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CLINICAL AND MOLECULAR CHARACTERISATION OF AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE (ARPKD) IN AFRIKAANS FAMILIES

Lindsay Ann Lambie

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Science in Medicine in Genetic Counselling

Johannesburg, South Africa, 2009

DECLARATION

I, Lindsay Ann Lambie, declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in Genetic Counselling in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Lindsay Ann Lambie

1st day of September 2009

PRESENTATIONS ARISING FROM THIS STUDY

Poster Presentations

F Essop, P Thomson, L Lambie , H Joubert, L M Guay-Woodford and A Krause (2005) A Founder Mutation Identified in Afrikaner Families with Autosomal Recessive Polycystic Kidney Disease (ARPKD). 11th Biennial Congress of the Southern African Society of Human Genetics, Muldersdrift, South Africa, 13-16 March 2005

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ABSTRACT

Autosomal recessive polycystic kidney disease (ARPKD; MIM263200) is a severe recessively inherited disease of the kidneys and biliary tract, with an incidence of approximately 1 in 20000 in non-isolated populations. It has a variable clinical spectrum from neonatal demise (in 30-50%) to survival into adulthood. ARPKD is caused by mutations at a single locus, polycystic kidney and hepatic disease 1 (*PKHD1*), with over 270 pathogenic mutations described to date. The high rate of compound heterozygosity in affected individuals has made genotype-phenotype correlations difficult. A common missense mutation, p.M627K, in exon 20 of *PKHD1* was identified previously on the majority of ARPKD disease associated alleles in the Afrikaans population of South Africa suggesting the presence of a founder effect.

The aim of this study was to describe the clinical phenotype of ARPKD in Afrikaans speaking individuals found to be homozygous for the common mutation, and to compare this phenotype to previously described cohorts of patients with ARPKD, known to harbour a spectrum of mutations. This descriptive study used retrospective data collected from records of patients with ARPKD at Johannesburg and Pretoria Academic Hospitals. Twenty seven individuals from 24 families were included in the study.

Marked clinical variability was demonstrated within this subject group supporting the limitation of genotype-phenotype correlation described worldwide. ARPKD was diagnosed at a median age of 27 days, older than a North American cohort (NAC) born after 1990 (median age of 1 day). The majority (93%) of subjects in this study were diagnosed with chronic renal

insufficiency (CRI) and hypertension (HT), indicating the renal morbidities to be more common than noted in previous studies, but occurring at a later median age (1.4 years vs 13.5 days in the NAC). This may indicate a trend toward milder expression of renal morbidities in the present study. Portal hypertension was also diagnosed more frequently (81%) than in previous studies but at a younger median age (1.3 years vs 2.8 years), although with similar complication rates. Overall statistical correlation was found between the renal and hepatic related morbidities in this study, indicating that progression of the condition is not organ specific. A survival rate of 89% at one year is comparable to previous studies with similar patient ascertainment.

This cohort represents the largest series of patients affected by ARPKD with a common mutation, described to date. The findings will provide for more accurate, specific and informative genetic counselling in families with ARPKD and may present a resource for future studies of modifier genes and environmental influences on the phenotypic expression of ARPKD.

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ABBREVIATIONS

ADPKD: Autosomal dominant polycystic kidney disease

ARPKD: Autosomal recessive polycystic kidney disease

CHF: Congenital hepatic fibrosis

CRI: Chronic renal insufficiency

ESRF: End stage renal failure

FH: Familial hypercholesterolaemia

GFR: Glomerular filtration rate

GR: Growth retardation

NAC: North American cohort

NHLS: National Health Laboratory Service

OMIM: Online Mendelian Inheritance in Man

PHT: Portal hypertension

PKD1: Polycystic kidney disease locus 1

PKHD1: Polycystic kidney and hepatic disease locus 1

RRT: Renal replacement therapy

UAB RPKDCC: University of Alabama Recessive Polycystic Disease Core Center

UTI: Urinary tract infection

1 INTRODUCTION

Autosomal recessive polycystic kidney disease (ARPKD; MIM263200) is a severe recessively inherited disease of the kidney and biliary tract. The principal, invariable features are cystic dilatation of the renal collecting ducts, and malformation of the ductal plate with portal tract fibrosis. The clinical presentation and progression is variable, although most cases present in infancy with an estimated 30% mortality rate in the first year (Zerres et al. 1996). The causative gene is polycystic kidney and hepatic disease 1 (*PKHD1*) (MIM 606702), and there is no evidence for locus heterogeneity (Zerres et al. 1994). However, the presence of allelic heterogeneity, with more that 270 mutations described to date in the ARPKD-PKHD1 Mutation Database, Aachen University (2008), has made genotype-phenotype studies challenging.

The suggestion of an unusually high frequency of ARPKD in the Afrikaans speaking population of South Africa (Lombard et al. 1989), led to the finding in 2002 of a common haplotype in the majority of these patients (Professor A Krause, Division of Human Genetics, NHLS and University of the Witwatersrand, unpublished data), suggesting the presence of a founder mutation. Recent identification of a common mutation (Guay-Woodford et al. 2005) in the Afrikaans speaking population confirmed this theory.

The aim of the present study was to describe the clinical phenotype of ARPKD in the Afrikaans speaking population, particularly in those patients found to be homozygous for the

common mutation, and to compare this phenotype to those found in previous studies of more heterogenous groups of patients with ARPKD, known to harbour a spectrum of mutations.

In this introduction, the clinical presentation and diagnosis of ARPKD is discussed. The molecular cause and spectrum of mutations is mentioned, with emphasis on the events leading to the discovery of a common mutation in the Afrikaans population. The objectives, limitations and uniqueness of the present study are outlined.

1.1 INCIDENCE

The incidence of ARPKD is estimated to be 1:20 000 world-wide (reviewed in Zerres et al. 1998b), in non-isolated populations, with a heterozygote frequency of 1:70. However, incidence figures vary widely depending on the study population. Kaariainen (1987) found the birth prevalence of polycystic kidney disease in the Finnish population to be in the region of 1:8000 (which may have included some early onset cases of autosomal dominant polycystic kidney disease), while Zerres et al (1984) initially estimated an incidence of 1:40 000 in West Germany for ARPKD. Although no evidence illustrates an ethnic predominance, ARPKD has been reported rarely in non-Caucasian populations (reviewed in Guay-Woodford 1996). In a series of South African paediatric patients with renal failure, ARPKD was only found in the Caucasian children (Thomson 1997). On the basis of the autosomal recessive mode of inheritance the sex distribution is considered equal, but a few reports have suggested a female predominance (Gang and Herrin 1986, Kaariainen 1987).

1.1.1 Incidence in the Afrikaans Speaking Population

Lombard et al. (1989) estimated an incidence of 1:11 000 based on the live birth rate, in the Afrikaans speaking population of the, then, Transvaal Province of South Africa. This translated to a carrier rate of 1:53 in this group, and provided evidence of an unusually high frequency of ARPKD in the Afrikaans population.

1.2 CLINICAL FEATURES OF ARPKD

1.2.1 Clinical Presentation

The majority of cases of ARPKD present in infancy (Cole et al. 1987), and they are often identified in utero or at birth, hence the previously used term, infantile polycystic kidney disease. Blyth and Ockenden (1971) originally proposed the existence of four distinct groups of patients (perinatal, neonatal, infantile and juvenile), postulating causation by different genetic mutations. It has since become clear that there is significant overlap clinically between the groups, and sibships discordant for age, presentation and clinical course have been reported (Gang and Herrin 1986, Kaplan et al. 1989a, Deget et al. 1995).

The most common presenting clinical features of ARPKD include enlarged palpable kidneys, enlarged liver, respiratory failure, hypertension and urinary tract infections (UTI) (Zerres et al. 1996). The gross in situ anatomy of an affected infant is shown in Figure 1.1.

Standard diagnostic criteria for ARPKD do not exist but with various modifications, the following are usually used (Zerres et al. 1996, Guay-Woodford and Desmond 2003):

- Typical ultrasound features of ARPKD (enlarged echogenic kidneys with poor corticomedullary differentiation), AND
- At least one of
    - Patho-anatomic diagnosis of ARPKD in an affected sibling
    - Absence of renal cysts in the renal ultrasounds of both parents (aged >30)
    - Clinical or histopathologic evidence of hepatic fibrosis
    - Parental consanguinity suggesting autosomal recessive inheritance

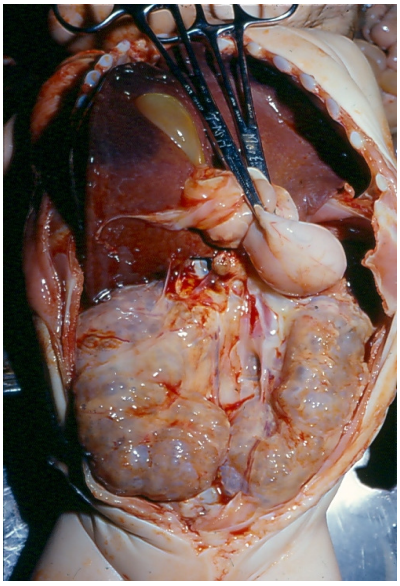


Figure 1.1: Post mortem anatomy of an infant affected with ARPKD*

*The kidneys are massively enlarged but maintain their reniform shape with, in this case, normal fetal lobulation. The liver is also enlarged. Photograph courtesy of Prof PD Thomson

1.2.1.1 Prenatal features and diagnosis

Affected fetuses may have bilaterally enlarged echogenic kidneys and oligohydramnios on fetal ultrasound, but these features are often not seen until late in the second trimester (Zerres et al. 1998a) since nephrogenesis is only completed at around 34 weeks gestation.

Oligohydramnios, if present, results in development of the Potter sequence: pulmonary hypoplasia, characteristic facies and deformities of the limbs (Osathanondh and Potter 1964). Massive abdominal distention due to nephromegaly and hepatosplenomegaly has resulted in birth dystocia (Blythe and Ockenden 1971). In as many as 70% of cases, however, the precise diagnosis can only be made postnatally (Roume and Ville 2004) and thus fetal sonography is not considered a reliable method for definitive prenatal diagnosis (Zerres et al. 1998a).

1.2.1.2 Neonatal presentation

Severely affected neonates most often present with respiratory difficulties secondary to pulmonary hypoplasia resulting from oligohydramnios, attributable to poor renal output in utero. Respiratory compromise is exacerbated by abdominal distension due to enlarged kidneys, and may result in death (Guay-Woodford 1996). Despite poor renal function, death from renal failure is rare at this stage (Blyth and Ockenden 1971, Kaplan et al 1989b). In those neonates whose respiratory status does not deteriorate, improvement is noted in their renal function (Kaplan et al. 1989a). As many as 30-50% of affected neonates are thought to die soon after birth due to respiratory insufficiency (Zerres et al 1998a).

1.2.1.3 Infancy and childhood features

For those infants surviving the neonatal period a wide range of renal and hepatic related morbidities can evolve, although with marked variability. These include renal failure, hypertension, and portal hypertension (Guay-Woodford 1996).

Surviving neonates usually have decreased glomerular filtration rates (GFR) (Guay-Woodford 1996), but Cole et al. (1987) showed a gradual improvement in renal function over the first 6

months in neonatal survivors, and then a decrease after the first year of age. The rate of decline is variable and the consequences of renal insufficiency (including growth failure, anaemia and osteodystrophy) appear. As treatment for end stage renal failure (ESRF) improves, prolonging survival, the hepatic complications may become the predominant clinical picture.

Hypertension is the most significant cause of morbidity and mortality among those presenting with ARPKD in infancy (Guay-Woodford 1996). It affects the majority of patients either at initial presentation or later in the clinical course, and may be severe in young infants requiring aggressive treatment to prevent cardiac hypertrophy and failure. Regression of hypertension has been documented but the mechanism of this has not been explained (Gagnadoux et al. 1989). Tubular function abnormalities, with hyponatraemia as a prominent feature in infants with ARPKD, are found (Kaplan et al. 1989a), and are thought to result from an inability to maximally dilute the urine. Metabolic acidosis is not, however, a significant clinical feature (Kaariainen et al. 1988).

A small minority of individuals present as older children or adolescents, providing a diagnostic challenge. These patients typically have congenital hepatic fibrosis with relatively mild renal medullary ectasia. Their clinical features are mainly a consequence of their portal hypertension (Guay-Woodford 1996), and include hepatosplenomegaly, hypersplenism, bleeding oesophageal varices and portal thrombosis. ARPKD patients presenting in early childhood may also occasionally have dominant liver involvement, while older patients may

not have signs of portal hypertension at presentation. Hepatic synthetic function is usually normal, as the lesion in ARPKD is confined to the biliary tree. Ascending cholangitis is a recognized complication that may result in hepatic failure (Guay-Woodford 1996).

ARPKD is rarely diagnosed in adults (Blyth and Ockenden 1971, Fonck et al. 2001), and the clinical course of these patients is not well documented (Adeva et al. 2006). Adeva et al. (2006) studied a cohort of 65 patients of whom a third presented in adulthood (>20 years). The initial manifestations in these patients were diverse and include renal morbidities (hypertension, renal insufficiency) and hepatic morbidities (hypersplenism, upper gastrointestinal bleeding and cholangitis).

While recent advances in neonatal care and paediatric renal replacement therapy have altered the prognosis for patients with ARPKD, the natural history of the condition still requires further characterization (Guay-Woodford and Desmond 2003).

1.2.1.4 Co-morbidity correlations

Data from Bergmann et al. (2005) suggest a positive correlation between renal and hepatobiliary morbidities, in contrast to the organ-specific progression of disease suggested by Guay-Woodford and Desmond (2003) who found no significant correlation between age of diagnosis of chronic renal insufficiency (CRI) and portal hypertension. This controversy will be explored further in the cohort of homozygous patients included in the present study.

1.2.1.5 Survival and renal survival

Mortality in the first year of life is reported to be between 9 and 24% (Cole et al. 1987, Kaplan et al. 1989a, Gagnadoux et al. 1989, Zerres et al. 1996). Kaplan et al (1989a) reported the overall survival at one and 15 years to be 79% and 46% respectively. More recently, Guay-Woodford and Desmond (2003) reported overall patient survival in their cohort of ARPKD patients born after 1990, as 85.8% at one month, 78.6% at one year and 74.6% at five years. Altogether 58% of the deaths occurred in the first month of life and they were related to respiratory insufficiency. Of those who survived the first month, 87% were alive at 5 years. Neonatal ventilation requirements were a strong negative predictor of long term survival.

Roy et al. (1997), in their cohort of 52 neonatal survivors with ARPKD, reported actuarial renal survival rates of 86% at one year and 67% at 15 years of age, taking the end point as glomerular filtration rate < 10ml/min/1.73m^2, or the start of dialysis or renal transplant, or death due to end stage renal failure. Bergmann et al. (2005) found a comparable renal survival rate of 66% at 15 years.

1.2.1.6 Gender differences

Zerres et al. (1996) found that girls had significantly more severe disease with respect to frequency of urinary tract infections (UTI), growth retardation and renal function, and a higher mortality rate than boys with ARPKD. The survival probability at one year was 94% for male patients and 82% for female patients. Aside from an increased frequency of UTIs in girls, no gender differences were detected in the study by Guay-Woodford and Desmond (2003).

1.2.1.7 Sibships

Kaplan et al. (1989a) reported sibships in six out of eight families as discordant for age and mode of onset while Kaariainen (1987) noted consistency among siblings with perinatal diagnosis, but more variability among other families. Using the now outdated classification of Blyth and Ockenden (1971), dividing ARPKD into perinatal, neonatal, infantile and juvenile forms, 11 out of 20 sibships studied by Deget et al. (1995) showed different subtypes among affected siblings. In seven families the subtypes were adjacent while only four families showed major intrafamilial differences in onset of disease. Eight families however were markedly discordant for age at death, suggesting that longevity can often not be predicted from the course of an affected sibling. In contrast to the proposals of Blythe and Ockenden (1971) however, this study supported the argument for allelic rather than locus heterogeneity. More recently, Bergmann et al. (2005) reported marked intrafamilial variability in phenotype in 20 of 48 pedigrees with more than one affected child (neonatal death in one and survival into childhood in another sibling).

1.2.2 Pathoanatomical features

Renal involvement in ARPKD is bilateral and mainly symmetrical, although the gross appearance varies with age and extent of involvement. In the neonate the kidneys are large (up to 10 times normal weight) but are reniform (Blyth and Ockenden 1971). Cortical extension of fusiform collecting ducts is seen on cut section, and the corticomedullary junction is obscured. Microscopically, the cysts are of collecting duct origin, with proximal nephron segments being structurally normal. The renal pathoanatomical features are shown in Figure 1.2. Dysplastic elements are not seen (Guay-Woodford 1996). In patients surviving beyond the neonatal

period, increase in renal size and cystic involvement is less extensive, but cysts may expand to up to 2cm in size, and are more spherical (Blyth and Ockenden 1971). Interstitial fibrosis becomes apparent and kidney size can decrease.

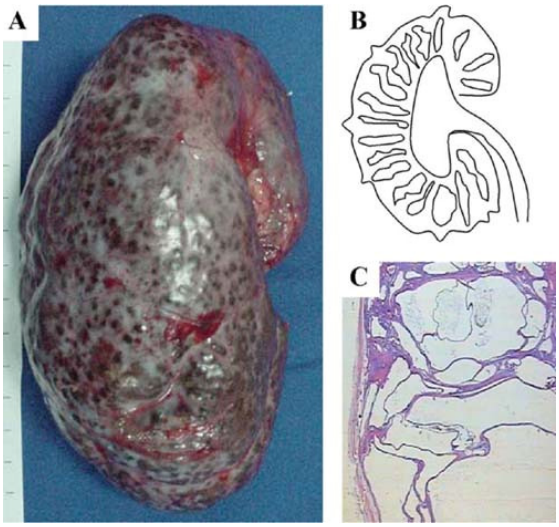


Figure 1.2: Renal pathology of autosomal recessive polycystic kidney disease.

A, Kidney of a 2-year-old ARPKD-affected child. Macroscopically, the organ displays multiple small cysts on its surface and is severely enlarged, measuring ~15 cm in its longitudinal axis (Gift of Dr. Francisco Tibor Dénes). B, Drawing representing the diffuse and radial distribution of dilated collecting ducts throughout the cortex and medulla. C, ARPKD renal histology, showing dilation of collecting ducts. Image reproduced from Menezes and Onuchic (2006)

In ARPKD the liver size may be normal or increased, with a firm texture. Cut section reveals fibrous septa often linking the portal tracts. Microscopically, the bile ducts are increased in number and there is a varying degree of portal tract fibrosis, small portal vein hypoplasia and hepatic arteriolar prominence. Hepatocytes are normal, and gross cysts are unusual, although bile ductules are often dilated (reviewed in Guay-Woodford 1996). There are reports of frank

cystic dilatation of the entire biliary tree in ARPKD, a condition usually referred to as Caroli's disease (Guay-Woodford 1996).

1.2.3 Differential Diagnosis

Autosomal dominant polycystic kidney disease (ADPKD) is the most important differential diagnosis, and normal parental renal ultrasounds can virtually exclude this diagnosis (Zerres et al. 1998a). Glomerulocystic kidney disease (GCKD) and diffuse cystic dysplasia fall into the complex group of diffuse cystic renal diseases along with ARPKD and ADPKD. They often present in early life and are variably associated with biliary dysgenesis, but are distinguished on sonography, family history and histopathological study (Guay-Woodford et al. 1998). Other conditions that require consideration include tuberous sclerosis, juvenile nephronophthisis, nephroblastomatosis, bilateral renal vein thrombosis, radiocontrast nephropathy and renal involvement in conditions like Meckel-Gruber or Bardet-Biedl syndrome (Zerres et al. 1998a). Congenital hepatic fibrosis (CHF) can be associated with several of the above as well as Jeune, short rib polydactyly and Ivemark syndromes (Zerres et al. 1984), and has been occasionally observed in ADPKD. Congenital hepatic fibrosis may also be seen with Caroli's disease (also seen in ARPKD) and choledochal cysts.

1.2.4 Prenatal diagnosis

Definitive prenatal diagnosis by fetal ultrasound is not reliable (Zerres et al. 1998a). Ultrasonographic features include increased echogenicity and bilateral symmetrical renal enlargement with difficulty in visualization of the fetal bladder. Oligohydramnios may not be present, and renal enlargement may not be detected until the second half of pregnancy (Roume and Ville 2004).

Haplotype-based prenatal analysis for pregnancies at-risk for ARPKD was shown to be reliable in all 65 cases assessed by Zerres et al. (1998a), with the proviso of an accurate clinico-pathologic diagnosis in a previous sibling, and exclusion of cystic renal disease in both parents. Direct mutation analysis of the *PKHD1* gene (discussed further below) is a newer option for prenatal diagnosis, with the first cases described by Zerres et al. (2004), in families where prenatal diagnosis by means of linkage analysis was not possible.

1.2.5 Management

Initial management of severely affected infants requires stabilization of respiratory function which is compromised by both pulmonary hypoplasia and abdominal distention due to nephromegaly. Nephrectomy may be considered, with some potential benefit (MacRae Dell et al. 2001). Renal function must be closely monitored, particularly with regard to the urine concentrating defects and associated hyponatraemia. These patients are at risk of significant dehydration with intercurrent illness (Guay-Woodford 1996).

Hypertension is usually managed with angiotensin-converting enzyme (ACE) inhibitors, but may require several medications (Cole et al. 1987). Growth should be optimized with aggressive nutrition, and may require nasogastric or gastrostomy tube feeding. Urinary tract infections should be treated and other tract abnormalities excluded.

The treatment of chronic renal failure (CRF) in paediatric patients is detailed and discussion thereof is too extensive for this text, but in general renal replacement therapy (RRT) in the

form of dialysis (peritoneal or haemodialysis) or renal transplant is indicated when children become symptomatic or growth failure does not respond to medical management (which may include recombinant growth hormone) (Guay-Woodford 1996). Specific to ARPKD, pretransplant splenectomy may be indicated if hypersplenism is severe, and nephrectomy may be required if the kidneys are massively enlarged.

Close monitoring for the complications of portal hypertension is necessary, with melena or haematemesis suggesting the presence of oesophageal varices requiring sclerotherapy or variceal banding. Serial ultrasound and Doppler flow studies can be used to monitor the portal hypertension. Patients must also be monitored for the development of hypersplenism (Guay-Woodford 1996). Portocaval shunting and even liver transplantation are therapeutic options. Cholangitis can be a difficult problem and must be treated aggressively, with antibiotics.

The psychosocial stresses related to this condition, on both the child and the family, must not be underestimated, and should be monitored and managed appropriately. Ultimately management requires a multidisciplinary team which includes the paediatrician, paediatric nephrologist, hepatologist, specialized nurses, dieticians, genetic counsellor and psychologist or social worker.

1.3 MOLECULAR GENETICS OF ARPKD

1.3.1 PKHD1

The ARPKD locus was localized to chromosome region 6p21-cen by genetic linkage analysis in 16 families in 1994 (Zerres et al. 1994). This localization was confirmed and refined to a

3.8cM interval in families with the severe perinatal form of ARPKD (Guay-Woodford et al. 1995). Zerres et al. (1998a) found no evidence for locus heterogeneity in this condition. Further refinement of the locus and exclusion of a number of candidate genes followed (reviewed in Harris and Rossetti 2004). Ward et al. (2002), using a newly described rat model (the PCK rat), then identified the human orthologue of *Pck*, *PKHD1* within the ARPKD candidate region. Parallel to this, using a positional cloning approach utilizing ARPKD patients, Onuchic et al. (2002) independently identified and cloned the *PKHD1* gene. These studies have provided the basis for direct genotyping in ARPKD.

The *PKHD1* gene is an extremely large gene, extending over about 472kb, with the longest open reading frame comprising 67 exons and encoding a protein of 4074 amino acids (Onuchic et al. 2002 and Ward et al. 2002). Expression studies by these groups suggested that *PKHD1* and its murine analogue undergo complex alternative splicing patterns, and showed high levels of expression of *PKHD1* in adult and fetal kidney, and lower levels in pancreas and liver.

The predicted protein (fibrocystin/polyductin) represents a putative integral membrane protein with an as yet unknown function, localized to the primary cilia and basal body (Wang et al. 2004).The conserved domains suggest a role as a receptor in collecting duct and biliary differentiation (Ward et al. 2002).

1.3.2 Spectrum of Mutations

A number of comprehensive mutation screens have been performed (Bergmann et al. 2003, Furu et al. 2003, Rossetti et al. 2003) and to date, more than 270 mutations have been described in the *PKHD1* gene (ARPKD-PKHD1 Mutation Database, Aachen University 2008), the majority of which are private. Detection rates of up to 85% have been described in the more severely affected groups, with lower rates in the more moderate cases, which may indicate inclusion of clinical phenotypes not representing ARPKD (Harris and Rossetti 2004). An explanation of locus heterogeneity is not supported by linkage studies (Zerres et al. 1998b), and the incomplete detection may be explained by intronic mutations and large scale rearrangements not detected by the methods used in the above studies (Harris and Rossetti 2004).

Mutations are spread thoughout the gene with no evidence of clustering at specific sites (Furu et al. 2004), and include missense, nonsense, frameshift insertion or deletion and in frame deletions as well as mutations altering splicing.

Thus, the size and complexity of the *PKHD1* gene in combination with marked allelic heterogeneity and compound heterozygosity makes gene-based molecular diagnostics complicated, and not routinely available.

1.3.2.1 Recurrent mutations

The most commonly reported mutation, c.107C>T (T36M), described in a number of studies, accounts for about 15-20% of mutated alleles (Bergmann et al. 2005). It is unclear whether this is a mutational hotspot or whether it is due to an ancestral change in the Central European population. Evidence seems to indicate that this change is due to a frequent mutational event because the C>T change is at a mutagenic CpG dinucleotide, and there are different associated haplotypes (Bergmann et al. 2005). Consugar et al. (2005) however found a common haplotype in 14 out of 15 alleles with this mutation and estimated an origin for the mutation about 49 generations ago.

Two founder mutations (R496X and V3471G) comprise about 60% of *PKHD1* mutations in the Finnish population (Bergmann et al. 2003), and a specific mutation, 9689delA, is seen in the Spanish population (Rossetti et al. 2003), on a single haplotype. Other mutations found in multiple studies are I222V, 3761delCCinsG and I2957T, with preliminary haplotype analysis indicating the changes to be the result of ancestral mutations (Rossetti et al. 2003).

1.3.3 Genotype-Phenotype Correlations

Bergmann et al. (2003) drew preliminary genotype-phenotype correlations for the type of mutation present. All patients with two truncating mutations demised early (within the neonatal period), indicating that longer term survival requires the presence of at least one missense mutation. Other studies by Furu et al. (2003), Rossetti et al. (2003) and Bergmann et al. (2004) confirmed this finding. Bergmann et al. (2005), in the first study reporting long-term outcome of ARPKD patients with defined *PKHD1* mutations, reported a series of 164 neonatal

survivors over a mean observation period of six years, and identified no families with truncating mutations on both parental alleles.

Missense mutations may range in effect from complete loss of function alleles (similar in effect to a truncation mutation), to hypomorphic alleles allowing for a milder clinical course (Bergmann et al. 2005), but efforts to assess the genetic contribution of single missense mutations to the observed phenotype are hampered by the marked allelic heterogeneity at *PKHD1*. However, attempts are being made to categorise individual mutations into severe and moderate or mild changes (Bergmann et al. 2005), comparing the renal phenotypes of individuals carrying the same set of PKHD1 mutations. Homozygotes for the same amino acid substitutions would be most useful in this regard, but such patients are rare and no large groups have been described to date. The group of patients involved in the present study is unique in this regard.

The missense mutation R3482C has been seen in two consanguineous Israel-Arab families with perinatal demise of five affected children, while affected individuals in a family with the T36M mutation in the homozygous state showed intrafamilial phenotypic variability (Bergmann et al. 2003).

1.3.4 Modifier Genes

Recent molecular advances have not answered questions around the factors that modulate gene expression. The variability in clinical phenotype, found even within families, is likely to be

influenced by modifier genes as well as the environment. Although a human homologue has not yet been identified, a modifying gene complex on mouse chromosome 4 is suggested (Mrug et al. 2005). Research in this area is active at the University of Birmingham, Alabama, USA.

1.4 ARPKD IN THE AFRIKAANS SPEAKING POPULATION

1.4.1 Incidence and Carrier Rate

Lombard et al. (1989) investigated 28 families with ARPKD from Pretoria and Johannesburg hospitals, and found 92% of these families to be Afrikaans speaking, indicating that ARPKD was significantly more common in Afrikaans speakers than in the local English speaking community. Based on national statistics for the Transvaal province in 1985, they estimated a point prevalence of 1:26 000 for ARPKD. Using the live birth rate, the birth prevalence of ARPKD was found to be 1:11 000, the gene frequency 0.0096 and the carrier rate 1:53, in this group.

1.4.2 Founder Effect

The first description of ARPKD in South Africa was by Isdale and Thomson (1973) who discussed a series of seven patients at the Transvaal Memorial Hospital for Children, with an emphasis on the major clinical features. A decade later they then reported a group of 26 patients from Afrikaans families with ARPKD (Thomson and Isdale 1984). Lombard et al. (1989) postulated that the reason for the relatively high frequency of ARPKD in the Afrikaans speaking population in the Transvaal was a result of founder effect.

1.4.2.1 A Brief History of the Afrikaners

South African Afrikaans speaking individuals are descendants of relatively small numbers of predominantly Dutch, German and French immigrants who settled in the Cape of Good Hope between 1652 and 1701. The settlement arose after the Dutch East India Company established a refreshment station there (Nurse et al. 1985). From a group of 1265 individuals in 1701, this population grew to 15000 by the turn of the century (Christopher 1976). The majority of this expansion was related to large 18th century sibships with high survival potential compared to those of Europe at the same time. The rapid growth was thus not immigration dependent and the founder population was small (Nurse et al. 1985). It was during this time that the language of Afrikaans developed as a dialect of Dutch, spoken in the Cape.

This population growth resulted in a slow development of pressure for more land, and the Caucasoid population began to spread. Later German and British immigrants mixed very little with the now 'Afrikaners'. The Great Trek represented a later radiation outward from the Eastern frontier of the Cape Colony and involved greater numbers and more rapid movement than the earlier movements. The Afrikaners, threatened by the liberalism of the new British colonial administration, insecure about conflict and 'squeezed out' by their own expanding population, hoped to restore their cultural and political unity, and in the decade following 1835, about 12 000 migrated to the interior, organized into numerous'trek parties' under individual leaders, many of which remained geographically and socially isolated through the

next century. They established the republics of the Transvaal and Orange Free State (South African History Online 2008).

1.4.2.2 'Founder' conditions in the Afrikaners

In the Afrikaners as a founder population, there are a number of dominant and recessive conditions found to be relatively common. Classically described is porphyria variegata, a dominant disease due to a deficiency of protopophyrin oxidase. Through detailed genealogical studies the disease has been traced back to a Dutch couple who arrived at the Cape in 1688 (Dean 1971). This condition is common in the white and mixed ancestry Afrikaans speaking populations and a founder mutation, R59W, has been identified (Meissner et al. 1996).

Other genetic conditions that have been found to be unusually frequent in the Afrikaans speaking population include familial hypercholestrolaemia (FH) (Jenkins et al. 1980), lipoid proteinosis and sclerosteosis (Botha and Beighton 1983).

1.4.3 Exclusion of Linkage to 16p

A study carried out in the Department of Human Genetics, NHLS, Johannesburg, by Ramsay et al. (1988) using 14 individuals affected with ARPKD from 12 South African families, 10 of whom were of Afrikaans origin, and 10 affected individuals from eight British families, excluded linkage of the ARPKD gene to the α-globin gene locus on 16p. The α-globin gene locus had been previously linked to a gene for autosomal dominant polycystic kidney disease (ADPKD) (Reeders et al. 1987), and this gene was subsequently shown to be *PKD1* (OMIM +601313).

1.4.4 Haplotype Studies

Work in the Division of Human Genetics, NHLS and University of the Witwatersrand dating back to 2002 (Professor A Krause, Division of Human Genetics, NHLS and University of the Witwatersrand, personal communication), on DNA collected by Professor P. Thomson (then Head of Paediatric Nephrology) from ARPKD patients at Johannesburg Hospital, used extragenic linked microsatellite markers to confirm the presence of a common haplotype on the majority of disease associated chromosomes. Fifty disease chromosomes were studied, and using six markers closely linked to the *PKHD1* gene, a full founder haplotype was identified on 24 of these chromosomes, with a suggestive founder haplotype on a further nine. Thus, a common mutation was proposed to account for the majority of ARPKD alleles in the Afrikaans population.

1.4.5 Founder Mutation

Sharp et al. (2005) identified a homozygous missense mutation, p.M627K, in two unrelated South African patients of Afrikaner origin. Prior to the present study, DNA from a further 25 unrelated Afrikaner ARPKD patients (most of whom were used in the previous haplotype studies) was sent from the Division of Human Genetics, NHLS and University of the Witwatersrand, to Dr. Lisa M. Guay-Woodford, Division of Genetic and Translational Medicine, University of Alabama, Birmingham, USA for further analysis. Thirteen patients (52%) were found to be homozygous for the p.M627K substitution; five (20%) were heterozygous for p.M627K and a second *PKHD1* mutation (detected by L.Guay-Woodford); and one patient (4%) was heterozygous for the p.M627K missense change but no other

mutation was identified. Three patients (12%) were found to have other mutations elsewhere in *PKHD1* (compound heterozygotes), and in the remaining three patients (12%), no mutations were detected. Therefore, the p.M627 substitution was detected in 32/50 (64%) of the Afrikaner ARPKD alleles tested in that series (Guay-Woodford et al. 2005).

The p.M627K mutation occurs in exon 20 of the *PKHD1* gene, at nucleotide 1880. It is a missense mutation changing a thymidine to an adenine residue (T>A) and resulting in a peptide sequence change from methionine (M) to lysine (K) at position 627. Methionine is a polar, uncharged amino acid while lysine is positively charged, and the pathogenic consequence, on this basis, is likely to be significant. Conservation of the methionine amino acid at position 627 of the *PKHD1* protein can be demonstrated across species, further supporting the likelihood that a change in this position would alter the protein function. The potential functional protein change predicted by the mutation cannot at this stage be assessed, since position 357 to 728 of the *PKHD1* gene is a poorly characterised region.

1.5 STUDY OBJECTIVES

ARPKD is a relatively rare condition, and as such the number of families expected to be available for study is small, and then only about 1/3 are expected to be currently in contact with nephrology centres in Gauteng. The Nephrology divisions of Johannesburg and Pretoria Academic Hospital represent the only specialist centres for the care of children with renal disease in the Gauteng province. It is expected therefore, that the majority of children with chronic renal disease will have had contact with one of these centres during the course of their

illness owing to the need for specialist care, and the paucity of paediatric nephrologists in the private sector.

It is the writer's impression (supported by Lombard et al. 1989) that the majority of children assessed for ARPKD in these centres and referred for genetic assessment are of Afrikaans origin. Based on this perception, previous haplotype studies, and the recent confirmation of a disease causing founder mutation in the majority of Afrikaans patients with ARPKD, the aims and objectives of this study can be summarized as follows:

- To determine the frequency of the founder mutation in those Afrikaans patients shown to be clinically affected with ARPKD.
- To describe the clinical phenotype of Afrikaans patients with ARPKD, particularly those shown to be homozygous for the founder mutation.
- To compare the clinical phenotype of the local genetically homogenous group (homozygotes for the founder mutation), with published clinical data from genetically heterogenous groups, known to have a spectrum of mutations, in an attempt to understand the influence of genotype on the presentation and natural history of ARPKD, and indirectly the influence of environment and genetic modifiers on this condition.
- To assess the phenotypic variability of affected sibships within this cohort of homozygous patients.

1.6 LIMITATIONS OF THE STUDY

This study recruited patients that were referred to the Paediatric Nephrology Divisions at Johannesburg and Pretoria Academic Hospitals. Patients born in other centres and those not surviving the neonatal period, may not have been referred, and thus the number of severely clinically affected patients is likely to be underrepresented in this study. In addition, the private health care sector may be expected to manage a few of the ARPKD patients, but the paucity of paediatric nephrologists in this sector, makes this a minor limitation, as few patients would have been excluded for this reason.

This study involved collection of predominantly retrospective data from clinical records where multiple physicians, including paediatric registrars, may have been involved in patient care, and there may thus be inaccuracies in the data relying on clinical examination. Patients born in the 1970's and 1980's may not have had all the diagnostic evaluations available to patients born more recently. Many families of patients who had died or were lost to follow up could not be contacted to verify the extent of their Afrikaans ancestry, and thus assumptions were made based on the language spoken and the surname as to their ancestry.

In terms of the long term follow up of patients surviving into the third decade, many patients were lost to follow-up as they received further care in adult centres and in the private sector, where adult services are more widespread than those for paediatric patients.

1.7 UNIQUENESS OF THE STUDY

The majority of previous studies have focused either on the clinical aspects of cohorts of patients with ARPKD (Kaplan et al. 1989a, Deget et al. 1995, Zerres et al. 1996, Roy et al. 1997, Capsidona et al. 2003, Guay-Woodford and Desmond 2003), without describing mutational data, or on describing the molecular aspects and mutational spectrum of this condition (Bergmann et al. 2003, Rossetti et al. 2003, Bergmann et al 2004, Losekoot et al. 2005, Sharp et al. 2005). While attempts have recently been made to combine the clinical and molecular data (Furu et al. 2003, Bergmann et al. 2005, Adeva et al. 2006), the level of allelic heterogeneity and rate of compound heterozygosity has limited genotype-phenotype correlations. The subset of patients in this study is distinct in its homogeneity: the patients are homozygous for a common mutation arising on a common haplotype, due to founder effect. They thus represent a unique group providing an opportunity to describe the variability in presentation and natural history of ARPKD in these patients, and in the future, to study the modifiers that may account for the variability.

2 SUBJECTS AND METHODS

2.1 PATIENT ASCERTAINMENT

This descriptive study used retrospective data collected from records of patients diagnosed with ARPKD. Using the diagnostic coding schedules of the Nephrology Units at Johannesburg and Pretoria Academic Hospitals, as well as of the Genetic Counselling Unit and Molecular Genetics Laboratory of the Division of Human Genetics, NHLS and University of the Witwatersrand, a list of potential patients was compiled.

Patients who were in current contact with either the Nephrology Units or the Division of Human Genetics were contacted, and consent (and assent where applicable) was obtained for further record review as well as for the taking of blood samples from the affected family member/s. For patients who had died or had been lost to follow-up, record review was conducted and it was ascertained whether blood had previously been collected (during the mid 1990s) by Professor Peter Thomson, the then head of Paediatric Nephrology at Johannesburg Hospital. A number of the patients included in this study were part of the study by Lombard et al. (1989).

A conclusive diagnosis, for inclusion into the study, required ultrasound evidence of enlarged echogenic kidneys or compatible renal histopathology, and at least one of the following criteria:

a. Biopsy proven ARPKD in a sibling

b. Biliary fibrosis, based on clinical or histopathologic evidence

c. Sonographic evidence of the absence of renal cysts in the parents

d. Parental consanguinity

Of the 76 files reviewed, for which clinical information was available, 10 patients were excluded as they did not meet the above criteria. Of those patients (66) who met the criteria, 58 (88%) were considered to be Afrikaans.

2.2 DATA COLLECTION

Data were collected using the Pediatric Polycystic Kidney Disease Registry Primary Data Form, developed by Professor L Guay-Woodford (Guay-Woodford and Desmond 2003) (see Appendix A), with some modification, particularly with regard to recording the ethnic group of the patients. Caucasian patients were sub-classified as Afrikaans with both parents being Afrikaans; Afrikaans with one parent Afrikaans and the other parent uncertain/other Caucasian; or other Caucasian. Where possible, Afrikaans ancestry was confirmed at the time of taking consent. Otherwise, patients who were Afrikaans speaking or had an Afrikaans surname, were assumed to have Afrikaans origins.

The data collection form was completed using data from as many of the sources (hospital clinical file, genetic counselling file and molecular file) as were available for each individual. All the data were collected by the writer and entered manually onto the data collection form at the time of collection. For patients still in contact with the Nephrology or Genetics Units, a brief interview (based on the Primary Data Form) was conducted when consent was obtained, to supplement the data from the files. The parents were also offered further information and genetic counselling at the time of taking consent. In addition, the parents were asked to

indicate if they would like to be contacted if a specific mutation was identified in their child, for the purposes of further genetic counselling and possible carrier testing of other family members. Consent was obtained and blood was taken in the clinic setting (at either Johannesburg or Pretoria Academic Hospitals) at one of the patient's regular clinic visits, and where possible, blood was taken at the time of other routine blood sampling, to avoid additional discomfort.

For the purposes of confidentiality, all patients were assigned a numerical code, and for each patient for whom blood was available, or from whom a sample was collected as part of the study, this code was linked to a Molecular Genetics Laboratory assigned code which was family specific. Only the writer and her supervisors were able to view the originating patient name. Professor P Thomson also had access to patient names where clinical data required clarification.

Data gathered included the dates of birth, diagnosis, last contact and cause and date of death, where applicable. A history of affected siblings, parental consanguinity and prenatal diagnosis was obtained, as well as details regarding the neonatal period. Presence and dates of onset of renal and hepatic ARPKD related morbidities were recorded, as were results of initial radiologic studies and histopathology reports. Information relating to interventions, particularly with regard to renal replacement therapies and treatment of the complications of portal hypertension, was collected.

2.3 MOLECULAR DATA

Simultaneously with the clinical data collection, existing DNA samples, and those collected during the course of the study, from Afrikaans patients with ARPKD, were tested in the Molecular Genetics Laboratory of the Division of Human Genetics, NHLS and the University of the Witwatersrand for the presence, in the homozygous or heterozygous form, of the founder mutation, p.M627K. These data were then linked to the patients for whom clinical data had been collected.

Only those Afrikaans patients who met the diagnostic criteria and who were found to be homozygous for the founder mutation, were selected for further analysis (27 patients from 24 families).

2.4 STATISTICAL ANALYSIS

Data were transferred into a Microsoft Excel spreadsheet for initial analysis. Means and frequencies relating to the data were calculated in Excel. Further analysis of the Afrikaans patients who were homozygous for the founder mutation was conducted using the SAS (Statistical Analysis Software) statistical package version 9.0 with the assistance of a statistician, Mr Eustacius Musenge of the Epidemiology Data Centre of the University of the Witwatersrand.

Survival times were calculated using the Lifetest Procedure, from the date of birth to date of death or date of last contact, for those who were lost to follow-up. Renal survival times were calculated from the date of birth to the date of initiation of dialysis or renal transplant,

whichever occurred first. The Means Procedure was used to calculate median and quartile ranges for the age of diagnosis of co-morbidities, and associations and correlations between co-morbidities were conducted using a two-tailed Fisher's exact test and Pearson correlation coefficients, respectively. For all analyses a p value of <0.05 was deemed significant.

2.5 ETHICS APPROVAL

Ethics approval for this study was obtained from the Committee for Research on Human Subjects (Medical) of the University of the Witwatersrand, certificate number R14/49 Lambie dated 26 March 2004 (expiry date 27 February 2009), as well as from the Faculty of Health Sciences Research Ethics Committee of the University of Pretoria, certificate number 00002567 (dated 18 August 2004) (see Appendix C).

3 RESULTS

3.1 SUBJECTS

The files of 76 patients with a possible diagnosis of ARPKD were reviewed. Ten of these patients were excluded as insufficient data were found in the patient's file to enable the researcher to assess whether they met the inclusion criteria, or the diagnosis was incorrect. Of the 66 patients who met the inclusion criteria, 58 were thought to be Afrikaans (on the basis of their first language and surnames). Molecular data were available for 39 of the 58 Afrikaans individuals, from 36 families. Of the 39 individuals, 27 patients from 24 families (one family with data from two affected siblings and one family with data from three affected siblings), were homozygous for the founder mutation (p.M627K). Ten patients were compound heterozygotes for the p.M627K mutation and another mutation. The second mutation was identified in five of these patients (five different mutations), by Professor L Guay-Woodford (University of Birmingham, Alabama, USA) as part of a larger sequencing study (unpublished data), not discussed here, while the second mutation remains unidentified in the other five. Only two of the 39 Afrikaans patients did not carry the p.M627K mutation on either chromosome. These findings are summarized in Figure 3.1. Of the 72 alleles tested from 36 unrelated individuals, 58 (81%) were found to carry the p.M627K mutation.

The 27 patients that were homozygous for the founder mutation formed the subject group of this study.

Of the eight patients who were not Afrikaans, three (from two families) were Indian, three (from two families) were of German descent, one was Portuguese and one was of unspecified Caucasian descent. There were no Black African patients identified in this series.

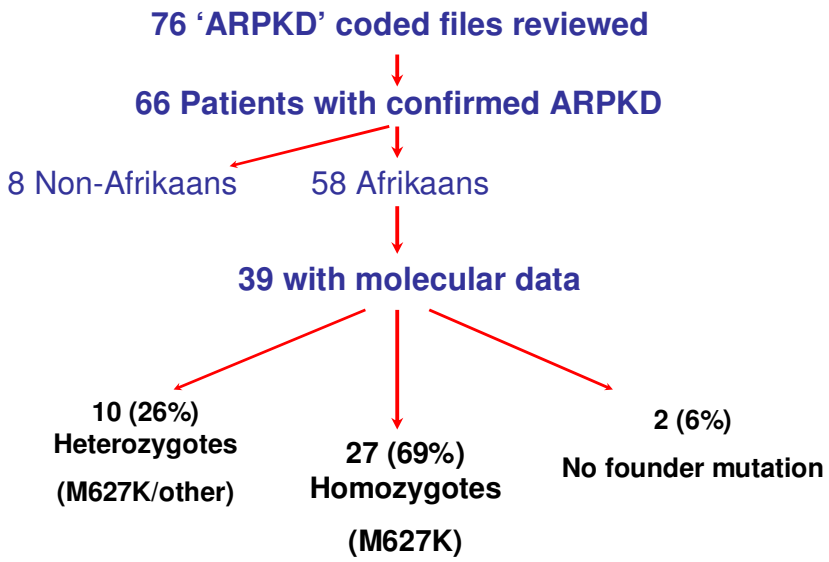


Figure 3.1: Subject ascertainment and classification

3.2 PREVALENCE OF ARPKD

The writer was unable to calculate the birth prevalence of ARPKD in the Afrikaners in the Gauteng province because of lack of availability of data on births by population group. The point prevalence in Gauteng, the province in which all the patients are or were resident was calculated using 2001 censorship data (Statistics South Africa 2008). Of the 746 761 Caucasians in Gauteng, 452 624 (57%) were Afrikaans. In 2001 there were 29 known patients with ARPKD living in Gauteng. The point prevalence was thus calculated to be 1 in 14 678. At almost 1 in 15 000 this is a slightly higher estimate than the 1989 point prevalence estimate

of 1 in 26 000 for the then Transvaal province (Lombard et al. 1989). Since the point prevalence is significantly lower than the incidence, a carrier frequency of 1 in 60, derived from the point prevalence, must be an underestimate. Based on the assumption of a 30-50% neonatal mortality, the birth prevalence could be between 1 in 7339 and 1 in 10275, and from this a rough estimate of the carrier frequency of between 1 in 43 and 1 in 51 is calculated.

3.3 COHORT CHARACTERISTICS

3.3.1 File review

The cohort for further analysis comprised 27 Afrikaans subjects (from 24 families) who were homozygous for the p.M627K mutation. All the subjects in the subset whose data were analysed had molecular files in the Molecular Laboratory of the Division of Human Genetics, NHLS, Johannesburg. Various combinations of clinical files from Johannesburg Hospital, Pretoria Academic Hospital and the Genetic Counselling Unit at the Division of Human Genetics, NHLS were also reviewed (Figure 3.2). Only five (20%) patients had previously been seen by the Genetic Counselling Unit.

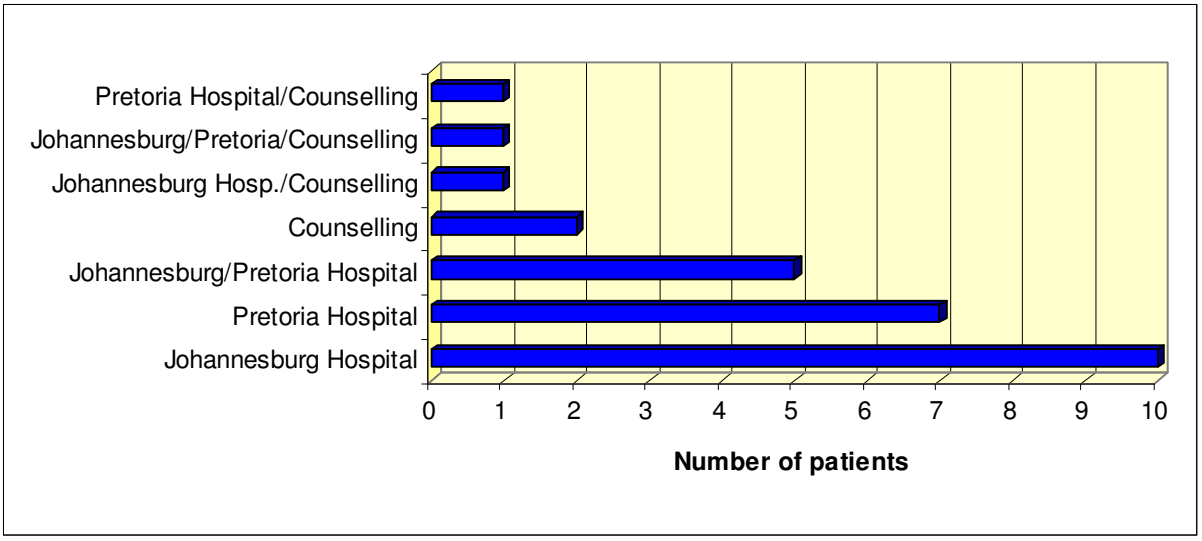


Figure 3.2: Source of clinical files reviewed

3.3.2 Characteristics of the subjects

The subjects were born between 1969 and 2002, and the record review produced a mean observation period (from date of diagnosis to date of death or last contact) of 14.04 years (SD 8.36 years) and a range of one day to 28.97 years. Figure 3.3 indicates the status of the subjects at the time of record review.

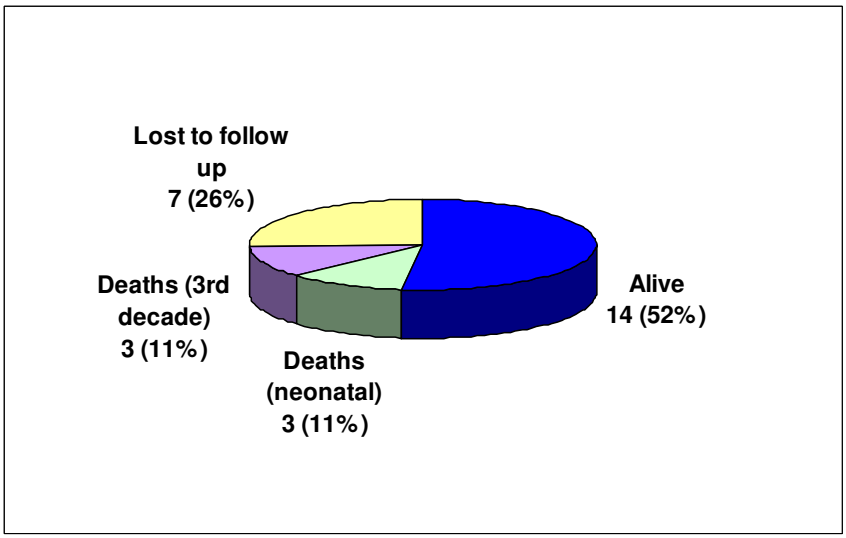


Figure 3.3: Patient Status at the time of record review

Eleven of the 24 families were in current contact with the nephrology units. For the remaining 13 families the record review was entirely retrospective, and existing DNA was used for mutation testing.

The mean age of the subjects at the time of last contact with the clinic (death or last visit to the clinic) was 14.37 years (SD 8.39 years) with a range of one day to 29.69 years.

Altogether, 15 (55.6%) of the patients were male and 12 (44.4%) were female, giving a male:female ratio of 1.25. All the patients were Afrikaans speaking and had both parents who were of Afrikaans origin. There was no history of consanguinity in any of the families, although in one, this information was not specifically available.

3.3.3 Family History

There was a family history of more than one child affected with ARPKD in four families. Clinical information was available for one sibling pair (who also had a third affected sibling on whom there was no information), and a set of three siblings. The other two families had two affected siblings, but clinical information was only available for one of the siblings. In one of these families there was an early demise, and in the other the Johannesburg Hospital file could not be located.

3.4 CLINICAL FEATURES

The clinical features of the 27 subjects are detailed in Table 3.1 and summarized in Figure 3.4.

Table 3.1 Clinical features of the 27 homozygous Afrikaans subjects with ARPKD

	Sex	Status	Age at diagnosis	Age of last contact	Affected sibling	Ventilation	Hypo-natraemia	Hypertension Age of dx	CRI Age of dx	UTI	PHT Age of dx	Variceal bleeding Age of dx	Hyper splenism Age of dx	Growth retardation	Dialysis Age of start	Variceal banding Age	Splenic shunt Age	Emboli-sation Age	Splenec-tomy Age	1st Renal graft Age	2nd Renal graft Age
1	M	Alive	41 days	7.7 years	No	No	Yes	54 days	54 days	No	54 days	54 days	54 days	No							
2	M	Unknown	170 days	29.4 years	Yes	No	No	10.3 years	10.3 years	No	10.3 years	10.3 years	10.3 years	Yes			14.0 years			15.7 years	15.7 years
3	M	Unknown	1.4 years	29.7 years	Yes	No	No	9 years	9 years	Yes	9 years	9 years	9 years	Yes						18.7 years	18.7 years
4	F	Unknown	48 days	27.3 years	No	No	No	1 year	1 year	Yes	1 year	1 year	1 year	No						15.8 years	15.8 years
5	F	Alive	1 day	4.6 years	No	Yes	Yes	1 day	1 day	Yes	1 day	1 day	1 day	Yes							
6	F	Unknown	46 days	17.7 years	No	No	No	0.5 years	0.5 years	Yes	0.5 years	0.5 years	0.5 years	No						17.8 years	17.8 years
7	F	Alive	27 days	13.2 years	Yes	Yes	Yes	1 day	1 day	No	1 day	1 day	1 day	No				6.1 years		6.2 years	6.2 years
8	M	Unknown	3 days	17.7 years	No	Yes	No	3 days	3 days	Yes	3 days	3 days	3 days	Yes	13.9 years			14.4 years	17.4 years	14.1 years	14.1 years
9	F	Unknown	1.1 years	16.5 years	Yes	Unknown	No	2.5 years	2.5 years	Yes	2.5 years	2.5 years	2.5 years	Yes	11.2 years		11.3 years		11.3 years	11.5 years	11.5 years
10	M	Dead	1 day	1 day	No	Yes	No			No				No							
11	M	Alive	2 days	15.3 years	No	No	No	1.1 years	1.1 years	Yes	1.1 years	1.1 years	1.1 years	Yes	13.6 years					13.5 years	13.5 years
12	F	Alive	0.9 years	9.8 years	No	No	No	1 year	1 year	Yes	1 year	1 year	1 year	No							
13	F	Alive	3.4 years	10.1 years	No	No	No	3.4 years	3.4 years	Yes	3.4 years	3.4 years	3.4 years	Yes					8.1 years		
14	M	Dead	1 day	1 day	No	No	No			No				No							
15	F	Alive	89 days	11.7 years	No	No	Yes	89 days	89 days	Yes	89 days	89 days	89 days	No							
16	M	Alive	29 days	11.8 years	No	No	No	104 days	104 days	No	104 days	104 days	104 days	Yes	11.8 years						
17	M	Dead	5 days	24.1 years	No	No	Yes	5 days	5 days	No	5 days	5 days	5 days	Yes	19.5 years		14.0 years			14.6 years	14.6 years
18	F	Alive	5 days	15.7 years	No	No	Yes	26 days	26 days	Yes	26 days	26 days	26 days	Yes		8.0 years					
19	M	Alive	143 days	13.9 years	No	No	No	117 days	117 days	No	117 days	117 days	117 days	No							
20	F	Dead	1 day	24.8 years	No	No	No	1.9 years	1.9 years	Yes	1.9 years	1.9 years	1.9 years	No	11.9 years		5.3 years			9.4 years	9.4 years
21	F	Dead	71 days	21.6 years	No	No	No	127 days	127 days	Yes	127 days	127 days	127 days	Yes			9.9 years			10.7 years	10.7 years
22	M	Alive	16 days	14.3 years	No	Yes	No	104 days	104 days	Yes	104 days	104 days	104 days	Yes						15.5 years	15.5 years
23	M	Alive	44 days	20.0 years	No	No	No	44 days	44 days	Yes	44 days	44 days	44 days	Yes						16.9 years	16.9 years
24	M	Unknown	1 day	12.0 years	No	Yes	Yes	1 day	1 day	Yes	1 day	1 day	1 day	Yes				7.2 years			
25	M	Dead	1 day	2 days	Yes	Yes	No	1 day	1 day	No	1 day	1 day	1 day	No							
26	M	Alive	2 days	12.5 years	Yes	No	Yes	1 day	1 day	Yes	1 day	1 day	1 day	Yes							
27	F	Alive	2 days	6.4 years	Yes	No	Yes	2 days	2 days	Yes	2 days	2 days	2 days	Yes							

3.4.1 Age of diagnosis

The age of diagnosis of ARPKD ranged from one day to 3.4 years with a mean of three months, but a median of 27 days, since half the subjects were diagnosed in the first month of life, and 12/27 (44.4%) in the first week. In only two subjects was the diagnosis suspected prenatally on fetal ultrasound. These subjects were siblings, their first sibling having died at two days of age of ARPKD.

3.4.2 Neonatal Characteristics

Seven subjects (7/27, 26%) required neonatal ventilation, of which two died in the first week of life, and one developed chronic lung disease. Hyponatraemia (defined as a serum sodium <130mmol/l) developed in nine subjects (9/27, 33%) at a median age of 17 days, with a range of one to 137 days.

3.4.3 Renal related morbidities

3.4.3.1 Hypertension

Hypertension was defined as the date of initiation of anti-hypertensive therapy. Hypertension was diagnosed in 25/27 subjects (92.6%). The two subjects who were not diagnosed with hypertension died on the first day of life, thus all those surviving the first day required anti-hypertensive therapy. The age of diagnosis of hypertension ranged from one day to 10.3 years, with a median of 122 days. All patients remained on anti-hypertensive therapy until demise or loss to follow-up, although the dosages and number of medications required for control were not analysed in this study to assess if the severity of the hypertension declined with age.

3.4.3.2 Chronic renal insufficiency (CRI)

CRI was defined as a glomerular filtration rate (GFR) of <75% of normal, adjusted for age, based on the estimated method of Schwartz et al. (1976). CRI was diagnosed in 25/27 subjects (92.6%). The same two patients, who died within the first 24 hours of life and were not diagnosed with hypertension, were not diagnosed with CRI either. The age range of diagnosis of CRI was the same as that for hypertension but the median age of diagnosis was 1.6 years.

3.4.3.3 Urinary tract infections (UTIs)

UTIs were diagnosed in 18/27 subjects (66.7%), with a range of presentation of first infections from 27 days to 17.1 years. Fourteen of the initial infections were documented as a cystitis, and the remaining four involved the upper urinary tract (pyelonephritis). All the pyelonephritides and 10 of the cystitis infections were culture proven. Thus 52% of subjects had had a culture proven UTI.

3.4.4 Hepatic morbidities

3.4.4.1 Portal hypertension (PHT)

PHT was diagnosed in 22/27 subjects (81.5%). The diagnosis was based on the presence of a persistent palpable splenomegaly of >2cm below the costal margin. The mean age of diagnosis was 2.8 years (SD 3.4 years), with the earliest at 40 days, and the latest at 11.2 years. Of the five subjects in whom splenomegaly was not detected, three died in the neonatal period, one was 17.7 years at loss to follow up and one was 13.9 years at last contact.

3.4.4.2 Variceal bleeding

Variceal bleeding was only documented in three subjects, at 5, 8.1 and 10 years respectively.

3.4.4.3 Hypersplenism

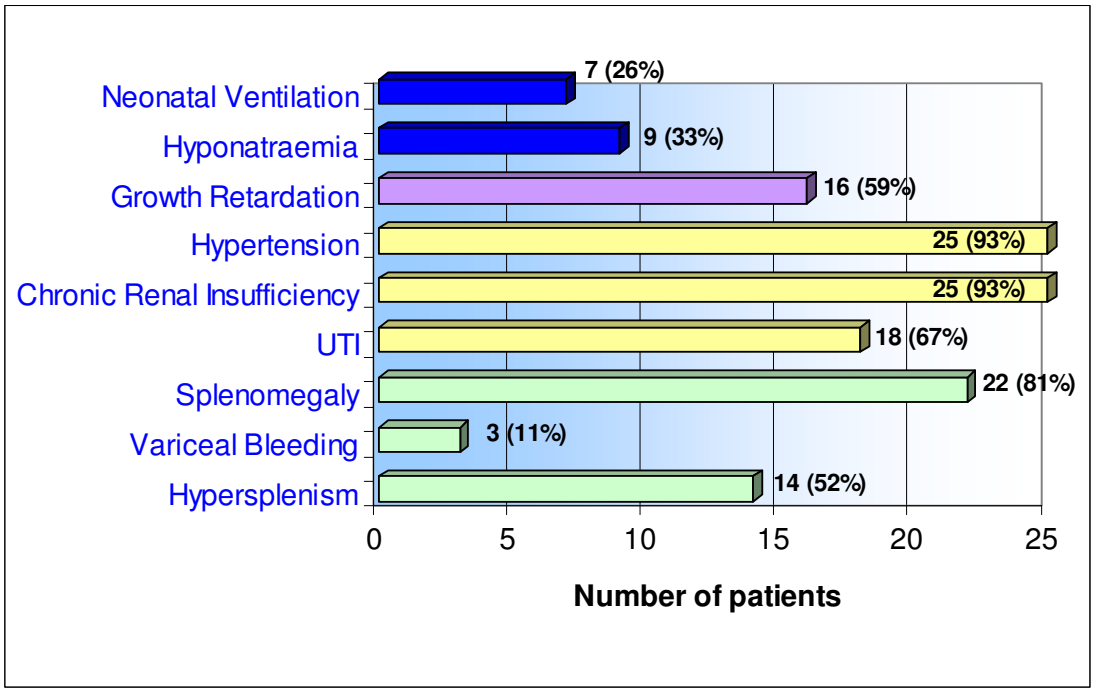
Hypersplenism, defined as a platelet count persistently <140x10^9/l, was found in 14/27 subjects (51.9%) at a mean age of 6.7 years (SD 4.3 years, with a range of 1.8 - 15.9 years).

3.4.4.4 Cholangitis

Cholangitis was only diagnosed in one subject.

3.4.5 Growth

Sixteen subjects (16/27, 59.3%) showed growth retardation (defined as a height for age <3rd centile on the CDC 2000 charts) (National Center for Health Statistics 2008). All of these patients had hypertension and CRI, and 10 (63%) required renal replacement therapy (RRT) (dialysis or renal transplant). Excluding the three patients who died in the neonatal period, of those eight patients who were not growth retarded, four required RRT.



Key: Neonatal morbidities

Growth

Renal morbidities

Hepatic morbidities

Figure 3.4: Summary of the clinical features of patients with ARPKD

3.4.6 Gender differences

As illustrated in Table 3.2, no significant differences were detected between males and females in terms of the various morbidities, except for UTIs which were found to be significantly more common in females.

Table 3.2: Morbidity in males and females with ARPKD

MORBIDITY	*MALES (n=15)*	*FEMALES (n=12)*	*P-VALUE**
Hyponatraemia	**4**	**5**	**0.4479**
Growth retardation	**10**	**6**	**0.4517**
Hypertension	**13**	**12**	**0.4872**
CRI	**13**	**12**	**0.4872**
UTI	**7**	**11**	**0.0192**
Portal hypertension	**11**	**11**	**0.3419**
Hypersplenism	**8**	**6**	**1.0000**

* Fisher's exact test for small samples (two-tailed)

3.5 CORRELATION AMONG ARPKD COMORBIDITIES

The writer investigated the correlations between renal (CRI and hypertension) and hepatic (splenomegaly) morbidities by age of diagnosis. Persistent splenomegaly was used as an indicator of raised portal venous pressure. Statistically highly significant correlations were noted overall and in the male subgroup between CRI and hypertension, between CRI and splenomegaly, and between hypertension and splenomegaly . Despite this, none of these correlations were statistically significant in the female subgroup (see Table 3.3).

Table 3.3: Correlation between renal (CRI and hypertension) and hepatic (splenomegaly) comorbidities

	CRI	*Hypertension*
CRI		0.68 (p=0.0002)
Male (13)		0.85 (p=0.0002)
Female (12)		0.15 (p=0.65)
Splenomegaly	0.71 (p=0.0003)	0.79 (p<0.0001)
Male (11)	0.93 (p<0.0001)	0.94(p<0001)
Female (11)	0.097 (p=0.78)	0.16 (p=0.64)

3.6 IMAGING FINDINGS

All except one subject, who died at 24 hours of age and whose diagnosis was made histopathologically, had renal imaging performed. This was done at a median age of 75 days (range one day to 11.5 years). Twenty-two of the 26 subjects had renal sonography performed, of which nine were reported to have echogenic kidneys without gross cysts, two had echogenic kidneys with gross cysts visible, and one had an incomplete record, reported as "in keeping with ARPKD". Of those patients who had initial imaging, but not sonography, three had intravenous pyelograms (IVPs) and one had computerized tomography (CT) of the abdomen, and all were reported as consistent with ARPKD.

Liver imaging was performed in 18 subjects and at a median age of 1.1 years (range 1 day to 9.4 years). Sixteen subjects had sonography performed, of which nine cases were reported as normal, six as "echogenic" and one reported visible 'cysts'. Two subjects had CT scans in

which the radiologist commented on dilated bile ducts in one and on the presence of ‘cysts’ in the liver in the other.

3.7 PATHOLOGIC FINDINGS

Only six of the 27 subjects had renal histopathological studies: two were performed at the time of autopsy, two at the time of nephrectomy, and two at biopsy (one open and one needle biopsy). All six had evidence of dilated collecting ducts and an absence of dysplastic elements and glomerular dilatation. Eight subjects had liver histopathological studies performed, six at biopsy (five of which were open), and two at autopsy. All were reported as either having biliary dysgenesis or fibrosis.

3.8 MANAGEMENT

3.8.1 Renal replacement therapy

Thirteen subjects (13/27, 48%) required renal replacement therapy, 12 of who had renal transplants (with or without dialysis) and one who remained on renal dialysis without a transplant at the time of last contact.

Six subjects (6/27, 22%) required renal dialysis and of these four had peritoneal dialysis only, one had haemodialysis only and one required both (at different times). Dialysis commenced at a mean age of 13.6 years (SD 3.1 years). Of the six subjects requiring dialysis, in three this was preceded by preemptive failed renal transplants, another two subsequently had successful renal transplants and one continues on dialysis at this time. Overall 12 subjects have had renal transplants, at a mean age of 13.7 years (SD 3.7 years, range 6.2 to 18.7 years at first graft). Five of the 12 were from living related donors and the remainder were cadaver transplants. Six

grafts were lost due to chronic rejection and the other six were functioning at the time of this study. Four subjects have had a second transplant, at a mean age of 18.3 years (SD 2.6 years), two of which were acutely and two were chronically rejected.

3.8.2 Interventions for hepatic related morbidities

Ten subjects (10/27, 37%) required a form of intervention for a hepatic related morbidity. One subject was recorded to have had variceal banding performed (at 8.1 years), five to have had splenic shunts, at a mean age of 10.9 years, and three to have had embolisation of the splenic artery, at a mean age of 9.2 years. Three patients had splenectomies, at a mean age of 12.3 years (including the one who had the banding and another who also had a shunt procedure).

3.9 SURVIVAL

3.9.1 Patient survival

Of the whole Afrikaans subject group (58 patients), 11 (19%) died at less than one month of age, while in the group homozygous for the p.M627K mutation (27 patients), three (11%) died within the first month of life. Table 3.4 illustrates the survival rates at different ages among the homozygous group.

Table 3.4: Survival rates based on age at last contact in the homozygous group

Age	*Male (n=15)*	*Female (n=12)*	*Overall*
1yr	12/15 (80%)	12/12 (100%)	24/27 (89%)
5yrs	12/15 (80%)	12/12 (100%)	24/27 (89%)
10yrs	12/15 (80%)	12/12 (100%)	24/27 (89%)
15yrs	12/15 (80%)	12/12 (100%)	24/27 (89%)
20yrs	12/15 (80%)	12/12 (100%)	24/27 (89%)
25yrs	9/15 (57%)	4/12 (33%)	13/27 (48%)

The overall mean survival was 21.45 years (SE 1.67 years), with a mean survival in males of 19.33 years (SE 2.88 years) and in females of 23.70 years (SE 1.24 years). The difference between male and female survival was not statistically significant. The Kaplan Meier survival graphs, based on age at last contact, are shown in Figure 3.5.

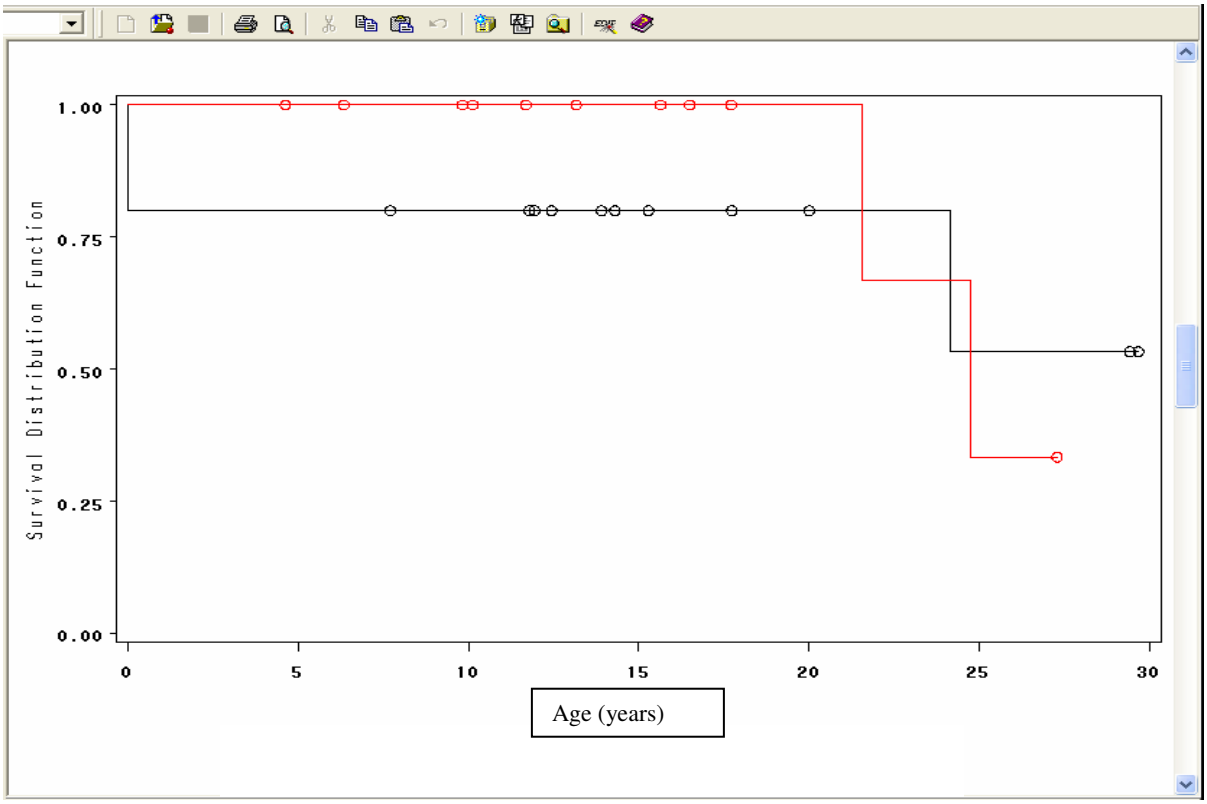


Figure 3.5: Kaplan Meier survival graphs for males and females

*○ (males)/○ (females) Last age of contact for subject still alive/ lost to follow up

3.9.2 Cause of death

Of the three neonatal deaths, all were male and all were due to respiratory failure. Two of these subjects were unsuccessfully ventilated and the other could not be resuscitated for mechanical ventilation. The three other documented deaths occurred at 24.2, 24.8 and 21.6 years of age, two due to renal failure (one of whom had had two, and the other, one renal transplant), and the third due to hepatic failure.

3.9.3 Renal survival

Renal survival was calculated from birth with an end point at the start of renal replacement therapy (dialysis or renal transplant). These data are presented in Table 3.5.

Table 3.5: Renal survival rates in the homozygous group

	Male (n=15)	*Female (n=12)*	*Overall*
5yrs	15/15 (100%)	12/12 (100%)	27/27 (100%)
10yrs	15/15 (100%)	10/12 (80%)	25/27 (91%)
15yrs	10/15 (67%)	6/12 (53%)	18/27 (61%)

The overall mean renal survival was 14.72 years (SE 0.75 years), with a mean survival in males of 15.61 years (SE 0.66 years) and in females of 13.54 years (SE 1.43 years). The difference between male and female survival was again not statistically significant. The renal survival curves are shown in Figure 3.6 below.

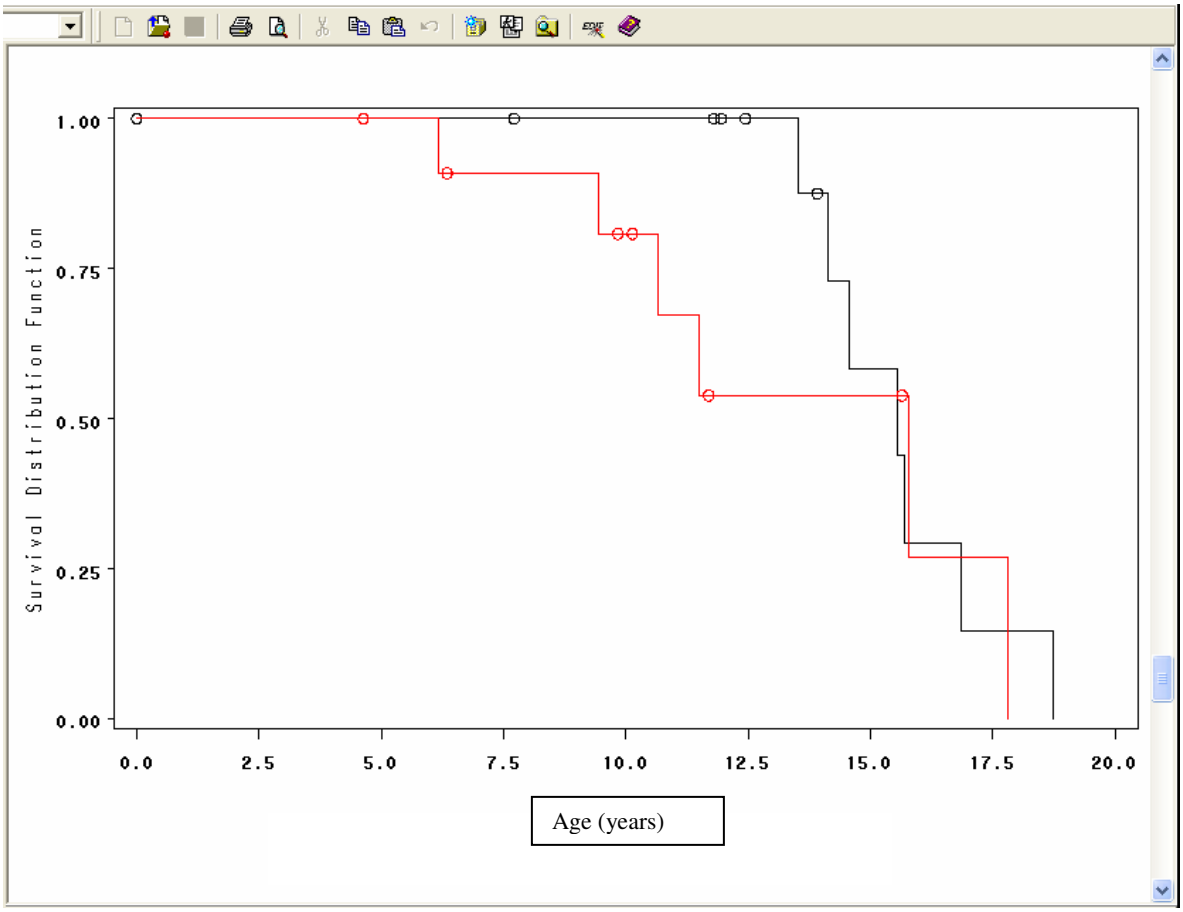


Figure 3.6: Kaplan Meier renal survival graphs for males and females

*○ (males)/○ (females) Last age of contact for subject still alive/ lost to follow up

3.10 SIBSHIPS

Of the four families in which more than one affected sibling was reported, only two families had sufficient data on more than one sibling for inclusion into the study. Detailed comparisons of the clinical features within sibships could thus not be made. However the ages of death or last contact were available for all those affected and the data are presented in Table 3.6.

Table 3.3: Survival differences in families with more than one affected sibling

	Sib 1	***Sib 2***	***Sib 3***
Family 1	Died at 5m*	Alive at 29y*	Alive at 29y*
Family 2	Died <1m	Alive at 12y*	Alive at 6y*
Family 3	Died <1m	Alive at 13y*	
Family 4	Alive at 15y*	Alive at 15y	

* Subjects included in the clinical analysis in this study

3.11 COMPARISON WITH PUBLISHED DATA

A comparison of some of the clinical characteristics was made with the largest group of patients described to date, that of the North American cohort (NAC) (Guay-Woodford and Desmond 2003). The patients in this cohort were subdivided into those born before 1990 and those born in or after 1990 (43 and 166 patients respectively). The latter group was used for the comparison in Table 3.7. Data from the NAC patients born before 1990 was not available for this comparison. The genotypes of the NAC patients were not described but they would have represented a wide variety of mutations, the majority being compound heterozygotes.

The Afrikaner cohort demonstrates a later median age of diagnosis of ARPKD (27 days vs. one day), as well as of hypertension (104 vs. 16 days) and CRI (515 vs. 16 days). Renal transplant was also performed at a later median age in the present cohort (14.6 vs. 4.4 years). In contrast, hepatic morbidity: PHT (splenomegaly) was diagnosed at an earlier median age in the Afrikaner cohort although a complication thereof (variceal bleeding) presented later (8.1 vs. 5.9 years).

Table 3.4: Comparison of clinical characteristics by age of onset of morbidity

Characteristic	*Afrikaner cohort (N=27) (Present study)* *Median (25th%-75th%)*	*North American cohort (N=166)* *(Patients born after 1990)* *Median (25th%-75th%)*
Diagnosis	27d (2-89)	1d (1-61)
Hyponatraemia	17d (3-65)	16d (1-46)
Hypertension	104d (5-378)	16d (5-165)
CRI	515d (79-1443)	13.5d (1-394)
Splenomegaly	1.3y (0.3-3.4)	-
Portal hypertension	-	2.8y (0.9-4.7)
Variceal bleeding	8.1y (5.0-10.0)	5.9y (4.0-6.0)
Renal transplant	14.6y (11.5-15.8)	4.4y (1.5-6.9)
Liver transplant	-	8.2y (5.4-10.0)
Death	10.8y (incl 3 NN deaths)	10d (1-93)

A more detailed comparison of our findings with nine previous studies, including the NAC, and completed between 1989 and 2005, can be found in Appendix B, based on the table presented in Bergmann et al. (2005). The lack of prenatal diagnosis in the present study is evident, compared to between 11% (Zerres et al. 1996) and 46% (Guay-Woodford and Desmond 2003) of diagnoses made prenatally in other studies. Growth retardation was diagnosed relatively more often in the present study (59%), compared to between 6% (Kaariainen et al. 1988) and 25% (Zerres at al. 1996) in other studies. Altogether 93% of subjects in the present study had CRI, but this is not directly comparable to criteria used to define renal failure or insufficiency in other studies. The survival rates at five years appear to be minimally higher in this study (89%) compared to other studies which report between 75% (Guay-Woodford and Desmond 2003) and 84% (Bergmann et al. 2005). While the mortality in

the first year in this study was 11%, other studies reported between 9% (Zerres et al. 1996 and Gagnadoux et al. 1999) and 26% (Roy et al. 1997).

4 DISCUSSION

4.1 ALLELE FREQUENCY

Collaborative studies between the Division of Human Genetics (NHLS and University of the Witwatersrand) and Professor L. Guay-Woodford (University of Birmingham, Alabama, USA) on 25 unrelated patients with a presumed diagnosis of ARPKD found the p.M627K mutation on 64 % of alleles tested (Guay-Woodford et al. 2005). In the present study, of 39 families (36 unrelated individuals) meeting the inclusion diagnostic criteria for ARPKD (some of whom may have been included in the earlier studies), the p.M627K mutation was found on 81% of alleles tested. The latter figure is likely to be more accurate since some of the patients tested initially were, on clinical review, found to have ADPKD or another diagnosis. While the present study utilized DNA from a number of the patients tested in 2005, the strict application of the inclusion criteria allowed for a diagnostically more specific group.

This finding represents the highest frequency of a single mutation in a specific population group documented worldwide, to date. The two mutations found recurrently in Finnish patients with ARPKD together account for 60% of disease associated alleles (Bergmann et al. 2003), and reports of the recurrent mutation documented in the Spanish population recorded only two individuals who were homozygous for that mutation (Rossetti et al. 2003).

The finding of the p.M627K mutation in 81% of ARPKD disease associated alleles in the Afrikaner population has important diagnostic implications: an Afrikaans patient presenting

with a suspected clinical diagnosis of ARPKD can be tested for this mutation. p.M627K, in the homozygous form, will confirm the diagnosis of ARPKD, and if found in the heterozygous form will be highly supportive of the diagnosis in conjunction with the clinical findings. Should the p.M627K mutation not be detected (as would be expected to occur in 3.6% of affected Afrikaans patients, in a population assumed to be in Hardy-Weinberg equilibrium), further investigation (invasive testing or sequence analysis of the *PKHD1* gene) may be indicated to confirm the diagnosis. The cost-effectiveness and relative ease of such a DNA based diagnostic test for this population is self evident.

The serious implications for those affected with ARPKD makes requests for prenatal diagnosis, for couples who have had a previously affected child, not uncommon. Linkage analysis, as mentioned in the introduction, has a number of limitations, including the need for DNA from a previously affected sibling and a confirmed histopathological diagnosis in that sibling. For affected siblings found to be homozygous for the p.M627K mutation, or in the absence of DNA from the sibling, where both parents are found to be carriers of this mutation, prenatal diagnosis can be offered more quickly, less expensively and more accurately than linkage analysis in the Afrikaans population.

4.2 PREVALENCE

4.2.1 ARPKD in the Gauteng Province

The estimated point prevalence in this study, of 1 in 14 678 based on 2001 censorship data (Statistics South Africa 2008) is higher than the 1 in 26 000 reported by Lombard et al. in 1989, for the then Western Transvaal. This difference may have a number of explanations.

The more recent point prevalence may reflect improved survival of patients with more advanced neonatal care and improved management of renal morbidities. Although the figure may partly reflect better patient ascertainment, this is unlikely as the original studies were conducted by the nephrologists who were themselves managing the majority of paediatric renal problems in the province. Alternatively, the changing provincial borders in the mid 1990's means that the population base for the estimations have changed making comparison more difficult. However, the majority of affected families tend to be concentrated close to the tertiary care centres in Johannesburg and Pretoria (in Gauteng), and these centres were also included in the then Western Transvaal..

The 2001 censorship data did not provide information on birth by population group. From the data presented in this study (point prevalence), a birth prevalence of between 1 in 7339 and 1 in 10275 based on a 30-50% neonatal mortality (Zerres et al. 1998b) can only be estimated. Again, these figures are slightly higher than the 1 in 11 000 estimated by Lombard et al. (1989).

Lombard et al. (1989) calculated a carrier frequency, based on the birth prevalence of ARPKD in the Afrikaans population of 1 in 53, only slightly lower than the estimation of between 1 in 43 and 1 in 51 arising from the present study. Assuming 81% of the ARPKD associated alleles in this population carry the p.M627K mutation, the carrier rate for this specific mutation would be expected to be in the region of 1 in 54 to 1 in 64 Afrikaans individuals in the Gauteng province. The Human Genetics Molecular Laboratory at the NHLS has subsequently

screened 98 random Afrikaans individuals for this mutation and found one p.M627K mutation (Professor A. Krause, Division of Human Genetics, NHLS and University of the Witwatersrand, personal communication). This small sample of screened individuals is of course insufficient to accurately determine a carrier rate, but 1/98 is not out of keeping with the above estimations.

4.2.2 ARPKD in the Rest of South Africa

Communication between the writer and consultants in Paediatric Nephrology in other referral centres in South Africa has raised some interesting observations. Dr P.Gajar at Red Cross Children's Hospital and Dr P.Nourse at Tygerberg Hospital, both in the Western Cape, report that they together currently manage six Afrikaans speaking patients of Caucasian origin, from five families, with ARPKD. One of these patients recently arrived from Gauteng and had been included in the present study. In addition, they manage two Afrikaans speaking patients of mixed ancestry, and two Caucasian patients with no Afrikaner ancestry. Prof J. Cronje, at Universitas Hospital in Bloemfontein reports that they manage no patients with ARPKD, while the same applies at King Edward Hospital in Durban. This is in contrast to the 18 patients currently managed at Johannesburg and Pretoria Academic Hospitals (most updated figures, April 2007). Again, using 2001 census data (Statistics South Africa website 2008), since more updated population statistics are not available, this indicates that ARPKD is presently 1.4 times more common in the white Afrikaners in Gauteng (population 1003860), compared to white Afrikaners in the Western Cape (population 461522).

4.2.2.1 ARPKD in Black South Africans

Of the 66 patients ascertained to have ARPKD only eight, from six families, were not of Afrikaans origin, and none were considered of to be of Black South African descent. This is in keeping with the findings of Thomson (1997). Communication between the writer and doctors at Chris Hani Baragwanath Hospital, where staff of that Nephrology Unit attend to the paediatric population of Soweto and serve mainly Black patients, revealed no additional confirmed cases of ARPKD. Again, early neonatal demises attributable to ARPKD may have not been diagnosed, but there remain no confirmed cases of ARPKD in Black South Africans.

4.2.3 Possible Causes for the Difference in Prevalence in Different Regions of South Africa

Lombard et al. (1989) also observed that there was an apparent concentration of ARPKD cases in the western Transvaal. A similar geographical clustering was observed by Torrington et al. (1984) with respect to FH. Torrington et al. (1984) associated FH with the religious affiliation of their patients to the Gereformeerde Kerk established in 1859. They discussed the formation, following the migration of Afrikaans speaking trekkers from the Cape in the mid 1800's, of a genetic isolate based on sociological and geographical factors, supporting the theory of founder effect in FH.

The finding of a founder mutation in the white Afrikaans patients with ARPKD in Gauteng, and the apparent concentration of patients in this region of the country may also be attributed to the initial rapid expansion of the population in the Cape and the subsequent discrete trek party migrations to the interior, with relative social isolation over the following decades. The

results of inbreeding in a small subgroup of Afrikaners, may have lead to expression of this recessive condition at increased frequency in a relatively localized area of the country.

The p.M627K mutation has not been described in any of the European populations from which the Afrikaners may have originated (ARPKD-PKHD1 Mutation Database, Aachen University 2008). More detailed and extensive haplotype studies might have the potential to date the origin of this mutation more accurately.

4.3 PATIENT ASCERTAINMENT

Determination of whether an individual is 'Afrikaans' is not always simple. Simply speaking the Afrikaans language or having an Afrikaans surname does not exclude more recent admixed ancestry and conversely, a surname that is not Afrikaans does not exclude a close Afrikaans ancestry. As an example, one pair of 'Afrikaans' siblings, who were found in a previous study (Onuchic et al. 2002) to be compound heterozygotes for an I2944fsX2949 mutation in exon 58 and an I222V mutation in exon 9, were, on direct questioning of the mother, found to be of recent German descent on both the maternal and paternal sides of the family, excluding them as descendents of the Afrikaans founder population. For the purposes of this study, where possible, Afrikaans ancestry was discussed with the parents. In the absence of such a history, documentation of languages spoken by the parents and surnames of the parents were used to classify the individuals. In the 24 families with homozygous p.M627K affected offspring, there was clear predominance of Afrikaans ancestry.

The likelihood of under representation of subjects who died in the postnatal period of ARPKD related complications, either undiagnosed or not referred for tertiary care, has been mentioned and is a significant limitation in fully describing this subject group of homozygous patients. However the paucity of facilities and specialists for treatment of the longer term renal complications of this condition means that the majority of survivors of the neonatal period are likely to have been ascertained by this study.

The availability of DNA for molecular analysis in only 39 of the 58 confirmed cases of ARPKD in Afrikaners is limiting, but could not be overcome as the majority of these patients had died years to decades previously.

Only the 27 subjects homozygous for the p.M627K mutation were chosen for the clinical component of the study, as one of the study objectives was to establish if the molecular homogeneity of the group in anyway made them distinguishable from the molecularly heterogeneous groups described previously. The patients who carry a single p.M627K mutation are compound heterozygotes for a number of different mutations, five of which were previously identified, and would have diluted the homogeneity of the former group. Of the five other mutations identified, all were different. The 10 compound heterozygotes remain an important resource for future modifier gene and genotype-phenotype correlation studies.

4.4 COHORT CHARACTERISTICS

The subject group of this study included patients born up to 39 years ago (in 1969) and thus caution is required in describing the clinical course of such patients, particularly when

comparing them with those born more recently, given the advances in neonatal intensive care, anti-hypertensive therapy and renal replacement therapy. However, the long mean observation period of 14 years in the present study is significant, as one of the largest long-term clinical studies (Bergmann et al. 2005) of 164 patients, reported a mean observation period of only six years.

About half the patients were alive at the time of the present study, and of these, 11 families were in contact with the nephrology units involved, allowing for the collection of more accurate clinical information, as well as for feedback to the families with the offer of carrier-testing in those at risk who request it. Just less than a quarter (six) of the patients had demised, and slightly more (seven) were lost to follow up, mainly as they transferred out of the confines of the paediatric setting into the more widespread adult nephrology services. Attempts were made, as far as possible, to trace these patients, but with little success.

The male:female ratio of patients of 1.25 is compatible with the autosomal recessive mode of inheritance in a group of this size.

4.5 CLINICAL FEATURES AND COMPARISON WITH PUBLISHED DATA

In discussing the clinical features of this subject group (homozygous for the p.M627K mutation) (N=27), the data will be compared with those of other clinical cohorts (see appendix B), particularly of the North American Cohort (NAC) (Guay-Woodford and Desmond 2003), the largest clinical cohort described to date. This cohort was divided into a younger group

(n=166) born in or after 1990 and an older group born before 1990 (n=43). The characteristics of the NAC of 166 patients born after 1990 are summarized in Table 3.7 in the results section of this report.

4.5.1 Age at diagnosis

A median age of diagnosis, in our cohort, of 27 days (and mean age of 120 days), compared to the median age of one day in the subset of the NAC, born after 1990 (46% of whom were diagnosed prenatally), may indicate a bias in the present study toward inclusion of subjects that survived the neonatal period. The older NAC (n=43), born before 1990, had a median age of diagnosis of 72 days, even later than that of the present study, possibly reflecting the same bias. Availability of prenatal ultrasound in the developed world, particularly subsequent to 1990 (when the younger NAC patients were born), as well as earlier referral to tertiary centers, could account for the earlier median age of diagnosis in that group. The quartile ranges of age at diagnosis are not dissimilar among the subjects in the present study and the younger NAC.

4.5.2 Renal related morbidities

Hyponatraemia was diagnosed in nine (33.3%) of the subjects in this study, compared to 26.5% in the younger NAC, with a very similar median age of diagnosis (17 vs 16 days of life). Kaariainen et al. (1988) who studied a cohort of 18 patients also diagnosed hyponatraemia in 33%, but Zerres et al. (1996) found hyponatraemia in only 7/115 (6%) of his West German patients. The explanation for this difference is unclear, but a larger proportion (25%) of Zerres' subjects was diagnosed with ARPKD at >1 year of age compared to 12% and 16% in ours and the younger NAC respectively, thus his figures may represent an underestimate of the frequency of hyponatraemia in this condition.

Almost all (93%) of our cohort, but only 65% of the younger NAC, developed hypertension. The NAC showed an early median age of diagnosis (16 days) compared to the present study's 104 days. The mean age of diagnosis of hypertension in the local group was 490 days (1.3 years). Again, a number of factors, including later referral and a wide range of age at diagnosis (latest at 10.3 years in the present study) make direct comparison problematic.

Similarly, CRI was found in 93% of our subjects, but in 42% of the NAC (similar in the older and younger cohorts). Bergmann et al. (2005) found CRI in 86% of their cohort of 164 subjects. In contrast to the median age of diagnosis of CRI in the younger NAC patients of 13.5 days, the older NAC patients were diagnosed with CRI at a median age of 3.7 years. The criterion for diagnosis of CRI remained unchanged thus this difference may reflect the later age of diagnosis of ARPKD in the older cohort. The subjects in the present study were diagnosed with CRI at a median age of 515 days (1.4 years), but a mean of 962 days (2.6 years), the increased mean reflecting the wide range of age at diagnosis (1 day to 10.3 years).

It is worth noting that the patient diagnosed with hypertension and CRI at 10.3 years was diagnosed with ARPKD at 0.46 years (6 months) of age and had constant follow-up – thus, the later mean age of diagnosis of HT and CRI in the present study is not entirely a result of late referral, but is also skewed by one individual who developed hypertension later in the course of the disease. The younger median age of diagnosis of CRI in the younger NAC (13.5 days), compared to 1.4 years in this study, may partly reflect earlier referrals and ascertainment, but

may also indicate a milder phenotypic expression of the renal morbidity in homozygotes for the p.M627K mutation. Further exploration of this suggestion is severely limited by the lack of consistent criteria among studies used to document the progression of renal disease.

Culture proven UTIs in 52% of our patients compared to the 18.5% in the younger NAC is likely to reflect the longer observation period in our patients.

4.5.3 Hepatic related morbidities

Splenomegaly was used as a clinical marker for portal hypertension (PHT) in the present study since a persistently enlarged spleen in the context of ARPKD is likely to indicate raised portal venous pressure. Hepatomegaly was considered a poor marker for portal hypertension, as it is not reflective of venous pressures in the portal system, but rather of the concomitant primary hepatic pathology. A clinical marker was chosen because the vast majority of subjects, particularly the older subjects, did not undergo detailed imaging or Doppler studies of the portal vein, and if they did, this was not performed serially. This is important as other studies, for example that of the Guay-Woodford and Desmond (2003), used sonographic evidence of hepatomegaly, splenomegaly and directional reversal of portal vein flow as their definition of PHT, and the results may therefore not be directly comparable.

The median age of onset of splenomegaly was 1.3 years in the 81% of subjects in our cohort who developed PHT, with a range of 40 days to 11.1 years, and an average age of 2.7 years. The median age is younger than that of the younger NAC (2.8 years), in whom only 15% and 34% of the younger and older cohorts respectively, had evidence of portal hypertension. The

study of Bergmann et al. (2005) showed evidence of portal hypertension in 44% of their patients and splenomegaly in 38%. No other study has recorded as high an incidence of PHT as the present study (see Appendix B). While this may indicate that patients with the homozygous p.M627K mutation are more likely to develop PHT, the progress and implications thereof are more difficult to assess, due to the limitations of the clinical marker in the present study, retrospective data and minimal investigation that could be performed locally over the last 30 years. In addition, our long observation period and bias toward long term survivors may be the reason splenomegaly was ultimately documented in such a high proportion of our subjects.

A complication of PHT, variceal bleeding, occurred in 10.8% of the older NAC patients and, comparably, 11% of the patients in the present study. The similar complication rate is unexpected, since the incidence of PHT (splenomegaly) is higher in the local series. This could indicate that the PHT in the local series has a milder, less complicated clinical course, or that the incidence of PHT in the present study has been overestimated, for reasons mentioned above.

4.5.4 Growth

Growth retardation (GR), defined as height for age <3rd percentile on the CDC 2000 growth charts (National Centre for Health Statistics 2008), was documented in 16/27 (59%) subjects in this study. Direct comparison with other studies in Appendix B is difficult because of the differing criteria used to define GR. Bergmann et al. (2005) showed GR in 16% of subjects, similar to the 18% found by Gagnadoux et al. (1999), but these studies used definitions of

<2SD and <4SD below the mean height for age, respectively. Guay-Woodford and Desmond (2003) found GR in 24% of their subjects (also using <2SD below mean height for age as their definition of GR). Zerres et al. (1996) found GR in 25% (height <3rd percentile for age) with evidence of reduced GFR in the majority (24/28) of patients with GR. Konrad et al. (1995) ascribed growth retardation in patients with ARPKD to the rapid deterioration in their renal function, but Guay-Woodford and Desmond (2003) in their NAC could not attribute the delayed growth to specific causal factors in all cases. All 16 subjects with GR in the present study had evidence of CRI and HT. Thus the particularly high incidence of GR in this, compared to other studies, may reflect the relatively high incidence of CRI which in turn, may be partly a function of the long observation period of this study.

4.5.5 Gender differences

In keeping with the NAC data of Guay-Woodford and Desmond (2003), UTIs were found to be significantly more common in females, a fact that is well appreciated in general, and not specific to ARPKD. The increased incidence in females is thought to be due to anatomical differences between the normal male and female urinary tracts (Elder 2007). Also in keeping with the NAC data, there were no significant differences in the frequency of any other ARPKD related morbidities in this study when stratified by gender. This contrasts with Zerres et al (1996) (N=115) who found both a higher mortality rate in females and increased frequency of growth retardation and more severe renal disease in females. No explanation was given for these differences, and data in the present study do not support these findings.

4.6 CORRELATION AMONG ARPKD COMORBIDITIES

It is interesting, although not entirely explicable, that correlations were found overall in our cohort between the two renal morbidities (CRI and hypertension) and the hepatic morbidity (splenomegaly), but these were not significant in affected females. The discrepancy may be because this analysis was done with such small numbers of patients and would need validation in a larger group. Overall though, it is possible to conclude that the progression of disease in the homozygous subject group does not appear to be organ specific.

Gagnadoux et al. (1989) found no relationship between renal and hepatic involvement in affected individuals and this was supported in the younger NAC (Guay-Woodford and Desmond 2003) by a lack of correlation between both renal morbidities and PHT. However, the large study (n=186) by Bergmann et al. (2005), as with the present study, did not support the findings in the NAC: renal morbidities were positively correlated with hepatic morbidities, and patients who appeared to exhibit an organ specific phenotype were regarded as exceptions. They suggest that genotype-phenotype correlations in patients with such organ specific presentations require further exploration, as does the role of genetic and environmental modifiers.

4.7 MANAGEMENT

4.7.1 Renal Replacement Therapy and Renal Survival

Renal transplant was performed in 12/27 (44%) subjects in the present study at a median of 14.6 years (mean of 13.7 years), older than the median of 4.4 years in the NAC born after 1990. Communication between the writer and Professor P Thomson, previously head of

Paediatric Nephrology at Johannesburg Hospital, indicates that the criteria used for renal transplant (similar to those used internationally) and the availability of resources do not account for the large difference. While the long observation period may account for some of the difference it is possible that a milder renal phenotype in the p.M627K homozygotes may partly explain the later need for renal transplant.

Mean renal survival (calculated from birth with an endpoint at the start of RRT, or death due to end stage renal disease), of 14.72 years in the present study is slightly longer than the 10.1 years reported by Bergmann et al. (2005) in a subset of patients (N=11) carrying missense mutations on both alleles, and twice as long as their subset of patients (N=16) carrying a truncating mutation on one of the mutated alleles. This again may indicate that homozygotes carrying the missense p.M627K mutation have, as a group, a less severe renal phenotype than the mutationally heterogenous groups described above. Bergmann's (2005) study also indicated that all patients carrying a truncating mutation on both alleles died shortly after birth, providing evidence of some degree of genotype-phenotype correlation.

4.7.2 Interventions for hepatic related morbidities

PHT resulting in hypersplenism that was clinically severe enough to require intervention occurred in 10 patients in the present study, including variceal banding in one, splenic shunts in five, embolisation of the splenic artery in three and splenectomy in three (two of whom had prior procedures). Details regarding interventions for hypersplenism were not available in other studies. It was thus not possible to compare the severity of the PHT in the present study with others, despite the apparent high incidence discussed in *4.5.3*.

Altogether 2.4% and 7% of the older (born before 1990) and younger patients, respectively, in the NAC (Guay-Woodford and Desmond 2003) received liver transplants. Liver transplant was not a therapeutic option available to the patients in the present study, and the number of patients who may have met criteria for liver transplant could not be assessed from the available data.

4.8 SURVIVAL

Three (11%) of the homozygous subjects died within the first month of life, with no further deaths in the remainder of the first year, translating to an 89% survival rate at one year. This is comparable to previous studies including that of Zerres (1996) who reported an identical survival rate at one year, Gagnadoux (1999), who reported a 91% survival rate at one year, and more recently, Guay-Woodford and Desmond (2003) who reported a 79% survival rate for the same period in their cohort of patients born after 1990. They suggested that recent therapeutic advances in neonatal and paediatric management have thus had little clinical impact on the outcome of patients with ARPKD and the present study supports this finding. The ascertainment of patients in the context of tertiary nephrology referral centers (in the majority studies included in Appendix B), must of course be considered in interpretation of the relatively good early survival rates. The under representation of patients dying in the perinatal or early neonatal period in all these studies cannot be accurately estimated.

Only Kaariainen et al (1988), working in Finland, demonstrated a lower survival rate of 19% (14/73 subjects) (see Appendix B), but it appears that this was a diagnostically broad and

difficult to classify group (including all polycystic kidney disease patients) as the analysis was made mainly from the Finnish death registers. When attempts were made to sub-classify the patients, survival to one year of the ARPKD patients was 42%, more in keeping with the estimate of Zerres (1998a) and supporting evidence of the bias toward long term survivors when nephrology centers ascertain patient cohorts.

Although a number of patients were lost to follow up during the first 20 years of observation, no further deaths were recorded in the present study until after 20 years of age and the mean survival was 21.45 years. This finding is difficult to compare to other published studies where the mean observation periods were shorter. In the older NAC patients (Guay-Woodford and Desmond 2003), born before 1990, no deaths after one year of age were reported. Survival beyond the first year of life, and more specifically the neonatal period, appears to be better than is generally perceived, as suggested by Guay-Woodford and Desmond (2003) and supported by the present study.

4.9 SIBSHIPS

There were 4 families in which more than one sibling was affected with ARPKD. Unfortunately, clinical data were not available for all of these patients (10), thus it was not possible to assess detailed differences within each sibship. However, in terms of survival, the discordance within the first three families was clear, with one sibling dying in the first six months and the other/s surviving into the second or third decades.

On initial observation, it may seem apparent that the first sibling tends to do worse, and that perhaps with increased awareness and earlier management, subsequent siblings are at a survival advantage. However the number of sibships (four) is too small to draw such a conclusion. In addition, in families 2 and 3, the first sibling is reported to have had severe pulmonary hypoplasia, which was not apparent in the next sibling. Sibling 1 in family 1 died of sepsis at 5 months, while his brothers had a relatively uncomplicated early course and were in fact only diagnosed with ARPKD at 0.5 and 1.3 years respectively.

This discordance among sibships has been well documented. Kaplan et al. (1989a) reported eight families, six of whom were discordant for age and mode of onset. This was in contrast to Kaariainen (1987) who noted familial consistency for perinatal presentation in 11 families. Of the twenty sibships studied by Deget et al. (1995), only nine sibling pairs demonstrated the same clinical subtype (according to Blyth's and Ockenden's (1971) classification of perinatal, neonatal, infantile and juvenile forms). Eight families of the 11 in which one sibling had died, showed markedly discordant ages of death with one death postnatally, and the second sibling surviving to the date of last examination (8 months to 14 years). The affected sibling with the early death was the first born in six of the eight families, but as with the present study the complications occurred early on in the first sibling, and the clinical course of the second was milder, indicating that the survival with increased birth order is unlikely to be due to opportunities for more aggressive treatment in the second child. More recently Bergmann et al. (2005) reported that overall the majority of sibships display comparable clinical courses with about 20% exhibiting gross intrafamilial variability in phenotype, in their unpublished

data of more than 100 pedigrees. In that study, however, gross variability with respect to survival was found in 20 out of 48 sibships (42%) studied. Of these 20, the firstborn faired worse in 14 (70%) of the sibships. They comment that these results may indicate a bias as a result of the study design requiring at least one neonatal survivor per family. The present study may also be biased toward this as primary ascertainment was usually of the longer surviving sibling through the nephrology clinic.

5 CONCLUSIONS

The pathogenic missense p.M627K mutation was detected in 81% of ARPKD disease associated alleles in the Afrikaner population. This significant finding supports the use of targeted mutation analysis in Afrikaans patients with a suspected clinical diagnosis of ARPKD, decreasing the need for renal biopsy and increasing the cost-effectiveness of diagnosis. Accurate prenatal diagnosis is feasible, without the need for a confirmed histological diagnosis in a sibling, and with increased accuracy compared to the previously used molecular method of linkage analysis.

Marked clinical variability was demonstrated within the subject group of affected individuals homozygous for the p.M627K mutation in the present study, supporting the limited genotype-phenotype correlation described worldwide, particularly among individuals with two missense mutations accounting for their disease. The findings in this study were compared to previously described cohorts representing diverse mutational spectrums and as with those studies, a wide degree of phenotypic variability was evident, despite the local genetic homogeneity. The limited genotype-phenotype correlation for this condition was further demonstrated by vast discordance in survival in three of the four affected sibships observed.

ARPKD was diagnosed at a median age of 27 days, older than a North American cohort born after 1990 (median age of one day). While the vast majority of subjects in the present study were diagnosed with CRI and HT, indicating the renal morbidities to be more common than in

previously described cohorts, these morbidities occurred at a later median age than in most studies, possibly indicating a trend to a milder expression of renal morbidity in homozygotes for the p.M627K mutation. Further support for this conclusion lies in the later median age of renal transplant (14.6 years vs 4.4 years in the NAC). PHT was diagnosed in the present study more frequently than in previous studies, at a younger median age, although with similar complication rates. Statistical overall correlation was found between the renal and hepatic related morbidities in this study, indicating that progression of the ARPKD is not organ specific in this homozygous subject group, in contrast with findings in the NAC but in keeping with other previous studies such as Bergmann et al. 2005. The survival rate of 89% at one year in the present study was not dissimilar to previous studies with similar patient ascertainment.

Correlation of an underlying molecular defect with the clinical course of a condition aims to improve both the care given to an affected family as well as provide a better understanding of the molecular pathogenesis of the condition. From the findings in this study, aside from a possibly milder course of the renal disease, and earlier onset of PHT, no genotype specific phenotype patterns are evident. The degree of intra-familial variability in terms survival further supports the limited correlation. Additional genetic and/or environmental factors must account for the variability seen in these patients with comparable genotypes.

This group represents a unique cohort and is the largest series of patients affected by ARPKD homozygous for a single mutation, described to date. The findings from this study in conjunction with the presence and availability of testing for the p.M627K mutation in the

Afrikaans population will provide for more accurate, specific and informative genetic counselling in families affected by ARPKD, as well as accurate, rapid and cost effective prenatal diagnosis in situations where it is requested.

Continued collaboration with the University of Alabama at Birmingham (USA) Recessive Polycystic Kidney Disease Core Center (UAB RPKDCC), under the directorship of Professor L Guay-Woodford will enable future studies to be undertaken. This subject group presents a valuable and unique resource for future studies of modifier genes and environmental influences on the phenotypic expression of ARPKD. The additional data from the group of ten individuals, compound heterozygotes for the p.M627K and another mutation, which was not analysed for the purposes of this study, may contribute to future genetic modifier studies.

Further follow up of the patients who remain in contact with the various nephrology units will provide additional valuable information regarding the natural history of ARPKD within a genetically homogenous setting. Finally, further studies should also aim to supplement the current data with newly diagnosed patients countrywide as well as existing patients in the Western Cape.

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Zerres K, Senderek J, Rudnick-Schoneborn S, Eggerman T, Kunze J, Mononen T, Kaanainen H, Kirfel J, Moser M, Buettner R and Bergman C (2004) New options for prenatal diagnosis in autosomal recessive polycystic kidney disease by mutation analysis of the *PKHD1* gene. Clinical Genetics 66:53-57

WEB RESOURCES

The URLs for data presented herein are as follows:

ARPKD-PKHD1 MUTATION DATABASE. http://www.humgen.rwth-aachen.de [Accessed 1 November 2008]

NATIONAL CENTRE FOR HEALTH STATISTICS. CDC Growth Charts (2000) www.cdc.gov/GrowthCharts [Accessed 1 November 2008]

SOUTH AFRICAN HISTORY ONLINE. http://www.sahistory.org.za [Accessed 1 May 2008]

STATISTICS SOUTH AFRICA. Census 2001 http://www.statssa.gov.za/census01/html/C2001Interactive.asp [Accessed 1 November 2008]

Appendix A: Pediatric Polycystic Kidney Disease Registry: Primary Form

PEDIATRIC POLYCYSTIC KIDNEY DISEASE REGISTRY

PRIMARY FORM (Page 1 of 4)

Patient ID #: ☐☐☐☐☐☐ **Name:** _____

Medical Record #: ☐☐☐☐☐☐☐ **Center:** ☐☐☐☐☐☐

month day year

Date of Birth: ☐☐☐☐☐☐☐☐ **Sex:** ☐ 1=Male 2=Female

Date of Diagnosis: ☐☐☐☐☐☐☐☐ **Race:** ☐ 1= AFRIK (BOTH PARENTS) 2=African 3=Asian 4=AFRIK (ONE PARENT) 5 = OTHER

Date of Last Contact: ☐☐☐☐☐☐☐☐ **Patient Status:** ☐ 1=Alive 2=Dead 9=Unknown

Date of Death: ☐☐☐☐☐☐☐☐

Immediate Cause of Death: ☐ 1=Respiratory failure 2=Sepsis 3=Renal failure 4=Variceal bleeding 5=Hepatic failure 6=Other; specify: _____

Family History of PKD: ☐ 1=Yes 2=No 3=Unknown *Please check one* (1=Yes 2=No) ☐ Sibling ☐ Mother ☐ Father ☐ Grandparents

Parental Consanguinity: ☐ 1=Yes 2=No 3=Unknown

PRENATAL

IF DIAGNOSED AT LESS THAN 30 DAYS OF AGE: Prenatal diagnosis: ☐ 1=Yes 2=No

If **Yes,** ☐ Ultrasound 1=Yes 2=No ☐ Genetic 1=Yes 2=No If **Yes,** Gestational age at diagnosis ☐☐ *weeks*

NEONATAL DATA

Gestational Age: ☐☐ *weeks*

Apgar Scores: 1 min. ☐☐

5 min. ☐☐

INTERVENTIONS

Ventilation	☐ 1=Yes 2=No
Conventional	☐ 1=Yes 2=No
High Frequency	☐ 1=Yes 2=No
Nitric Oxide	☐ 1=Yes 2=No
CAVH	☐ 1=Yes 2=No
Dialysis	☐ 1=Yes 2=No
ECMO	☐ 1=Yes 2=No
Nephrectomy	☐ 1=Yes 2=No
Unilateral	☐ 1=Yes 2=No
Bilateral	☐ 1=Yes 2=No

PEDIATRIC POLYCYSTIC KIDNEY DISEASE REGISTRY

PRIMARY FORM (Page 2 of 4)

Patient ID #: | | | | | | | **Name:** _____

Medical Record #: | | | | | | | | **Center:** | | | | | | |

PKD-ASSOCIATED MORBIDITY:

			Date of Diagnosis (month day year)
	Chronic Lung Disease (requiring supplemental oxygen)	☐ 1=Yes 2=No	
Renal:	Hyponatremia	☐ 1=Yes 2=No	
	Hypertension	☐ 1=Yes 2=No	
	Chronic renal insufficiency	☐ 1=Yes 2=No	
	Urinary tract infection	☐ 1=Yes 2=No	
	If **yes**, Cystitis	☐ 1=Yes 2=No	
	Pyleonephritis	☐ 1=Yes 2=No	
	If **yes**, culture proven?	☐ 1=Yes 2=No	
	Cyst Rupture/Bleed	☐ 1=Yes 2=No	
	Renal Stone	☐ 1=Yes 2=No	
Liver:	Portal Hypertension	☐ 1=Yes 2=No	
	Variceal Bleeding	☐ 1=Yes 2=No	
		☐ 1=Yes 2=No	
		☐ 1=Yes 2=No	
		☐ 1=Yes 2=No	
	Hypersplenism	☐ 1=Yes 2=No	
	Cholangitis	☐ 1=Yes 2=No	

Growth Retardation ☐ 1=Yes 2=No
(height < 2 SD for age)

Recurrent Pain ☐ 1=Yes 2=No
☐ If **Yes**, 1=Abdominal
2=Flank
3=Back

Last serum creatinine
☐☐.☐ mg/dl

PEDIATRIC POLYCYSTIC KIDNEY DISEASE REGISTRY

PRIMARY FORM (Page 3 of 4)

Patient ID #: ☐☐☐☐☐☐ **Name:** _____

Medical Record #: ☐☐☐☐☐☐☐ **Center:** ☐☐☐☐☐☐

INITIAL RADIOLOGIC STUDIES

Kidney:

Date: ☐☐☐☐☐☐☐☐ *month day year*

Imaging Study: ☐ 1=Yes 2=No

☐ Ultrasound ☐ CT scan with contrast ☐ CT scan without contrast

☐ Other; specify: _____

Result: ☐ 1=Echogenic kidneys without gross cysts 2=Echogenic kidneys with gross cysts

Liver:

Date: ☐☐☐☐☐☐☐☐ *month day year*

Imaging Study: ☐ 1=Yes 2=No

☐ Ultrasound ☐ CT scan

☐ Other; specify: _____

Result: ☐ 1=Normal 2=Echogenic liver 3=Dilated bile ducts

HISTOPATHOLOGY

Kidney:

Date: ☐☐☐☐☐☐☐☐ *month day year*

Pathology: ☐ 1=Autopsy 2=Open biopsy 3=Needle biopsy 4=Nephrectomy

Result: ☐ 1=Dilated collecting ducts 2=Glomerular dilation 3=Dysplastic elements

Liver:

Date: ☐☐☐☐☐☐☐☐ *month day year*

Pathology: ☐ 1=Autopsy 2=Open biopsy 3=Needle biopsy

Result: ☐ 1=Normal 2=Biliary dysgenesis

PEDIATRIC POLYCYSTIC KIDNEY DISEASE REGISTRY

PRIMARY FORM (Page 4 of 4)

Patient ID #: ☐☐☐☐☐☐ **Name:** _____

Medical Record #: ☐☐☐☐☐☐☐ **Center:** ☐☐☐☐☐☐

INTERVENTIONS

Medications:		Date Initiated *month day year*	Date Discontinued *month day year*
ACE inhibitor	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
Beta blocker	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
Calcium channel blocker	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
Diuretic	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
Growth hormone	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
Recombinant erythropoietin	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
Other; specify: _____		☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
Supplemental feeding: NG	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
GT	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐
Parenteral	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐

Dialysis ☐ 1=Yes 2=No ☐ If **YES,** 1=hemodialysis 2=peritoneal dialysis 3=both

Date Initiated ☐☐☐☐☐☐☐☐ Date Discontinued ☐☐☐☐☐☐☐☐

		Date Performed
Variceal banding:	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐
Splenic shunting:	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐
Splenectomy:	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐
Kidney Transplantation:	☐ 1=Yes 2=No	Date Performed
Kidney: Graft #1	☐ 1=LRD 2=cadaver	☐☐☐☐☐☐☐☐
graft function	☐ 1=Yes 2=No	
graft loss	☐ 1=Acute rejection 2=Chronic rejection 3=Infection 4=Other	
		Date Performed
Graft #2	☐ 1=LRD 2=cadaver	☐☐☐☐☐☐☐☐
graft function	☐ 1=Yes 2=No	
graft loss	☐ 1=Acute rejection 2=Chronic rejection 3=Infection 4=Other	
		Date Performed
Liver Transplantation:	☐ 1=Yes 2=No	☐☐☐☐☐☐☐☐

DATA ABSTRACTOR _____ **DATE SURVEY COMPLETED:** ☐☐☐☐☐☐☐☐

Appendix B: Summary of findings of previous studies compared with the Afrikaans homozygous cohort

	The present study(2006)	***Bergmann et al (2005)***	***Guay-Woodford (2003)***	***Capsidona et al (2003)***	***Roy et al (1997)***	***Zerres (1996)***	***Gagnadoux et al(1999)***	***Kaplan et al(1989)***	***Kaariainen et al(1988)***	***Cole et al(1997)***
Patients,n	27	186(164)	166	31	52	115	33	55	18	17
m:f	1.18		0.96			1.35	N/A	1.39	N/A	N/A
Age at diagnosis	0% prenatal	23% prenatal	46% prenatal	32% prenatal		11% prenatal				Diagnosed in 1st year
	50% <1 month	31% <1 month	27% < 1 month	23% < 1 month		41% <1month	33% <1month	45% <1month	72% <1month	
	38% 1-12 months	16% 1-12 months	11% 1-12 months	19% 1-12 months	85% <1 year	23% <= 1 year	55% <18months	45% <=1year	6% <=1year	
	12% >1year	30% >1year	16% >1year	26% >1year	15% >1year	25% >1year	12% 6-11 years	10% >1year	22% >1year	
Hyponatraemia	33%	NA	26%	10%	NA	6%	18%		33%(6/18)	NA
Growth Retardation	59%	16% <2SD	24%	NA	NA	25%	18% (6/33)<4SD	NA	6% (1/18) <2.5SD	NA
Renal Function	93% (CRI)	86% GFR<3rd	42% GFR<3rd	51% GFR<80		72% GFR<3rd	42% GFR<80	58% creat>100	82%GFR<90	35%GFR<40
		29%ESRD by 10y	13%ESRD	16%ESRD	33%ESRD by 15y	10%ESRD	21%ESRD			29%ESRD
Hypertension (drug treatment)	93%	76%	65%	55%	60% (by 15 years)	70%	76%(25/33)	65%	61%(11/18)	100%
Evidence of portal hypertension	81% splenomegally	44%	15%	37%	23% (8/35)	46%	39%(13/33)	47%	11% (2/18)	35%(6/17)
		38% splenomegally								
Survival rate		85% 1y	79% 1y	87% 1y	NA	89% 1y	91% 1y (30/33)	79% 1y	19% 1y (14/73)	88% 1y
	89% 5y	84% 5y	75% 5y	80% 9y		88% 3y		51% 10 y		
	89% 20yr	82% 10yr						46% 15y		
Death rate in 1st year	11%	8% (survivors of 1st month)	15%	13%	26% (12/47)	9%(10/115)	9%	24%	22% (4/18) survivors of 1st month	12%(2/17)
Renal survival rate	100% 5yr	86% 5y								
	90% 10yr	71% 10yr								

Appendix C: Consent forms and ethics clearance

STUDY OF AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE IN AFRIKAANS FAMILIES

Information sheet

Dear Parent

My name is Dr Lindsay Lambie. I work in the Department of Paediatrics, Renal Unit, at Johannesburg Hospital and with the Division of Human Genetics at the National Health Laboratory Service and University of the Witwatersrand.

We are doing a study to describe the clinical features of patients diagnosed with autosomal recessive polycystic kidney disease (ARPKD), and to find out if there is a common gene fault (mutation) that causes this problem in Afrikaans families. As you know, your child has been diagnosed with this condition.

We know that ARPKD is a rare inherited disease, and that all affected individuals are not equally affected – some are more severely affected than others. To get the disease, a child needs to inherit this gene fault from both parents, and will have two faults. The parents are both well because they only have one fault each.

Worldwide ARPKD affects 1 in 20 000 people, but in the Afrikaans population ARPKD occurs more commonly. This is thought to be because the disease was present among the small number of settlers who originally came to South Africa, and today's Afrikaans population grew from that small group.

The gene that causes ARPKD has recently been found, but so far, the specific fault in Afrikaans families has not been identified.

Since some patients are more severely affected than others, and yet many may have the same specific genetic fault, there must be other factors as well, that determine the severity of each patient. We would therefore also like to see if a common specific fault can be connected to certain clinical features.

You do not have to take part in this study, but if you agree, we would ask whether you would be prepared to allow us to look at your child's hospital and / or genetic counselling files. This personal information will remain confidential, and will not be passed on to anyone without further written consent. The information will be coded (only I will know from whom it originated) and compared with other patients who have ARPKD. To make sure we have complete and accurate information, I may need to ask you a few questions, related to your child's condition, after looking at the records. This would take about 30 minutes and would be done at your convenience.

In addition, if you agree to take part in the study, we would ask to obtain a sample of blood from your child, as well as both the parents. We will need about 10ml (2 teaspoonsful) of blood from each individual. I, Dr Lindsay Lambie, will do the procedure. The procedure is safe, and there is only a slight prick as the needle is placed through the skin. If your child requires any routine blood sampling for ongoing management, we could collect the study sample at the same time, thus not requiring an extra prick.

These blood samples will be numbered, and sent to the laboratory, where the genetic material (DNA) will be extracted. This DNA will be used to look for the genetic fault that causes ARPKD. We will also ask that once the study is complete, we may store the rest of the blood, again this will be coded to protect your privacy. If you specifically agree, we may use these samples for future research, related to ARPKD.

Remember that this study is completely voluntary, and you may choose not to take part, or may withdraw at any time. This carries no penalty, and will not influence your child's ongoing treatment, or any genetic counselling or assistance you may require in future.

This study may not provide any direct benefit to yourself or your child, but may help us understand more about ARPKD, and benefit those with the condition in future, in terms of more accurate genetic counselling, and perhaps prenatal diagnosis. However, if we are able to identify the specific gene fault, we could, at your request, let you know these results. These results may not be informative and may not benefit you directly. The results would not change the treatment or diagnosis of your child.

If you have any questions about the study, you can contact myself at telephone number 082 787 7134, or Professor Amanda Krause at 011 489 9224.

This study has been approved by the Postgraduate and Ethics Committees of the University of the Witwatersrand.

If you agree to participate, please could you read and sign the attached consent forms (each parent should sign a separate consent form).

STUDY OF AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE IN AFRIKAANS FAMILIES

Consent form

I,_____, parent of _____, have read and understood the attached information sheet, and agree to participate in this study.

I :

1. Give permission for review of my child's clinical records, and understand that the information will be kept confidential.
2. Agree to a short interview, related to my child's condition, in order for you to complete the records.
3. Agree to collection of a sample of blood from me, and from my child, to be used for this study.

I do / do not (delete whichever is not applicable) wish to be informed of any results. If so, a counseling session will be arranged, in about 9 months time.

I do / do not (delete whichever is not applicable) give permission for the blood samples to be stored, and used in further studies related only and directly to ARPKD.

I understand that participation in this study is voluntary, and we may withdraw at any time. A decision not to participate will not influence future management in any way. I also understand that participation in this study will provide no direct benefit to myself or my child, and will not change the ongoing management or diagnosis.

Signed: _____ (Parent)

Contact Telephone number: _____

Date: _____

Witness: _____

Date: _____

STUDY OF AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE IN AFRIKAANS FAMILIES

Assent form

Hello

My name is Dr Lindsay Lambie. I work in the Department of Paediatrics at Johannesburg Hospital, with children who have kidney problems. We are doing a study about the type of kidney disease that you have. We are trying to find out what exactly causes this problem, and why some children with this disease are more sick than others.

To do this, we need to look at your hospital files, talk with your parents, and take a small sample of blood (about 10ml, which is the same as 2 teaspoonsful) from your arm. We can try to do this at the time we take your other blood tests, if you need them. If not, we will just take blood for this. As you know, when we take blood, it is a quick prick, which is uncomfortable as the needle goes through the skin, but does not cause any harm.

All the information from your files is private (no one else will be allowed to see them).

This study will not help you directly – it will not help to make you better. Your treatment will continue just as before. This study will help doctors to understand your problem better, and may help other families in the future.

Please let us know if you agree to taking part in this study.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Lambie

CLEARANCE CERTIFICATE **PROTOCOL NUMBER M040222**

PROJECT Clinical and molecular characterisation of autosomal recessive polycystic kidney disease (ARPKD) in Afrikaans families.

INVESTIGATORS	Dr L A Lambie
DEPARTMENT	Paediatrics
DATE CONSIDERED	04.02.27
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 04.03.26 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof A Krause

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES