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Renal cell carcinoma of acquired cystic disease in 2 patients with TSC2/PKD1 contiguous gene syndrome

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Renal cell carcinoma of acquired cystic disease in 2 patients with *TSC2/PKD1* contiguous gene syndrome

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

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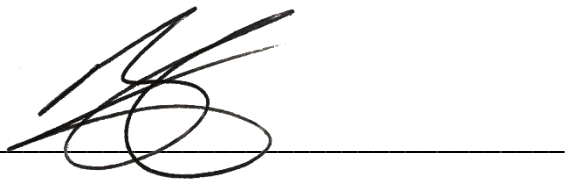
DECLARATION

I, Brett Stephen Mansfield, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Medicine (MMed) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



Brett Mansfield

15th day of October in 2020

DEDICATION

For my wife, Taryn. Forever supportive and always inspirational.

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Many thanks are extended to Dr Caroline Dickens who provided guidance in the interpretation of the raw genetic data.

Dr Claudia Do Vale is also acknowledged for her input into the two clinical cases which are described.

ABSTRACT

Introduction

The genes for tuberous sclerosis complex and autosomal dominant polycystic kidney disease lie adjacent to each other on chromosome 16p13.3. Rarely, a large deletion can result in a contiguous gene syndrome which leads to early, severe polycystic kidney disease and the development of end stage renal disease (ESRD) in the third decade of life. Acquired cystic disease is most frequently seen in the dialysis population and predisposes the affected individual to acquired cystic disease associated renal cell carcinoma (ACD-RCC), a new subtype of renal cell carcinoma (RCC).

Methods

Two patients with phenotypic characteristics of *TSC2/PKD1* contiguous gene syndrome with ESRD who developed ACD-RCC will be described. The Affymetrix® OncoScan® chromosomal microarray was performed on DNA extracted from whole blood from both participants to assess for genetic changes across the genome. The NanoString® nCounter® Cancer CN Assay was used to identify copy number variations (CNVs) in 87 common cancer genes.

Results

No mutation was identified by conventional genetic testing to account for the *TSC2/PKD1* contiguous gene syndrome in both participants. Mutations in the BRCA2, KRAS and MDM4 genes were common to both patients and identified by means of the NanoString® nCounter® Cancer CN Assay. One participant was found to have CNVs in 3p22.1 and 3q26.2, important loci in RCC.

Discussion

The absence of a mutation in *TSC2/PKD1* contiguous gene syndrome occurs in 10-25% of affected individuals. The most common cause of which is somatic mosaicism. Further genetic testing would be required to confirm mosaicism in each of the cases described. Alteration observed in the three cancer causing genes (BRCA2, KRAS and MDM4) were

present in both cases, however, these are not known to predispose to RCC. One participant was found to have CNVs in 3p22.1 and 3q26.2, important susceptibility loci in RCC which may have contributed to cancer development. Further, targeted gene sequencing studies are needed to identify mutations that may predispose individuals with ESRD to ACD-RCC.

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LIST OF ABBREVIATIONS

ACD	Acquired cystic disease
ACD-RCC	Acquired cystic disease-associated renal cell carcinoma
aCGH	Array-based comparative genomic hybridization
ADPKD	Autosomal dominant polycystic kidney disease
AMACR	α-methyl-acyl-coenzyme A
BRCA2	Breast cancer gene 2
CD10	Cluster of differentiation 10
CK7	Cytokeratin 7
CMA	Chromosomal microarray analysis
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CNV	Copy number variation
CPGR	Centre for Proteomic and Genomic Research
DNA	Deoxyribose nucleic acid
E-cadherin	Epithelial cadherins
EDTA	Ethylenediaminetetraacetic acid
EMA	Epithelial membrane antigen
ESA	Erythropoietin stimulating agent
ESRD	End-stage renal disease
FISH	Fluorescence-*in situ*-hybridization
GTPase	Guanosine triphosphatase
GWAS	Genome-wide association study
Kbp	Kilobase pairs
KRAS	Kirsten rat sarcoma viral oncogene homolog
LOH	Loss of heterozygosity
MDM-4	Mouse double minute-4
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
MYC	Myelocytomatosis; a proto-oncogene
NGS	Next generation sequencing
OMIM	Online Mendelian Inheritance of Man
PCR	Polymerase chain reaction
RCC	Renal cell carcinoma
SNP	Single-nucleotide polymorphism

TSC	Tuberous sclerosis complex
WHO	World Health Organization

CHAPTER 1: INTRODUCTION & BACKGROUND

1.1 Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder with highly variable disease manifestations, of which, hamartomatous lesions of the skin, kidneys, lungs, heart and brain are the most common.[1] The incidence of TSC is in the region of 1 in 5800 and 1 in 10000 live births.[1–3] Point prevalence estimates of the syndrome lie between 3.8 and 8.8 per 100 000 population.[3–6]

1.1.1 Diagnosis of TSC

Vogt, in 1908, described the syndrome as a triad of features – mental retardation, epilepsy and facial angiofibromas (adenoma sebaceum).[7] However, more than half of patients with TSC exhibit normal cognitive ability and a quarter do not have epilepsy.[8]

While the teaching of Vogt's triad of features has persisted, a more comprehensive set of major and minor criteria for the diagnosis of TSC has been developed.[1] Clinical diagnostic criteria developed at the International Tuberous Sclerosis Complex Consensus Conference in 2012 include 11 major features and 6 minor features (Table 1).[1] A definite diagnosis is made when 2 major or 1 major with 2 or more minor features are present.[1] A possible diagnosis is given by 1 major or 2 or more minor features.[1] Alternatively, a definite diagnosis may be made following the identification of a pathogenic mutation in either the *TSC1* or the *TSC2* gene.[1]

Table 1. Clinical diagnostic criteria for tuberous sclerosis complex (adapted from Northrup *et al* 2012)

Major criteria	Minor criteria
• Hypomelanotic macules (≥3; 5mm diameter) • Angiofibromas (≥3) • Ungual fibromas (≥2) • Shagreen patch • Retinal hamartomas • Cortical dysplasias • Subependymal nodules • Subependymal giant cell astrocytoma • Cardiac rhabdomyoma • Lymphangioleiomyomatosis • Angiomyolipomas (≥2)	• Confetti skin lesions • Dental pits (>3) • Intraoral fibromas (≥2) • Retinal achromic patch • Multiple renal cysts • Non-renal hamartomas

1.1.2 Genetics and pathophysiology of TSC

TSC is inherited in an autosomal dominant fashion but has a high rate of spontaneous mutation.[1] Mutations in either of two tumour suppressor genes, *TSC1*, which encodes for the protein hamartin and is found on chromosome 9q34, or *TSC2*, which encodes for tuberin and is found on chromosome 16p13.3, results in the disease.[1] Despite being genetically heterogenous, mutations in either of the implicated genes results in a phenotypically indistinct syndrome.[4] Approximately 80% of mutations in TSC are de novo or sporadic.[6] Mutation of the *TSC2* gene is four times more prevalent than in the *TSC1* gene in the setting of de novo mutations.[6] Prevalence of mutations in *TSC1* and *TSC2* is approximately equal among those with inherited TSC.[6] Approximately 10-25% of patients with TSC do not have a mutation identified by genetic screening, and thus, clinical diagnostic criteria are relied upon in this instance.[1] The absence of a mutation does not, however, exclude a diagnosis.[1]

The multisystem involvement of TSC demonstrates the important role that the gene products, tuberin and hamartin, play in cellular proliferation and regulation.[9] Since their discovery, downstream effects on the mammalian target of rapamycin (mTOR) pathway have been identified.[10] The mTOR pathway is important for many cellular processes such as transcription, translation, nutrient uptake and cell cycle progression.[10] Hamartin and tuberin bind together to form a tuberin-hamartin complex which inhibits GTPase (an enzyme which hydrolyzes guanosine triphosphate to guanosine diphosphate).[10] Inhibition of GTPase leads to a reduction in the stimulation of mTOR.[10] Inactivation of both alleles for either *TSC1* or *TSC2* leads to unregulated stimulation of the mTOR pathway and this leads to tumour development.[10]

Clinical manifestations of TSC have variable penetrance and, as a result, the severity of impairment is also variable.[10] Thus, while individuals may have the same or similar genotype, they may exhibit markedly different phenotypes of varying severity.[10] Individuals with *TSC2* mutations are more likely to have severe disease manifestations, as compared to those with *TSC1* mutations.[10]

1.1.3 Renal disease in TSC

Renal disease is a major cause of mortality in TSC.[11–13] The commonest renal manifestations are renal cysts and angiomyolipomas, which may co-exist.[13–15] The TSC genotype is a predictor for renal involvement, with *TSC2* mutations exhibiting a greater incidence and severity of angiomyolipomas and renal cysts, when compared to individuals harbouring the *TSC1* mutation.[13] Individuals with TSC but without an identifiable mutation tend to have a less severe disease phenotype, especially with respect to the typical dermatological features (hypomelanotic macules and shagreen patches), seizures and the cognitive manifestations of the disease.[13,16] By contrast, renal manifestations were as commonly seen in individuals without an identifiable mutation as they were in those with a *TSC2* mutation.[17]

Angiomyolipomas are found in the renal cortex and comprise adipocytes, smooth muscle cells and have abnormal vasculature.[13] Multiple, bilateral angiomyolipomas may occur in up to 70% of adults.[5] Complications of angiomyolipomas may include haemorrhage and mass effect.[13]

The risk for haemorrhage increases with increasing size and vascularity of the tumour.[13] The mass effect of large angiomyolipomas may lead to pain or discomfort or lead to compression of nearby structures.[13] Complications are treated either by arterial embolization of the tumour or by surgical excision.[13] Very large tumours (greater than 4cm) may be prophylactically embolized in asymptomatic patients.

Renal cysts are seen in approximately 30% of patients with TSC.[13] Cysts are usually single or few in number and are more frequently found in those with the *TSC2* mutations.[18] End stage renal disease (ESRD) is not common.[15] A French study found ESRD to occur in approximately 1 in 100 TSC patients.[15] This occurs as a result of nephron loss due to angiomyolipoma and renal cyst enlargement.[15,19]

1.2 Autosomal Dominant Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is the commonest of the inherited renal cystic diseases.[20] Prevalence rates vary around the world, but typically the disease is found in between 1 in 400 and 1 in 4033 of the population.[6,20] Two genes have been identified, namely *PKD1* on chromosome 16p13.3 which accounts for around 85% of cases, and *PKD2* on chromosome 4q21 accounting for the remainder.[20]

ADPKD is the fourth most common cause for ESRD worldwide, accounting for 5-10% of cases.[21] Half of all individuals with *PKD2* mutations develop renal failure by the age of 70 years, whereas almost all individuals with *PKD1* mutations will have renal failure by this age.[20] There is, however, marked phenotypic variability in ADPKD and the genotype-phenotype is not completely understood.[21]

The *PKD1* gene encodes for polycystin-1, which functions as a receptor and adhesion molecule in the primary cilium.[20,21] The *PKD2* gene encodes for polycystin-2 which function as a non-selective cation channel involved in the transport of calcium ions and is found in the primary cilium as well as the endoplasmic reticulum.[20,21] Both gene products are membrane proteins that likely form a complex within the primary cilium.[20]

The diagnosis of ADPKD is usually made with imaging (some cases are diagnosed by genetic testing) and typical findings would be bilaterally enlarged kidneys with multiple cysts.[21] For an individual with a family history of ADPKD, a diagnosis can be made if a total of ≥3 cysts are observed between the ages of 15 and 39.[21] In older individuals between the ages of 40 and 59, at least 2 cysts need to be observed in each kidney before a diagnosis of ADPKD is made.[21] The absence of cysts effectively excludes the diagnosis in individuals over the age of 40.[21]

Around 10-15% of individuals with ADPKD will not have a family history of the disease.[21] A proportion of these cases are as a result of *de novo* mutations or mosaicism.[21] In this instance, a diagnosis of ADPKD can be made if imaging reveals bilaterally enlarged kidneys with >10 cysts in each kidney.[21]

Genetic testing is only recommended where there is diagnostic uncertainty or when a definite diagnosis is required, such as in the case of a related living donor.[21]

1.3 *TSC-2/PKD-1* contiguous gene syndrome

The *PKD1* gene lies adjacent to the *TSC2* gene on chromosome 16 and, rarely, in approximately 2-3% of cases of TSC, large genomic deletions can result in a contiguous gene syndrome.[10,19] Almost all cases in which TSC and ADPKD have co-existed have been reported to be due to an identified deletion spanning both *PKD1* and *TSC2* genes, however, Woerner and colleagues (2006) described an unusual situation in which a family with both ADPKD and TSC, did not have *TSC2/PKD1* contiguous gene syndrome, but rather mutations on *TSC1* and *PKD2* genes.[22] This situation represents the need to consider screening *TSC1, TSC2, PKD1* and *PKD2* for subtle mutations in patients who present with less severe disease and at a later stage in life.[22]
The *TSC2/PKD1* contiguous gene syndrome, however, results in early, severe renal cystic disease and carries a poor prognosis for renal survival.[13,19] Those with the syndrome progress to ESRD earlier, typically in the third to fourth decade of life.[23,24]

1.4 Acquired cystic disease (ACD)

In 1977, the Oxford histopathologist Mike Dunnill, reported on 30 necropsies of patients who had undergone longstanding haemodialysis for ESRD.[25] Fourteen patients were noted to have extensive cystic disease of both kidneys.[25] Dunnill and colleagues described two important consequences of this cystic change – haemorrhage into cysts and tumour formation.[25] Of the 14 patients with ACD, 6 were found to have tumours.[25] While Dunnill was able to successfully establish a link between renal cell carcinoma and ESRD, the prevalence reported was disproportionately high.

ACD, by definition, is the presence of at least 3 cysts in a kidney of a patient with renal failure who has not had cysts prior to the onset of renal failure.[26] However, individuals with ACD generally have many more than 3 cysts and, at autopsy, cysts have been found to occupy at least 25% of the kidney parenchyma.[26] The kidneys are generally of a normal size or only slightly enlarged, but not to the size of those seen in ADPKD.[26]

The pathogenesis of ACD is not completely understood.[27] Nephron loss from any disease may lead to compensatory hypertrophy of parenchyma manifesting as cysts.[27]

Cysts are sometimes found in patients with ESRD prior to the initiation of dialysis but ACD is more readily found among the dialysis population, and in particular, those undergoing haemodialysis.[26] Length of time on dialysis (more than 3 years) was the greatest risk factor for the development of ACD.[18] The prevalence of ACD among the dialysis population varies with the population group being sampled but ranges from 20-90%.[18]

1.5 Renal cell carcinoma (RCC)

RCC represents approximately 2% of cancer diagnoses globally and is more prevalent among developed countries.[28] The incidence of RCC is increasing across all countries but mortality has remained unchanged and, in some developed nations, has been decreasing.[28] The Global Cancer Observatory estimated 403 262 new cases and 175 098 deaths due to RCC in 2018.[29]

The incidence tends to increase with age and men are twice as likely to be affected than women.[28]

The most established risk factors for the development of RCC include obesity, hypertension and smoking.[28] Further associations have been established via epidemiological studies and these include chronic kidney disease, haemodialysis and acquired cystic kidney disease.[17–19,21]

A number of syndromes have specific germline mutations which lead to an increased risk of RCC.[28,31] These include von Hippel-Lindau syndrome, succinate dehydrogenase-associated kidney cancer, Birt-Hogg-Dube syndrome and tuberous sclerosis complex, among others.[28,31] RCC occurring below the age of 45, may suggest the presence of a hereditary predisposing syndrome[28]

Additionally, genome-wide association studies have identified a total of 13 loci which increase susceptibility to the development of RCC.[31] These include loci on chromosome regions 1p32.3, 2p21, 2q22.3, 3p22.1, 3q26.2, 8p21.3, 8q24.21, 10q24.33-q25.1, 11q13.3, 11q22.3, 12p11.23, 12q24.31 and 14q24.2.[31–35]

Genes located at some of these loci have been found to be tumour suppressor genes or oncogenes which influence cancer growth. However, at some loci, no known cancer genes are identified and the underlying mechanisms to explain cancer development in these instances is not, yet, understood.[32]

By way of example, the gene *EPAS1* is found on chromosome 2p21 and encodes for the hypoxia-inducible factor 2α (HIF-2α) which is implicated in the upregulation of both vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR).[32] In the same study, a region at 11q13.3, was also associated with RCC, but this locus does not contain any known genes.[32]

Gudmundsson *et al* (2013) showed that a mutation at 8q24.21, a locus previously associated with other organ cancers, resulted in an increased susceptibility to RCC in a GWAS study of an

Icelandic population group.[35] This risk variant was located in a region which interacts with the *MYC* oncogene.[35]

The risk of RCC has not been established in ADPKD.[18] While case reports and small case series have been reported in the literature, the risk for malignancy has not been found to be greater than that for sporadic RCC which occurs among the general population.[18]

The most common neoplasms in the kidneys of individuals with TSC are angiomyolipomas, which are frequently benign.[18] Rarely, variants of angiomyolipomas may undergo malignant transformation, the incidence of which is not known.[18] RCC is an uncommon finding in TSC.[14] Nevertheless, it occurs in approximately 2-4% of patients.[28,36] The *TSC2-PKD1* contiguous gene syndrome, which leads to renal failure at an earlier age, has been reported as having a greater risk for the development of RCC.[18]

The incidence of RCC is increased in patients with ESRD, with numerous histological subtypes having been reported.[37] In one of the largest studies evaluating the spectrum of malignancy in ESRD, Tickoo *et al* (2006) described two main groups of RCC – one of which was the sporadically occurring type (papillary, clear cell and chromophobe histological subtypes) and the other was RCC unique to ESRD.[38] The latter group consisted of the most common type of tumour, which the authors named "acquired cystic disease-associated renal cell carcinoma", and a less common subtype, "clear-cell papillary RCC of end stage kidneys".[38]

Acquired cystic disease-associated renal cell carcinoma (ACD-RCC) has since been recognized as a distinct entity among the different types of RCC.[26,39] In 2016, it was incorporated into the WHO's Classification of Tumours of the Urinary System and Male Genital Organs.[40] The incidence of ACD-RCC is in the order of 1.6 to 8%.[18] This figure represents a prevalence approximately 100 times that of sporadically occurring RCC among the general population.[26,41]

The mechanisms by which ACD-RCC develops are incompletely understood. Cancer development is thought to occur because of cellular damage from an altered cellular milieu in the setting of ESRD.[30] Calcium oxalate crystals, frequently found in ACD, obstruct tubules

and are deposited in the interstitium and have been implicated in the development of ACD-RCC.[26,30] Accumulation of oxalate crystals leads to the development of oxygen free radicals and subsequent damage and alterations to DNA, thus predisposing to cancer development.[26] The exact pre-cursor lesions thought to lead to the development of malignancy have not been identified but almost all tumours appear to arise from cysts.[26]

Compared with sporadic cases of RCC, the tumours of ACD-RCC are smaller, multifocal and more likely to be bilateral.[18] While the tumours of ACD-RCC are generally said to be more indolent, Takebayashi *et al* (2000) noted that 5 of 17 patients (29%) with high grade ACD-RCC had tumour doubling times of just 6 months.[18,42] Very few cases of metastatic spread have been reported.[18,38]

Histologically, the tumours of ACD-RCC have a microcystic, macrocystic, tubulocystic, cribriform and papillary architecture with eosinophilic, granular cytoplasm containing dysplastic nuclei.[30,38] There is a high prevalence of calcium oxalate crystals which is a notable distinguishing feature more in keeping with ACD-RCC.[18,38]

Immunohistochemistry is an additional means by which ACD-RCC is differentiated from the other sub-types of RCC.[30] The most reported pattern expressed includes CD10 positivity, cytokeratin 7 (CK7) is negative or focally positive and positive staining for α-methyl-acyl-coenzyme A (AMACR or racemase).[30,39] Cytokeratin stains for AE1/AE3 are positive.[30,39]

1.6 Study Aims

This study reports on two rare cases of *TSC-2/PKD-1* contiguous gene syndrome both of whom developed ACD-RCC.

Genetic tests were used to confirm the clinical suspicion of the syndrome and attempt to identify additional genetic mutations which may have contributed to the risk for development of ACD-RCC.

CHAPTER 2: METHODOLOGY

2.1 Study Design

This study involved a retrospective review of patient hospital records as well as a prospective patient interview, physical examination and blood sample all of which took place at a single study visit.

2.2 Study Setting

The study took place in the Division of Nephrology of the Department of Internal Medicine at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). DNA extraction took place at the Department of Internal Medicine Laboratory.

2.3 Study Participants

Two patients known to the Division of Nephrology at CMJAH were invited to participate in the study.

2.4 Ethical Considerations

Written, informed consent was obtained from both study participants and ethics approval received from the University of the Witwatersrand's Human Research Ethics Committee prior to any study-related activities commenced. Institutional approval to conduct the study and access patient hospital records was obtained from the Charlotte Maxeke Johannesburg Academic Hospital management.

2.5 Funding

Funding for genetic studies of the two participants was graciously received from the University of the Witwatersrand Faculty of Health Sciences' Faculty Research Committee.

2.6 DNA Extraction

A 10ml blood sample from each patient was collected in EDTA tubes. DNA was extracted from the blood using a modified salting-out method, one which is routinely employed at the University of Witwatersrand Department of Internal Medicine laboratory. Once extracted, DNA was assessed for quality by electrophoresis and by measuring the A260/A280 and A260/A230 ratios on a NanoDrop spectrophotometer. DNA samples require an OD (A260/280) ratio value >1.8 and OD (A260/A230) ratio >1.9.

2.7 DNA Analysis

DNA samples were sent to the Centre for Proteomic and Genomic Research (CPGR) in Cape Town where the Affymetrix® OncoScan® assay was used to obtain a genome-wide profile. This assay requires at least 100ng of DNA per sample, with a concentration of at least 20ng/µl. The OncoScan® assay has been used for identifying genetic aberrations such as insertions and deletions, large copy-number variations (CNV), loss of heterozygosity as well as the detection of somatic mutations.[43]

Chromosomal microarray analysis (CMA) has an advantage over real time polymerase chain reaction (PCR) and fluorescence-*in situ*-hybridization (FISH) in that it allows for simultaneous detection of mutations across the genome, while FISH and PCR are designed to detect a limited number of genetic variants.[43]

Chromosomal microarray analysis includes two methods. Firstly, array-based comparative genomic hybridization (aCGH), is a method used to map DNA sequence copy differences between two differentially labelled genomic DNA samples (a study and a control sample).[43] Secondly, the single-nucleotide polymorphism (SNP)-array utilizes a reference population instead of a control sample.[43] Combining both methods maximizes coverage of the genome and allows for a higher yield of variant detection.[43]

Bioinformatics specialists at the CPGR provided advice on raw data extraction, quality control analysis and statistical analysis.

A NanoString® nCounter® Cancer CN Assay (NanoString Technologies, Seattle, Washington) was performed on each sample as well as three control samples from non-affected, apparently healthy individuals which were used as comparators. This assay allows for copy number quantification of 87 genes which are commonly amplified or deleted in cancer. A list of the genes can be found in Appendix A.

CHAPTER 3: RESULTS

3.1 Case Report 1

At 27 years of age, a female patient (denoted as “Participant 1” in further references to this case) presented to her local general practitioner with loss of appetite, malaise and oliguria. Investigations revealed severe renal failure and she was immediately transferred to a nearby tertiary academic hospital.

She was noted to have clinical features of tuberous sclerosis complex, namely multiple facial angiofibromas (Figure 1), periungal fibromas, a Shagreen patch (Figure 2) and dental pits. She had no history of seizures or cognitive impairment, having completed high school, and did not report a family history of inherited diseases.



Figure 1. Facial angiofibromas (adenoma sebaceum). Photograph used with patient consent.



Figure 2. Shagreen patch – a connective tissue naevus located on the lumbosacral flank.

Photograph used with patient consent.

An initial ultrasound demonstrated markedly enlarged, polycystic kidneys (this has been shown as a sagittal magnetic resonance image (MRI) in figure 3). She was commenced on haemodialysis before converting to peritoneal dialysis two months later.



Figure 3. Sagittal MRI showing bilateral, enlarged, polycystic kidneys

Seven years later, at the age of 34, the erythropoietin stimulating agent (ESA) she was receiving was stopped due to a rising haemoglobin and haematocrit. However, when her haemoglobin continued to rise despite cessation of the ESA, concerns for the development of RCC prompted further investigation.

An MRI of her abdomen demonstrated a solid mass (1.8 x 1.5 x 1.7 cm) in the upper pole of the right kidney.

A right nephrectomy was performed. The kidney measured 15 x 9.5 x 6.5 cm. No normal renal parenchyma was identified. There were numerous cysts, with the largest cyst measuring 4 cm in diameter. A well circumscribed tumour (2 x 2 x 1 cm) was present in the upper pole and a second well circumscribed tumour (1.5 x 1.5 x 1 cm) was noted in the lower pole.
Both tumours had central areas of haemorrhage.

Sections of the tumour in the upper pole showed a glandular, cystic and pseudopapillary growth pattern. The tumour cells had abundant eosinophilic, granular cytoplasm and contain oval nuclei.

Sections of the second tumour revealed a cystic, solid and pseudopapillary growth pattern with tumour cells displaying an abundant eosinophilic, granular cytoplasm and containing oval nuclei. Cytoplasmic vacuoles with calcium oxalate crystals are noted.

Table 2. Immunohistochemical staining of tumours (participant 1)

A/E 1/3	Focal positive staining in tumour cells
CK7	Negative
AMACR	Diffusely positive in tumour cells
E-cadherin	Negative

Histopathological assessment, together with immunohistochemical staining (Table 2) of both tumours suggested a diagnosis most compatible with acquired-cystic disease renal cell

carcinoma. While the immunohistochemical staining pattern presented in table 2 was common to both tumours, the tumour located in the lower pole also stained positive for MNF116 (broad spectrum cytokeratin stain), EMA (epithelial membrane antigen) and E-cadherin (epithelial cadherins).

3.2 Case Report 2:

A 32-year-old female (denoted as “Participant 2” in further references to this case), known with both TSC (Figure 4) and ADPKD had been on continuous ambulatory peritoneal dialysis since developing ESRD six years earlier. She was admitted with flank pain and haematuria which was thought to be due to the rupture of a renal cyst. A computed tomography scan of the abdomen, however, revealed a large heterogenous right renal mass.



Figure 4. Hypomelanotic macule. Photograph used with patient consent.

A nephrectomy was performed. The kidney measured 12.5 x 10 x 6.7cm. A large, well circumscribed, solid and multiloculated mass (measuring 6.5 x 5 x 5cm) was found involving the superior pole of the kidney. This mass was haemorrhagic and necrotic and lay adjacent to the renal pelvis and renal sinus. The remainder of the kidney parenchyma was occupied by multiple, dilated cysts varying in size.

Table 3. Immunohistochemical staining of tumour (participant 2)

A/E 1/3	Positive
CK7	Negative
CD10	Positive
AMACR	Diffusely positive in tumour cells
E-cadherin	Negative
EMA	Negative
Vimentin	Positive

Histological and immunohistochemical findings were most in keeping with ACD-RCC.

3.3 Genetic Analyses

3.3.1 *Chromosomal microarray*

A chromosomal microarray (CMA) performed on the DNA from both participant samples is represented as a karyoview to demonstrate all CNVs across the genome (Figure 5).

CNVs spanning regions common to both participants were found on chromosomes 3, 14, 16, 17, 20, 22 and X. These CNVs together with their type (loss of heterozygosity or gain), exact location, size (in kilobase pairs) and their respective Online Mendelian Inheritance in Man (OMIM®**)** genes are shown in tables 4-10.

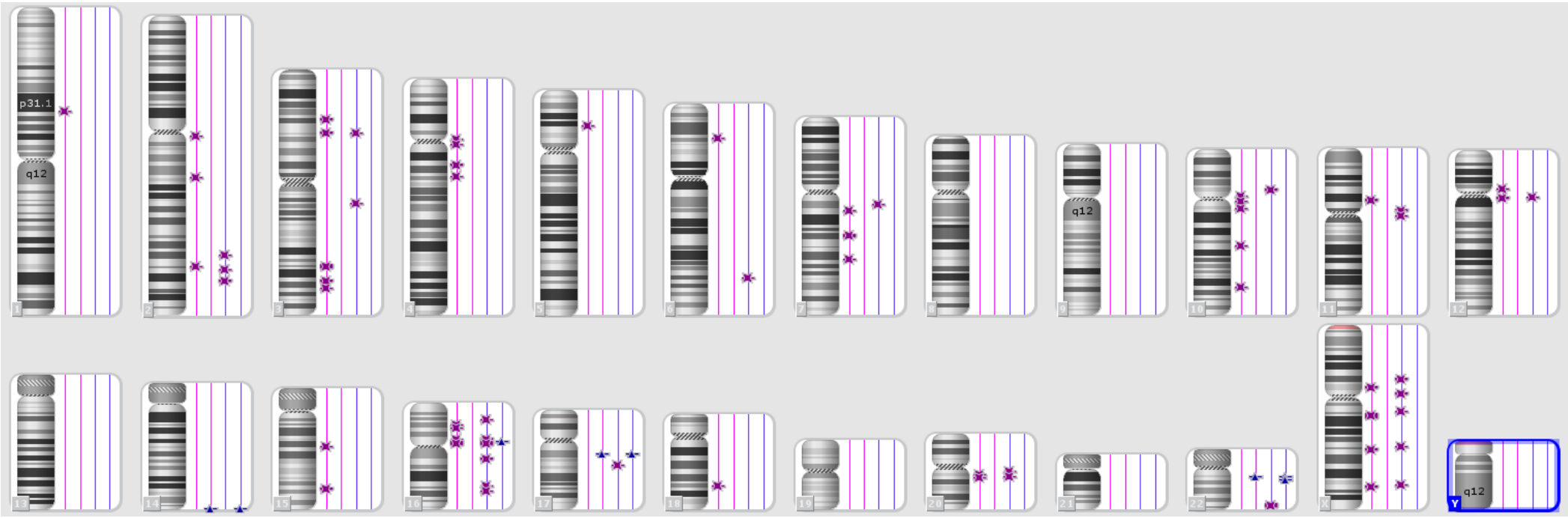

Figure 5. Karyoview of CMA showing all CNVs across the genome for both participants. CNVs for Participant 1 are shown along the pink lines, while CNVs for Participant 2 are shown along the blue lines. Asterisks mark the site of a CNV.

Table 4. CNV common to both participants located on chromosome 3

	Type	Region	Size (kbp)	OMIM® Genes
Participant 1	LOH	3p21.31-21.2	1988	SEMA3F (601124), GNAT1 (139330), SLC38A3 (604437), GNAI2 (139360), SEMA3B (601281), IFRD2 (602725), HYAL3 (604038), NAA80 (607073), HYAL1 (607071), HYAL2 (603551), TUSC2 (607052), RASSF1 (605082), ZMYND10 (607070), NPRL2 (607072), CYB561D2 (607068), TMEM115 (607069), CACNA2D2 (607082), CISH (602441), MAPKAPK3 (602130), DOCK3 (603123), MANF (601916), RBM15B (612602), DCAF1 (617259), GRM2 (604099), PARP3 (607726), GPR62 (606917), PCBP4 (608503), ACY1 (104620), RPL29 (601832), DUSP7 (602749), POC1A (614783)
Participant 2	LOH	3p21.2	1432	DOCK3 (603123), MANF (601916), RBM15B (612602), DCAF1 (617259), GRM2 (604099), PARP3 (607726), GPR62 (606917), PCBP4 (608503), ACY1 (104620), RPL29 (601832), DUSP7 (602749), POC1A (614783)

Table 5. CNV common to both participants on chromosome 14

	Type	Region	Size (kbp)	OMIM® Genes
Participant 1	Gain	14q32.3	539	FAM30A
Participant 2	Gain	14q32.3	689	FAM30A

Table 6. CNV common to both participants on chromosome 16

	Type	Region	Size (kbp)	OMIM® Genes
Participant 1	LOH	16p11.2	1429	ITGAL (153370), PRR14 (617423), FBRS (608601), SRCAP (611421), PHKG2 (172471), RNF40 (607700), BCL7C (605847), CTF1 (600435), FBXL19 (609085), ORAI3 (610930), SETD1A (611052), HSD3B7 (607764), STX1B (601485), STX4 (186591), ZNF668 (617103), PRSS53 (610561), VKORC1 (608547), BCKDK (614901), KAT8 (609912), PRSS8 (600823), PRSS36 (610560), FUS (137070), PYCARD (606838), TRIM72 (613288) PYDC1 (615700), ITGAM (120980), ITGAX (151510), ITGAD (602453), COX6A2 (602009), ARMC5 (615549), TGFB1I1 (602353), SLC5A2 (182381), AHSP (605821), ZNF267 (604752)
Participant 2	Gain	16p11.2	961	TP53TG3 (617482)
Participant 1	LOH	16p11.2-11.1	4040	TP53TG3 (617482)
Participant 2	LOH	16p11.2-11.1	4124	ZNF267 (604752), TP53TG3 (617482)

Table 7. CNV common to both participants on chromosome 17

	Type	Region	Size (kbp)	OMIM® Genes
Participant 1	Gain	17q12	368	CCL4 (182284), TBC1D3B (610144), CCL3L3 (609468), CCL3L1 (601395), CCL4L1 (603782), CCL4L2 (610757)
Participant 2	Gain	17q12	368	CCL4 (182284), TBC1D3B (610144), CCL3L3 (609468), CCL3L1 (601395), CCL4L1 (603782), CCL4L2 (610757)

Table 8. CNV common to both participants on chromosome 20

	Type	Region	Size (kbp)	OMIM® Genes
Participant 1	Gain	20q11.23	1050	DSN1 (609175), SAMHD1 (606754), RBL1 (116957), RPN2 (180490), GHRH (139190), SRC (190090), BLCAP (613110), NNAT (603106), CTNNBL1 (611537)
Participant 2	Gain	20q11.23	1124	DLGAP4 (616191), MYL9 (609905), TGIF2 (607294), SLA2 (606577), NDRG3 (605273), DSN1 (609175), SAMHD1 (606754), RBL1 (116957), RPN2 (180490), GHRH (139190), SRC (190090)

Table 9. CNV common to both participants on chromosome 22

	Type	Region	Size (kbp)	OMIM® Genes
Participant 1	Gain	22q11.22	306	GGTLC2 (612339), MIR650 (615379)
Participant 2	Gain	22q11.22	254	GGTLC2 (612339), MIR650 (615379)

Table 10. CNV common to both participants on chromosome X

	Type	Region	Size (kbp)	OMIM® Genes
Participant 1	LOH	Xq26.3	1853	CT45A1 (300648), CT45A3 (300794), CT45A5 (300796), CT45A6 (300797), CT45A2 (300793), SAGE1 (300359), SLC9A6 (300231), FHL1 (300163), MAP7D3 (300930), BRS3 (300107), HTATSF1 (300346), VGLL1 (300583), CD40LG (300386), ARHGEF6 (300267), RBMX (300199), GPR101 (300393)
Participant 2	LOH	Xq26.3	1288	MOSPD1 (300674), RTL8C (300213), ZNF75D (314997), ZNF449 (300627), CT45A1 (300648), CT45A3 (300794), CT45A5 (300796), CT45A6 (300797), CT45A2 (300793), SAGE1 (300359), SLC9A6 (300231), FHL1 (300163)

CNVs involving known RCC susceptibility loci were specifically searched for. Participant 1 was found to have a 2468 kbp LOH CNV on chromosome 3p22.1 as well as a 1999 kbp LOH mutation spanning chromosome 3q26.2-q26.31.

No CNVs were identified in the regions coding for *TSC1* (9q34.13), *TSC2, PKD1* (16p13.3) nor *PKD2* (4q22.1).

3.3.1 *NanoString® nCounter® Cancer CN Assay*

An assay quality control check of the samples revealed a co-efficient of determination for the positive control probes of R^2 = 0.99. Thereafter, restriction fragmentation controls were performed using a set of four DNA controls that, when added to the DNA sample prior to fragmentation, assess the efficiency by which enzymatic digestion and heat denaturation takes place.

The DNA targets for probes labelled “restriction site A” and “restriction site B” contain an *Alu*I enzyme restriction site such that, after complete digestion, the target site will be cleaved by the enzyme and low probe count will be observed. The DNA targets for probes labelled “restriction site C” and “restriction site D” do not contain *Alu*I enzyme sites. As a result, probe counts will be generated even in the absence of fragmentation. If the DNA sample is not denatured prior to hybridization, low counts for “restriction site C” and “restriction site D” probes will be observed.

When the genomic DNA sample is completely digested with *Alu*I enzyme and denatured, one should observe at least a 10-fold difference in counts between “restriction site probes A and B” and “restriction site probes C and D”. A more than 28-fold difference between restriction sites was noted across all sampled DNA.

Raw counts of genes were normalized to the geometric mean to correct for DNA quality and input across samples. Copy numbers were estimated for each probe relative to three reference (control) samples. Copy number estimates were then generated by determining the ratio of counts from test samples to the counts of the three reference samples and calculating copy numbers relative to that reference sample.

Analysis of results of the NanoString® nCounter® Cancer CN Assay identified 3 CNVs common to both reported cases as compared to control samples. A gain in CNV on chromosome

12p12.1 in the region encoding for the KRAS gene (molecular location: 25285835-25285922) was observed in both patients. There was a loss in CNV on chromosome 13q13.1 in the region encoding for the BRCA2 gene (molecular location: 31797932-31798017). A loss in CNV was also observed at chromosome 1q32.1 (molecular location: 202763368-202763467) of which the protein product is MDM-4.

All CNVs identified with the NanoString® nCounter® Cancer CN Assay are shown in figures 6 and 7 below.

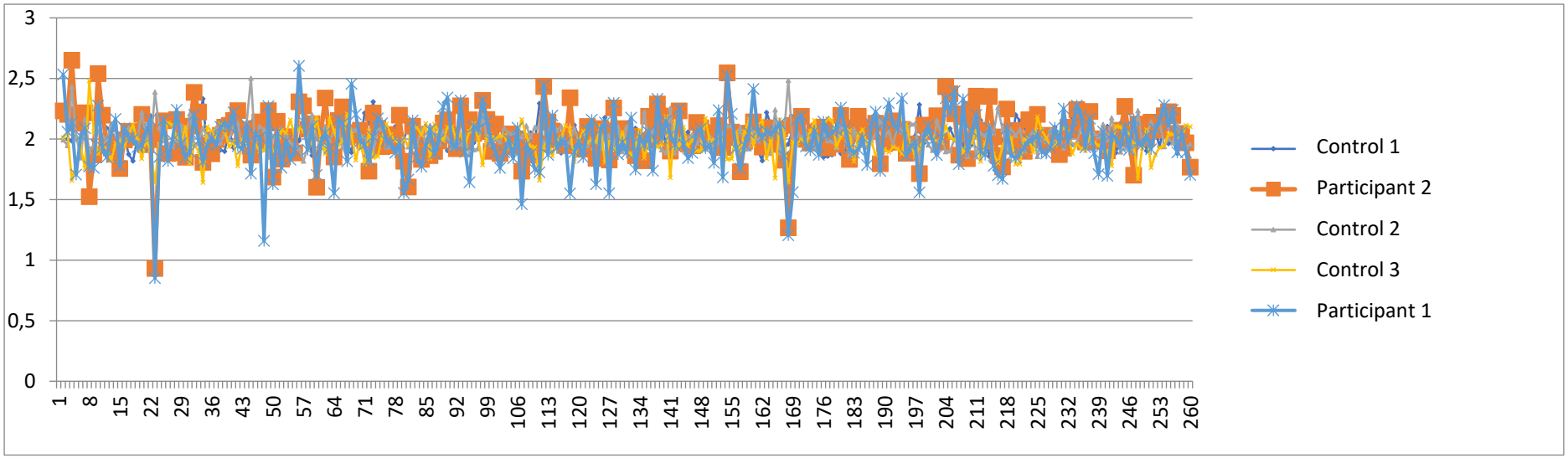

Figure 6. CNVs for 87 common cancer genes in 2 cases of ACD-RCC in *TSC2-PKD1* contiguous gene syndrome as compared to 3 healthy controls.

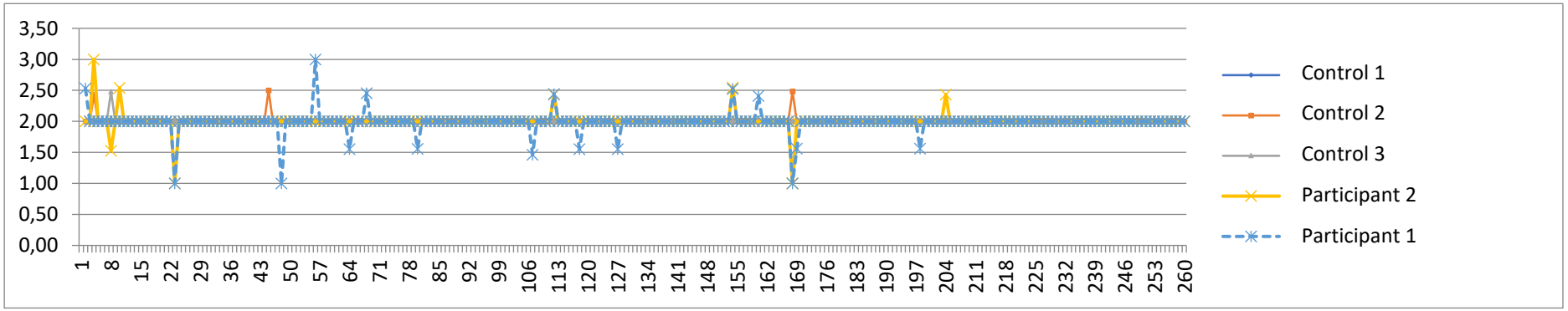

Figure 7. Graph showing only significant CNVs (losses <1.5 and gains >2.5) for 87 common cancer genes in 2 cases of ACD-RCC in *TSC2-PKD1* contiguous gene syndrome as compared to 3 healthy controls.

CHAPTER 4: DISCUSSION

In this study, we have reported on two individuals with phenotypic features of the *TSC2/PKD1* contiguous gene syndrome who developed ESRD in the third decade of life. Both patients developed ACD-RCC following several years of dialysis.

No mutation was identified on chromosome 16p13.3 in both cases. Further to this, no mutations were identified in any of the *TSC1*, *TSC2*, *PKD1* or *PKD2* genes. In addition to technical failures, there are a few possibilities which may explain the absence of mutations.

In 10-25% of cases of TSC, no mutation is identified by conventional genetic testing.[1,44] A study by Tyburczy *et al* (2015) evaluated 53 cases of TSC in which no mutation was identified and found that the majority of these cases (85%) were accounted for by somatic mosaicism, and to a lesser extent by intronic mutations.[44] A less favoured hypothesis is that of a third, as yet unidentified TSC gene.[44,45]

Mosaicism has also been described as one of the causes for no mutation being identified in the *TSC2/PKD1* contiguous gene syndrome.[46,47] In one study, 7 of 25 patients with *TSC2/PKD1* contiguous gene syndrome were found to have mosaicism.[47] Rarely, separate mutations involving *TSC1* and *PKD2* genes may lead to a clinical syndrome which is phenotypically indistinct from the *TSC2*/*PKD1* contiguous gene syndrome.[22]

Some of the techniques used for identifying mosaicism include deep next generation sequencing (NGS) and Sanger sequencing.[45] NGS applies new methods and approaches to the detection of mutations and increases the yield of detection of pathogenic mutations.[45] When somatic mosaicism is suspected and conventional genetic testing has not identified a mutation, further DNA testing of other tissues is warranted as well as the use of these alternative techniques.

Copy number variants (CNV) are an important means by which normal or pathogenic variations in the human genome are identified.[48] The clinical interpretation of CNVs does, however, pose a challenge. One needs to determine which variants are benign and inconsequential, and which are pathogenic, and thus leading to disease.[48]

Two susceptibility loci which predispose to the development of RCC were identified in participant 1, namely mutations in the regions 3p22.1 and 3q26.2.[31] Mutations in 3q26.2 are found in 15% of patients with clear cell RCC.[31] No mutations known to predispose to RCC were identified in participant 2.

The NanoString® nCounter® Cancer CN Assay performed in this study allowed us to identify CNVs across 87 commonly deleted or mutated cancer genes. Both patients were found to have CNVs in 3 potentially pathogenic regions, namely MDM-4, BRCA-2 and KRAS.

The MDM-4 (mouse double minute-4) gene is found on chromosome 1q32.1 and encodes a protein that inhibits p53, a tumour suppressor gene with abilities to arrest the cell cycle and promote apoptosis.[49,50] Inhibition of p53 is important in tumour development, progression and metastasis, thus overexpression of MDM-4 contributes to tumour formation.[49] The association between p53 and CNVs is a strong one and is seen across the cancer spectrum.[50] p53 is rarely expressed in RCC.[50]

The BRCA-2 gene is a tumour suppressor gene located on chromosome 13q12.[51] Pathogenic mutations in this gene render affected individuals at high risk of both breast and/or ovarian cancer but is not recognized as a risk factor for renal cell carcinoma.[51] However, mutations are increasingly being observed in other cancers, such as lung, prostate and pancreatic cancer, and thus the role of BRCA-2 in the aetiology of RCC cannot be excluded.[51]

RAS mutations are found in up to 30% of all cancers.[52] Kirsten RAS (KRAS) is the most frequent mutation in the oncogene family of RAS mutations and encodes a protein implicated in a number of malignancies, including lung cancer, colorectal cancer, breast cancer and pancreatic cancer.[52] A single nucleotide substitution is responsible for activating the mutation.[52] KRAS is not recognized as a predisposing mutation for the development of RCC.[52]

CHAPTER 5: CONCLUSION

In conclusion, this dissertation has described two exceptionally rare cases in which three types of cystic kidney disease was found – that of tuberous sclerosis complex, autosomal dominant polycystic kidney disease and acquired cystic disease related to dialysis. Both patients developed acquired cystic disease-associated renal cell carcinoma necessitating nephrectomy.

Genetic analysis revealed CNVs in 3 common cancer genes (KRAS, BRCA-2 and MDM-4) in both patients, however, none of the genes are known to lead to an increased risk for RCC. One study participant had mutations in 2 of 13 known loci resulting in susceptibility to RCC. Further studies are needed to evaluate for susceptibility loci which may play a role in the development of ACD-RCC among the dialysis population.

In both individuals, no mutation could be identified to genotypically explain the syndrome. This occurs in 10-25% of cases and is usually due to somatic mosaicism. Further genetic studies would be required to confirm this.

Additionally, due to the heterogenous nature of ACD-RCC tumours, molecular testing in the form of targeted next generation sequencing of tumour tissue may provide further information on mutations acquired by the tumours.

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APPENDIX A

NanoString® nCounter® Cancer CN Assay Gene List							
AKT2	C8orf4	DCC	GAB2	MAGI3	MYC	PDGFRA	TERT
AKT3	CCND1	DCUN1D1	GPC5	MAP2K4	MYCL1	PIK3CA	TP53
APC	CCND2	DYRK2	GRB2	MAP3K5	MYCN	PRKCI	TP73
AR	CCNE1	E2F3	HMGA2	MAPK7	NCOA3	PTEN	TRAF2
AURKA	CDK4	EEF1A2	IGF1R	MCL1	NF1	PTPRD	VEGFA
BBC3	CDK6	EGFR	IRS2	MDM2	NKX2-1	RB1	WHSC1L1
BCL2L1	CDKN1A	ERBB2	ITGB4	MDM4	NKX2-8	PEG4	WT1
BCL2L2	CDKN2A	FADD	JUN	MELK	OROAV1	REL	YAP1
BIRC2	CDKN2C	FGFR1	KDR	MET	PARK2	RPS6KB1	YWHAZ
BRCA1	CRKL	FHIT	KIT	MITF	PAX9	SHH	ZNF217
BRCA2	CSMD1	FOXO1	KRAS	MYB	PDE4D	SKP2	

Further information regarding the assay can be obtained from www.nanostring.com.

APPENDIX B

All genes identified in both participants by means of the NanoString® nCounter® CN Assay are displayed in the table below. Highlighted values represent significant CNV findings (losses <1.5 and gains >2.5).

Gene	Location	Participant 1	Participant 2
TP73-1	chr1:3602323-3602398	2,231982	2,53303
TP73-2	chr1:3618806-3618881	2,20322	2,059844
TP73-3	chr1:3635526-3635605	2,650622	2,136267
MYCL1-1	chr1:40133428-40133522	2,130982	1,705846
MYCL1-2	chr1:40138157-40138251	2,147701	2,023819
MYCL1-3	chr1:40140830-40140923	2,215414	2,08786
CDKN2C-1	chr1:51208860-51208959	1,524824	1,774036
CDKN2C-2	chr1:51208985-51209084	1,870013	1,759946
CDKN2C-3	chr1:51211185-51211284	2,540445	2,275166
JUN-1	chr1:59019662-59019761	2,197491	1,931497
JUN-2	chr1:59021204-59021279	1,976378	1,848393
JUN-3	chr1:59022170-59022254	1,924166	2,046772
MAGI3-1	chr1:113768227-13768296	1,874349	2,166528
MAGI3-2	chr1:113879304-13879403	1,755418	1,777388
MAGI3-3	chr1:113994548-13994647	1,993388	2,088895
REG4-1	chr1:120140232-20140331	2,063742	2,009042
REG4-2	chr1:120146328-20146425	2,013602	2,002392
REG4-3	chr1:120151633-20151720	2,061178	1,934053
MCL1-1	chr1:148816829-48816928	2,203669	1,946279
MCL1-2	chr1:148817556-48817635	1,953935	2,064298
MCL1-3	chr1:148817804-48817888	1,99526	2,158008
MDM4-1	chr1:202763368-02763467	0,930503	0,851183
MDM4-2	chr1:202773602-02773701	2,054177	1,850247
MDM4-3	chr1:202784295-02784394	2,149634	2,155668
AKT3-1	chr1:241773455-41773554	1,880387	1,81828
AKT3-2	chr1:241906439-41906536	1,886627	1,989781
AKT3-3	chr1:242035384-42035483	2,16123	2,240682
MYCN-1	chr2:16001194-16001286	1,972583	1,962376
MYCN-2	chr2:16001697-16001791	1,847961	1,845727
MYCN-3	chr2:16002411-16002505	2,105679	1,894206
REL-1	chr2:60966136-60966235	2,383605	2,19827
REL-2	chr2:60974704-60974803	2,222019	1,985854
REL-3	chr2:60991209-60991303	1,806563	1,811634
FHIT-1	chr3:59797020-59797109	1,947318	1,951911
FHIT-2	chr3:60098286-60098372	1,877742	2,007337
FHIT-3	chr3:60419678-60419777	1,994708	1,951807
MITF-1	chr3:69914121-69914220	2,03061	2,101333

Gene	Location	Participant 1	Participant 2
MITF-2	chr3:69997161-69997260	2,096024	2,115824
MITF-3	chr3:70077697-70077786	2,112436	2,100116
PRKCI-1	chr3:171427028-71427127	2,152662	2,221744
PRKCI-3	chr3:171492743-71492836	2,234872	1,941166
PIK3CA-1	chr3:180415123-80415222	2,032118	1,91971
PIK3CA-2	chr3:180423950-80424049	2,075957	2,116905
PIK3CA-3	chr3:180432391-80432478	1,865901	1,714325
DCUN1D1-1	chr3:184148431-84148518	2,086732	2,034633
DCUN1D1-2	chr3:184163826-84163925	2,140915	2,010465
DCUN1D1-3	chr3:184178796-84178895	1,981375	1,159425
PDGFRA-1	chr4:54790278-54790365	2,234181	2,272152
PDGFRA-2	chr4:54819525-54819624	1,684328	1,626231
PDGFRA-3	chr4:54849381-54849480	2,14453	2,053103
KIT-1	chr4:55225010-55225109	1,833208	1,762292
KIT-2	chr4:55259887-55259986	2,018447	1,982583
KIT-3	chr4:55289855-55289948	1,909543	1,828389
KDR-1	chr4:55645279-55645369	1,884327	1,901293
KDR-2	chr4:55665006-55665100	2,306497	2,604407
KDR-3	chr4:55680917-55681016	2,273203	2,143635
TERT-1	chr5:1310215-1310284	2,08153	1,909886
TERT-2	chr5:1327132-1327206	2,12577	2,106619
TERT-3	chr5:1345100-1345175	1,602222	1,702679
SKP2-1	chr5:36204032-36204126	2,047787	1,967796
SKP2-2	chr5:36209814-36209913	2,337084	2,049738
SKP2-3	chr5:36216363-36216462	2,020823	1,923449
PDE4D-1	chr5:58311135-58311222	1,850472	1,552487
PDE4D-2	chr5:58337667-58337754	2,151963	2,160088
PDE4D-3	chr5:58365055-58365154	2,264561	2,126774
APC-1	chr5:112087874-12087970	2,015125	1,814971
APC-2	chr5:112136660-12136754	2,053507	2,454379
E2F3-1	chr6:20520166-20520264	2,030508	2,203302
E2F3-2	chr6:20555305-20555404	2,072614	1,982371
E2F3-3	chr6:20587986-20588067	1,983963	1,953513
CDKN1A-1	chr6:36760296-36760371	1,734532	1,823102
CDKN1A-2	chr6:36760902-36760995	2,213064	2,020256
CDKN1A-3	chr6:36761312-36761391	2,088284	2,170682
VEGFA-1	chr6:43848194-43848281	1,936957	2,131027
VEGFA-2	chr6:43853829-43853909	2,036819	1,984511
VEGFA-3	chr6:43858876-43858960	1,943476	1,965936
MYB-1	chr6:135547914-35548013	1,994803	1,886255
MYB-2	chr6:135563069-35563163	2,197518	1,968254
MYB-3	chr6:135576682-35576778	1,815309	1,554781
MAP3K5-1	chr6:136946961-36947060	1,603469	1,665477
MAP3K5-2	chr6:137035896-37035995	2,105963	2,15328
MAP3K5-3	chr6:137133957-37134056	2,039144	1,849568

Gene	Location	Participant 1	Participant 2
PARK2-1	chr6:161840833-61840932	1,831216	1,77148
PARK2-2	chr6:162373001-62373100	1,941373	1,943989
PARK2-3	chr6:162937714-62937813	1,86139	2,077439
EGFR-1	chr7:55072102-55072181	1,897972	1,878696
EGFR-2	chr7:55130363-55130434	1,983374	1,995809
EGFR-3	chr7:55188580-55188673	2,132521	2,266232
CDK6-1	chr7:92102617-92102714	2,157741	2,342409
CDK6-2	chr7:92192438-92192525	2,030943	1,949211
CDK6-3	chr7:92279486-92279585	1,919703	1,939613
MET-1	chr7:116136002-16136089	2,274837	2,317363
MET-3	chr7:116214262-16214361	1,972459	1,892908
SHH-1	chr7:155289361-55289435	2,15929	1,643871
SHH-2	chr7:155293246-55293317	2,045667	2,101646
SHH-3	chr7:155296679-55296752	2,062317	2,084119
CSMD1-1	chr8:2797891-2797990	2,318572	2,318921
CSMD1-2	chr8:2950237-2950336	2,161042	2,146474
CSMD1-3	chr8:3214313-3214412	1,896768	1,98307
WHSC1L1-1	chr8:38259715-38259807	2,123425	1,884987
WHSC1L1-2	chr8:38289874-38289973	1,880705	1,761586
WHSC1L1-3	chr8:38301851-38301941	1,952239	1,890891
FGFR1-1	chr8:38393783-38393874	2,042595	1,991805
FGFR1-2	chr8:38413246-38413333	1,939907	1,839512
FGFR1-3	chr8:38432656-38432745	2,001099	2,091907
C8orf4-1	chr8:40130342-40130441	1,732992	1,461745
C8orf4-2	chr8:40130490-40130589	1,889396	1,950382
YWHAZ-1	chr8:102005665-02005764	1,867811	1,873733
YWHAZ-2	chr8:102016293-02016392	1,980309	1,748848
YWHAZ-3	chr8:102026544-02026631	1,960468	1,7249
MYC-1	chr8:128818714-28818790	2,433016	2,441646
MYC-2	chr8:128820773-28820868	2,13857	1,870045
MYC-3	chr8:128821228-28821321	2,065441	2,194403
PTPRD-1	chr9:8346725-8346824	1,993479	1,9233
PTPRD-2	chr9:8518998-8519086	1,976772	1,986051
PTPRD-3	chr9:8685055-8685139	1,946281	1,972111
CDKN2A-1	chr9:21963369-21963468	2,340609	1,550564
CDKN2A-2	chr9:21964186-21964285	1,943834	1,89273
CDKN2A-3	chr9:21965829-21965928	1,992136	1,98096
MELK-2	chr9:36619719-36619818	1,914601	1,849032
MELK-3	chr9:36657010-36657101	2,104029	2,077904
TRAF2-1	chr9:138914852-38914925	2,007091	2,158438
TRAF2-2	chr9:138925356-38925439	1,841006	1,625251
TRAF2-3	chr9:138937951-38938044	2,085553	2,076201
PTEN-1	chr10:89622208-89622302	2,069136	2,137688
PTEN-2	chr10:89665411-89665493	1,82774	1,552141
PTEN-3	chr10:89706094-89706193	2,256366	2,29741

Gene	Location	Participant 1	Participant 2
WT1-1	chr11:32371946-32372045	1,975693	1,872325
WT1-2	chr11:32389362-32389461	2,084893	1,976197
WT1-3	chr11:32399750-32399849	1,971057	1,886306
CCND1-1	chr11:69166461-69166560	2,021962	2,175531
CCND1-2	chr11:69170198-69170280	1,861209	1,747497
CCND1-3	chr11:69173802-69173877	1,984142	2,019358
ORAOV1-1	chr11:69192231-69192301	1,81937	1,949019
ORAOV1-2	chr11:69195254-69195353	2,187906	2,056576
ORAOV1-3	chr11:69198440-69198539	1,992169	1,737621
FADD-1	chr11:69728086-69728161	2,289596	2,331257
FADD-2	chr11:69729344-69729423	2,099816	2,008728
FADD-3	chr11:69729830-69729919	2,189796	2,146818
GAB2-1	chr11:77626666-77626749	1,900245	2,030468
GAB2-2	chr11:77707489-77707575	2,044229	1,953437
GAB2-3	chr11:77788065-77788164	2,231075	2,250415
YAP1-1	chr11:101500813-101500906	1,971167	1,954606
YAP1-2	chr11:101545947-101546046	1,960657	1,842065
YAP1-3	chr11:101593600-101593690	1,943345	1,884401
BIRC2-1	chr11:101729215-101729314	2,137378	2,051425
BIRC2-2	chr11:101739387-101739486	2,093408	2,085178
BIRC2-3	chr11:101751234-101751321	2,051604	1,922928
CCND2-1	chr12:4255700-4255794	1,980757	1,946674
CCND2-2	chr12:4266788-4266877	2,016647	1,803097
CCND2-3	chr12:4276394-4276476	2,109502	2,237374
KRAS-1	chr12:25261832-25261919	1,928019	1,682542
KRAS-2	chr12:25275152-25275241	2,546541	2,523358
KRAS-3	chr12:25285835-25285922	2,055934	2,206882
CDK4-1	chr12:56428625-56428714	2,013321	1,92112
CDK4-2	chr12:56430637-56430731	1,730046	1,756783
CDK4-3	chr12:56430858-56430945	1,980357	1,951815
HMGA2-1	chr12:64520510-64520597	2,047567	2,049682
HMGA2-2	chr12:64573245-64573328	2,141094	2,412839
HMGA2-3	chr12:64628802-64628886	2,010642	2,060433
DYRK2-1	chr12:66329702-66329771	1,933728	2,01264
DYRK2-2	chr12:66332211-66332310	2,092369	2,062604
DYRK2-3	chr12:66335651-66335738	1,954856	2,04374
MDM2-1	chr12:67498111-67498194	1,957423	2,073165
MDM2-2	chr12:67504615-67504714	2,083968	2,129915
MDM2-3	chr12:67518205-67518304	1,824188	1,859472
BRCA2-1	chr13:31797932-31798017	1,266796	1,204602
BRCA2-2	chr13:31828061-31828160	2,112903	1,562417
BRCA2-3	chr13:31863547-31863624	2,133753	2,158622
FOXO1-1	chr13:40041119-40041218	2,186529	2,190107
FOXO1-2	chr13:40085696-40085795	1,998285	1,939021
FOXO1-3	chr13:40128074-40128173	1,966788	1,905697

Gene	Location	Participant 1	Participant 2
RB1-1	chr13:47791833-47791930	2,055985	2,067311
RB1-2	chr13:47865403-47865492	2,065617	1,869441
RB1-3	chr13:47937609-47937708	2,095951	2,14319
GPC5-1	chr13:90980155-90980251	1,926558	2,030024
GPC5-2	chr13:91591905-91591989	2,010192	2,06
GPC5-3	chr13:92170317-92170414	1,954262	2,07444
IRS2-1	chr13:109210026-109210110	2,19499	2,25763
IRS2-2	chr13:109221410-09221497	2,092547	2,088021
IRS2-3	chr13:109233159-109233228	1,832016	1,92195
BCL2L2-1	chr14:22847449-22847524	2,042074	1,887008
BCL2L2-2	chr14:22847588-22847672	2,187243	1,913992
BCL2L2-3	chr14:22851661-22851760	2,08458	2,012074
NKX2-1-1	chr14:36057200-36057271	2,066927	1,784287
NKX2-1-2	chr14:36057430-36057500	2,088569	2,122514
NKX2-8-1	chr14:36120550-36120625	2,128956	2,226295
NKX2-8-2	chr14:36120900-36120982	1,794961	1,73588
PAX9-1	chr14:36202567-36202656	1,962374	2,133408
PAX9-2	chr14:36208293-36208392	2,101147	2,297054
PAX9-3	chr14:36213906-36214005	2,151058	2,123562
IGF1R-1	chr15:97040030-97040129	1,972761	2,084609
IGF1R-2	chr15:97164717-97164804	2,079514	2,33535
IGF1R-3	chr15:97284983-97285082	1,883282	1,862175
TP53-1	chr17:7514574-7514654	1,939274	1,941048
TP53-2	chr17:7521469-7521553	1,958752	1,971348
TP53-3	chr17:7528081-7528180	1,714397	1,560111
MAP2K4-1	chr17:11875724-11875823	2,113518	2,01017
MAP2K4-2	chr17:11924608-11924707	2,092529	2,105445
MAP2K4-3	chr17:11973304-11973398	2,059019	1,986814
MAPK7-1	chr17:19223237-19223327	2,191856	1,867405
MAPK7-2	chr17:19224105-19224204	1,975203	2,010273
MAPK7-3	chr17:19226491-19226580	2,431064	2,336387
NF1-1	chr17:26474985-26475084	2,373256	2,222035
NF1-2	chr17:26588947-26589046	2,206518	2,396482
NF1-3	chr17:26700783-26700882	1,866433	1,791164
ERBB2-1	chr17:35112859-35112934	2,184183	2,328316
ERBB2-2	chr17:35124948-35125029	1,838067	1,897383
ERBB2-3	chr17:35135268-35135347	2,245334	2,06431
BRCA1-1	chr17:38497832-38497931	2,353866	2,19768
BRCA1-2	chr17:38511129-38511228	2,072472	1,896735
BRCA1-3	chr17:38528808-38528907	1,98242	1,870004
RPS6KB1-1	chr17:55328200-55328299	2,351039	2,087941
RPS6KB1-2	chr17:55346679-55346778	2,018069	1,778543
RPS6KB1-3	chr17:55351204-55351293	1,864205	1,70002
GRB2-1	chr17:70835466-70835565	1,769816	1,67079
GRB2-2	chr17:70864411-70864501	2,246782	2,086803

Gene	Location	Participant 1	Participant 2
GRB2-3	chr17:70894369-70894463	1,939725	1,863025
ITGB4-1	chr17:71235454-71235534	2,070427	1,843592
ITGB4-2	chr17:71249483-71249574	1,979065	1,886789
ITGB4-3	chr17:71261870-71261948	1,898137	1,9334
DCC-1	chr18:48203915-48204014	2,157976	1,988768
DCC-2	chr18:48759887-48759980	2,005464	1,995698
DCC-3	chr18:49235727-49235826	2,203421	2,035657
CCNE1-1	chr19:34996365-34996462	1,997328	1,893816
CCNE1-2	chr19:35000303-35000396	2,029832	1,879631
CCNE1-3	chr19:35005181-35005262	1,965619	1,930629
AKT2-1	chr19:45434936-45435017	1,981893	2,095097
AKT2-2	chr19:45447104-45447181	1,870356	1,972235
AKT2-3	chr19:45459758-45459852	1,923368	2,252611
BBC3-1	chr19:52417146-52417223	2,096736	1,979827
BBC3-2	chr19:52418885-52418972	2,07145	2,081554
BBC3-3	chr19:52422518-52422598	2,243437	2,275475
BCL2L1-1	chr20:29723120-29723219	2,151249	2,190397
BCL2L1-2	chr20:29743874-29743971	1,975753	1,930676
BCL2L1-3	chr20:29768021-29768115	2,226693	2,168118
NCOA3-1	chr20:45686809-45686905	1,980843	1,882122
NCOA3-2	chr20:45700129-45700228	2,001719	1,71034
NCOA3-3	chr20:45712217-45712316	1,992091	2,060991
ZNF217-1	chr20:51622866-51622960	1,901616	1,697304
ZNF217-2	chr20:51626779-51626878	2,039553	2,03662
ZNF217-3	chr20:51631781-51631874	2,002315	2,007873
AURKA-1	chr20:54380758-54380852	2,072298	1,932395
AURKA-2	chr20:54387730-54387829	2,269636	2,099598
AURKA-3	chr20:54395073-54395172	2,007575	1,910665
EEF1A2-1	chr20:61591105-61591192	1,701647	2,163332
EEF1A2-2	chr20:61595035-61595122	2,123542	1,964594
EEF1A2-3	chr20:61598804-61598881	2,02687	1,950241
CRKL-1	chr22:19605393-19605480	2,002882	2,028826
CRKL-2	chr22:19618876-19618975	2,138137	1,915815
CRKL-3	chr22:19631240-19631331	2,075758	2,082147
Xp11.3	chrX:42638800-42638899	2,07574	2,156349
Xp11.21	chrX:55020685-55020784	2,192509	2,27958
AR-1	chrX:66690656-66690755	2,225756	2,109982
AR-2	chrX:66771034-66771133	2,194861	2,240211
AR-3	chrX:66856290-66856369	2,045737	1,888738
Xq13.1	chrX:71869531-71869630	2,026269	2,058866
Xq21.31	chrX:87113332-87113431	1,967832	1,888264
Xq21.32	chrX:93197592-93197691	1,767009	1,704056