Genetic background and phenotype of autosomal dominant polycystic kidney disease in eastern Finland

Doctoral dissertation

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary diseases, with a frequency of 1 in 1000 in the general population. ADPKD is characterized by the formation of fluid-filled cysts in both kidneys that leads to progressive renal failure. It is a heterogeneous genetic disorder resulting from mutations in at least two genes, the polycystic kidney disease type 1 (PKD1) gene or the polycystic kidney disease type 2 (PKD2) gene. In Finnish ADPKD families the phenotype and genetic background has not previously been characterized. Therefore, in this study 20 families with ADPKD (including 125 affected and 92 unaffected family members) from Eastern Finland were identified and investigated.

The haplotype analysis showed that 16 families had defects in the PKD1 gene and one family in the PKD2 gene. Three families could not be classified because of uninformative haplotypes. The entire coding regions of PKD1 and PKD2 genes in 17 families with ADPKD were screened by single strand confirmation polymorphism analysis, and direct sequencing.

A higher prevalence of hepatic cysts, subarachnoid haemorrhage (SAH) or cerebral aneurysms, proteinuria and hematuria was found in patients with PKD1 compared with healthy relatives. Patients with PKD1 more often had haemodynamically significant mitral regurgitation (grade 2 or 3), mitral valve prolapse and left ventricular hypertrophy (LVH) compared with control subjects. Insulin resistance was significantly associated with left ventricular mass index (LVMI) in patients with PKD1 independently of other factors. Patients with PKD1 had a faster progression of their kidney disease than patients with PKD2. However, the rate of progression varied substantially between the PKD1 families.

Mutations co-segregating with ADPKD were identified in all 16 families having PKD1 in haplotype analysis. Of these mutations 6 were insertions/deletions, 5 nonsense mutations, and 5 missense mutations. For the PKD2 gene the R322Q mutation was found in one family. With the exception of one mutation (I845S in PKD1) all mutations were novel. The mutations did not show a strong correlation with the phenotype but mutations associated with SAH or brain aneurysm were located more often at the 5' end of the PKD1 gene than at the 3' end of the PKD1 gene.

National Library of Medicine Classification: WJ 358
Medical Subject Headings: polycystic kidney, autosomal dominant/epidemiology; polycystic kidney, autosomal dominant/genetics; polycystic kidney, autosomal dominant/complications; phenotype; mutation; haplotypes/genetics; chromosomes, human, pair 16; proteins/genetics; polymorphism, single-stranded conformational; intracranial aneurysm; subarachnoid hemorrhage; proteinuria; hematuria; Finland
To Juha, Iina and Vilma
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Kuopio, December 2003

Anne Lumiaho
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADPKD</td>
<td>Autosomal dominant polycystic kidney disease</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>Ratio of peak early to peak late flow velocity</td>
</tr>
<tr>
<td>CGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EPSS</td>
<td>Mitral valve end point septal separation</td>
</tr>
<tr>
<td>ESRD</td>
<td>End stage renal disease</td>
</tr>
<tr>
<td>FS</td>
<td>Fractional shortening</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostasis model assessment</td>
</tr>
<tr>
<td>ICA</td>
<td>Intracranial aneurysm</td>
</tr>
<tr>
<td>IVS</td>
<td>Ventricular septal thickness in diastole</td>
</tr>
<tr>
<td>LR-PCR</td>
<td>Long-range-polymerase chain reaction</td>
</tr>
<tr>
<td>LRRs</td>
<td>Leucine-rich repeats</td>
</tr>
<tr>
<td>LVH</td>
<td>Left ventricular hypertrophy</td>
</tr>
<tr>
<td>LVIDD</td>
<td>Left ventricular internal dimension in diastole</td>
</tr>
<tr>
<td>LVIDS</td>
<td>Left ventricular internal dimension in systole</td>
</tr>
<tr>
<td>LVMi</td>
<td>Left ventricular mass index</td>
</tr>
<tr>
<td>MRA</td>
<td>Magnetic resonance angiography</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
</tr>
<tr>
<td>PKD1</td>
<td>Polycystic kidney disease type 1</td>
</tr>
<tr>
<td>PKD2</td>
<td>Polycystic kidney disease type 2</td>
</tr>
<tr>
<td>PW</td>
<td>Posterior wall thickness in diastole</td>
</tr>
<tr>
<td>RAAS</td>
<td>Renin-angiotensin-aldosterone system</td>
</tr>
<tr>
<td>SAH</td>
<td>Subarachnoid haemorrhage</td>
</tr>
<tr>
<td>SSCP</td>
<td>Single-strand conformation polymorphism</td>
</tr>
<tr>
<td>TSC2</td>
<td>Tuberous sclerosis type 2 gene</td>
</tr>
</tbody>
</table>
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9. ORIGINAL PUBLICATIONS
1. INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease. In the white population, approximately 1 in 1000 persons carries a gene defect for this condition. (1) ADPKD is characterized by the formation of fluid-filled cysts in the kidneys. These cysts grow progressively in size and number with age, and lead to end-stage renal disease (ESRD) in approximately 50% of the patients by the age of 60 years. (2,3)

At least two genes are responsible for the disease. The gene for polycystic kidney disease type 1 (PKD1) is located on chromosome 16p13.3, and accounts for approximately 85% of all cases with ADPKD. (4-6) The gene for polycystic kidney disease type 2 (PKD2) maps to chromosome 4q21-23 and accounts for most of the remaining cases. (7,8) However, in some families ADPKD is not linked to either of these genes, suggesting the existence of at least one more locus (PKD3). (9) Mutations in the PKD1 gene cause a more severe disease with a higher risk of the progression to ESRD than mutations in the PKD2 gene. (10) Furthermore, the progression varies between affected members of different families, as well as between different members of the same family. (11)

Although renal cysts and renal failure are the major clinical characteristics of ADPKD, this disease also has multiple extrarenal manifestations including hypertension, extrarenal cysts, cerebral aneurysms and cardiac valve involvement.

In Finnish ADPKD families the prevalence of these extrarenal disorders has not previously been investigated. Because the Finnish population is relatively homogeneous, it is possible that only a few founder mutations could explain the ADPKD in the Finnish population and thus it could be hypothesized that the phenotype of this disease vary less between Finnish ADPKD families than it varies among ADPKD families from other populations.
In this study we carefully assessed the genetic background and the phenotypic characteristics of 20 families with ADPKD from the eastern part of Finland.
2. REVIEW OF THE LITERATURE

2.1. Genetic background of ADPKD

2.1.1. PKD1 and PKD2 genes

In 1985, ADPKD was linked to chromosome 16p13.3. (4) The PKD1 gene was identified in 1994 (5) and fully characterized in the following year. (6) The PKD1 gene is one of the largest genes described in the human genome covering 52kb of genomic DNA, containing 46 exons, and encoding a 14kb mRNA. (6,12,13) Approximately 70% of the gene’s length (exons 1-33) is replicated at least 3 times at another locus on chromosome 16 (5,14) and each copy has approximately 95% similarity with the PKD1 gene. In addition, the PKD1 gene has two long polypyrimidine tracts in introns 21 and 22.

The PKD2 gene was localised to chromosome 4q13-23 in 1993, and it accounts for 10-15% of ADPKD cases. (8) PKD2 is associated with a milder phenotype than PKD1, since the mean age of onset of ESRD among patients with PKD2 is 69 years compared with 53 years in patients with PKD1. (10) The genomic structure of the PKD2 gene is not as complex as that of the PKD1 gene. The PKD2 gene is a single-copy gene that spans approximately 68 kb of genomic DNA and encodes a messenger RNA (mRNA) of 5.4 kb composed of 15 exons.

2.1.2. Polycystin-1 and -2

The protein product of the PKD1 gene is polycystin-1. It is a very large cell membrane glycoprotein including 4303 amino acids. (15) It contains a large extracellular, N-terminal domain (approximately 2,500-amino acids), 11 transmembrane domains, and a rather short cytoplasmic COOH-terminus. (5,6) Figure 1 shows the structure of polycystin-1. Polycystin-1 includes two leucine-rich repeats (LRRs), cell-wall and stress-response component (WSC), a set of
16 immunoglobulin (ig)-like PKD repeats (PKD-domain), a C-type lectin domain, low-density lipoprotein-A domain (LDL-A), a receptor for egg jelly domain (REJ), a G-protein coupled receptor proteolytic site domain (GPS) and the first cytoplasmic loop contains a lipooxygenase homology 2 domain (PLAT domain). (5,6,12,16-19) The cytoplasmic portion of polycystin has an α-helical coiled-coil structure made of 5 heptad repeats. (20)

Polycystin-2, the protein product of PKD2 gene, is a 968-amino acid integral membrane protein having six membrane-spanning domains and intracellular amino and carboxyl termini (Figure 1). (21) Polycystin-2 has a 25% sequence identity and 50% similarity to polycystin-1. The interaction between the COOH termini of the polycystin-1 and polycystin-2 has been demonstrated via domains that are known as coiled-coil domains. (20, 22)

Figure 1. Schematic illustration of the structures of polycystin-1 and -2 (modified from Peters et al. (23))
Structural features of polycystin-1 suggest that it participates in the regulation of cell-cell and cell-matrix interactions in renal tubular epithelial cells. (24,25) Polycystin-1 and polycystin-2 are shown to function together as a part of a multiprotein membrane-spanning complex (26) and to initiate signal transduction. It is possible that PKD1 functions as a receptor with as yet unknown ligands. By binding to PKD2, PKD1 transmit signals to the interior of the cell through a mechanism that may involve ion channel activity. (15) In addition, recent evidence has shown that polycystin-1 participates in complexes containing E-cadherin and α-, β- and γ-catenin. (26) Taken together, polycystin-1 and -2 may have an important role in signalling pathways, controlling cell proliferation and differentiation. (27)

Both polycystin-1 and -2 are widely expressed in embryonic and adult tissue and organs. (28-32) Expression of these proteins have been detected during embryonic development in the morula and blastocyst stages, and both are required for normal development. (33,34) In adults polycystin-1 and -2 are expressed in hepatic biliary and pancreatic ducts, breast ductal epithelium, bladder urothelium, skin, brain astrocytes, bronchiolar epithelium, vascular endothelium and smooth muscle cells. (26) The expression of polycystin-1 is higher in fetal kidneys than in adult kidneys (28,29,35) suggesting a role for PKD1 in the development of the kidneys. In contrast, the strongest expression of polycystin-2 persists into adulthood, indicating a role for polycystin-2 in the maintenance of mature tubular structure. (36) Polycystin-1 is expressed in the tubular epithelium with the strongest expression observed in the distal tubules and collecting ducts. (35,37-39) Polycystin-2 is predominantly expressed in the distal convoluted tubules. Polycystin-1 is localised both in the cytoplasm and on the cell-surface. (28,35) Polycystin-2 is located primarily in the endoplasmic reticulum, although it can also be found in the plasma membrane. (40)

Recently, homologs of the PKD genes have been identified (PKDL, PKD2L and PKDREJ). (41) The protein products of these three genes are polycystin-L, polycystin-2L and polycystin-REJ, respectively. The PKDL gene was localised to chromosome 10q24-25 (42), the PKD2L gene to chromosome 5q31 (43) and
the PKDREJ gene to chromosome 22q13.3. (44) The PKDL gene contains 16 exons and polycystin-L 805 amino acids. It shows a 50% identity and an approximately 70% similarity to PKD2. Polycystin-L functions as calcium-modulated nonselective cation channel. (42,45) The localisation of polycystin-L predominantly to the apical region of the principal cells of the inner medullary collecting ducts suggests that it is unlikely to be a candidate gene for ADPKD. Polycystin-REJ is 2253 amino acids long and its expression is restricted to the testis. Polycystin-2L includes 613 amino acids and is expressed in striated muscle, brain, retina and testis. In spite of striking similarity with known PKD genes, PKDL, PKDREJ and PKD2L have not been found to be linked to ADPKD. (42,45,46)

2.1.3. Mutations in the PKD1 gene

Altogether 204 different mutations in families with PKD1 have been found so far (Table 1). With very few exceptions, every PKD family has a different mutation and no clear mutation hotspot has been identified. (47,48) Mutations include missense/nonsense mutations (51%), small deletions (25%), splicing mutations (9%), small insertions (7%) and gross deletions (5%) (Table 1).

Until recently, most of the described mutations have been located within the single-copy region of the PKD1 gene (exons 33 to 46), mainly because it was the only region of the gene that could be easily investigated using conventional methods. (49) In recent studies the methodological problems have been solved by using long-range polymerase chain reaction (PCR) protocols coupled with locus-specific primers. (48-50) Rossetti et al. (51) developed a method to amplify the entire coding region of the PKD1 gene and screened for mutations in 131 unrelated patients with ADPKD, using the protein-truncation test and direct sequencing. A detection rate of 52.3% was achieved in 155 families. Mutations were distributed throughout the gene, from exon 1 to exon 46, with no clear hotspot.
Table 1. Mutations of the PKD1 gene (modified from the Human Gene Mutation Database, [http://uwcm.ac.uk/uwcm/mg/search](http://uwcm.ac.uk/uwcm/mg/search))

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>Total number of mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide substitutions (missense/nonsense)</td>
<td>105</td>
</tr>
<tr>
<td>Nucleotide substitutions (splicing)</td>
<td>18</td>
</tr>
<tr>
<td>Nucleotide substitutions (regulatory)</td>
<td>0</td>
</tr>
<tr>
<td>Small deletions</td>
<td>51</td>
</tr>
<tr>
<td>Small insertions</td>
<td>15</td>
</tr>
<tr>
<td>Small indels</td>
<td>0</td>
</tr>
<tr>
<td>Gross deletions</td>
<td>11</td>
</tr>
<tr>
<td>Gross insertions &amp; duplications</td>
<td>2</td>
</tr>
<tr>
<td>Complex rearrangements (including inversions)</td>
<td>2</td>
</tr>
<tr>
<td>Repeat variations</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>204</strong></td>
</tr>
</tbody>
</table>

2.1.4. Mutations in the PKD2 gene

Mutations in the PKD2 gene were first reported in 1996. (21) Different gene defects have been reported over the entire sequence of the gene. Fifty-eight mutations in families with PKD2 have been identified so far, and virtually all have been premature chain terminations that are predicted to result in functional loss of PKD2. (21,52-56) The spectrum of mutations identified includes missense/nonsense mutations (40%), small deletions (31%), small insertions (12%) and splicing defects (10%) (Table 2). No clustering of mutations has been described, and only a few mutations have been found to occur in more than one family. (57)
Table 2. Mutations of the PKD2 gene (modified from the Human Gene Mutation Database [http://uwcm.ac.uk/uwcm/mg/search])

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>Total number of mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide substitutions (missense/nonsense)</td>
<td>23</td>
</tr>
<tr>
<td>Nucleotide substitutions (splicing)</td>
<td>6</td>
</tr>
<tr>
<td>Nucleotide substitutions (regulatory)</td>
<td>0</td>
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<tr>
<td>Small deletions</td>
<td>18</td>
</tr>
<tr>
<td>Small insertions</td>
<td>7</td>
</tr>
<tr>
<td>Small indels</td>
<td>1</td>
</tr>
<tr>
<td>Gross deletions</td>
<td>1</td>
</tr>
<tr>
<td>Gross insertions &amp; duplications</td>
<td>1</td>
</tr>
<tr>
<td>Complex rearrangements (including inversions)</td>
<td>1</td>
</tr>
<tr>
<td>Repeat variations</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58</strong></td>
</tr>
</tbody>
</table>

2.1.5. Somatic second hits in the PKD genes

Intra-familial phenotype variability has been documented in patients with ADPKD. Furthermore, fewer than 5% of all nephrons develop cysts, even though all cells carry the same germline mutation. (16,58-60) A possible explanation could be the “two-hit” model for cystogenesis which means that the formation of cysts requires a somatic mutation (second hit) in addition to a germline mutation (first hit). (61,62) It is assumed that the normal PKD gene acts to suppress epithelial cell growth and that inactivation of the gene leads to an abnormal phenotype. (26)

2.2. Mechanism of cyst formation in ADPKD

Cyst formation begins with focal ballooning of tubules. Cell proliferation and the accumulation of fluid within the cysts are responsible for the growth and
enlargement of cysts. (63,64) As a consequence, blood vessels and nephrons are pushed aside, and remodelling of the extracellular matrix occurs. Because the number of functioning nephrons declines and the renal parenchyma is replaced by cysts and fibrotic tissue, renal failure develops.

Epidermal growth factor (EGF) and cyclic AMP (cAMP) regulate proliferation. (65,66) cAMP stimulates ADPKD kidney cells to proliferate. In contrast, cAMP inhibits the proliferation of normal kidney cells. However, the specific molecular mechanisms that are responsible for the change in proliferative response to cAMP are not known. (26)

In addition to cell proliferation, fluid accumulation and basement membrane remodelling also contribute to cyst growth in ADPKD. (26) In the early stage the cysts are connected to tubules and the fluid in the cysts is a derivative of the glomerular filtrate. (67) When the cysts become disconnected from the tubules, accumulation of fluid within these cysts most probably occurs by transepithelial chloride secretion across the cystic epithelium. (65) Apoptosis may play an important role in this process. (26) In addition to its effect on cell proliferation, cAMP is also a central component in the stimulation of apical chloride secretion and a driving force for the accumulation of cyst fluid. Increased levels of intracellular cAMP activate protein kinase A (PKA) to increase the permeability of the apical membrane to chloride.

The two-hit mechanism, by which the normal PKD1 or PKD2 alleles are mutated, is possibly a prerequisite for the initiation of cyst growth. Because polycystin-1 and -2 have been shown to function together as a part of a multiprotein membrane-spanning complex involved in cell-cell or cell-matrix interactions (26), both proteins possess the potential to regulate signalling through a number of different pathways. These pathways (heterotrimeric G-proteins, protein kinase C, mitogen-activated protein kinases, AP-1 transcription factor and β-catenin) can lead to changes in gene expression affecting not only cycle regulation and apoptosis, but also cellular differentiation. (26) In addition, polycystin-2 may mediate calcium flux. (26)
2.3. Mouse models for ADPKD

In the first animal model for ADPKD, the mutant allele was created to mimic a known truncating mutation of the human exon 34 of the PKD1 gene. (68) Heterozygous mice (PKD1\textsuperscript{+/−}) did not develop classic ADPKD, although a few cysts were found in older animals. However, homozygous animals (PKD1\textsuperscript{−/−}) developed massive glomerular, tubular and pancreatic cysts and died perinatally as a result of pulmonary hypoplasia. In a second PKD1 knockout model, exon 4 was mutated. Homozygous animals died in utero with massive oedema. In the third model (33), portions of exons 43 and 45 and the entire exon 44 were replaced by the neo cassette. The homozygous mouse embryos had vascular leaks, rupture of blood vessels and massive haemorrhage leading to death in utero. Therefore, polycystin-1 is likely to play a role in the structural integrity of blood vessels.

For PKD2, several different mutant PKD2 alleles have been developed. (62) Mice homozygous for an unstable WS25 PKD2 allele (PKD2\textsuperscript{WS25}) were born alive but developed kidney and liver cysts 6-14 weeks after birth. (62) Some of the animals had moderately severe disease, whereas others had few if any cysts. In one case, the first exon was targeted for disruption, resulting in a true null allele incapable of making any protein (PKD2\textsuperscript{−/−}). Mice heterozygous for this allele (PKD2\textsuperscript{+/−}) developed occasional renal and hepatic cysts that lacked PKD2 protein as revealed by PKD2 antibody staining. PKD2-null embryos developed progressive total body oedema and focal haemorrhages (69) and died in utero between 13.5 embryonic day.

2.4. Extrarenal manifestations of ADPKD

2.4.1. Hypertension

Hypertension is a common finding in patients with ADPKD. It occurs in 50-75% of the patients and even before there is any decrease in kidney function. (70,71)
However, in a Scandinavian population the prevalence of hypertension has been reported to be only 41%. (1) A high prevalence of hypertension has been found also in children with ADPKD (18-22%). (72,73) Hypertension is an important risk factor for cardiovascular death, which is the most frequent cause for mortality in these patients. (74) Furthermore, ADPKD patients with hypertension progress much faster to ESRD than those without hypertension. (3,75) Patients with PKD2 are less likely to develop hypertension (odds ratio 0.25 (95% CI 0.15-0.42)) (10) compared with patients with PKD1 and they develop hypertension at a higher age than patients with PKD1 (49.7 vs. 34.8 years). (76)

Activation of the renin-angiotensin-aldosterone system (RAAS) caused by cyst expansion and local renal ischaemia has been suggested to be an important factor in the development of hypertension in patients with ADPKD. (77) Graham et al. (78) observed an increased number of renin granules in the juxtaglomerular apparatus and also an abnormal distribution of renin-containing cells located along the arterioles and within cyst walls. In addition, increased amounts of tubular immunoreactive renin in kidney specimens and an increased renin concentration in cyst fluid has been reported. (79) Furthermore, the RAAS is more stimulated in hypertensive patients with ADPKD than in patients with essential hypertension. (80)

There are no long-term, prospective, randomised studies investigating the impact of reduction of blood pressure on the progression of ADPKD. In the Modification of Diet In Renal Disease (MDRD) study (81) the patients with ADPKD had no benefit from a lower than the standard blood pressure goal. However, the patients included in that study were at a late stage of their disease. In the Angiotensin-Converting-Enzyme Inhibition In Progressive Renal Insufficiency Study (82) benazepril had a beneficial effect on the disease progression in patients with various renal disease but not in patients with ADPKD. It is however noteworthy that the patients with ADPKD had renal insufficiency. Schrier et al. (83) investigated in a prospective, randomised, 7-year study in ADPKD patients the cardiac and renal effects of rigorous
(<120/80 mmHg) versus standard (135-140/85-90 mmHg) blood pressure control on ADPKD. They found no statistically significant difference in renal function between the two groups. However, left ventricular mass index (LVMI) decreased more in the rigorous group (35%) than in the standard group (21%, \( p < 0.0001 \)).

Because the RAAS plays an important role in the development of hypertension in ADPKD, ACE-inhibitors may be the optimal mode of treatment in hypertensive patients. In the prospective study by Ecder et al. (84) patients who were treated with diuretics had a faster loss of renal function compared with patients who were taking ACE-inhibitors only. In addition, in the subgroup analysis in the study by Schrier et al. (83) patients receiving enalapril had a significantly greater decrease in left ventricular hypertrophy (LVH) than the patients in the amlodipine group. Furthermore, an epidemiological study of kidney survival in patients with ADPKD demonstrated a significant slowing of renal progression that was associated with significantly lower mean arterial pressure and increased use of ACE inhibitors in a later cohort (1992 to 2001) compared with an early cohort (1985-1992). (85) In contrast, in another study by Ecder et al. (86) no difference in renal function was observed between the enalapril and amlodipine groups after five years. However, in the latter study ACE-inhibition, but not treatment with calcium channel blockers, had an antialbuminuric effect. Kanno et al. (87) reported that the decrease in creatinine clearance was smaller in patients on calcium channel blocker treatment than in patients on ACE inhibitors ( \( p < 0.05 \)). Taken together, an early and efficient treatment of hypertension may be important to slow down renal progression to prevent cardiovascular mortality. However, the number of randomized, prospective studies is too low to verify that ACE inhibitors are superior to other antihypertensive medications. In addition, a major limitation of the previous studies is a rather low number (24-75) of studied patients.
2.4.2. Liver cysts

Cysts in the liver are the most common extrarenal manifestations of ADPKD. Such cysts derive from progressive dilatation of small clusters of bile ducts and are lined by biliary epithelium. (80, 89) As cysts grow they become disconnected from the biliary duct. (90) Polycystin-1 is expressed both in hepatocytes and in the biliary epithelium during the first four years of life. (29) The prevalence of liver cysts increases with age, being very rare before the age of 20 years, but reaching 70% at the age of 60 years or more. (91, 92) However, approximately 25% of patients with ADPKD do not develop cysts in the liver. (93) Those patients with the most severe renal cystic disease and the worst renal function have the most severe liver cystic disease. (92, 94) Both patients with PKD1 and patients with PKD2 have a high prevalence of liver cysts (7.5-70%). (7, 8, 76, 95)

Liver cysts are likely to be larger and more numerous in women than in men. (91, 92) In addition, a large number of pregnancies increase the risk of hepatic cysts. (94) An increase in the size of liver cysts after menopause in ADPKD women who receive estrogen replacement therapy indicates that estrogens may play an important role in the development the polycystic liver disease. (93)

Liver cysts never cause liver failure either in male or female patients, and the liver enzymes and bilirubin in the serum are usually within normal limits (96,97), because the liver parenchyma itself remains normal. However, patients with massive cystic liver disease can have elevated alkaline phosphatase and transaminase levels. (98) In addition, liver cyst infection can increase liver enzymes and bilirubin. (99)

Several authors have suggested that liver cyst infections occur more often among ADPKD patients on haemodialysis than among ADPKD patients not requiring renal replacement. (92, 96) In contrast, in one study liver cyst infection was found in only 3% of 229 ADPKD patients with ESRD. (100) Furthermore, intracystic haemorrhage is another complication of polycystic liver disease seen
in patients with ADPKD. Congenital liver fibrosis also affects a small number of families with typical ADPKD. (101)

2.4.3. Intracranial aneurysms

Patients with ADPKD are at risk of developing intracranial aneurysm (ICA), and cerebral aneurysmal rupture is the most severe extrarenal complication of ADPKD. The prevalence of asymptomatic ICA in ADPKD has been estimated to be about 8% in three prospective series of 226 patients. (102-104) In the general population the prevalence of ICA is 1%. (105) Prevalence estimates are not available separately for PKD1 and PKD2 families. Mean age at rupture is 41 years, a decade earlier than sporadic rupture in the general population, but close to that observed in familial aneurysms (42 years). (106,107) Death because of subarachnoid haemorrhage (SAH) occurs in about 6% of patients with ADPKD. (74)

There is evidence for familial clustering of ICA, suggesting that genetic factors are important in the aetiology. The prevalence ranges from 5% in patients with no family history of ICA to 21% in those with a positive family history of ICA rupture. (100,102,104) Polycystin-1 and -2 are expressed in vascular smooth muscle and endothelium indicating that both proteins may be involved in the vascular manifestations of ADPKD. (108,109) In addition, mouse models with targeted mutations in PKD1 and PKD2 developed vascular leakage and blood-vessel rupture, indicating a role for polycystins in vascular wall integrity. (33, 69) The fact that ICA is not present in all affected family members suggests that other factors, in addition to type of mutation, play a role in the formation of ICA. Such factors could be somatic mutations in the normal PKD allele as well as in modifying genes.

There are only little sparse available regarding the natural history of ICA in patients with ADPKD. The risk of rupture increases with increasing size of the aneurysm, but, also aneurysms smaller than 5 mm can rupture. (110) In the study by Belz et al. (111) twenty ADPKD subjects with ICA were followed for 15
years. Five of the 20 (25%) subjects with ICA developed new ICAs in different locations and two of these subjects showed an increase in size of an existing ICA. However, only one subject experienced recurrent rupture of ICA. In the study by Huston et al. (104) 18 ADPKD patients having 15 asymptomatic, small (1.5-6.5 mm) ICAs were followed for a mean of 33 months with magnetic resonance angiography (MRA) examinations. None of the aneurysms changed in size or ruptured, and no de-novo aneurysms were formed. In addition to size of aneurysms, a family history of ICA and previous SAH have been associated with ICA rupture. (112) However, there are no studies about the association of mutations in the PKD1 and PKD2 genes and the risk of ICA rupture.

Because ADPKD patients with a family history of ICA have a higher prevalence of ICA and may be at a higher risk for rupture, screening with MRA is recommended. (112) MRA should be performed every 5 years if findings are initially negative and every 2-3 years following a prior ICA rupture (113), because patients with a prior history of ICA rupture have a high rate of recurrence of ICA and subsequent bleeding. Patients without a family history of ICA are not recommended for screening.

Another cause of cerebrovascular accidents in ADPKD patients is intracranial arterial dolichoectasia (arterial elongation and distension). This condition has a prevalence of 2-2.5% in patients with ADPKD, which is 10 times more frequent than in the general population (114). Furthermore, in the study by Belz et al. (115) patients with ADPKD had significantly more nonaneurysmal vascular central nervous system events than did subjects without ADPKD (3 vs. 1.6%, p = 0.05, respectively).

In Finnish ADPKD families there is only scarce information available on the prevalence of ICA. One study concerning familial ICA included 85 families without ADPKD and six families with ADPKD from Eastern Finland. (107) Thirty-eight of 438 individuals from 85 families without ADPKD and 2 of 22 individuals from families with ADPKD had an incidental aneurysm.
2.4.4. Cardiovascular manifestations

2.4.4.1. Valvular disorders

Cardiovascular disorders are frequently associated with ADPKD. One study (116) reported a higher prevalence of cardiac valvular abnormalities in patients with ADPKD compared with healthy relatives and controls. In these three groups the prevalence of mitral prolapse was 26, 14 and 2% (p < 0.0005), the prevalence of mitral regurgitation was 31, 14 and 9% (p < 0.005) and the prevalence of aortic regurgitation 8, 3 and 1% (p < 0.02), respectively. Another study describing cardiac findings were described in one large family with ADPKD, also reported a high prevalence of cardiac valvular abnormalities. (117) Moreover, a high prevalence of mitral valve prolapse has previously been reported in children with ADPKD. (118) It is noteworthy that ADPKD children with mitral valve prolapse also had more severe renal lesions (>10 cysts) than did ADPKD children without the valvular abnormality. In Finnish patients with ADPKD the prevalence of cardiac valvular abnormalities has not previously been studied.

Contradictory results have also been reported with respect to the prevalence of valvular abnormalities in patients with ADPKD. In normotensive patients with ADPKD the prevalence of cardiac valvular abnormalities was not increased compared with the general population. (119, 120) Ritz et al. (121) found a 17% prevalence of mitral regurgitation in patients with ADPKD, but none of the patients had mitral valve prolapse. In the study by Bardaji et al. (122) patients with ADPKD were divided into normotensive, hypertensive and those undergoing haemodialysis. In non-dialysis patients the prevalence of mitral valve prolapse and aortic and/or mitral regurgitation was 4.3 and 8.6%, respectively. The majority of valvular abnormalities occurred in dialysis patients, and were in general related to annular mitral calcification or aortic valve calcification.
The primary cause of cardiac valvular abnormalities in patients with ADPKD remains unexplained. Myxomatous degeneration with loss and disruption of collagen has been found in the histological examination of mitral and aortic valve tissue in patients with ADPKD. (123) Because the protein of the PKD1 gene, polycystin 1, and the protein of the PKD2 gene, polycystin 2, are expressed in cardiac myocytes and in valvular myofibroblasts of patients with ADPKD, it is possible that defects in the PKD1 or PKD2 genes could explain the myxomatous degeneration of the mitral valve tissue.

2.4.4.2. LVH

Several studies have reported an increased frequency (18-24%) of LVH in patients with ADPKD, and it has been related to a high prevalence of hypertension in such patients. (116,117,124) In the study by Chapman et al. (124) LVH was present in 48% of hypertensive ADPKD subjects, and LVMi was correlated with systolic blood pressure. Bardaji et al. (122) further reported that systolic blood pressure is the most important factor related to LVM in dialysis and non-dialysis ADPKD patients.

A high prevalence of LVH has also been reported in normotensive patients with ADPKD. (124) One study suggested that even normotensive patients with normal renal function have increased LVH. (120) In addition, young normotensive ADPKD patients have been shown to have higher LVM that is closely related to ambulatory systolic blood pressure. (125) Furthermore, a study including children and young adults with ADPKD showed that ambulatory daytime and night time blood pressure and LVMi were higher in patients with ADPKD than in controls. (126)

Alongside the systolic blood pressure, diastolic blood pressure, increased renal volume and impaired renal function are related to LVH. (124) Activation of the RAAS due to cyst expansion and local renal ischaemia probably play an important role in the development of hypertension in ADPKD, (127) and angiotensin II, a key player of the RAAS, has been suggested to be one of the
major pathogenic factors in myocardial hypertrophy. (128) In this respect it is interesting that Eder et al. (129) showed that ACE inhibition in hypertensive ADPKD patients provided long-term reversal of LVH. Furthermore, modifying genetic factors, such as the ACE gene deletion polymorphism was related to LVH in normotensive ADPKD patients. (130) Lower hematocrit values have consistently been associated with LVH in predialysis and dialysis patients. (131) Polycystin-1 and polycystin-2 are expressed in cardiac myocytes and smooth muscle cells, indicating that gene defects may be important factors in the development of LVH in patients with ADPKD.

There is some evidence that insulin resistance is related to LVH in humans, but the results have not been consistent. (132-136) In addition, increased LVM has been described in endocrine diseases such as acromegaly (137), hypothyroidism (138) and obesity (139,140) which are characterized by insulin resistance and hyperinsulinaemia.

Vareesangthip et al. (141) demonstrated that ADPKD patients without renal failure were insulin resistant and had compensatory hyperinsulinaemia. This could explain the increased risk of post-transplant diabetes in patients with ADPKD. (142) Insulin resistance in ADPKD patients might be due either to abnormal PKD protein or to abnormalities in membrane cytoskeleton. (143) However, the relationship between insulin resistance and LVMI in patients with ADPKD has not been evaluated yet.

2.4.5. Other clinical manifestations

Mild proteinuria often occurs during the course of ADPKD, but nephrotic range proteinuria is uncommon. (144) The prevalence of proteinuria has been reported to be 14% in children with ADPKD (72), 34% in adult ADPKD patients without renal failure (145) and from 70 to 80% in ADPKD patients with advanced disease. (146) In the study by Chapman et al (147), proteinuria was found in 18% and microalbuminuria in 41% of patients with ADPKD. Both proteinuria and microalbuminuria were associated with an increased mean
arterial pressure and impaired renal function. Furthermore, the presence of hypertension or proteinuria at the time of the clinical diagnosis of ADPKD has been associated with poorer renal survival. (148)

Hematuria is a common symptom of ADPKD. In a study by Gabow et al. 42% of patients experienced at least one episode of gross hematuria and 23% of them had experienced more than six episodes. (149) In addition, patients with ADPKD have a high prevalence of urinary tract infections, because 50-75% of all patients have at least one symptomatic urinary tract infection at some stage. (1) The frequency of renal stone disease ranges from 8 to 36% in different studies. (1,150-152)

In a Canadian study 13% of women with ADPKD had ovarian cysts (153), but contradictory results have also been reported. In the study by Stamm et al. (154), cysts in the ovaries were no more frequent in patients with ADPKD than in control subjects. (155) In a Finnish study (156) ADPKD was not associated with cystic ovaries either, but postmenopausal women with ADPKD had an increased mean ovarian volume compared with postmenopausal women without ADPKD ($p < 0.01$). Pancreatic cysts are another frequent extrarenal manifestation of ADPKD (5-10%). (144) In Japanese subjects the prevalence of pancreatic cysts was 7%. (157) A high prevalence (82%) of colon diverticula has been reported in dialysis patients with ADPKD. (158) Hernias are more frequent in ADPKD patients than in the general population. (159)

2.4.6. Progression of ADPKD

The age at onset of ESRD in patients with ADPKD is highly variable ranging from childhood to old age. (3) Approximately 50% are alive without ESRD at the age of 60 years. (2, 3) In addition, the prognosis varies between affected members of different families, as well as between different members of the same family. In adults, several risk factors for faster progression have been identified, including mutations in the PKD1 gene, male gender, a younger age at
diagnosis. the presence of hypertension and hematuria. larger kidneys. and a younger age at incipient renal failure. (3, 75, 160-162)

Patients with mutations in the PKD1 gene show faster progression than patients with mutations in the PKD2 gene (median age of death or onset of end-stage renal disease has been reported to be 53 vs. 69 years, respectively). (10) Patients with PKD2 are less likely to have hypertension, urinary tract infection, and hematuria. (10) The PKD1/PKD2 distribution has so far not been studied in Finnish patients with ADPKD. Therefore, data on progression of the disease in Finnish patients with either PKD1 or PKD2 are not available.

Modifying genes that are not directly associated with APKD might have an effect on the progression. (23) Intrafamilial variability and the presence of modifying loci in mouse mutants indicated that genetic factors, other than germ line mutations in the PKD1 or PKD2 genes, might affect disease progression. (23) Interestingly, progressive ADPKD is associated with overexpression of proto-oncogenes and a variety of genes coding for growth factors, chemokines, metalloproteinases and apoptosis, interstitial accumulation of types I and IV collagen, laminin, fibronectin, macrophages and fibroblasts. (163) Cyst activation factor may also be important for progression factor. (163) It is likely that the cysts themselves are involved in the progression of the renal dysfunction, although their number and size may not be the major determinant. (163) Interstitial fibrosis probably plays an important role in the progression of ADPKD as well.

Environmental factors may also be associated with disease progression. An effect of low protein diets to ameliorate progression has been observed in mice with a slowly progressive type of PKD2. (164) In animals, diet affects the loss of renal function, and the growth of cysts is reduced if mice are kept in a germ-free environment. (165, 166) Administration of diets with low potassium or high protein content also increased renal size, serum urea nitrogen and fibrosis index in Hannover-Sprague Dawley rats. (167)
2.5. Genotype/phenotype correlation

There are only few studies investigating genotype-phenotype correlation in patients with ADPKD. The only clear evidence available involves large deletions disrupting the PKD1 gene and the adjacent tuberous sclerosis type 2 (TSC2) gene. The TSC2 gene is located immediately adjacent to the 3′ region of the PKD1 gene. (16, 168) The two genes lie tail-to-tail with their ends overlapping by several base pairs. Because of deletions that include both genes patients have a severe form of ADPKD and tuberous sclerosis. (168, 169)

Mouse models with targeted mutations in PKD1 and PKD2 or a PKD1 transgene have been described. (33, 62, 68, 69, 170) PKD1<sup>L</sup>/<sup>L</sup> mice developed renal, hepatic, and pancreatic cystic disease and they also developed gross oedema and subcutaneous haemorrhage, which may be caused by a defect in vascular wall integrity. (33) However, PKD1<sup>del34</sup>/<sup>L</sup> developed renal, hepatic and pancreatic cystic disease without oedema or haemorrhage. (68) PKD1<sup>L</sup>/<sup>L</sup> and PKD1<sup>del34</sup>/<sup>L</sup> did not have cardiac abnormalities. In contrast, PKD1<sup>del17</sup>-<sup>21</sup>βgeo mice (171) developed cardiovascular defects including double outflow right ventricle, disorganised myocardium and abnormal atrioventricular septation. PKD2 mutant mice (69) presented themselves with major defects in their cardiac development. Therefore, mouse models suggest that the location of the mutation may influence the phenotype.

Location of mutations within the PKD1 and PKD2 genes further influences the clinical outcome in patients with ADPKD. A study of 10 large PKD1 families in South Wales suggested that phenotype differences exist between PKD1 families. (11) In another study, 7 out of 10 families with very early-onset disease and/or aneurysms had mutations in exon 15 (47), and three of them had an identical deletion. A large study regarding genotype/phenotype relationships including 80 families with PKD1 (172) showed that the position of the PKD1 mutation, but not the type, was significantly associated with earlier ESRD. Patients with mutations in the 5′ region of the PKD1 gene had significantly more severe disease than patients with a mutation in the 3′ region. In addition,
mutations in the 5' region of the PKD1 gene have been reported to be associated more commonly with vascular disease than mutations located in the near 3' region. (173)

A similar genotype/phenotype relationship has been observed in families with PKD2. Consequently, the location of mutations within the PKD2 gene also influences the clinical outcome. (174) Mutations in the 5'-end, however, were not shown to be associated with a more severe phenotype. (55) In addition, a study based on 71 families with PKD2 mutations (175) reported that the location of PKD2 mutations did not influence the age of onset of ESRD, but patients with splice site mutations nonetheless had milder renal disease than patients with other mutations. In Finnish patients with ADPKD such a phenotype/genotype relationship has not been previously evaluated.

2.6. ADPKD in Finnish patients

There is little information available in the literature about ADPKD in Finnish patients. In a retrospective, epidemiological study including 82 children with PKD from 69 families (176) it was shown that 11 families had ADPKD, 14 had autosomal recessive PKD, and 44 were sporadic cases. In another study the clinical features of 18 children with ADPKD and 73 children with recessive PKD were characterized. (177) The study by Reeders et al. (178) involved four families with ADPKD from Finland. All four families had PKD1.
3. **AIMS OF THE STUDY**

This study was undertaken to investigate the genetic background and phenotype characteristics of ADPKD in Finnish subjects. The following questions were addressed:

1. What is the PKD1/PKD2 distribution in Finnish patients with ADPKD?

2. What are the clinical manifestations of ADPKD in patients from Eastern Finland?

3. What are the mutations associated with ADPKD in Eastern Finland?

4. Are there genotype/phenotype relationships in Finnish patients with ADPKD?
4. SUBJECTS AND METHODS

4.1 Subjects

Twenty index patients with ADPKD from the Kuopio University Hospital region were identified by nephrologists. All available relatives of the index patients were identified and recruited based on a questionnaire completed by the index patient. Subjects were eligible to participate if they were more than 14 years old. A total of 125 affected and 92 unaffected family members were recruited into the studies from these families.

All subjects gave informed consent, and the study was approved by the Ethics Committee of the University of Kuopio and the Kuopio University Hospital.

Study I: According to haplotype analysis sixteen families were linked to the PKD1 gene and one family to the PKD2 gene. Three families were excluded because of lack of informativity for classification. Therefore, the final study population for study I consisted of 109 patients (50 men, 59 women; age 44±1 years), with defects in the PKD1 gene, 10 (4 men, 6 women; age 51±5 years) with defects in the PKD2 gene and 79 unaffected family members from the PKD1 and PKD2 families (32 men, 47 women; age 40±2).

Study II: The study population consisted of the aforementioned 16 PKD1 families, including 109 PKD1 patients and their 73 unaffected family members. In addition, 73 control subjects (32 men, 41 women; age 45±2 years) of similar age and gender as the patients with PKD1 were recruited from a previous study (199). None of them had a family history of ADPKD.

Study III: The study included the aforementioned 16 PKD1 families. Three patients with PKD1 and 3 healthy relatives were excluded because of missing LVMi. Finally, the study population consisted of 106 PKD1 patients (48 men, 58 women; age 43 ±2) and 70 unaffected family members (28 men, 42 women; age 41±2).
Study IV: This study population originally consisted of 16 PKD1 families from previous studies I, II and III. However one family was excluded from this study since based on final haplotype analysis we could not be sure if it was a true PKD1 family. In addition, one family (2 affected and 2 healthy relatives), which was originally excluded from the studies I, II and III turned out to be a PKD1 family after additional haplotyping and it was therefore included in the study IV. Therefore the final study population consisted of 16 PKD1 families (102 PKD1 patients; 43 men, 59 women; age 43±2 years) and 71 their unaffected family members (28 men, 43 women; age 40±2 years). In addition, 50 subjects without a family history of ADPKD served as controls in genetic analyses.

4.2. Assessment of clinical characteristics

The subjects were classified as hypertensives if systolic blood pressure was equal or more than 160 or diastolic blood pressure was equal or more than 95 mmHg (the 1993 WHO criteria) (179) or if they were on antihypertensive medication. The age at onset of ESRD was defined as the age when dialysis or renal transplantation due to renal failure became necessary. Albuminuria (proteinuria) was defined as a urinary albumin excretion rate greater than 30 µg/min. Any history of renal calculi, urinary tract infections or ruptured cerebral aneurysms was ascertained by the nephrologists during the interview of each family member. To evaluate the rate of progression of ADPKD, retrospective data of creatinine values were collected from medical records.

4.3. Methods

4.3.1. Abdominal ultrasonography

Abdominal ultrasound examination was performed by a radiologist using an Aloka SSD-1200 or SSD-2000 3.5 MHz convex probe in all cases, except for healthy control subjects. A diagnosis of ADPKD was made when
ultrasonography showed at least two cysts in one kidney and one cyst in the other kidney, and the patients had a positive family history of ADPKD. (180)

4.3.2. MRA (Studies I and IV)

MRA of the head was performed to evaluate the presence of ICA by using a standard superconducting 1.5-T whole-body system (Magnetom Vision; Siemens Medical Systems, Erlangen, Germany). Time-of-flight MRA and T2- and proton density-weighted transaxial slices of the brain were performed on all index patients and on those patients with ADPKD whose first-degree relatives had an ICA.

4.3.3. Echocardiography (Studies II, III and IV)

All subjects underwent a M-mode echocardiography with a Hewlett Packard Sonos 2500 ultrasound system (Hewlett-Packard Company, Andover, MA, USA) using a 2.5 MHz transducer. M-mode measurements were performed from the parasternal short axis view according to the recommendations of the American Society of Echocardiography. (181) Measurements included the evaluation of left ventricular end diastolic and end systolic diameter, left ventricular septal and posterior wall thickness at end-diastole, aortic root, and left atrial diameter. Mitral valve E point septal separation (EPSS) was measured as the distance between the anterior mitral leaflet and the interventricular septum at the E point. (182) Left ventricular fractional shortening was calculated as the percentage of change in the internal left ventricular dimensions between systole and diastole. The left ventricular mass index (LVMI) was calculated using the Penn equation and corrected for body surface area. (183) LVH was considered to be present if LVMI was > 131 g/m² in men and > 100 g/m² in women. (184)

Doppler signals were recorded from the apical 4-chamber view with a 3-5 mm sample volume placed at the level of mitral leaflet tips parallel to maximal
mitral inflow. The midpoints of the darkest portion of the Doppler velocity waveforms were digitised and the following indexes were calculated: 1) peak flow velocity of early left ventricle (LV) filling, 2) peak flow of late LV filling and 3) ratio of peak early to peak late flow velocity (E/A ratio). (185) The echocardiographic criteria for mitral valve prolapse were: grade 0 = no leaflet bowing, grade 1 = mild bowing of the anterior and posterior mitral valve leaflets with coaptation on the left ventricular side of the annulus, grade 2 = moderate bowing of the posterior leaflet with mild bowing of the anterior leaflet with coaptation at the level of the annulus, and grade 3 = severe bowing of both leaflets with coaptation on the left atrial side of the annulus. (186,187) We graded the severity of colour Doppler-detected regurgitation according to previously described qualitative methods. (188) Mitral regurgitation was graded as grade 1 when the flow disturbance was localized to the area immediately behind the mitral valve, grade 2 when the disturbance was dispersed and extended to the midportion of the left atrium and grade 3 when the disturbance was well dispersed and detected at the posterior left atrial wall. Narrow jets with moderate or deep penetration into the left atrium were graded as grade 1.

4.3.4. **$^{51}$Cr-EDTA clearance (Studies I and II)**

Renal function was measured by $^{51}$Cr-EDTA- clearance in PKD1 patients and their healthy relatives. After a baseline serum sample was taken a standard dose of 150 μCi of $^{51}$Cr- EDTA was administered intravenously. Blood samples were drawn at 90, 150, 210 and 270 min after the injection to determine the decrease in $^{51}$Cr radioactivity. Glomerular filtration rate (GFR) was estimated as previously described (189). Values for $^{51}$Cr- EDTA clearance were corrected for body surface area (ml/min/1.73 m²). Renal insufficiency was considered to be present if GFR was below 80 ml/min/1.73m² or renal transplantation had been done. The magnitude of renal insufficiency was considered severe if GFR was < 30 ml/min/1.73m², and mild if it was 30-80 ml/min/1.73m². Normal renal function was defined as GFR > 80ml/min/1.73m². (190)
4.3.5. Determination of insulin sensitivity (Study III)

All laboratory specimens were drawn after a 12-hour fast at 8 am. Fasting plasma glucose was determined by the glucose oxidase method (Glucose Auto & Stat II GA-1120 analyzer, Daiichi, Kyoto, Japan). Fasting plasma insulin was determined from samples stored at -70°C by a commercial double-antibody solid-phase radioimmunoassay (Phadeseph Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden). The homeostasis model of insulin resistance (HOMA IR) was used as a measure of insulin resistance. (191) The formula for the HOMA IR follows:

\[
\text{HOMA IR} = \frac{\text{Fasting insulin (µU/mL)} \times \text{Fasting glucose (mmol/L)}}{22.5}
\]

4.3.6. Genotyping

DNA was extracted from peripheral blood leukocytes by salting-out method. Haplotype analysis was performed in 20 ADPKD families to test for a linkage to the ADPKD locus on chromosome 16p13.3. The families were typed using three microsatellite markers HBAP1, KG8 and CW2 for PKD1 and five microsatellite markers D4S1534, D4S1563, D4S1544, D4S414 and D4S423 for PKD2. (192-195) Polymerase chain reaction amplification was carried out in a 10 µl volume containing 35-50 ng of genomic DNA, 0.2 pmol/l of each primer, 10 mmol/l Tris-HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l of MgCl₂, 0.1% Triton X-100, 200 µmol/l dNTPs, 0.1 units of DNA polymerase (Dynazyme DNA polymerase, Finnzymes, Espoo, Finland). Conditions for amplification were an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55-63°C for 30 s and extension at 72°C for 30 s with final extension at 72°C for 4 min. The fluorescently Cy5-labelled PCR-products were electrophoretically separated on 6% ReproGel Long Read using an automated laser fluorescence ALFexpress DNA sequencer (Pharmacia Biotech AB, Uppsala, Sweden). Two internal size markers were included in each lane and at least one previously genotyped sample was included in each run to confirm
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correct allele sizing. The alleles were identified by the ALFwin Fragment
Analysers 1.00 (Amersham Pharmacia Biotech AB, U, S).

4.3.7. Long-range polymerase chain reaction (Study IV)

Four pairs of primers were used to specifically amplify the repeated part of
PKD1 (accession number L39891). The ~2.2 kb polymerase chain reaction
(PCR) for exon 1 was performed as previously described. (196) The primers for
PKD1 exons 2 – 15 have been also reported. They amplify a ~13.6 kb product
(197). PKD1-c5179F (5’-TAGGGATGCCACCAACGTCTCC-3’, genomic
position 29078 – 29099) and PKD1-c8902R (5’-AACGGAGTTGGCGGAGTTTG-3’, genomic position 38211 – 38230) were
used for a ~9.1 kb product of exons 15 – 23 and primers PKD1-2khF (5’-GCAAATACACCGTCTCCAC-3’, genomic position 38058 – 38076) and PKD1-
34R (5’-CTCCTCTGGCAATCCCCCT-3’, genomic position 44469 – 44488)
amplified exons 23 – 34 in a ~6.4 kb fragment. A total of 12.5 ng of genomic
data was used in long-range-PCR (LR-PCR) reactions in a 12.5 μl volume. LR-
PCRs were performed using 0.25 units Elongase enzyme mix (GibcoBRL,
USA), manufacturers buffer B, 400 μM dNTPs, 0.4 μM of each primer and in
~13.6 kb and ~9.1 kb long reactions 5 % DMSO. The PCR-conditions were
denaturation at 94 °C for 2 min, 35 cycles at 94 °C 15 s, annealing 68 °C (for
~13.6 kb), 59 °C (for ~9.1 kb) or 62 °C (for ~6.4 kb) 30 s and elongation at
68 °C for 10 – 15 min, final extension at 68 °C for 7 min. Exon 1 was amplified
from 25 ng of genomic DNA by 0.3 units of Expand High Fidelity PCR System
enzyme mix (Roche, Germany) with the provided buffer and 200 μM dNTPs,
1.5 mM MgCl2, 0.3 μM each primer and 5 % DMSO in a volume of 12.5 μl. The
conditions were as described in Phakdeekitcharoen et al. (196) The LR-PCR
products were diluted 1:105 to prevent the genomic contamination and 1 μl of
the dilution was used as a template for 25 μl nested PCR. For the nested PCR
were either Dynazyme DNA polymerase (Finnzymes, Finland) or Expand High
Fidelity PCR System was used with or without 1.5 % DMSO. With Dynazyme we used 100 μM dNTPs, 1.5 mM MgCl and 0.2 μM each primer in a manufacturers buffer. In the Expand High Fidelity PCR System the reaction mix contained 0.2 mM dNTPs, 1.5 mM MgCl and 0.3μM each primer in the provided buffer. The conditions for nested PCR were denaturation at 94 °C for 3 min, 35 cycles at 94 °C for 30 s, annealing at 60 - 69 °C for 30 s and elongation at 72 °C for 2 min and final extension at 72 °C for 4 min.

4.3.8. Single-strand conformation polymorphism (SSCP) (Study IV)

All 15 exons and intron-exon junctions of the PKD2 gene and exons 34 – 46 apart from exon 42 of the PKD1 gene were amplified in a volume of 6 μl with the polymerase chain reaction. The primers for the PKD2 gene have been previously reported (198) and the primers for the unique region of the PKD1 gene were designed by us (available by request). The reaction mix contained 50 ng of genomic DNA, 0.5 μM each primer, 100 μM dNTPs (100 μM dATP, dTTP, dGTP; 75 μM dCTP), 1.5 mM MgCl, 0 – 2.5 % DMSO, 0.12 units of Dynazyme DNA polymerase and 0.24 μCi of [α-33P] dCTP. The PCR conditions were denaturation at 94 °C for 3 min, 35 cycles at 94 °C for 30 s, annealing at 55 – 72 °C for 30 s, elongation at 72 °C for 30 s - 1 min and final extension at 72 °C for 4 min. The PCR fragments were digested with the appropriate restriction enzyme when they were longer than 265 bp.

The dilution of the PCR reactions was 0-4 fold with 0.1 % SDS, 10 mM EDTA and then 1:1 with loading buffer (95 % formamide, 20 mM EDTA, 0.05 % bromophenol blue, 0.05 % xylene cyanol). Denatured samples were electrophoresed on a 6 % non-denaturing polyacrylamide gel (acrylamide/N,N-methylene-bis-acrylamide ratio 49:1) containing 10 % glycerol for 4 – 6 h at two different temperatures: 29 °C and 38 °C. The gel was autoradiographed at -20 °C up to two weeks. When aberrantly migrating bands were found in both samples of the family, all family members were screened with SSCP to verify
the segregation of the variant with the disease. The mutations were sequenced and confirmed from all affected subjects by restriction enzyme or direct sequencing.

4.3.9. Direct sequencing (Study IV)

The whole repeated part of the PKD1 gene and exon 42 (primers and PCR conditions for exon 42 were takes from Perrichot et al. (199)) were screened with direct sequencing. PCR fragments were purifled by GFX PCR DNA and gel band purification kit (Amersham Pharmacia Biotech, USA). The sequencing reactions were performed by ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) according to manufacturers guidelines. The products were purified by Autoseq G-50 -columns (Amersham Pharmacia Biotech, USA) from the excess of the fluorescent labels and sequenced by ABI Prism 310 or ABI Prism 3100 Genetic Analyzer (Applied Biosystems, USA).

4.3.10. Statistical analysis

The unpaired t-test was used to assess differences between patients with mutations in the PKD1 and PKD2 genes and healthy relatives for continuous variables and chi-square test for dichotomous variables (SPSS/WIN programs version 9.0, SPSS Inc. Chicago, Ill). Comparisons among the three groups (PKU1, healthy relatives, and control group) were performed if the p value for the analysis of variance (ANOVA) for continuous variables and chi-square test for dichotomous variables was less than 0.05 (Study II). The reciprocal of serum creatinine was calculated and then linear logistic regression analysis was applied to analyse the progression of renal function over time (Study I). Univariate logistic regression analysis was used to determine variables significantly associated with mitral valve prolapse, mitral regurgitation, LVMI and LVH, and univariate linear regression analysis, to determine variables
associated with EPSS (Studies II and III). Multivariate linear regression was used to assess relationship between insulin sensitivity and LVMI (Study III). Regression analysis in PKD families was done using the program ASSOC (S.A.G.E., version 2.2, Ohio). This program allows the quantitative trait to have a familial correlation among individuals. The likelihood for the pedigree is computed with a linear regression model in which the quantitative trait is the dependent variable and the genetic variation and discrete and/or continuous covariates are independent variables. Residual variation is modelled assuming an additive polygenic pattern of correlation among relatives. P-value less than 0.05 was considered statistically significant.
5. RESULTS

5.1. Clinical characteristics and the progression of the disease in Finnish patients with ADPKD (Study I)

Table 3 shows the clinical characteristics of healthy relatives, patients with PKD1 and patients with PKD2. Patients with PKD1 and PKD2 did not differ significantly from each other. However, patients with PKD2 were older than healthy relatives ($p < 0.05$). There were no significant differences between any of the study groups with respect to gender distribution and body mass index. Both systolic and diastolic blood pressure were higher in patients with PKD1 than in healthy relatives ($p = 0.001$ and $p < 0.001$, respectively), but there was no difference in between healthy relatives and patients with PKD2. The prevalence of hypertension (systolic blood pressure $\geq 160$ mmHg or diastolic blood pressure $\geq 95$ mmHg in the sitting position or the use of antihypertensive medication) was higher in the PKD1 group and in the PKD2 group than in the group of healthy relatives ($71.3$ in PKD1 patients, $80.0$ in PKD2 patients and $26.9\%$ in healthy relatives, respectively; $p < 0.001$ and $p = 0.001$). In addition, patients with PKD1 had been diagnosed with hypertension earlier than healthy relatives ($36.3$ vs. $46.4$ years, $p < 0.001$). Patients with PKD1 and PKD2 had a lower GFR ($71.6$, $74.1$ and $96.2$ ml/min/1.73m$^2$, respectively) and higher creatinine level ($142.8$, $98.5$ and $83.8$, µmol/l respectively) than healthy relatives ($p < 0.001$). About $10\%$ of patients with PKD1 and none of PKD2 patients had ESRD.
Table 3. Clinical characteristics of subjects included in the study

<table>
<thead>
<tr>
<th></th>
<th>Healthy relatives (N = 79)</th>
<th>PKD1 (N = 109)</th>
<th>PKD2 (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marriages</td>
<td>32/47</td>
<td>50/59</td>
<td>4/6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.9 ± 1.8</td>
<td>43.1 ± 1.4</td>
<td>51.4 ± 4.9*</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.6 ± 0.6</td>
<td>25.4 ± 0.4</td>
<td>28.1 ± 1.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>133.9 ± 2.3</td>
<td>144.2 ± 1.9 **</td>
<td>138.7 ± 4.7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>83.3 ± 1.4</td>
<td>91.6 ± 1.2 ***</td>
<td>91.0 ± 4.1</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>26.9</td>
<td>71.3 ***</td>
<td>80.0**</td>
</tr>
<tr>
<td>Age at diagnosis of hypertension</td>
<td>46.4 ± 2.3</td>
<td>36.3 ± 1.3 ***</td>
<td>41.9 ± 4.5</td>
</tr>
<tr>
<td>GFR (ml/min/1.73m²)</td>
<td>96.2 ± 1.6</td>
<td>71.6 ± 3.3 ***</td>
<td>74.1 ± 7.1***</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>83.8 ± 1.3</td>
<td>142.8 ± 11.5 ***</td>
<td>98.5 ± 6.6***</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>-</td>
<td>34.3 ± 1.4</td>
<td>40.0 ± 4.5</td>
</tr>
<tr>
<td>End stage renal disease (%)</td>
<td>0</td>
<td>10.1**</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: PKD1 = polycystic kidney disease type 1; PKD2 = polycystic kidney disease type 2, GFR = glomerular filtration rate, *p < 0.05, **p < 0.01 and ***p < 0.001 vs. healthy relatives.

Figure 2. The prevalence of hepatic cysts, SAH or brain aneurysm, proteinuria and gross hematuria among healthy relatives (open bar), patients with polycystic kidney disease type 1 (black bar) and patients with polycystic kidney disease type 2 (hatched bar). **p < 0.01 and ***p < 0.001 vs. healthy relatives.
Patients with PKD1 had a higher prevalence of SAH or brain aneurysm (12 vs. 0 vs. 1%, p < 0.01), proteinuria (23 vs. 0 vs. 1%, p < 0.01) and hematuria (30 vs. 0 vs. 5%, p < 0.001) than PKD2 patients and healthy relatives (Figure 2). PKD2 patients (90%) on the other hand had more hepatic cysts than healthy relatives (3%) and PKD1 patients (60%).

The rate of progression of kidney disease was assessed by reciprocal serum creatinine values as shown in Figure 3. Patients with PKD1 showed a faster progression than patients with PKD2. It is of note that the progression rate varied substantially between the PKD1 families.

![Figure 3](image_url)

**Figure 3.** Average decline in 1/serum creatinine in a year in 16 PKD1 families.

5.2. **Cardiac abnormalities in patients with PKD1 (Study II)**

M-mode and Doppler echocardiographic findings for the 109 patients with PKD1, 73 unaffected family members and 73 healthy control subjects are reported in Table 4.
### Table 4. M-Mode and Doppler echocardiographic findings

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Healthy relatives</th>
<th>PKD1 patients</th>
<th>ANOVA/χ² test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic root (mm)</td>
<td>32.0±0.8</td>
<td>31.7±0.5</td>
<td>33.3±0.5</td>
<td></td>
<td>0.133</td>
</tr>
<tr>
<td>Left Atrium (mm)</td>
<td>34.5±0.5</td>
<td>35.0±0.6</td>
<td>36.6±0.6 **</td>
<td></td>
<td>0.033</td>
</tr>
<tr>
<td>LVIDD (mm)</td>
<td>48.8±0.7</td>
<td>50.1±0.6 #</td>
<td>52.6±0.6 ***</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVIDS (mm)</td>
<td>31.4±0.6</td>
<td>32.2±0.6</td>
<td>33.9±0.6 **</td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>IVS (mm)</td>
<td>9.4±0.2</td>
<td>10.0±0.2 #</td>
<td>10.7±0.2 ***</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PW (mm)</td>
<td>9.4±0.4</td>
<td>8.9±0.2</td>
<td>9.5±0.2</td>
<td></td>
<td>0.153</td>
</tr>
<tr>
<td>EPSS (mm)</td>
<td>4.9±0.2</td>
<td>5.8±0.2 **</td>
<td>6.7±0.4 ***</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mitral annulus (mm)</td>
<td>34.3±0.7</td>
<td>34.6±0.6</td>
<td>37.1±0.5 ***</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>FS (%)</td>
<td>35.9±0.6</td>
<td>36.2±0.6 #</td>
<td>35.8±0.6 ###</td>
<td></td>
<td>0.946</td>
</tr>
<tr>
<td>Peak E (cm/s)</td>
<td>69.2±1.7</td>
<td>68.3±2.0</td>
<td>63.5±1.6</td>
<td></td>
<td>0.054</td>
</tr>
<tr>
<td>Peak A (cm/s)</td>
<td>55.5±1.0</td>
<td>50.2±1.9</td>
<td>50.0±1.4</td>
<td></td>
<td>0.876</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.3±0.05</td>
<td>1.3±0.07</td>
<td>1.2±0.05</td>
<td></td>
<td>0.292</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>66.7±3.1</td>
<td>73.2±2.7 #</td>
<td>84.3±2.9 ***</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVH (%)</td>
<td>4.2</td>
<td>5.7 #</td>
<td>18.9 **</td>
<td></td>
<td>0.002</td>
</tr>
</tbody>
</table>

Abbreviations: LVIDD = left ventricular internal dimension in diastole; LVIDS = left ventricular internal dimension in systole; IVS = ventricular septal thickness in diastole; PW = posterior wall thickness in diastole; EPSS = mitral valve end point septal separation; FS = fractional shortening; LVMI = left ventricular mass index; LVH = left ventricular hypertrophy. **p < 0.01, ***p < 0.001 vs. control subjects and # p < 0.05, ### p < 0.001 vs. PKD1 patients. The results are given as means ± SEM or %.

The left atrium was larger in the PKD1 group than in the control group (p < 0.01). Left ventricular diameter during diastole (LVIDD) and systole as well as the thickness of the intraventricular septum (IVS) were also significantly larger in the PKD1 group compared to those in the control group (p < 0.001, p < 0.01 and p < 0.001 respectively). In addition, LVIDD and IVS in the PKD1 group were larger than in healthy relatives (p < 0.05 for both). Furthermore, the mitral valve end point septal separation (EPSS) and the diameter of the mitral annulus were larger in the PKD1 group than in the control group (p < 0.001 for...
both). EPSS was also higher in healthy relatives than in control subjects (p < 0.01). In univariate linear regression analysis systolic blood pressure, age and GFR were significantly associated with EPSS. LVMI and the prevalence of LVH were significantly higher in the PKD1 group (p < 0.001 and p < 0.01, respectively) and in the group of healthy relatives (p< 0.05 for both) than in the control group. In univariate logistic regression analysis systolic blood pressure, age and GFR were significantly associated with LVH in patients with PKD1.

**Figure 4.** The prevalence of mitral-valve prolapse (upper part) and mitral regurgitation (lower part) (grade 2-3) among control subjects (open bar), healthy relatives (hatched bar) and patients with PKD1 (black bar). *p < 0.05 and **p < 0.01 vs. controls
As shown in Figure 4 the PKD1 group had a higher prevalence of mitral valve prolapse than the control group (25.7 vs. 9.6%, p < 0.01). The prevalence of mitral valve prolapse did not differ between healthy relatives and control subjects (13.7 vs. 9.6%). The prevalence of haemodynamically significant mitral regurgitation (grade 2 or 3) (Figure 4) was higher in the PKD1 group than in the control group (12.8 vs. 2.7%, p < 0.05). No difference was found between healthy relatives and controls.

The prevalence of grade 2 or 3 aortic regurgitation was 8.3% in the PKD1 group, 4% in the healthy relatives and 2.7% in the control group, but the differences were not statistically significant. LVH was found in 18.9% of PKD1 subjects, 5.7% of healthy relatives and 4.2% of control subjects. In univariate logistic regression analysis systolic blood pressure and the severity of renal insufficiency were related to mitral regurgitation and LVH in subjects with PKD1.

5.3. Insulin resistance and LVH in patients with PKD1 (Study III)

LVMi was significantly higher in patients with PKD1 than in healthy relatives (84.3 vs. 73.2 g/m², p < 0.01). As shown in Figure 5 insulin resistance was related to LVMi in patients with PKD1 (β = 0.385, p < 0.001) in univariate regression analysis. In multiple linear regression analysis insulin resistance correlated significantly with LVMi in healthy relatives (β = 1.672, p < 0.01) and in patients with PKD1 (β = 1.139, p < 0.05) independently of age (β = 0.543, p < 0.01 and β = 0.680, p < 0.05), weight (β = 0.539, p < 0.001 and β = 0.056, p > 0.05), systolic blood pressure (β = 0.283, p < 0.05 and β = 0.002, p > 0.05) and albuminuria (β = -0.239, p > 0.05 and β = 0.002, p < 0.01) (Table 5).
Figure 5. Association of insulin resistance measured with the homeostasis model of insulin resistance (HOMA IR) with left ventricular mass index (LVMI) in patients with PKD1 (univariate linear regression analysis)

Table 5. Factors significantly associated with left ventricular mass index in healthy relatives and in patients with PKD1 (multiple linear regression analysis)

<table>
<thead>
<tr>
<th></th>
<th>Healthy relatives</th>
<th>Patients with PKD1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.543</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.539</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.283</td>
<td>0.013</td>
</tr>
<tr>
<td>Albuminuria (μg/min)</td>
<td>-0.239</td>
<td>0.331</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.672</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Abbreviations: HOMA IR = the homeostasis model of insulin resistance.
5.4. Genetics and phenotypic characteristics of ADPKD in Finns (Study IV)

In Study IV we screened the entire coding region of the PKD1 and PKD2 genes in 17 Finnish families with ADPKD with a long-range polymerase chain reaction, single strand confirmation polymorphism analysis, and direct sequencing.

We identified mutations co-segregating with ADPKD in all 16 families determined to have PKD1 by haplotype analysis (Table 6). Pathogenic mutations included three deletions (6945 del T, 12354-12363 del, 12752-12768 del), two insertions (966 ins C, 11064 ins C) and one combination of a missense mutation and an insertion (V873A + 2830 ins. C) leading to truncated proteins. Furthermore, we identified five nonsense mutations (Q1020X, Q1117X, S3384X, W3574X and Q4004X). Probable pathogenic mutations included three missense mutations (L845S in one family, V3138M in two families, and A3954P in two families). A family having PKD2 had a missense mutation R322Q in the PKD2 gene. All these mutations, with the exception of L845S, are novel mutations. None of these missense mutations were found in 100 chromosomes of healthy control subjects. Mutations did not have a strong correlation with the phenotype but mutations associated with renal insufficiency and SAH or brain aneurysm were more often located at the 5' end of the PKD1 gene than at the 3' end of the PKD1 gene.

We also analyzed phenotypic characteristics of the mutation carriers (Table 7). In all families the mutation cosegregated with the disease. In four of 16 families having mutations in the PKD1 gene we found anticipation (onset of dialysis at least 10 years earlier in the offspring of any parent-offspring pair). Hypertension was present in the majority of affected patients (69.6 %), and renal insufficiency was present in 47.5 % of affected patients. Liver cysts were also very common (60.4 % of affected patients). Mitral regurgitation occurred in 38.8 % and mitral prolapse in 28.1 % of patients. SAH or brain aneurysm were found in 5 of 16 families (31.2 %).
In two families (1 and 4) having the A3953P mutation the clinical phenotype was quite similar with the exception of SAH and brain aneurysms which were found only in Family 1. In Families 3 and 6 having the V3138M mutation hypertension and renal insufficiency were common, but liver cysts were found only in Family 3. In Family 7 having the L045S mutation phenotypic characteristics in affected patients were quite severe, because they often had hypertension, renal insufficiency, liver cysts, cardiac valvular abnormalities and SAH or brain aneurysms.

In a single family having the R322Q mutation in the PKD2 gene, hypertension, renal insufficiency, and liver cysts were common (Table 7). In contrast, the patients belonging to this family rarely had mitral valve abnormalities, and no SAH or brain aneurysm was found in any of the 10 affected family members. When the patients having the definite pathogenic disease causing mutations (insertion/deletion, nonsense mutation) were compared with patients having the missense mutation no statistically significant differences were found in the frequency of hypertension (71.2 vs. 67.4 %), renal insufficiency (44.1 vs. 51.2 %), liver cysts (62.5 vs. 57.5 %), mitral regurgitation (40.0 vs. 37.2 %), mitral prolapse (25.0 vs. 32.5 %), or SAH or brain aneurysm (18.2 vs. 28.6 %).
Table 6. Mutations in families with autosomal dominant polycystic kidney disease

<table>
<thead>
<tr>
<th>Pathogenic mutations in PKD1</th>
<th>Family</th>
<th>Exon</th>
<th>Nucleic acid change</th>
<th>Codon change</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>5</td>
<td>CCG CCA → CCC GCC</td>
<td>366 ins C*</td>
<td>Frameshift</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>GTC TGC CCT → GCC GTG CCC</td>
<td>V873A+2830 ins C *</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>13</td>
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<td>Q1020X</td>
<td>Nonsense termination</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>CAG → TAG</td>
<td>Q1117X</td>
<td>Nonsense termination</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>ATC CAG → ACC AGG</td>
<td>3945 del T</td>
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</tr>
<tr>
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<td>31</td>
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<td>S3384X</td>
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<td>9</td>
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<td>37</td>
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<tr>
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<td>45</td>
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</tr>
<tr>
<td>10</td>
<td>46</td>
<td>GCC TCG GAT GCC TCG GAC CCC TCC</td>
<td>12752-12768 del*</td>
<td>Frameshift</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Probable pathogenic mutations PKD1</th>
<th>Family</th>
<th>Exon</th>
<th>Nucleic acid change</th>
<th>Codon change</th>
<th>Consequence</th>
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</thead>
<tbody>
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<td>L845S</td>
<td>Missense</td>
<td></td>
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<tr>
<td>3</td>
<td>27</td>
<td>GTG → ATG</td>
<td>V3138M</td>
<td>Missense</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>GTG → ATG</td>
<td>V3138M</td>
<td>Missense</td>
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<td>43</td>
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<td>A3653P</td>
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</tr>
<tr>
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<td>GCC → CCC</td>
<td>A3653P</td>
<td>Missense</td>
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<th>PKD2</th>
<th>Family</th>
<th>Exon</th>
<th>Nucleic acid change</th>
<th>Codon change</th>
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<td>R322Q</td>
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* Positions in Acc. Num. L32
Table 7. Mutations and phenotypic characteristics in families with autosomal dominant polycystic kidney disease (no. of patients with a complication/all affected patients)

<table>
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<tr>
<th>Family</th>
<th>Mutation</th>
<th>Cosagg-relation</th>
<th>Anti-</th>
<th>Hyper-</th>
<th>Renal insuff.</th>
<th>Liver Cysts</th>
<th>Valvular abnormalities</th>
<th>SAH or brain aneurysm</th>
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<td></td>
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<td></td>
<td></td>
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<td>Mr</td>
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<td>17</td>
<td>6945 delT</td>
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<td>No</td>
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<td>9/10</td>
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</tbody>
</table>

NA = not available, Mr = mitral regurgitation, Mp = mitral valve prolapse, SAH = subarachnoid haemorrhage.
6. DISCUSSION

6.1. Study subjects

Twenty families with ADPKD from the Kuopio University Hospital region were identified. One hundred and twenty five affected and 92 unaffected family members were recruited from these families. Since the Finnish population is relatively homogeneous, the study population is likely to be representative of ADPKD patients in Finland. However some of patients with ADPKD could be asymptomatic, and therefore it is possible that not all patients with ADPKD in the Kuopio University Hospital region had been identified and included in our study. Especially patients with PKD2 have a milder form of the disease, and therefore the percentage of patients with PKD2 may be underrepresented in our study. Thus, the prevalence of ADPKD in the Finnish population cannot be estimated reliably according to our study.

In Study II 73 control subjects of similar age and gender as patients with PKD1 were recruited from a previous study. (200) None of them had a family history of ADPKD. The subjects were evaluated with a detailed medical history, physical examination, and routine laboratory tests. All of them were non-smokers, and none was on cardiovascular medication. Therefore, the control subjects may be healthier than the general population.

The diagnosis of ADPKD was made when ultrasonography showed at least two cysts in one kidney and one cyst in the other kidney, and the patients had a positive family history of ADPKD. (180) Other definitions for ADPKD have been suggested. According to Ravine et al. (201) the minimum requirement for the diagnosis of ADPKD in individuals less than 30 years are two cysts, between 30-60 years more than five cysts, and above the 60 years at least eight cysts bilaterally.
6.2. Extrarenal manifestations in Finnish patients with ADPKD (Study I)

The phenotype/genotype relationship of patients with ADPKD has not previously been evaluated in Finnish patients. The PKD1/PKD2 distribution (94%/6%) in Finnish families with ADPKD was in agreement with previous studies. (202) Also, the phenotype of ADPKD in Finnish patients resembled that previously reported (Table 8). (10,76) The age at onset of ESRD was similar in all three studies (50 vs. 53 vs. 53 years, Lumiaho et al. (study I), Hateboer et al (10), Torra et al. (76) In addition, the prevalence of hepatic cysts and macrohaematuria did not differ from that reported in other European PKD1 populations. (10, 76) In contrast, we did not find any difference between PKD1 patients and healthy relatives with respect to urinary tract infection or nephrolithiasis. Furthermore, the prevalence of hypertension was higher in our study population. One reason for these different findings could be the older age of the patients with PKD1 in our study than in the study by Torra et al. (76) In the study by Hateboer et al. (10) the age of study population was not reported. In addition, the prevalence of essential hypertension is higher in the Finnish population than in the English or Spanish populations, (203) which could partly contribute to the high prevalence of hypertension in patients with PKD1. However, the age of the patients at the time of diagnosis of hypertension was almost the same in our study population as the age of patients with PKD1 in the study by Torra et al. (76)

The prevalence of ICA in patients with PKD1 was 12% in our study, which is in accordance with other studies. (102-104) The limitation of previous studies is the lack of classification of patients into the subgroups of ADPKD (PKD1, PKD2, or neither). Because we performed MRA only on index patients and those patients whose first degree relatives had ICA, a selection bias is obvious. In addition, the number of PKD2 patients was small with all, patients from a single family. Therefore any conclusions regarding the prevalence of ICA in patients with PKD2 cannot be drawn.
Significant intrafamilial phenotypic variation has been described, suggesting that modifying factors and environment factors influence disease progression. In addition, interfamilial phenotypic differences have been reported previously. In line with previous studies (11,172) we found interfamilial differences with respect to renal disease (Figure 2). The rate of progression of kidney disease evaluated by reciprocal creatinine values varied between PKD1 families in our study. In contrast, in the study by Hateboer et al. (11) and Rossetti et al. (172) the prognosis (progression) of kidney disease was evaluated by calculating the time from birth to ESRD by using the Kaplan-Meier method.
<table>
<thead>
<tr>
<th></th>
<th>Torra et al. (76)</th>
<th>Hateboer et al. (10)</th>
<th>Jurniaho et al. (Study I)</th>
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<tr>
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<td>PKD1 (N = 146)</td>
<td>PKD2 (N = 20)</td>
<td>PKD1 (N = 333)</td>
</tr>
<tr>
<td>Age at ESRD (years)</td>
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<td>73</td>
<td>53</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>47</td>
<td>40</td>
<td>54</td>
</tr>
<tr>
<td>Age at diagnosis of HTA (years)</td>
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<tr>
<td>Hepatic cysts (%)</td>
<td>58</td>
<td>60</td>
<td>NA</td>
</tr>
<tr>
<td>Macronematuria (%)</td>
<td>NA</td>
<td>NA</td>
<td>24</td>
</tr>
<tr>
<td>Urinary Tract infection (%)</td>
<td>35</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Nephrolithiasis (%)</td>
<td>18</td>
<td>15</td>
<td>7</td>
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</table>
6.3. Cardiac abnormalities in patients with PKD1 (Studies II and III)

In our study we found a relatively high prevalence of mitral valve prolapse (25.7%), mitral regurgitation (12.8%) and LVH (18.9%) in patients with PKD1 (Table 9). Mitral regurgitation was related to systolic blood pressure and the severity of renal insufficiency in PKD1 patients. LVH was associated with albuminuria and systolic blood pressure. In addition, insulin resistance was associated with LVMI independently of other factors known to increase LVMI. Mitral prolapse did not correlate with elevated blood pressure or severity of renal insufficiency.

Because patients with PKD1 have a more severe disease compared with that of PKD2 patients, it could be expected that patients with PKD1 have a higher prevalence of cardiac valve abnormalities than PKD2 patients. Thus, the lack of genotyping of patients into subgroups of ADPKD (PKD1, PKD2, or neither) in previous studies is a serious limitation. In our study there was only one family with PKD2. Therefore, we could not reliably estimate the prevalence of cardiac abnormalities in patients with PKD2. In the study by Timio et al. (117) a high prevalence of cardiac abnormalities was found in one family. Although the family was not genotyped, it seems very likely that it was linked to the PKD1 gene.

Our results concerning valvular disorders in patients with PKD1 are in agreement with the study by Hossac et al. (116) and Timio et al. (117) (Table 9). In the studies by Hossac et al. (116) and Timio et al. (117) grade 1 regurgitations were also included. Since grade 1 regurgitation is not haemodynamically significant, we decided to present only grade 2 and grade 3 regurgitations. In our study patients with PKD1 did not have more grade 2 or 3 aortic regurgitations than the control group. However, if grade 1 regurgitations were also included in the statistical analyses the prevalence was significant, similar to the studies by Hossac et al. (116) and Timio et al. (117).

In contrast to our study, both Hossac et al. (116) and Timio et al. (117) showed a higher prevalence of mitral valve prolapse, mitral regurgitation and
aortic regurgitation in unaffected family members compared with control subjects. However, in their study the age range was wide, from 4 to 74 years and 5 to 76 years, respectively. Since patients under 30 years can be affected although the ultrasonography findings are negative (201), it is possible that some of healthy relatives may have had ADPKD. However, other contradictory results have also been reported. Bardaji et al. (122) did not observed a higher prevalence of cardiac valvular abnormalities in patients with ADPKD, but it is likely that the lack of genotyping and differences in methods may explain some of these discrepancies.

LVH has been suggested to be an important extrarenal manifestation of ADPKD. Two earlier studies (116,117) reported a 18-24% prevalence of LVH in patients with ADPKD. The prevalence of LVH in our study (19%) was not different from previous studies, although it is noteworthy that one study (124) reported an even higher prevalence of LVH in patients with ADPKD. However no information is available concerning the occurrence of LVH separately in patients with PKD1 or PKD2.

Bardaji et al. (122) reported that systolic blood pressure was the most important factor related to LVM in dialysis and non-dialysis ADPKD patients. They suggested that cardiac involvement in patients with ADPKD is mainly due to hypertension. In the present study systolic blood pressure, age and GFR were significantly associated with LVH in patients with PKD1 in univariate logistic regression analysis. However, a high prevalence of LVH has been reported also in normotensive patients with ADPKD (23%) (124) and also in patients with normal renal function. (120) Since the relationship between LVH and blood pressure is modest other mechanisms could be involved in the development of LVH in patients with ADPKD.

Is screening with echocardiography indicated in patients with ADPKD? Almost all mitral prolapses were grade 1 in our study, and a grade 1 mitral prolapse is not an indication for the prophylaxis of endocarditis. In addition, the prevalence of a combination of mitral regurgitation and mitral prolapse, which could become more severe with age, was not higher in patients with PKD1
compared with control subjects. However, mitral regurgitations and LVH increased with ageing and we found a relatively high prevalence of haemodynamically significant mitral regurgitation and LVH. In addition, mitral regurgitation was associated with systolic blood pressure and GFR. Thus, based on our data echocardiography may not be indicated for young patients with ADPKD unless they have renal insufficiency, hypertension or a positive finding on cardiac auscultation. However, the screening with echocardiography in older patients and patients with renal insufficiency or hypertension may be recommended.

Table 9. Prevalence of cardiovascular abnormalities in patients with ADPKD (Hossack et al. (117), Timio et al. (118) and Bardaji et al. (122)) and in patients with PKD1 (Lumiaho et al. study II).

<table>
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<tbody>
<tr>
<td></td>
<td>n = 163</td>
<td>n = 228</td>
<td>n = 130</td>
<td>n = 109</td>
</tr>
<tr>
<td>Mitral prolapse (%)</td>
<td>26</td>
<td>25</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Mitral regurgitation (%)</td>
<td>31</td>
<td>30</td>
<td>17</td>
<td>13* (35*)</td>
</tr>
<tr>
<td>Aortic regurgitation (%)</td>
<td>8</td>
<td>19</td>
<td>11</td>
<td>8* (17*)</td>
</tr>
<tr>
<td>LVH (%)</td>
<td>16</td>
<td>24</td>
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<td>10</td>
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</table>

\* grade 2 or 3 regurgitation, \* grade 1, 2 or 3 regurgitation

The relationship between LVH and insulin resistance in patients with ADPKD has not been previously evaluated. Because insulin resistance has been related to LVH (132), and patients with ADPKD have been suggested to be insulin resistant (141) we therefore hypothesized that insulin resistance could be an important determinant of LVMI in patients with PKD1. Although in the present study HOMA-IR and fasting insulin did not differ between patients with PKD1 and healthy relatives, LVMI was associated with insulin resistance in PKD1 patients. The association of insulin resistance with LVMI was strengthened if blood pressure and albuminuria were omitted from statistical analyses. Thus,
the relationship between ADPKD and LVMI is partly, but not totally, related to albuminuria and blood pressure.

In a previous study Vareesanghip et al. (141) found a difference in insulin resistance between patients with ADPKD and normal subjects using the insulin tolerance test. In our study we used the IOMA model which, may explain the difference between the two studies. However, in the study by Vareesanghip et al. (141) LVMI was not measured, and the relationship between insulin resistance and LVMI could not be evaluated.

How can insulin resistance contribute to LVH in patients with ADPKD? Several mechanisms are possible. First, hyperinsulinaemia has been shown to increase angiotensin II stimulated aldosterone production (204) which could increase blood pressure. Second, angiotensin II has cardiac, growth-promoting effects independently of blood pressure (205, 206). Third, insulin has been shown to activate the sympathetic nervous system in patients with essential hypertension (207), and LVH has been suggested to be related to sympathetic nervous system activity (208). Fourth, hyperinsulinemia can induce in vitro cardiac cell growth through the stimulation of insulin-like growth factor-1 receptors. Fifth, since myocardial protein degradation is inhibited during hyperinsulinaemia in insulin-resistant subjects, this antiproteolytic action may be a mechanism by which hyperinsulinaemia could contribute to the development of LVH. (209) However, the role of insulin and glucose metabolism in the development of LVH is still unclear.

Taken together, hypertension is one of the most important factors behind the development of LVH and mitral regurgitation. In addition, there are multiple other factors, including insulin resistance, that also contribute to the development of LVH. Furthermore, mutations in the PKD1 or PKD2 genes may influence the cardiovascular phenotype. Especially mitral valve prolapse may be a characteristic finding in patients with ADPKD. Since all affected family members with the same germline mutation do not have mitral prolapse, other factors such as modifying genes and environmental factors must be involved. Further studies are needed to elucidate if the type or location of mutation in the
PKD1 or PKD2 genes influence the cardiovascular phenotype in patients with ADPKD.

6.4. Genetics and phenotype characteristics of ADPKD in Finns (Study IV)

Our study is the first to evaluate the genotype of Finnish patients with ADPKD. Because the Finnish population is genetically homogenous (210) we hypothesised that only few founder mutations would be found in patients with ADPKD as have been found in several other monogenic diseases studied in the Finnish population. However, almost all families had a different mutation and the distribution of insertions/deletions, nonsense mutations, and missense mutations, one third of each, was quite similar to what has been reported in previous studies. In line with this, the progression of kidney disease varied widely between the PKD1 families, suggesting the presence of several mutations in the PKD1 gene in Finns. Only one of our families had a mutation in the PKD2 gene and 16 families mutations in the PKD1 gene. Three families had uninformative haplotypes. Only one mutation of the 16 mutations in Finnish families with ADPKD has been reported previously. The proportion of patients having mutations in the PKD1 (94%) and PKD2 genes (6%) is in agreement with previous studies. (7, 200)

Our aim was not only to perform DNA screening, but also establish a correlation between the genotype and phenotype. We recruited our families from the Kuopio University Hospital region (about 250 000 inhabitants) and therefore our patients are representative of patients with ADPKD living in the study area. Clinical phenotype was characterized in all affected and unaffected patients of each pedigree with ultrasound examination of kidneys, liver and aortic and mitral valves, and MRA of cerebral arteries.

According to previous studies the location of the mutation within the PKD1 and PKD2 genes may influence the clinical outcome. However, in our study there was no obvious correlation between the severity and location of the
mutation and phenotype, perhaps with the exception of vascular disease. In our study 3 of 5 affected families with SAH or brain aneurysms had a mutation in the 5 position, which has been reported to be associated more commonly with vascular disease. (173) Watnick et al. (47) identified three separate families with aneurysms and/or very-early-onset disease that have an identical mutation (5224delI2) in exon 15 of the PKD1 gene. However, in our study only one family with aneurysms had a mutation in exon 15 (6945delT). In contrast, the prevalence of other complications, renal insufficiency (52.0 vs. 51.4 %), liver cysts (58.0 vs. 63.0 %), and mitral regurgitation (38.0 vs. 39.6 %) was quite similar in families with vascular disease compared with other families.

We did not find significant correlations between nucleotide positions of the mutations and the progression of kidney disease or the age at onset of dialysis. However, with respect to frameshift and nonsense mutations in PKD1, patients with mutations in exons from 5 to 15 tended to have renal insufficiency more often than patients having mutations in exons from 31 to 46 (53.1 vs. 37.5%), although the difference was not statistically significant. These results are in agreement with findings by Rosselli et al., (172) who reported that patients with mutations in the 5’ region of the PKD1 gene (nucleotide < 7812) had lower age of onset of end-stage renal disease compared with patients having mutation in the 3’ region (53 vs. 56 years, p=0.025). Also in our study the presentation of the disease was highly variable even within individual families. This suggests that other genetic and environmental factors may modify disease presentation and progression.

6.5. Concluding remarks

We have studied phenotype and genotype characteristics in a cohort of ADPKD patients from a geographical region of 250 000 inhabitants in eastern Finland. The phenotype of ADPKD in Finnish patients and the distribution between PKD1 and PKD2 did not differ from that previously reported. Similar frequencies of extrarenal complications like liver cysts, hypertension, cardiac valvular
abnormalities. LVH and SAH were found compared with other populations. The absence of ICA in all affected family members indicates that other factors than germline mutations may play a role in the formation and rupture of ICA.

This is the first study investigating cardiac manifestations in ADPKD patients with a uniform genotype. We found increased prevalence of mitral valve prolapse, mitral regurgitation and LVH in patients with PKD1 compared with healthy controls. Since we identified only one family with PKD2, the prevalence of cardiac manifestations in patients with PKD2 is only suggestive, and further research is needed to determine if PKD2 patients have less cardiac abnormalities than patients with PKD1. In our study insulin resistance, a major cardiovascular risk factor, was associated with LVH independently of other factors. Therefore, insulin resistance could contribute to increased cardiovascular mortality in patients with ADPKD. In addition to the mutation in the PKD1 gene, modifying genes might also predispose to the development of LVH. Therefore identification of these genes is important.

We expected to find a founder mutation in the PKD1 and PKD2 genes as has been reported for several other monogenic disease in the Finnish population. However our results support previous findings that almost all ADPKD families have their own mutations. In this report all mutations, with the exception of one, were novel. The size and replicated segments of the PKD1 gene makes screening extremely difficult. Only one previous study has reported findings on the screening of the entire coding region of both PKD1 and PKD2 genes.

In accordance with previous studies considerable inter- and Intrafamilial variability was observed in the phenotype of ADPKD in these Finnish families. The mutations identified did not have any clear correlation with the phenotype. There are several possible reasons for this observation. First, the loss of heterozygosity and acquired somatic second hits could explain the finding. Second, genetic locus heterogeneity has been a major determinant of interfamilial heterogeneity in earlier studies, because disease associated with mutations in the PKD1 gene appears to be more severe compared with that related to mutations in the PKD2 gene. Third, allelic heterogeneity and
modifying genes might also influence the phenotype. Because the number of families and family members were relatively small in the present investigation, further studies are needed to determine whether the nature of the underlying mutation influences the phenotype. It is important to identify genes modifying the progression of ADPKD, which could help in developing treatment modalities to slow or prevent the progressive cyst enlargement.

Why is it important to know the genetic background of ADPKD? The role of polycystin-1 and -2 in cyst formation and other disease manifestations is not clear. The identification of mutations in the PKD1 and PKD2 genes could help to understand how mutations in these genes affect the structure and the function of the proteins. DNA testing may be also useful in the confirmation of a clinical diagnosis in children, especially in those having an unclassified phenotype and in distinguishing ADPKD from autosomal recessive polycystic kidney disease. In addition, DNA testing could turn out to be useful in the evaluation of a potential kidney transplant donor, especially if a donor is a relative and thereby possibly have ADPKD. If one could identify specific mutations predisposing to disease progression or development of ICA, one could screen for patients at high risk. Dissecting out the genetic basis of ADPKD will ultimately help us to develop therapeutic modalities for this disease.

This work has provided new information about the phenotype and genetic background of ADPKD in Finland. The results form a basis for future studies of genotype/phenotype correlations. It will be particularly important to study the genetics of ADPKD in other parts of Finland to elucidate whether similar mutations are found in other areas and whether phenotype differences within and between families are explained by modifying genes or environmental factors.
7. SUMMARY

In Study I the phenotype of ADPKD in Finnish patients was evaluated. The relative proportion of PKD1 and PKD2, and the phenotype of ADPKD were similar in Finnish patients compared with previous studies in other populations. The progression of kidney disease differed substantially among PKD1 families.

In Study II cardiovascular abnormalities in patients with PKD1 were investigated. Mitral-valve prolapse was a characteristic finding in patients with PKD1. In contrast, mitral regurgitation and left ventricular hypertrophy are likely to be secondary to elevated blood pressure in these patients.

Study III showed that insulin resistance is correlated with LVMI in patients with PKD1. In addition, albuminuria may be a contributing factor to LVH in patients with PKD1.

In Study IV the entire coding region of the PKD1 and PKD2 genes in 17 Finnish families with ADPKD were screened. Almost all families had a different mutation. Mutations associated with renal SAH or brain aneurysm were located more often at the 5' end of the PKD1 gene than at the 3' end of the PKD1 gene.
8. REFERENCES


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183 Devereux RB. Echocardiography, hypertension, and left ventricular mass. Health Psychol 1988; (Suppl 7):89-104.


