CHARACTERIZATION OF POLYCYSTIC KIDNEY DISEASE IN THE LEWIS POLYCYSTIC KIDNEY RAT

Jada YENGKOPIONG
BSc [HONS] – BIOCHEMISTRY; MSc [MED] – PHARMACOLOGY AND THERAPEUTICS; MBSc – BIOMEDICAL SCIENCES; GRADUATE CERTIFICATE IN RESEARCH MANAGEMENT

FACULTY OF HEALTH SCIENCES
DIVISION OF VETERINARY AND BIOMEDICAL SCIENCES
MURDOCH UNIVERSITY
AUSTRALIA

THIS THESIS IS PRESENTED TO MURDOCH UNIVERSITY IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
APRIL 2010
Declaration

I, Jada Pasquale YENGKOPIONG, hereby declare that the work on which this thesis is based is my own account of my research and contains as its main content work, which has not previously been submitted for a degree at any tertiary institution.

Jada YENGKOPIONG
Dedication

To my father LOKU with love and gratitude
To my mother JUKA with thanks for the gift of life
To my wife TOSIKI with thanks for the gift of love
To my sons GUBEK, SWAKA and WANI with love for showing me my childhood
Acknowledgements

I have always given thoughts to moments that have changed my life. None is so central like being at Murdoch University. This, I consider a turning point in my life. While I take pride in the study reported in this thesis because it is the work of my own hands, and therefore I am the author of the thesis, it is also the result of the dedicated effort of many individuals, some of whom deserve special mention. First of all, my supervisors Dr. David Miller of Murdoch University and Dr. Kylie Munyard of Curtin University of Technology who offered their time to guide me to this point, because of their love to see students reach their academic potential. I would like to thank Professor Graham Wilcox, the Head of School of Veterinary and Biomedical Sciences and Professor Graham O’Hara, the Dean of Postgraduate Students in a special way for their invaluable advice without which this study would be like a blind alley. I must also thank Dr. Trish Fleming and Dr. Casta Tungaraza for their ever-encouraging advice. Many thanks go to Dora Li for assisting me in the initial stages of the PCR analyses and Prof. Grant Morahan from the Western Australian Institute of Medical Research for his generous advice in this study. Of course, there are other people who are not mentioned here but have helped me in various ways and specially for introducing me to appreciate the genetic causes of polycystic kidney disease (PKD). To them, a big thank you.
I am indebted to Murdoch University in many ways and especially for having given me the opportunity to investigate the role of genes in the development of PKD. I received the Murdoch scholarship to carry out this research and I am truly grateful for it. It would be nearly impossible to do this study without the scholarship.

I would like to thank my wife, Lilly TOSIKI, first for having introduced me to Murdoch University, and second for having the patience to let me do what I wanted most. When it all became confusing, she whispered into my ears, “yes you can”. I also want to thank my sons Gubek, Swaka and Wani who would always welcome me home and tell me, “good job baba”.

I want to mention here early that whether or not this thesis is accepted, I am confident I shall be acquitted of having acted recklessly. In all I have done, I have had a conviction for the faith in me and I was driven by the reasons that guide all of us in research: - First to passionately investigate the causes of PKD, and second, to progress the advancement of science in the understanding of PKD. Autosomal recessive polycystic kidney (ARPKD) that has become the theme of this thesis requires a quick response and all the scientific community is called upon to partake in finding a cure for it. Whether or not ARPKD is a form of neoplasm, the gene that leads to its development can be interfered with so that its transmission in successive generations is not evident. Can we find the gene and a cure for ARPKD? YES WE CAN!
**Acronyms and Abbreviations**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ADPKD</td>
<td>Autosomal dominant polycystic kidney disease</td>
</tr>
<tr>
<td>AR</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>ARPKD</td>
<td>Autosomal recessive polycystic kidney disease</td>
</tr>
<tr>
<td>BC1</td>
<td>Backcross 1</td>
</tr>
<tr>
<td>BN/ssArc^-^-</td>
<td>Brown Norway</td>
</tr>
<tr>
<td>bpk</td>
<td>BALK/c polycystic kidney</td>
</tr>
<tr>
<td>CAML</td>
<td>Calcium ion modulating cyclophilin ligand</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosylmonophosphate</td>
</tr>
<tr>
<td>CHF</td>
<td>Congenital hepatic fibrosis</td>
</tr>
<tr>
<td>C1</td>
<td>Chloride anion</td>
</tr>
<tr>
<td>cM</td>
<td>CentiMorgan</td>
</tr>
<tr>
<td>cpk</td>
<td>Congenital polycystic kidney</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>ESRD</td>
<td>End stage renal disease</td>
</tr>
<tr>
<td>F2</td>
<td>Second filial generation</td>
</tr>
<tr>
<td>FC</td>
<td>Fibrocystin</td>
</tr>
</tbody>
</table>
inv: Inversion of embryonic turning
jck: Juvenile cystic kidney
jcpk: Juvenile congenital polycystic kidney
Kat: Kidney, anemia, testis
LEW/SsNAr<sup>c-/c</sup>: Lewis
LOD: Logarithm of odds
LPK/SsNAr<sup>c+/c</sup>: Lewis polycystic kidney
LRS Likely Ratio Statistics
mTOR: Mammalian target of rapamycin
Na+: Sodium cation
NaCl: Sodium chloride
NPH: Nephronophthisis
orpk: Oak ridge polycystic kidney
PC-1: Polycystin-1
PC-2: Polycystin-2
PCD: Primary cilia dyskinesia
pck: Polycystic kidney
PCR: Polymerase chain reaction
PCV: Packed cell volume
pey: Polycystic kidney
PKD: Polycystic kidney disease
PKD-1: Polycystic kidney disease 1 gene in human
PKD-2: Polycystic kidney disease 2 gene in human
PKD-3: Polycystic kidney disease 3 gene in human
Pkd-1: Polycystic kidney disease 1 gene in murine models
Pkd-2: Polycystic kidney disease 2 gene in murine models
PKHD-1: Polycystic kidney and hepatic disease 1 gene in human
Pkhd-1: Polycystic kidney and hepatic disease 1 gene in murine models
QTL: Quantitative trait locus
RAAS: Renin-aldosterone-angiotensin system
RFLP: Restriction fragment length polymorphism
RGD: Rat Genome Database
RISC: RNA induced silencing complex
RNAi: Ribonucleic acid interference
SNP: Single nucleotide polymorphism
SNS: Sympathetic nervous system
SSR: Simple sequence repeat
TBE: Tris-base-Boric acid-EDTA
TBM: Tubular basement membrane
TRP: Transient receptor protein
TSC2: Tubular sclerosis
TSP: Total solid protein
UV: Ultraviolet light
V2R: Vasopressin-2-receptor
WKY/NArc: Wistar Kyoto
wpk: Wistar polycystic kidney
$\chi^2$: Chi Square
Summary

Polycystic kidney disease (PKD) is a life-threatening disorder that affects millions of people all over the world. The disease is usually inherited, but it can also be acquired and it leads to development of many cysts in the kidneys, liver, pancreas, brain, spleen, ovaries and testes. The major types of inherited PKD are autosomal dominant (AD) and autosomal recessive (AR) polycystic kidney disease. ADPKD is caused by mutations in PKD-1 (polycystin-1), PKD-2 (polycystin-2) or PKD-3 (polycystin-3) and it is mostly diagnosed in adults. ARPKD is caused by mutation in the polycystic kidney and hepatic disease gene 1 (PKHD-1, fibrocystin) and it is commonly diagnosed in neonates and infants. In murine models of the disease, the Pkd-1, Pkd-2 and Pkhd-1 genes are the homologs of human PKD genes and mutations in these genes cause PKD that resembles the human PKD. The common clinical features of PKD in all animal species are: development of bilaterally enlarged cystic kidneys, development of extra-renal cysts, development of higher systolic blood pressure, development of anemia, and deterioration of the kidney functions, leading to end stage renal disease.

In the present study, a spontaneous mutation occurred in the Lewis rat strain and this resulted in development of PKD in the mutant rats. Mating experiments between the mutant rats, now referred to as Lewis Polycystic Kidney rats (LPK/SsNAr+/-), produced all progeny with cystic kidneys. Unlike other forms of PKD, the PKD in this rat model did not lead to infant deaths and it did not lead to development of extrarenal cysts. Furthermore, the inheritance of the disease, the chromosome and
the quantitative trait locus (QTL) that harbors the mutation responsible for the
disease were not known. For these reasons, the inheritance of the disease in the
LPK/SsNArct+/+ rats was determined. Genetic mapping to identify the region that
controls the PKD phenotypes using 92 polymorphic simple sequence repeat markers,
covering the genome of the 20 rat autosomes, was carried out. Linkage analyses
between marker genotypes and phenotypic trait data in 152 F2 and 139 BC1 progeny
was performed and the QTL identified.

The PKD was inherited as an ARPKD, controlled by a recessive mutation in a single
gene (F2: PKD = 42, non-PKD = 110, \( \chi^2 = 0.53; \) BC1: PKD = 63, non-PKD = 76, \( \chi^2 \\
= 0.18, P > 0.05 \)). The PKD rats developed larger cystic kidneys: F2: %K/B weight = 
3.48 ± 0.30, n = 42; BC1: %K/B weight = 4.53 ± 0.20, n = 67, than the non-PKD
rats, F2: %K/B weight = 0.79 ± 0.01, n = 110; BC1: %K/B weight = 0.85 ± 0.02, n =
72, (P < 0.001). The PKD rats developed higher systolic blood pressure: F2: SBP =
160 ± 3 mmHg, n = 42; BC1: SBP = 164 ± 2 mmHg, n = 67, than the non-PKD rats,
F2: SBP = 117 ± 3 mmHg, n = 110; BC1: SBP = 116 ± 1 mmHg, n = 72, (P <
0.001). The PKD rats developed anemia: F2: PCV = 0.44 ± 0.01, n = 31; BC1: PCV \\
= 0.44 ± 0.01, n = 47, compared to the non-PKD rats, F2: PCV = 0.48 ± 0.00, n = 69;
BC1: PCV = 0.50 ± 0.00, n = 55, (P < 0.001). The PKD rats were less able to
concentrate urine: BC1: urine P/C ratio = 1.31 ± 0.15, n = 58 compared to the non-
PKD rats, urine P/C ratio = 0.76 ± 0.07, n = 65, (P < 0.001).
In the BC1 progeny, the PKD trait linked to marker D10Rat43 on chromosome 10q21, giving a LOD score of 7.9 with D10Rat218, (P = 0.00001). In the F2 progeny, the PKD trait linked to marker 10Rat26 on chromosome 10q21, giving a LOD score of 5.1 with D10Rat43, (P = 0.00001). All the other phenotypic traits in the BC1 and the F2 progeny also linked to the same QTL, and D10Rat43 and D10Rat26 are the peak markers, spanning a total genetic distance of 23.76 cM on rat chromosome 10q21.

The QTL region that controls the PKD phenotype does not contain the Pkhd-1 gene known to be responsible for ARPKD in well-characterized murine models. The QTL maps to human chromosome 5q34-5q35 and to mouse chromosomes 11C and 18B1. The location of the QTL in 10q21 has excluded the LPK/SsNArc+/+ locus as a candidate homolog for PKHD-1, which was located on human chromosome 6p21-p12. It has also excluded the wpk locus, which maps to rat chromosomes 5q13 and 10q25, and the bpk locus, which maps to mouse chromosome 10, the cpk locus, which maps to chromosome 12 and the orpk locus, which maps to chromosome 14, as homologs.

The candidate genes located in the QTL are the methionine adenosyltransferase IIβ, kidney injury molecule 1, gamma-aminobutyric acid receptor gamma 2, pituitary tumor-transforming gene 1, C1q, and tumor necrosis factor 2, eukaryotic translation initiation factor 4 gamma 1 and cyclin J-like. The proteins encoded by these genes are not homologous to, do not have common domains with fibrocystin, except for a signal peptide domain found between methionine adenosyltransferase IIβ, kidney
injury molecule 1 and fibrocystin. However, the genes are important in signal transduction, cell growth, cell proliferation, apoptosis and cell differentiation. Mutations in these genes were previously linked to cellular aberrations and tumors in various human organs.

In conclusion, the results presented in this thesis support the general hypothesis that a recessive mutation in a single gene was responsible for the development and inheritance of PKD in the LPK/SsNArcre/+ rats. The ratios: 42:110 in the F2 progeny and 67:72 in the BC1 progeny for PKD to non-PKD rats do not significantly deviate from Mendelian segregation ratios of 1:3 in the F2 and 1:1 in the BC1, and therefore, support the requirement for the inheritance of a recessive mutation controlled by one gene. The QTL mapped is novel and it was not previously linked to ARPKD in other murine models. It is therefore predicted that a new gene is responsible for ARPKD in LPK/SsNArcre/+ rats or an unknown mechanism is responsible for the development of the disease. Chromosome 10q21 is now targeted for fine genetic mapping to identify the actual gene that causes ARPKD. Once the gene is identified, sequencing of the gene in both the PKD and the non-PKD rats will be carried out to identify the type of mutation.
Table of Contents

Declaration..................................................................................................................i

Dedication ................................................................................................................... ii

Acknowledgements ..................................................................................................... iii

Acronyms and Abbreviations .......................................................................................v

Summary ..................................................................................................................... viii

Table of Contents ...................................................................................................... xii

List of Figures ............................................................................................................. xix

List of Tables .............................................................................................................. xxi

CHAPTER I ................................................................................................................... 1

  General Introduction ................................................................................................. 1

    1.1: Introduction ........................................................................................................ 1

    1.2: Mechanism in Development of Cysts in PKD patients ...................................... 2

    1.3: Diagnosis and Treatment of Polycystic Kidney Disease .................................... 3

    1.4: Animal Models Used in the Study of Polycystic Kidney Disease ....................... 4
CHAPTER II..............................................................................................................................................6

Literature Review ........................................................................................................................................... 6

2.1: Introduction ..............................................................................................................................................6

2.2: Murine Models of Polycystic Kidney Disease ...................................................................................... 12
  2.2.1: Rat Models of Polycystic Kidney Disease ......................................................................................13
  2.2.2: Mouse Models of Polycystic Kidney Disease ................................................................................14

2.3: Enlargement of Cystic Kidneys ............................................................................................................17
  2.3.1: Proliferation of Epithelial Cells Adjacent to the Point of Lesion ....................................................18
  2.3.2: Fluid Secretion in Normal and Cystic Renal Epithelial Cells .........................................................18
  2.3.3: Accumulation of Fluids and Enlargement of the Cysts .................................................................19
  2.3.4: Restructuring of Tubular Basement Membrane ............................................................................21

2.4: Genetic Mutations in Autosomal Dominant Polycystic Kidney Disease ...........................................21
  2.4.1: Two-hit Mechanism to Explain Cyst Formation ............................................................................21
  2.4.2: Patients with Polycystic Kidney Disease in Australia ....................................................................24
  2.4.3: Involvement of Polycystin-1 and Polycystin-2 on Cell Membranes ..............................................26
  2.4.4: Expression of Polycystin-1 and Polycystin-2 .................................................................................30
  2.4.5: Development of Cysts in ADPKD ..................................................................................................32

2.5: Autosomal Recessive Polycystic Kidney Disease .................................................................................36
  2.5.1: Structure and Function of Fibrocystin Protein ..............................................................................39
  2.5.2: Molecular Structure of Cilia and Arrangement of Axoneme ..........................................................41
  2.5.3: Polycystic Kidney Disease and Cilia-related Infertility ..................................................................44

2.6: Co-morbidity of Polycystic Kidney Disease .........................................................................................44
  2.6.1: Polycystic Kidney Disease and Hypertension ..............................................................................45
  2.6.2: Polycystic Kidney Disease and Anemia .........................................................................................47
  2.6.3: Polycystic Kidney Disease and Kidney Deterioration ...................................................................48
2.7: Treatment of Patients with Polycystic Kidney Disease ........................................ 48
  2.7.1: Renal Replacement Therapy in Patients with PKD ........................................ 49
  2.7.2: Dialysis and Infection in Patients with End Stage Renal Disease .................... 50
  2.7.3: Kidney Transplant in Patients with End Stage Renal Disease ........................ 50
  2.7.4: Vasopressin-2-receptor Antagonists in the Treatment of PKD ....................... 52
  2.7.5: Mammalian Target of Rapamycin Inhibitors .............................................. 54
  2.7.6: Ribonucleic Acid Interference ......................................................................... 55
2.8: Inheritance of PKD in the Lewis Polycystic Kidney Rats ....................................... 56
2.9: Genetic Mapping and Linkage Analysis of the PKD Locus .................................... 57
  2.9.1: Logarithm of Odds (LOD) Score ....................................................................... 60
2.10: The Study Plan ..................................................................................................... 62
  2.10.1: Crossbreeding to Determine the Inheritance of PKD ...................................... 62
  2.10.2: Backcross Studies for Mapping and Linkage Analysis ................................... 63
  2.10.3: Gene Identification in the Lewis Polycystic Kidney Rats ................................. 65
2.11: The Hypothesis ..................................................................................................... 67
2.12: The Aim and Objectives ........................................................................................ 67
  2.12.1: The Aim of the Study ....................................................................................... 67
  2.12.2: The Objectives of the Study ............................................................................ 68
CHAPTER III ........................................................................................................................................ 69

Determination of Inheritance of Polycystic Kidney Disease ...................................................... 69

3.1: Introduction ................................................................................................................................ 69

3.2: Materials and Methods ............................................................................................................... 70

3.2.1: Breeding of Animals ............................................................................................................... 70

3.2.2: Rat Strains to Determine the Inheritance of PKD ................................................................. 70

3.2.3: Experiment to Raise Second Filial (F2) Generation ............................................................... 71

3.2.4: Experiment to Confirm the Mode of Inheritance ................................................................. 72

3.2.5: Recording of Phenotypic Traits ............................................................................................ 73

3.2.5.1: Measurement of Systolic Blood Pressure ........................................................................ 73

3.2.5.2: Urine Collection and Analysis .......................................................................................... 73

3.2.5.3: Blood, Kidney and Liver Collection ................................................................................... 74

3.2.5.4: Blood Analysis .................................................................................................................. 75

3.2.5.5: Histological Analysis of Sections ..................................................................................... 75

3.2.5.6: Statistical Analysis ........................................................................................................... 76

3.3: Results .......................................................................................................................................... 76

3.3.1: Gross and Microscopic Anatomy ........................................................................................... 78

3.3.2: Body and Kidney Weights ..................................................................................................... 81

3.3.3: Systolic Blood Pressure ........................................................................................................ 83

3.3.4: Blood Sample Analysis ........................................................................................................ 85

3.3.5: Urine Sample Analysis .......................................................................................................... 90

3.4: Discussion .................................................................................................................................... 95
CHAPTER IV......................................................................................................................... 104

Determination of Quantitative Trait Locus Associated with ARPKD .............. 104

4.1: Introduction ......................................................................................................................... 104

4.2: Materials and Methods ..................................................................................................... 105
   4.2.1: The SSR Markers Chosen to Map the Trait Locus ..................................................... 105
   4.2.2: DNA Extraction and PCR Analysis .......................................................................... 109
   4.2.3: Scoring of Genotypes and Linkage Analysis ............................................................. 110
   4.2.4: Identification of Candidate Genes Controlling Phenotypic Traits ......................... 111

4.3: Results .................................................................................................................................. 111
   4.3.1: Gel Map and Scores ................................................................................................. 113
   4.3.2: Interval Mapping ....................................................................................................... 115
   4.3.3: Linkage Analysis and Epistatic Interaction ............................................................... 124
   4.3.4: The QTL that Controls the PKD Phenotypes ............................................................ 128
   4.3.5: The Candidate Genes in the QTL Region ................................................................. 129

4.4: Discussion ........................................................................................................................... 130
CHAPTER V ................................................................................................................................. 137

Analysis of Proteins Encoded by the Candidate Genes in the QTL .................. 137

5.1: Introduction ....................................................................................................................... 137
5.2: Materials and Methods ................................................................................................. 138
5.3: Results ............................................................................................................................. 140

5.3.1: Mapping Analysis ....................................................................................................... 141
5.3.2: Multiple Sequence Alignment ................................................................................... 142
5.3.3: Phylogenetic Analysis ................................................................................................ 143
5.4: Discussion ....................................................................................................................... 145
CHAPTER VI................................................................................................................. 150

General Discussion ........................................................................................................... 150

6.1: Discussion .................................................................................................................. 150

6.2: Future Direction ......................................................................................................... 159

6.3: General Conclusion .................................................................................................... 160

References .......................................................................................................................... 162

Appendix A ......................................................................................................................... 196

Appendix B .......................................................................................................................... 220

Appendix C .......................................................................................................................... 237
List of Figures

Figure 2-1: The Kidneys of Rats without PKD and with PKD .................................................. 11
Figure 2-2: PKD Cyst Enlargement ............................................................................................ 20
Figure 2-3: The Two-Hit Model in ADPKD ................................................................................. 23
Figure 2-4: Polycystin-1 Traversing the Membrane ................................................................. 27
Figure 2-5: Polycystin-2 Traversing the Membrane ................................................................. 28
Figure 2-6: The Interaction of PC-1 and PC-2 .......................................................................... 31
Figure 2-7: ADPKD in the Human Renal Nephron ................................................................. 33
Figure 2-8: A Coronal Section of Kidney from a PKD Rat ......................................................... 34
Figure 2-9: A Cross Section of Kidney Cortex ..................................................................... 35
Figure 2-10: ARPKD in the Collecting Ducts of the Human Nephron ..................................... 37
Figure 2-11: Molecular Structure of Fibrocystin .................................................................... 40
Figure 2-12: A Cross Section of Cilia with 9 + 2 and 9 + 0 Structure ..................................... 42
Figure 2-13: Cellular Changes Associated with PKD ............................................................ 53
Figure 2-14: Pathway to Identify the PKD Gene in the LPK/SSNARC+/+ Rat ....................... 66
Figure 3-1: Gross and Microscopic Anatomy in Non-PKD Kidneys ........................................ 78
Figure 3-2: Gross and Microscopic Anatomy of PKD Kidneys .............................................. 79
Figure 3-3: Microscopic Anatomy of Liver from PKD and Non-PKD Rats ............................. 80
Figure 3-4: Mean Body Weight of PKD and Non-PKD Rats .................................................... 81
Figure 3-5: Percentage Relative Kidney/Body Weight ............................................................ 82
Figure 3-6: Mean Systolic Blood Pressure of PKD and Non-PKD Rats .................................... 83
Figure 3-7: Systolic Blood Pressure and Percentage Kidney/Body Weight ............................ 84
Figure 3-8: Packed Cell Volume in PKD and Non-PKD Rats .................................................. 85
Figure 3-9: Correlation between PCV and Percentage K/B Weight ........................................ 86
Figure 3-10: Plasma Total Solid Protein in PKD and Non-PKD Rats ....................................... 87
Figure 3-11: Plasma Creatinine of PKD and Non-PKD Rats .................................................... 88
Figure 3-12: Plasma Urea of PKD and Non-PKD Rats ............................................................. 89
FIGURE 3-13: URINE PROTEIN OF PKD AND NON-PKD RATS .................................................. 90
FIGURE 3-14: URINE CREATININE IN PKD AND NON-PKD RATS ............................... 91
FIGURE 3-15: URINE PROTEIN/CREATININE RATIO IN PKD AND NON-PKD RATS .......... 92
FIGURE 3-16: URINE SPECIFIC GRAVITY OF PKD AND NON-PKD RATS ...................... 93
FIGURE 3-17: CORRELATION BETWEEN SPECIFIC GRAVITY AND PERCENTAGE K/B WEIGHT .......... 94
FIGURE 4-1: CHROMOSOME 1 AND SSR MARKERS CHOSEN ......................................... 106
FIGURE 4-2: GENOTYPE FOR MARKER D10Rat43 IN 22 BC1 DNA .................................. 113
FIGURE 4-3: GENOTYPE FOR MARKER D10Rat26 IN 22 F2 DNA .................................. 114
FIGURE 4-4: INTERVAL MAPPING BETWEEN THE PKD TRAIT AND THE BC1 GENOTYPE ........ 115
FIGURE 4-5: INTERVAL MAPPING BETWEEN SYSTOLIC BLOOD PRESSURE AND THE BC1 GENOTYPE ... 116
FIGURE 4-6: INTERVAL MAPPING BETWEEN PERCENTAGE KIDNEY/BODY WEIGHT AND BC1 GENOTYPE ....... 117
FIGURE 4-7: INTERVAL MAPPING BETWEEN PACKED CELL VOLUME AND THE BC1 GENOTYPE ........... 118
FIGURE 4-8: INTERVAL MAPPING BETWEEN PLASMA TOTAL SOLID PROTEINS AND THE BC1 GENOTYPE ........ 119
FIGURE 4-9: INTERVAL MAPPING BETWEEN PLASMA UREA AND THE BC1 GENOTYPE ........... 120
FIGURE 4-10: INTERVAL MAPPING BETWEEN PLASMA CREATININE AND BC1 GENOTYPE ........ 121
FIGURE 4-11: INTERVAL MAPPING BETWEEN URINE SPECIFIC GRAVITY AND BC1 GENOTYPE ........ 122
FIGURE 4-12: INTERVAL MAPPING BETWEEN URINE PROTEIN/CREATININE RATIO AND THE BC1 GENOTYPE 123
FIGURE 4-13: CHROMOSOME 10 AND QTL CONTAINING THE LPK/SsNarC+/locus .................. 128
FIGURE 5-1: MAPPING QTL TO HUMAN AND MOUSE CHROMOSOMES .......................... 141
FIGURE 5-2: CURATED PROTEIN SEQUENCES .......................................................... 142
FIGURE 5-3: EVOLUTIONARY RELATIONSHIPS ......................................................... 143
FIGURE 6-1: MANHATTAN PLOT .................................................................................. 151
FIGURE 6-2: SUMMARY: DEVELOPMENT OF ARPKD IN THE LPK/SsNarC+/locus RATS .......... 153

XX
List of Tables

**TABLE 2-1:** GENETIC HETEROGENEITY OF NPH, SENIOR-LÖKEN, JOUBERT AND MECKEL-GRUBER SYNDROMES 9

**TABLE 3-1:** THE CHI SQUARE STATISTICS ................................................................. 77

**TABLE 4-1:** THE SSR MARKERS USED TO MAP CHROMOSOMES 1 TO 20 .................. 107

**TABLE 4-2:** LINKAGE ANALYSIS AND LOD SCORE IN BC1 WITH LPK/SSNARc+/- LOCUS GENOTYPE .......................... 124

**TABLE 4-3:** LINKAGE ANALYSIS IN BC1 WITHOUT LPK/SSNARc+/- LOCUS GENOTYPE ........................................... 126

**TABLE 4-4:** EPISTATIC INTERACTION BETWEEN LOCUS ........................................ 127

**TABLE 4-5:** THE CANDIDATE GENES AND PROTEINS ASSOCIATED WITH THE LPK/SSNARc+/- LOCUS ................ 129

**TABLE 5-1:** FIBROCYSTIN PROTEINS FROM THE RAT AND MOUSE .......................... 139

**TABLE 5-2:** COMPARISON OF THE PROTEINS, THEIR FAMILIES AND DOMAINS ........ 144