled by a recessive mutation in a single gene (F2: PKD = 42, non-PKD = 110, $\chi^2 = 0.53$; BC1: PKD = 67, non-PKD = 72, $\chi^2 = 0.18$, $P > 0.05$) and that the disease was inherited as autosomal recessive polycystic kidney disease (ARPKD). The rats with PKD developed larger fluid-filled cystic kidneys, higher systolic blood pressure, and anemia. However, there were no extrarenal cysts and no pup deaths. Mapping studies and linkage analyses associated the disease phenotypes in both the F2 and BC1 rats to chromosome 10q21–q26, giving a maximum LOD score of 7.9 ($P = 0.00001$) between peak markers D10Rat180 and D10Rat26.

Conclusion: The quantitative trait locus on chromosome 10q21–q26 does not contain the *Pkhd-1* gene, and it is different from quantitative trait loci that control ARPKD in other murine models. The candidate genes located in the quantitative trait locus are important in signal transduction, cell growth, cell proliferation, and cell differentiation. Although expression of PKD in the LPK rat shares common phenotypic traits with ARPKD caused by mutation in *Pkhd-1*, the gene responsible for ARPKD in the LPK rat is different and unknown, or a mechanism yet to be identified is responsible for development of the disease.

Keywords: polycystic kidney disease, quantitative trait locus, signal transduction, cell proliferation

Introduction

Polycystic kidney disease (PKD) is a group of heterogeneous, genetically inherited human renal disorders that affects millions of people all over the world.[1,2] The disease leads to development of numerous fluid-filled cysts in the kidneys. Extrarenal cysts in the liver, pancreas, blood vessels, spleen, testes, and ovaries are common in humans and animals that develop PKD.[1,3] Furthermore, research has shown that dysfunction of genetic loci in different chromosomes in different animal species is associated

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with the development of PKD.[3–5] In some cases, the disease is a result of multiple mutations in the same gene.[6] Based on the genetic expression of PKD, it was found that genetic modifier loci, somewhere along the chromosomes, influenced the development and progression of the disease in different animal species.[5,7–9]

Genetic analyses established that PKD is inherited either as an autosomal recessive (ARPKD) or autosomal dominant (ADPKD) disease.[1,10] In human studies, the incidence of ADPKD is estimated at one per 500–1000 live births,[11,12] while that of ARPKD is one per 2000–14,000 live births.[13] ARPKD is caused by multiple mutations in *PKHD-1*[14,15] located on human chromosome 6p21.1–p12.[16] The *PKHD-1* gene consists of 86 exons, assembled into alternative spliced transcripts, and the longest transcript consists of 67 exons, which encodes for fibrocystin/polyductin.[17–19]

ADPKD is a result of mutations in two genes, *PKD-1*, which accounts for 85% of cases and *PKD-2*, which accounts for 10%–15% of the cases.[20] The *PKD-1* gene encodes for polycystin-1 and *PKD-2* encodes for polycystin-2.[21] A small percentage of patients with PKD, which is not associated with mutation in *PKD-1* or *PKD-2* genes, has been identified and the gene is now called *PKD-3*.[22] However, this gene is not mapped to any chromosome. In addition to PKD, which leads to development of cysts in the kidneys, there is also nephronophthisis with phenotypes that resemble the PKD phenotypes. However, this disease is a result of mutations in at least eight genes which are different from the *PKD* genes.[23]

ARPKD is an infantile disease,[24] ADPKD has an adult onset,[25] while nephronophthisis may be inherited as a dominant or recessive trait.[23] These diseases lead to development of extrarenal cysts, high systolic blood pressure, anemia, and eventually renal failure.[16,18,26] In all cases of PKD, congestive heart failure is a feature that cannot be avoided.[27,28]

To the present, many murine models have been identified and used to study and understand the development and progression of PKD.[10,14,29] However, the polycystic kidney (PCK) rat model seems to present with PKD phenotypes that resemble human PKD phenotypes.[30,31] In the current study, PKD in the Lewis polycystic kidney (LPK) rat, the newest model, is characterized. This model arose from the Lewis (LEW/SsNArc$^{-/-}$) strain that spontaneously developed enlarged cystic kidneys at the Animal Resources Center of Western Australia in 2003. Mating experiments between brothers and sisters with enlarged cystic kidneys produced 100% progeny with bilaterally enlarged fluid-filled cystic kidneys.[32] However, there were no extrarenal cysts and the disease did not lead to any pup deaths. This colony is now called the Lewis polycystic kidney (LPK/SsNArc$^{+/+}$) rat. However, it was not clear whether the disease was inherited as a recessive or dominant trait. Equally, it was not known which chromosome or locus within the chromosome carried the mutated gene responsible for the disease phenotypes. Furthermore, it was not clear how many genes or mutations were involved in development of the disease.

Materials and methods

Mating experiments

Mating experiments were performed between mutant LPK/SsNArc$^{+/+}$ male rats and nonmutant Brown Norway (BN/ssArc$^{-/-}$) and Wistar Kyoto (WKY/NArc$^{-/-}$) female rats to raise second filial (F2) and backcross 1 (BC1) progeny, respectively. The Brown Norway and Wistar Kyoto rat strains are genetically distant from the LPK rat strain. Both strains were used in the study to understand the effect of genetic background of the two female rat strains on the phenotypic expression of PKD. The experiments were performed according to the guidelines of the animal ethic committees of Murdoch University and the Animal Resources Center of Western Australia.

Phenotypic trait analysis

The progeny were earmarked and recorded. They were palpated once a week from immediately after birth until euthanasia to determine which rats had enlarged kidneys. Enlarged kidneys were confirmed on euthanasia and the presence of cysts was established on histological examination of the kidneys. Systolic blood pressure was measured when the rats were 12 weeks old by the tail-cuff method using the NIBP controller (ADI Instruments, Castle Hill, NSW, Australia). At least three measurements were taken, and the average measurement was used in statistical analysis. Urine was collected from the BC1 progeny using metabolic cages and its chemistry was determined.

Euthanasia

Euthanasia of the rats was carried out using a carbon dioxide and oxygen gas mixture, in proportions of 80:20, respectively. After euthanasia, the rats were weighed and the mass recorded in grams. Blood was removed by cardiac puncture into lithium-heparin tubes. The rats were opened on the ventral side along the linear alba and kidneys, and sections of liver were removed. Each kidney was weighed, the mass was recorded, and the kidneys and sections of liver

were fixed in 4% formaldehyde solution. Sections of the liver and tail tips were stored at −80°C for DNA extraction.

Packed cell volume

The packed cell volume was determined in 2 mL of blood at the Department of Pathology, Murdoch University, with a microhematocrit technique, using an Haeraeus Biofuge hemocentrifuge, according to the manufacturers' instructions (Randox Laboratories Ltd, Crumlin, UK).

Blood chemistry

Plasma total solid protein was estimated on ethylenediamine tetra-acetic acid using a refractometer according to the manufacturer's instructions (Randox Laboratories Ltd). Plasma creatinine was determined in the same way as for urine creatinine, except that no dilution of samples was carried out. Plasma urea was measured on the Randox Daytona, with the reaction catalyzed by urease enzyme (Randox Laboratories Ltd). The ammonia produced during the reaction combined with α-oxoglutarate and hydrogenated nicotinamide adenine dinucleotide (NADH), in the presence of glutamate-dehydrogenase, to yield glutamate and NAD^+. The NAD^+ produced was measured in μmol/L using an ultraviolet method.

Urine chemistry

Urine was collected from each BC1 rat between 10 am and 4 pm using metabolic cages. To 20 μL of urine samples, blank and standard solution, 1000 μL of Pyrogallol red was added. This was mixed and incubated at 37°C for 5 minutes. The absorbance of the samples (A_{sample}) and the standard ($A_{standard}$) was measured against the reagent blank at 600 nm using a spectrophotometer. Protein concentration was calculated according to the instructions of the manufacturers (Randox Laboratories Ltd):

$$\text{Protein concentration (g/L)} = A_{sample} \times [\text{standard}]/A_{standard}$$

where [standard] is the concentration of the standard solution in g/L, A_{sample} is the absorbance of the sample, and $A_{standard}$ is the absorbance of the standard solution.

Urine creatinine was determined by the following procedure. To 20 μL of urine sample (diluted 1 in 20 with distilled water) was added sodium hydroxide and picric acid, as per the instructions provided by the manufacturers (Randox Instruments). Creatinine, in the presence of sodium hydroxide solution, reacts with picric acid to form a colored complex. The rate of formation of the complex was measured using a colorimetric method. The urine protein/creatinine ratio was calculated according to the instructions of the manufacturer:

$$\text{Protein/creatinine ratio} = 8840[\text{protein}]/[\text{creatinine}]$$

where [protein] is the concentration of protein in g/L and [creatinine] is the concentration of creatinine in g/L.

Histological examination of tissues

Histological examination of the sections of kidney and liver tissues was carried out at the Department of Histology, Murdoch University. The tissues were paraffin-embedded and sectioned (4 μm thickness) using a microtome. The sections were fixed on glass slides, stained with hematoxylin and eosin, and viewed under a light microscope and the images were digitalized using a camera (Olympus, Perth WA, Australia).

Genetic analysis

Simple sequence repeat markers

The simple sequence repeat markers used for the mapping studies were chosen from the rat genome database (http://rgd.mcw.edu/). One simple sequence repeat marker was taken from the extreme ends of each chromosome and two or more markers between. In total, 150 simple sequence repeat markers distributed across the 20 rat autosomes were screened in the study, but only 96 were found to be informative, and therefore used in the mapping studies. The simple sequence repeat markers used in the study to map chromosomes 1 to 20 are shown in Table 1.

DNA extraction and PCR analysis

The extraction of DNA from liver tissue was carried out according to the instructions provided in the Standard Tissue Kit Protocol® (QIAamp DNA Mini Kit, Qiagen, Valencia, CA). The DNA was quantified using a NanoDrop®, ND-1000 (Biolab Inc, Lawrenceville, GA).

The polymerase chain reaction (PCR) was performed in a total volume of 10 μL containing 10% (w/v) Cresol Red solution; PCR buffer [6.7 mM Tris-HCl, pH 8.8, 1.66 mM $(NH_4)_2SO_4$, 0.045% Triton X-100, 0.02 mg/mL gelatin]; 0.25 mM of total dNTPs; 10 pM each of forward and reverse primers; 1 U Taq polymerase; 1.5 mM $MgCl_2$, and 20 ng/μL genomic DNA template in the reaction mixture. The amplification of DNA was performed using touchdown conditions, with an initial denaturation temperature of 94°C for 3 minutes, followed by 8 cycles at 94°C for 30 seconds, 63°C for 30 seconds and 72°C for 30 seconds, and the temperature decreased to 55°C, one cycle/1°C. A further 30 cycles

Table 1 Simple sequence repeat markers used to map the 20 rat autosomes, rat genome database identity, the chromosomes and their total length in cM, the name of the marker, the motif of the markers, the distance of each marker from the top of the chromosome in cM, the predicted fragment size in base pairs for each marker, and the start and stop position of each marker in the chromosomes

ID	Chr	Length (cM)	Name	Motif	cM	Size	Start	Stop
39666	1	149.25	D1Rat246	(CA)n	0.04	227	2436832	2437062
40798	1		D1Rat256	(GT)n	28.34	207	53871755	53872145
37930	1		D1Rat183	(GT)n	65.46	226	131123181	131123541
39264	1		D1Rat194	(GT)n	134.6	124	246528823	246528943
36896	2	112.4798	D2Rat9	(CA)n	12.71	130	33495861	33496242
38266	2		D2Rat136	(CA)n	43.34	215	121385356	121385565
40898	2		D2Rat228	(GT)n	70.05	134	181071573	181071715
35340	2		D2Rat70	(GT)n	112.5	172	254933191	254933362
35981	3	94.2199	D3Rat56	(GT)n	2.47	180	3577905	3578102
41476	3		D3Rat193	(CA)n	3.49	147	10016016	10016172
40968	3		D3Rat194	(GT)n	3.53	199	10311363	10311552
42639	3		D3Rat231	(GT)n	3.57	224	9925540	9925751
40854	3		D3Rat192	(GT)n	4.62	235	12470687	12470922
37166	3		D3Rat110	(CA)n	32.82	121	42729639	42729760
38316	3		D3Rat68	(CA)n	76.61	199	153994739	153995233
38244	3		D3Rat132	(CA)n	91.49	208	165064454	165064659
36305	4	102.2399	D4Rat4	(CA)n	0.04	182	3237190	3237361
41182	4		D4Rat168	(CA)n	37.41	142	77155286	77155422
40642	4		D4Rat195	(GT)n	65.69	215	143875115	143875322
38580	4		D4Rat140	(GA)n	98.81	146	182740817	182741162
40250	5	105.6599	D5Rat125	(GT)n	9.22	234	17702961	17703194
38548	5		D5Rat82	(CA)n	26.04	240	45822600	45822846
39248	5		D5Rat72	(CA)n	63.77	226	120983238	120983466
38574	5		D5Rat111	(CA)n	103.9	225	30418090	30418319
35963	6	85.1598	D6Rat46	(GT)n	1.15	144	13286615	13286759
39972	6		D6Rat132	(GT)n	39.81	221	52753065	52753434
37376	6		D6Rat87	(GT)n	56.75	173	98742972	98743161
39052	6		D6Rat101	(CA)n	81.77	120	135964407	135964844
35382	7	88.5699	D7Rat36	(CA)n	0.04	180	1526177	1526344
35237	7		D7Rat35	(GT)n	3.9	125	20307479	20307622
38322	7		D7Rat63	(CA)n	4.6	246	14454482	14454831
35068	7		D7Rat32	(CA)n	11.42	143	21790655	21790795
40050	7		D7Rat152	(GT)n	17.91	131	25926004	25926410
39016	7		D7Rat103	(CA)n	25.4	200	44462790	44463229
36932	7		D7Rat17	(CA)n	57.96	135	100258077	100258647
36193	7		D7Rat3	(GT)n	86.23	128	137404381	137404493
36039	8	85.2699	D8Rat58	(CA)n	0	174	3039648	3039822
38690	8		D8Rat98	(GT)n	38.06	214	53665152	53665540
41310	8		D8Rat130	(CA)n	57.27	174	98986085	98986263
37976	8		D8Rat69	(CA)n	83.04	148	125922482	125922636
35695	9	79.5198	D9Rat44	(CA)n	1.33	176	2948882	2949051
37416	9		D9Rat70	(GT)n	23.24	190	21667066	21667255
41600	9		D9Rat110	(CA)n	64.74	174	94217517	94217673
37268	9		D9Rat75	(CA)n	76.1	212	104531509	104531801
37464	10	95.4399	D10Rat95	(GT)n	0.04	217	6380753	6381051
41524	10		D10Rat218	(CA)n	4.5	143	9092033	9092176
38756	10		D10Rat180	(CA)n	4.6	170	19148316	19148638
35001	10		D10Rat43	(TG)n	24.27	150	23428127	23428280
36319	10		D10Rat26	(TG)n	51.03	167	72105962	72106120
35627	10		D10Rat12	(TG)n	76.13	170	99198838	99198997
34941	10		D10Rat7	(CA)n	87	173	105886241	105886414
35665	10		D10Rat4	(GT)n	95.44	164	107033560	107033716
37070	11	41.6299	D11Rat28	(GT)n	2.55	246	13185810	13186049
39084	11		D11Rat93	(CA)n	29.84	240	67930910	67931151
40938	11		D11Rat56	(GT)n	35.73	195	77880883	77881082

(*Continued*)

Table 1 (*Continued*)

ID	Chr	Length (cM)	Name	Motif	cM	Size	Start	Stop
38800	11		D11Rat43	(GT)n	41.63	234	85039892	85040103
40394	12	54.0099	D12Rat89	(GT)n	2.13	139	2957061	2957210
37272	12		D12Rat28	(GT)n	20.46	120	16312645	16312771
37830	12		D12Rat36	(GT)n	42.14	200	33216127	33216334
38410	12		D12Rat53	(GT)n	51.79	134	43863148	43863270
40188	13	44.1698	D13Rat108	(CA)n	0.29	219	21828843	21829056
38990	13		D13Rat58	(GT)n	26.04	249	71116479	71116738
40656	13		D13Rat131	(GT)n	33.98	147	80755428	80755583
39262	13		D13Rat86	(CA)n	40.75	209	91410706	91411049
36890	14	712399	D14Rat2	(GT)n	0	160	2350091	2350247
40852	14		D14Rat90	(CA)n	34.67	111	73956596	73956701
41138	14		D14Rat94	(GT)n	49.47	235	89192602	89192873
39030	14		D14Rat38	(GT)n	63.96	200	99079814	99080020
36516	15	66.5498	D15Rat1	(GT)n	1.63	280	8988164	8988442
36189	15		D15Rat17	(GT)n	33.51	178	45042674	45042874
40252	15		D15Rat101	(GT)n	53.95	157	38968338	38968494
41308	15		D15Rat107	(CA)n	65.4	151	55591156	55591299
40498	16	45.5099	D16Rat107	(GT)n	2.32	133	7776021	7776427
38428	16		D16Rat53	(GT)n	28.33	133	66606252	66606369
39010	16		D16Rat34	(GT)n	33.98	238	72653395	72653827
36608	16		D16Rat48	(CA)n	45.51	150	79850041	79850195
36051	17	47.5999	D17Rat1	(CA)n	3.67	166	5583326	5583494
36011	17		D17Rat17	(GACA)n	28.16	168	42344830	42344987
37998	17		D17Rat96	(GT)n	37.14	205	78253719	78254071
41084	17		D17Rat131	(CA)n	47.54	201	93347783	93347990
41120	18	52.3799	D18Rat112	(GT)n	2.48	223	6489051	6489519
39444	18		D18Rat96	(GT.GA)n	16	247	41105037	41105280
34975	18		D18Rat13	(TTTC)n	38.79	149	61728726	61728877
39278	18		D18Rat44	(GT)n	52.38	210	86863206	86863411
37042	19	48.1398	D19Rat19	(CT)n	2.24	140	7813537	7813671
35881	19		D19Rat11	(GT)n	22.52	128	31605037	31605157
40110	19		D19Rat64	(GT)n	38.05	243	46434967	46435202
43138	19		D19Rat5	(GT)n	46.36	157	54509577	54509737
37490	20	48.2299	D20Rat21	(GA)n	0	170	1674648	1675005
35327	20		D20Rat10	(GT)n	23.96	156	33642621	33642779
38304	20		D20Rat19	(GT)n	37.79	178	45070473	45070798
37792	20		D20Rat29	(CA)n	43.54	241	48055659	48055886

at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds were carried out. A final extension at 72°C for 5 minutes was allowed.

The PCR products were separated using 8% polyacrylamide (acrylamide/bis-acrylamide solutions 40% w/v) gel, and electrophoresis was carried out for 20 hours using 1 × Tris-base-boric acid-ethylenediamine tetra-acetic acid buffer. The potential difference for electrophoresis was set at 4 volts/cm and the gels were stained with ethidium bromide solution (E1510, Sigma, St Louis, MO, 10 mg/mL). The image of the gel was visualized using ultraviolet light on a transilluminator.

Scoring of genotypes and linkage analyses

The alleles amplified by each simple sequence repeat marker in all the progeny were independently scored by three people either as the homozygote parent A (lpk/lpk) or the heterozygote parent H (lpk/wky) in the BC1 (Figure 3) and either as the homozygote parent A, homozygote parent B (bn/bn), or heterozygote parent H (lpk/bn) in the F2 progeny (data available on request). When there was no agreement in the score because the alleles were not informative, the fragment was scored as a dash. Linkage analysis between the genotype and the phenotype to identify the quantitative trait locus that controlled the disease phenotypes in the LPK rats was carried out using Map Manager QTX20b software.[33]

A minimum $\log_{10}$ likelihood ratio (LOD) score of 3.6 was used to declare the presence of a quantitative trait locus,[34] and the additive effect for each quantitative trait locus was calculated.[33,35] The markers that showed LOD scores $>$ 3 in

Table 2 Number of progeny in F2 and BC1 with PKD and without PKD, and the Chi square statistics

Generation	Progeny	E	O	Ratio	χ^2	*P*-value
F2	152	38	42	1:03	0.53	~0.99
BC1	139	69.5	67	1:01	0.18	~0.97

Notes: χ^2, Chi square values; O, observed number of rats with PKD; E, expected number of rats with PKD; F2, second filial generation; BC1, backcross 1 generation.
Abbreviation: PKD, polycystic kidney disease.

the BC1 genotype were used in mapping and linkage analyses of the 152 F2 DNA samples.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences version 16 (SPSS Inc, Chicago, IL). One-way analysis of variance and multiple comparison tests with post hoc analysis of the combined male and female data sets were carried out, and significance was taken at $P < 0.05$, unless otherwise stated.

Results

Segregation ratios showed the inheritance of a recessive mutation in a single gene (Table 2). The cysts were only found in the kidneys of PKD rats and not in kidneys of non-PKD rats (Figure 1).

The blood and urine chemistry showed that the presence of cysts in the kidneys led to deterioration in kidney function. Consequently, the kidneys were unable to concentrate urine. Furthermore, the kidneys were unable to regulate the concentration of the osmolytes. An increase in systolic blood pressure (Figure 2A), a decrease in packed cell volume (Figure 2B), and a decrease in urine specific gravity (Figure 2C) strongly suggest that deterioration of kidney function due to development of cysts was responsible for the changes in these phenotypic traits. There was a highly significant difference in the measured clinical parameters between PKD and non-PKD in both male and female rats ($P < 0.0001$). However, there were no significant differences in the measured parameters between male and female rats with or without PKD (Table 3).

In linkage and mapping analyses, the genotypes of BC1 progeny (Figure 3) and F2 (not shown) associated with a

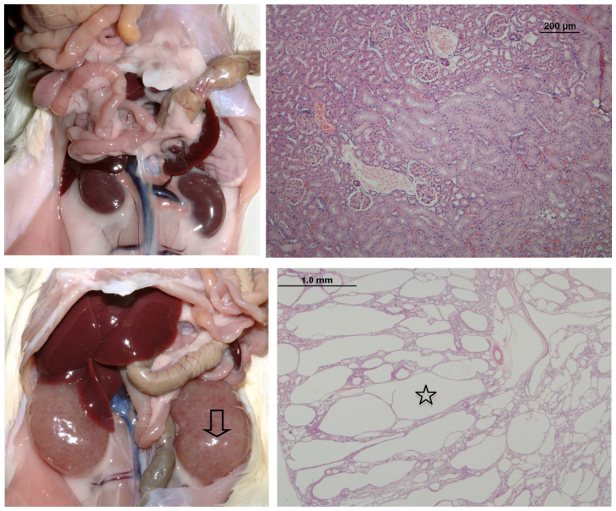

Figure 1 Gross and microscopic anatomy of kidneys from a BC1 rat that did not develop PKD (above) and gross and microscopic anatomy of kidneys of a BC1 rat that did develop PKD (below).
Notes: The arrow shows the fluid-filled cystic kidney and the star shows the cysts in a cross section of the kidney. Magnification 400×.
Abbreviation: PKD, polycystic kidney disease.

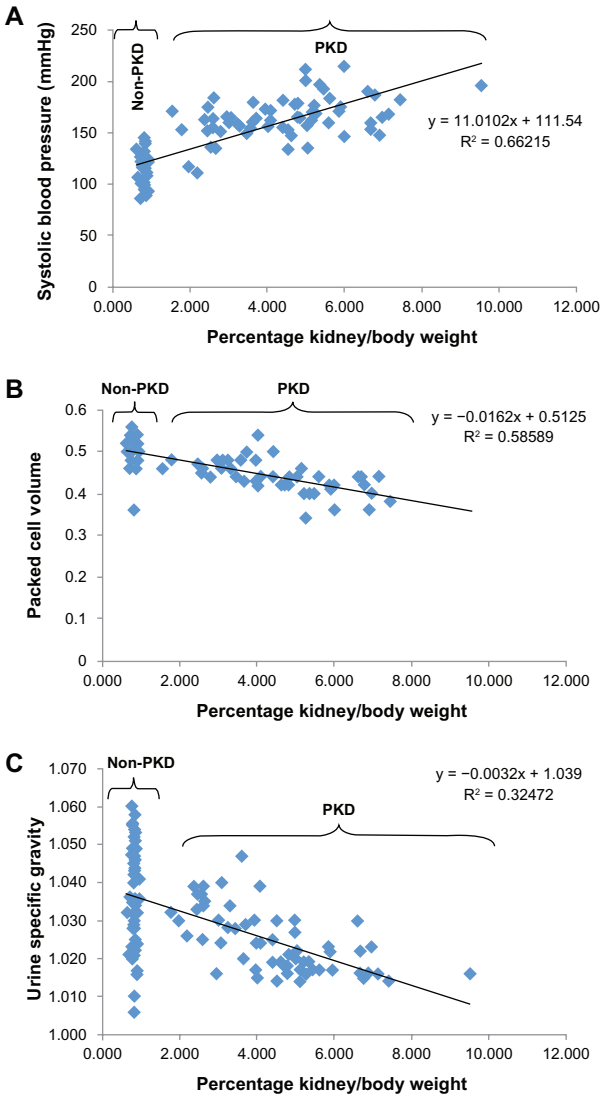

Figure 2 (**A**) Linear relationship between systolic blood pressure and percentage relative kidney/body weight, $R^2 = 0.66$, $P < 0.01$. (**B**) Linear relationship between packed cell volume and percentage kidney/body weight, $R^2 = 0.59$, $P < 0.01$. (**C**) Linear relationship between urine specific gravity and percentage kidney/body weight, $R^2 = 0.32$, $P < 0.01$.

Table 3 Phenotypes for PKD and non-PKD in PKD and non-PKD progeny

Generation	N	SBP (mmHg)[a]	%RK/BW[a]	PCV (L/L)[a]
F2 (non-PKD)				
Males	51	117.12 ± 1.45	0.80 ± 0.03	0.47 ± 0.01 (33)[b]
Females	58	116.29 ± 1.81	0.79 ± 0.01	0.48 ± 0.00 (36)[b]
F2 (PKD)				
Males	17	167.35 ± 5.65***	3.99 ± 0.50****	0.43 ± 0.02 (14)[b]***
Females	26	155.46 ± 3.74***	3.15 ± 0.37***	0.45 ± 0.01 (17)[b]***
BC1 (non-PKD)				
Males	38	118.76 ± 2.22	0.87 ± 0.04	0.50 ± 0.01 (26)[b]
Females	38	114.71 ± 1.74	0.82 ± 0.03	0.50 ± 0.00 (29)[b]
BC1 (PKD)				
Males	28	165.00 ± 3.28***	4.54 ± 0.30***	0.43 ± 0.01 (19)[b]***
Females	35	168.14 ± 2.94***	4.53 ± 0.26***	0.44 ± 0.01 (28)[b]***

Notes: [a]Data is presented as Mean ± SEM; [b]number of rats in each group of PCV phenotype; ***$P < 0.0001$ between PKD and non-PKD in both male and female rats.
Abbreviations: SBP, systolic blood pressure in mmHg; %RK/BW, percentage relative kidney/body weight; PCV, packed cell volume (L/L); PKD, polycystic kidney disease.

quantitative trait locus region on chromosome 10, and this quantitative trait locus maps to rat chromosome 10q21–q26 (Figure 4 for chromosome 10 only). In this figure, the values for the likelihood ratio statistics were found to be significant (8.4), very significant (13.5), or highly significant (21.3) as previously described.[33] In both the BC1 and the F2, the phenotypic traits linked to the same quantitative trait locus (Figure 5). In this figure, the phenotypes of the BC1 progeny link to a large genetic region, flanked by D10Rat180 and D10Rat26. The phenotypes of the F2 progeny link to a small genetic region within the BC1 genetic region, flanked by D10Rat43 and D10Rat26 ($P < 0.00001$). This finding confirms that the quantitative trait locus in this genetic region is responsible for the disease phenotypes in both the BC1 and F2 progeny. Presently, genetic mapping and linkage analyses of chromosome 10 using F2 traits are in progress to identify the actual quantitative trait locus and the gene responsible for the PKD phenotypes. As such, linkage results from the BC1 progeny only are discussed in this work.

Figure 6 shows a Manhattan plot where the negative $\log_{10}$ of the P values are plotted against the 20 rat autosomes. All the phenotypic traits are linked to the same quantitative trait locus region. This quantitative trait locus region maps to human chromosome 5q34–q35 and mouse chromosomes 11C and 18B1 (Figure 7). In mapping and linkage studies, LOD score and likelihood ratio statistics are commonly used and they are directly related. These are interchangeably used to identify an association between genetic regions that harbor sequence variants, which influence complex phenotypic traits under investigation.[33,36] The quantitative trait locus region maps to different human and mouse chromosomes (Figure 7), and the candidate genes located in the quantitative trait locus region are presented in Table 4.

Discussion

This study maps the quantitative trait locus associated with PKD traits in LPK rats. The major findings are that the PKD was a result of a recessive mutation in a single gene and the disease was inherited as ARPKD. The genetic locus that controlled the disease phenotypes was mapped to rat chromosome 10q21–q26. Mutation in this locus resulted in development of cysts in the kidneys of homozygous mutant rats. The presence of

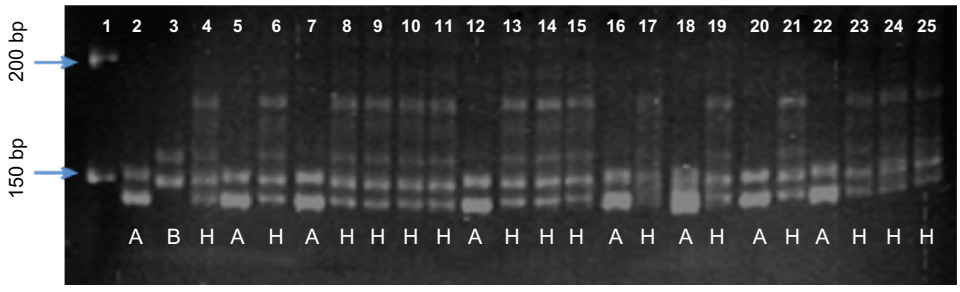

Figure 3 Genotypes for marker D10Rat43 in 22 BC1 DNA samples.
Notes: Lane 1, 50 bp molecular weight marker; lane 2, parent A (LPK/SsNArc$^{+/+}$); lane 3, parent B (WKY/NArc$^{-/-}$); lanes 4–25, BC1 progeny DNA samples. Parent B is included to show the segregation of alleles in parent A, parent B, and the heterozygote parent, H (LPK/SsNArc$^{+/+}$/WKY/NArc$^{-/-}$). Parent H is the female from the F1 progeny.

cysts in the kidneys led to hypertension, deterioration of kidney functions, development of anemia, and loss of homeostasis by the kidneys in all PKD rats. In this rat model, the inheritance of the disease was not accompanied by development of extrarenal cysts and the disease did not lead to any pup deaths.

It was established that all the F1 progeny (n = 291) from the two mating experiments did not develop cystic kidneys. This indicated that a recessive mutation in one gene was involved in the development of the disease, or mutations in several genes that range from dominant to recessive were involved but with interactive effect, making it unlikely that any of the F1 progeny developed the disease, unless a very large number of progeny was studied. The control of PKD phenotype by one gene was confirmed by the ratios of PKD to non-PKD in both the F2 and the BC1 progeny ($P > 0.05$).

The phenotypes of PKD in the LPK rats were similar in clinical presentation to the phenotypes of ARPKD in humans. However, there are also some fundamental differences in the two species. In both LPK rats and humans, homozygote individuals present with enlarged fluid-filled cystic kidneys. The cysts develop early in fetal life and in humans, enlarged kidneys can be observed in utero using computed tomography scanning.[1] In LPK rats, enlarged kidneys were palpated in the first week of birth.

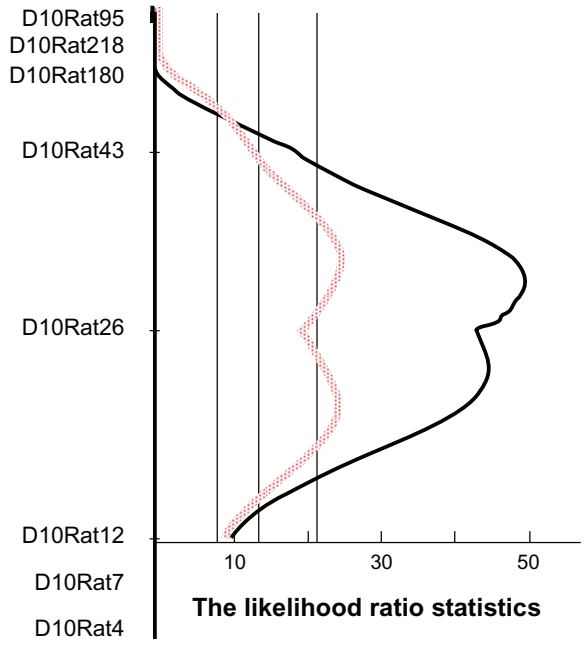

Figure 4 Interval mapping between the PKD phenotype and the BC1 genotypes.
Notes: The interval mapping is between the PKD phenotype and the marker genotypes of the BC1 DNA samples. The black curved line shows the likelihood ratio statistics and the red curved line is the regression coefficient. The vertical lines (from left to right) show chromosome 10, estimates for critical likelihood ratio statistic values for PKD phenotype: significant (8.4), very significant (13.5), and highly significant (21.3, $P < 0.05$).
Abbreviation: PKD, polycystic kidney disease.

In humans only, infants with ARPKD suffer from pulmonary hyperplasia and most die early in life.[37] In the LPK rats, there was no case of progeny death. This demonstrates that the progeny did not suffer from pulmonary hyperplasia. Furthermore, in human studies, affected individuals developed extrarenal cysts in the liver, spleen, pancreas, and other extrarenal organs.[38] However, not all the PKD rats developed extrarenal cysts. The lack of extrarenal cysts in PKD rats may not have been due to the effect of the genetic background of Brown Norway and Wistar Kyoto rats or the effect of modifier loci, as proposed in a study of Wistar polycystic kidney (wpk) rats,[37] but a result of a mutation in a different gene, which is still unknown. Numerous PKD studies in murine models have shown that genetic modifier loci influence the severity of the disease phenotypes.[5,7,9,39] In this study, it is also possible that an unknown mechanism yet to be identified is responsible for development of ARPKD in the LPK rats.

All the PKD rats developed high systolic blood pressure compared with their age-matched counterparts without

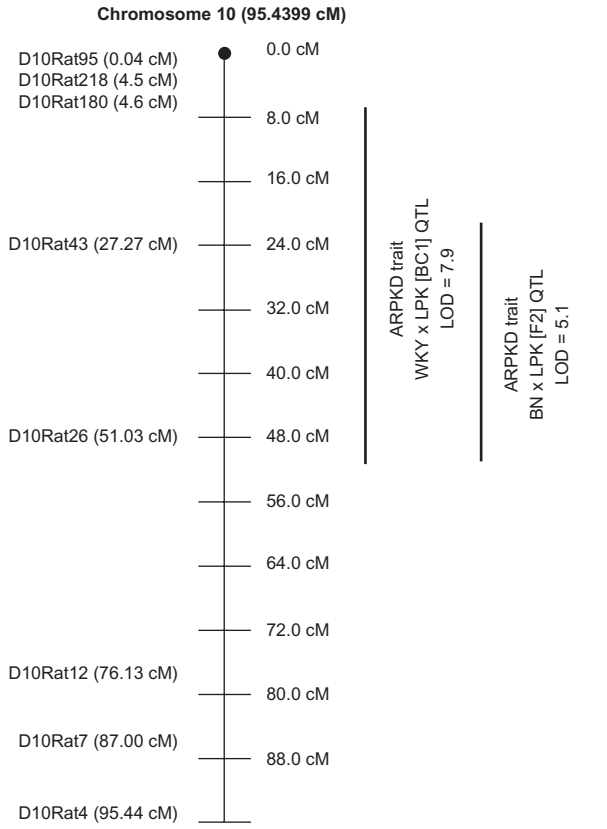

Figure 5 Chromosome 10 and quantitative trait locus containing the locus that controls the PKD phenotypes.
Notes: The quantitative trait loci and LOD scores for BC1 and F2 progeny. The two peak markers linked to the PKD phenotype in the two crosses are D10Rat43 and D10Rat26, and they are 23.76 cM apart.
Abbreviation: PKD, polycystic kidney disease.

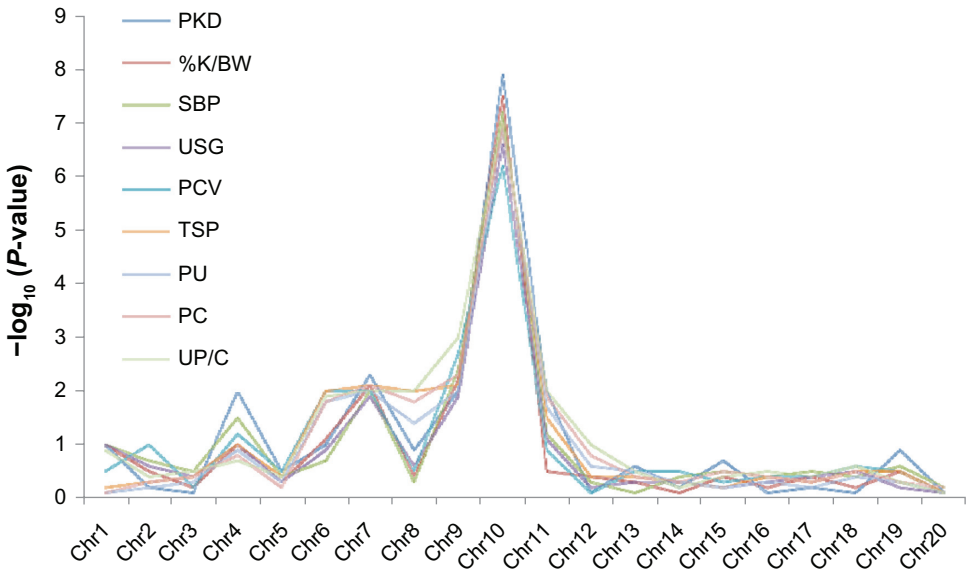

Figure 6 Manhattan plot. Negative $\log_{10}$ (*P* value) is plotted for each of the 20 rat autosomes.
Notes: All the phenotypic traits link to chromosome 10q21–q26, shown by the highest peaks. This genetic region is where the recessive mutation in a single gene is located and is responsible for ARPKD in the LPK rats.
Abbreviations: ARPKD, autosomal recessive polycystic kidney disease; LPK, Lewis polycystic kidney.

PKD, and this is a common feature in all PKD patients and animal models.[40] The development of higher systolic blood pressure in PKD rats preceded end-stage renal disease. This is similar to the development of higher systolic blood pressure in humans with PKD.[41] However, the mechanism involved in the development of higher systolic blood pressure due to development of PKD in kidneys is not well established in human studies, and is not well understood in LPK rats. However, stimulation of the renin-angiotensin-aldosterone system and increased sympathetic activity were suggested to influence systolic blood pressure[42,43] in all individuals who develop PKD.

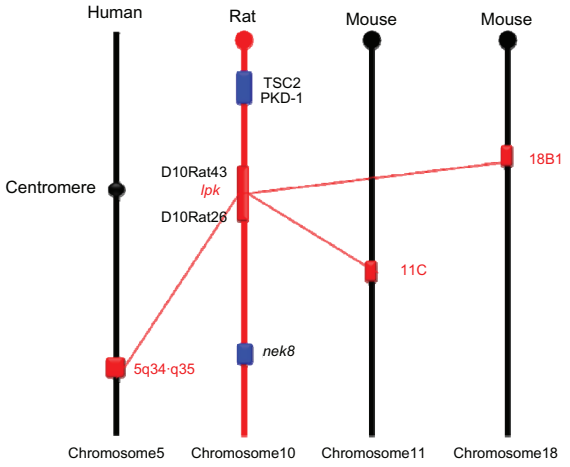

Figure 7 Quantitative trait locus on the LPK rat region 10q21–q26 mapped to human and mouse chromosomes.
Notes: The quantitative trait locus that controls the phenotype of ARPKD in the LPK/SsNArc$^{+/+}$ rats maps to human chromosome 5q34–q35 (*MAT2β* gene) and mouse chromosomes 11C (*Bcas3* gene) and 18B1 (*Rit2* gene).
Abbreviations: ARPKD, autosomal recessive polycystic kidney disease; LPK, Lewis polycystic kidney.

Measurement of packed cell volume is important in clinical studies to predict the development of anemia, which results from reduction in red blood cell mass or hemoglobin concentration, or both.[44] In this study, measurement of packed cell volume was necessary to define the effect of development of renal cysts on kidney function. Decreased packed cell volume in the PKD rats showed that the development of cysts in the kidney affected the physiological and homeostatic functioning of the kidneys.

Mapping and linkage analyses associated the PKD phenotypes in the LPK rats to a quantitative trait locus region on rat chromosome 10q21–q26. Mapping and linkage studies are commonly used to identify quantitative trait loci associated with particular traits.[45–48] In this study, the same methods were applied to identify the quantitative trait locus responsible for the PKD traits in the LPK rats. It was established that all the phenotypic traits link to the same quantitative trait locus, giving a maximum LOD score of 7.9 between markers D10Rat180 and D10Rat26 in BC1. The LOD score of 5.1 between markers D10Rat43 and D10Rat26 in F2 confirms that this is the actual quantitative trait locus responsible for the disease phenotypes in the LPK rats. The high LOD scores in both BC1 and F2 provide strong evidence for linkage between the phenotypic traits and the genotypes located in the quantitative trait locus.[33]

Table 4 Candidate genes and proteins encoded by genes in the quantitative trait locus region linked to PKD traits on chromosome 10q21–q26

Protein	Gene	Gene ID
Methionine adenosyltransferase IIβ	*Mat2b*	683630
Kidney injury molecule 1	*KIM1*	689968
Gamma-aminobutyric acid A receptor gamma 2	*Gabrg2*	29709
Pituitary tumor-transforming 1	*Pttg1*	64193
C1q and tumor necrosis related factor 2	*C1qtnf2*	497886
Eukaryotic translation initiation factor 4 gamma 1	*Eif4g1*	497887
Cyclin J-like	*Ccnjl*	303050

Abbreviation: PKD, polycystic kidney disease.

There was a highly significant difference in the values for the likelihood ratio statistics in the quantitative trait locus associated with the PKD traits. The likelihood ratio statistics and LOD score are interchangeably used to find an association between a phenotype and a genotype, with the aim of identifying genomic regions that harbor sequence variants which influence complex disease phenotypes.[36] However, the likelihood ratio statistics technique is commonly used and is statistically convenient because its distribution is asymptotically a χ^2 distribution.[33]

A Manhattan plot also confirmed the association of disease phenotypes in the LPK rats to the locus on chromosome 10. Therefore, this study has provided significant evidence that this quantitative trait locus is the only genetic region responsible for the phenotypic trait variation in LPK rats. The location of the quantitative trait locus in this genetic region has excluded the genetic loci that controlled expression of ARPKD in previously studied murine models of the disease.[49,50] This clearly shows that the locus responsible for the disease is novel and different from the loci that contain *Pkhd-1*, the gene responsible for ARPKD in other models of the disease. This quantitative trait locus does not contain *Nek8*, located on rat chromosome 10, mutation of which was associated with development of ARPKD in other models.[51]

The quantitative trait locus on rat chromosome 10q21–q26 shares homology with human chromosomes 5q34–q35 and mouse chromosomes 11C and 18B. Human chromosome 5q34–q35 contains the *MAT2β* gene and mouse chromosomes 11C and 18B1 contain breast carcinoma-amplified sequence 3 and Ras-like without CAAX2, respectively. These genes are different from *Pkhd-1*, which is responsible for ARPKD in other murine models. However, the genes are important in signal transduction, cell proliferation, and cell differentiation,[52,53] and their mutations were previously linked to development of cancer in different human and rat tissues.[54–56] Nonetheless, there is no evidence in the present study to suggest that the ARPKD inherited in LPK/SsNArc$^{+/+}$ rats is a cancer of kidney tissue. The location of the quantitative trait locus on chromosome 10q21–q26, which maps to human chromosome 5q34–q35, has excluded previously the identified human locus on chromosome 6p21.1–p12[16] as a possible candidate in the development of ARPKD in LPK rats.

Conclusion

It has been established that a recessive mutation in a single gene at a quantitative trait locus region located on rat chromosome 10q21–q26 is responsible for ARPKD in LPK rats. Unlike other forms of ARPKD that result in liver-related morbidity and mortality, the ARPKD in this rat model is not associated with liver-related death. Accumulation of fluids in the cysts resulted in kidney enlargement, and with time, kidney homeostatic function deteriorated. Deterioration in kidney function resulted in an increase in systolic blood pressure, a mechanism to compensate for decreased renal perfusion. Because the quantitative trait locus that harbors the disease gene responsible for the phenotypic trait variation spans a large genetic region in chromosome 10, genetic mapping of the entire chromosome using a dense set of simple sequence repeat markers along the chromosome is underway to identify the actual quantitative trait locus and the gene involved in expression of the disease. In addition, it is recommended that expression studies be carried out to explain the reason for the phenotypic trait variation between LPK and other rat models in the development of ARPKD. Therefore, this study has created an opportunity to identify the mutated gene and the type of mutation responsible for ARPKD in LPK rats. It has also presented a possibility for more research with the aim of identifying therapeutic interventions to prevent and/or cure ARPKD.

Acknowledgments

Dora Li and Andrew Williams of Murdoch University are thanked for their assistance and technical support with this study.

Disclosure

The author reports no conflicts of interest in this work.

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