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Clinical evaluation of pediatric patients with solid tumours, for features of cancer predisposition syndromes in Gauteng, South Africa

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Clinical evaluation of paediatric patients with solid tumours, for features of Cancer Predisposition Syndromes in Gauteng, South Africa.

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A Research Report (in the format of a “submissible” paper) 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, in Medical Genetics

Johannesburg, 2022

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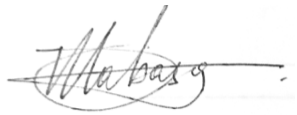
Declaration

I, **Njabulo Mabaso**, declare that this Research Report (in the format of a "submissible" paper) is my own, unaided work. It is being submitted for the Degree of Master of Medicine (MMed) in Medical Genetics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(signature of candidate)

22nd day of June in 2022 in Johannesburg

Dedication

This research report is dedicated to my family and my colleagues in the Division of Human Genetics, School of Pathology, University of the Witwatersrand.

Presentation arising from this research project

Njabulo Mabaso, Candice Feben, Lindiwe Lamola, Jennifer Geel, Gita Naidu, Janet Poole, and Amanda Krause. Development of a guideline to identify cancer predisposition syndromes in paediatric solid tumour patients in a low resource setting - Gauteng, South Africa. A poster was presented at Curating Clinical Genome meeting 2022, held virtually on 07 June 2022.

Research summary

Context: Malignant solid tumours are relatively uncommon in children when compared to adult cancer. The development of cancer in children provides an opportunity for understanding basic cancer pathogenesis on the background of minimal exposure to environmental carcinogens in a child's life. This study aimed at evaluating the factors that assist in determining whether the development of cancer in children is part of a cancer predisposition syndrome (CPS).

There is international consensus that about 8% - to – 10% of children with solid tumours harbour a genetic (familial or *de novo*) predisposition to cancer. There is also a growing interest in understanding childhood cancer predisposition to potentially develop novel therapies for treatment, as well as develop new approaches to diagnosing and prognosticating in childhood cancers.

Understanding genetic contribution to childhood cancer profiles would help manage other predicted health risks in the proband and also mitigate risks to other at-risk family members. Additionally, there is limited knowledge of childhood cancer profiles on the African continent. In this study, the researchers were aiming to determine the significance of genetic predisposition to childhood cancers in Gauteng, South Africa, as determined clinically, and contribute towards the development of clinical guidelines that can be utilized by paediatricians (or paediatric oncologists) in identifying patients that would benefit from a clinical genetics consultation. This study was nested in a larger study where detailed molecular genetic analysis will also be assessed and correlated with clinical features.

Objectives:

1. To obtain three-generation family histories from paediatric patients with solid tumours (non-haematological).
2. To utilize a clinical tick sheet designed by the researcher to identify clinical features relevant to inherited cancer predisposition syndromes (CPSs), including facial dysmorphic features, congenital abnormalities and benign tumours.
3. To assess whether there are criteria identifiable through family history, clinical examination, or histology that identify a likely CPS.

4. To develop clinical guidelines that will aid clinicians in identifying patients who need to be referred for further medical genetic assessment and testing.

Study Design, Settings and Patients:

A cross-sectional, descriptive study of 62 South Africanpatients with solid tumours, between the ages of 1 year and 18 years, recruited from Paediatric Oncology Clinics in tertiary academic hospitals within Gauteng, South Africa.

Main Outcomes Measured: Demographic characteristics (age, ethnicity and sex), personal medical history, family history, tumour histological features, growth and development, clinical features, unusual facial features and skin pigmentary changes. Ethnicity was measured because participants of African descent are often underrepresented in cancer studies.

Results: Among the 62 study participants, 55 were Africans, three were of mixed ancestry, and two each were Asians & Caucasians. The majority (63%) were diagnosed between 0 – 5 years of age ($p < 0.001$). Among participants, 44/62 (71%) patients were classified as 'likely CPS', whereas 18/62 (29%) were 'unlikely CPS'. Amongst 'likely CPS' cases, the majority of patients (33/44; 75%) were identified based on personal history (PH) criteria, whereas 9/44 (20.5%) were based on histology criteria and 2/44 (4.5%) were based on family history criteria. Amongst the "likely CPS'' cases, 33/44 (75%) patients had pigmentary abnormalities, 19/44 (43.2%) had macrocephaly, and 35/44 (79.5%) had syndromic features (recognized (n = 7) vs unrecognized (n = 28); highlighting that these outcome measures should not be underestimated in clinical practice.

Discussion: In this study we have demonstrated that clinically determining the possibility of a CPS in an individual or their family will help clinicians (paediatricians or paediatric oncologists) understand which of their patients (and their patient's families) will benefit from clinical genetics services. We propose a guideline, that histology should be considered first, followed by family history criteria and then lastly personal history criteria in determining the likely CPS status of an individual, for them to be considered for genetic evaluation. Whenever a positive germline genetic test result is found, heightened screening of the individual and their family (often referred to as 'at risk relatives') would be implemented to improve their general health outcomes — meaning early surveillance (i.e. to reduce mortality and morbidity from the familial

cancer syndrome). In order to streamline referral; the following steps (in order of priority) should be followed:

1. Histology in keeping with likely CPS – immediate referral for genetic testing or discuss with a medical geneticist on a genetic test to order.
2. Family pedigree analysis in keeping with likely CPS – refer for genetic testing or discuss with the genetic counsellor for selecting a genetic test.
3. Clinical examination suggestive of a genetic syndrome – refer to medical geneticist for evaluation and appropriate order of a genetic test.

We have further argued that family history and tumour histology alone are not sufficient, exclusively, to identify a "likely CPS" case; and that many require assessment by a medical geneticist to identify unusual features and diagnose underlying genetic syndromes. Specific clinical features that may predict a CPS remain to be correlated with molecular studies.

Conclusions: This study identified a high number of participants with likely CPS who would benefit from a clinical genetics assessment. In paediatric oncology, testing for an underlying CPS could benefit impacted families and aid in exercising their right to know a familial mutation. This study may be helpful for the management of solid tumours in children and their families.

Keywords: Cancer predisposition syndrome, paediatric solid tumours, genetics of paediatric cancer.

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I would like to thank my supervisors, Professor Amanda Krause, Dr Candice Feben and Dr Lindiwe Lamola, for their academic support and guidance throughout this research report. To my primary supervisor, Amanda, thank you for the continued mentorship that you have offered me over the past couple of years. Your dedication to your work and research is truly inspiring. Thank you for your continuous support in developing my research career.

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Special thanks to my partner, Fikile Mpofana and my parents Nhlanhla and Nozinto Mabaso for their support, prayers and love.

Lastly, I would like to thank the patients with solid tumours and their families for their willingness to participate, without whom this research would not have been possible.

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List of abbreviations

BWS	Beckwith-Wiedemann Syndrome
CA	Cancer
CALs	Café au Lait spots/ Macules
CHBAH	Chris Hani Baragwanath Academic Hospital
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CPS	Cancer Predisposition Syndrome
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
FH	Family History
FHC	Family History Criteria
GPV	Germline Pathogenic Variants
Hist	Histology
Kg	Kilogram
MEN2A	Multiple Endocrine Neoplasia type 2
MIPOGG	McGill Interactive Paediatric OncoGenetic Guidelines
Mm	Millimeter
MMR	Mismatch repair
NHLS	National Health Laboratory Service
NF1	Neurofibromatosis type 1
PH	Personal History
PHC	Personal history criteria
PHC1	Personal History Criteria type 1
PHC2	Personal History Criteria type 2

PHC3	Personal History Criteria type 3
PTEN	Phosphatase and Tensin Homolog gene
Rb1	Retinoblastoma 1 gene
RET	ret protooncogene receptor tyrosine kinase gene
SACCSG	South African Children's Cancer Study Group
SACTR	South African Children Tumour Registry
TREP	Italian Study on Rare Tumours in Paediatric Age

Research Report in the format of a “submissible” paper

CLINICAL RESEARCH ARTICLE

Clinical evaluation of paediatric patients with solid tumours, for features of Cancer Predisposition Syndromes in Gauteng, South Africa.

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Précis: Sixty-two South African patients with solid tumours were clinically evaluated (based on a personal and family history of cancer, histology of cancer and presenting clinical findings) for determining early signs/symptoms of a cancer predisposition syndrome (CPS) that would have benefited from referral to clinical genetics unit.

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Disclosure. The author reports no conflicts of interest in this work.

Abstract

Context: About 8% - to – 10% of children with solid tumours globally, harbor a genetic predisposition to cancer. There is limited knowledge of childhood cancer on the African continent. Early identification of a cancer predisposition syndrome (CPS) would define risk of further tumours as well as other non-cancer risks in a proband and also provide opportunities for cascade screening. The aim of this study was to evaluate the role of histology, family history and personal clinical criteria (as proposed by Goudie et al 2017) in identifying individuals with possible CPS in Gauteng, South Africa, and develop clinical guidelines that can be utilized by clinicians to refer patients for genetic evaluation or testing in a limited resource setting.

Methods: 62 South African children with solid tumours, ranging from 1-18 years old, were recruited from tertiary academic hospitals within Gauteng, South Africa. We evaluated personal & family history, performed clinical examination and file review (including review of tumour histology) to categorize a participant as likely to have a CPS or not.

Results: Among the participants, 44/62 (71%) patients were classified as 'likely CPS', whereas 18/62 (29%) were 'unlikely CPS'. Amongst 'likely CPS' cases, most patients 33/44 (75%) were identified based on personal history criteria (PHC), whereas 9/44 (20.5%) were based on histology criteria and 2/44 (4.5%) on family history criteria. Likely CPS participants were assessed for syndromic features of which 7 had a recognizable genetic syndrome (e.g. Beckwith Wiedemann, Proteus and Li Fraumeni syndromes) and 28 had syndromic features. Of the likely CPS cases identified using PHC, clinical examination identified 39/45 (86.7%); whereas 5/45 (11.1%) had bilateral or multifocal tumours and 1/45 (2.2%) had two primary tumours. Determining the likely CPS classification is important for directing appropriate genetic testing.

Discussion: Our results demonstrate that almost three quarters (3/4) of patients with paediatric solid tumours, may require some genetic evaluation (or testing) based on clinical assessment, family history or histology as guides for such a decision. We propose a guideline in which histology should be considered first, followed by family history criteria and lastly personal history criteria in determining the likely CPS status of an individual and the need for genetic evaluation (or testing).

Conclusion: We conclude that genetic evaluation (or testing) of patients identified as likely CPS, through our proposed algorithm could prove beneficial for the management of an index case (including their family). If further studies show the guideline to have a significant positive predictive value, then limiting genetic testing to those identified clinically as likely CPS would be more cost effective.

Keywords: Cancer predisposition syndrome, paediatric solid tumours, genetics of paediatric cancer

1. Introduction

Advances in cell biology and molecular genetics have revolutionized our understanding of cancer in recent years. All cancers are caused by genetic mutations in somatic cells, leading to aberrant cell division or loss of apoptosis (normal programmed cell death) (Harrington, 2016; Hegde & Chen, 2020). A small proportion of cancer is strongly linked to inherited germline mutations behaving as Mendelian traits. As a general principle, it is believed that cancers arise as the result of an accumulation of both inherited and somatic mutations in proto-oncogenes and tumour suppressor genes. If altered or mutated, tumour suppressor genes and DNA mismatch repair (MMR) genes significantly increase the likelihood of cancer development, either through an environmentally stimulated pathway or by progression from an inherited germline aberration (Turnpenny & Ellard, 2012). These germline variants give rise to cancer predisposition syndromes (CPS).

For the purposes of this study, a cancer predisposition syndrome (CPS) can be defined as a genetic condition (caused by an inherited or *de novo* germline genetic alteration) that places an individual at a significantly higher risk of developing cancer than individuals in the general population (Brodeur *et al.,* 2017; Scollon *et al.,* 2017). Features that could be suggestive of a CPS in an individual, based on the personal and/or family history, would include: (1) several close relatives (1st or 2nd degree) with the same cancer, (2) several close relatives with related cancers (e.g. breast, ovarian, bowel, and endometrial), (3) two family members with the same rare cancer, (4) unusually early age of onset of a particular cancer, (5) bilateral tumours in paired organs, (6) synchronous or successive tumours in an individual, and (7) tumours in two different organ systems in one individual (Turnpenny & Ellard, 2012).

By combining a thorough **personal and family history and a focused clinical examination**, the identification of a CPS in a child may be helpful to guide care and surveillance. The clinical benefits for the patient when a CPS is timeously diagnosed (even prior to any molecular confirmation) include the immediate commencement of key surveillance initiatives for the possible development of a tumour/s and the associated clinical and oncologic complications, which may be part of the particular CPS. Many cancer predisposing syndromes are inherited as autosomal dominant traits that are highly penetrant. This level of risk means that more aggressive means of screening with more frequent and earlier initiation of screening protocols are justified than would be acceptable for the population in general (Turnpenny & Ellard, 2012).

In addition to the personal and family history of cancer, according to Turnpenny and Ellard (2012), clinicians should also be aware of the **physical signs** that might indicate a specific CPS diagnosis in a child presenting with a tumour, for example – Peutz-Jegher syndrome (melanin spots around the mouth and lips), Cowden syndrome (macrocephaly, harmatomas, papillomatosis), neurofibromatosis type 1 (NF1) (café au lait macules, inguinal or axillary freckling, cutaneous neurofibromas)**.**

Other relevant clinical characteristics include physical malformations, cancer in young relatives, distinct cancer histologies, and unique mutation/methylation signatures in children with cancer, which may indicate an underlying CPS (Nguyen *et al.,* 2021). Due to variably identifiable clinical presentations and family histories, assessing CPS in paediatric tumours may prove difficult (i.e. not readily recognizable) for paediatric oncologists, especially in groups like Africans (Chan *et al.,* 2018). Identifying a CPS in a child might assist the clinician, affected child and the family in optimizing care and the overall management plan. Jongmans *et al.* (2016) and Postema *et al.* (2017) highlighted the key benefits to identifying patients that have a CPS, including: (1) modified treatment strategies, (2) improved surveillance measures, (3) knowledge for family members who are at increased risk for themselves, (4) opportunities for reproductive counselling and prenatal diagnosis, and (5) providing an answer for many parents' fundamental question: "why did my child get cancer?"

1.1. Prevalence and incidence of childhood cancer

1.1.1. International perspectives

Ferrari *et al.* (2006) published results of the "Italian TREP (Italian study on Rare Tumours in Paediatric Age) project", a national cooperative project on rare paediatric tumours. The study estimated that there are approximately 1800 new cases of childhood cancer a year in Italy. Furthermore, the international research agency estimated that in poor economies, the number of children who die from cancer in poor communities represents 60% of those diagnosed; the equivalent figure is 20% or less in developed countries of Europe and North America (Stefan *et al.*, 2015). Steliarova-Foucher *et al*. (2017) reported that around 300,000 children who presented aged 0 to 14 years are diagnosed with cancer around the world annually. Late diagnosis and poor surveillance of cancer syndromes could be important contributory factors to low cancer survival rates amongst children in Africa. Hence, the need to appropriately triage children with cancer predisposition syndromes (CPS) to medical genetics services for confirming a diagnosis, surveillance and provision of adequate care and treatment, as well as to identify at risk family members.

The above-mentioned studies concentrated mainly on the registries of paediatric cancer at a population level and did not specifically delineate cancers based on inheritance risk or any other form of cancer acquisition risk. Zhang *et al*. (2015) sought to define the prevalence and spectrum of heritable mutations that could potentially explain the index cancers in children. They concluded that germline mutations in cancer-predisposing genes were found in 8.5% of the children and adolescents with cancer. Family history did not predict the presence of an underlying CPS in most patients (Zhang *et al.,* 2015). Another group estimated that approximately 10% of children with cancer are affected by CPS, although they indicated possible under-reporting of cancer incidence in children (Goudie *et al.*, 2017). Grobner *et al.* (2018), in a German study, investigated the genomic alterations across childhood cancers and identified 7 – 8% of children in their cohort who had a predisposing germline variant. There are no comparable studies in South Africa or Africa.

1.1.2. African perspectives

Stefan *et al.* (2015) published the first report from the South African Children Tumour Registry (SACTR), describing the incidence of childhood cancer over 21 years between 1987 and 2007. The study estimated the average annual incidence of

childhood cancer at approximately 2340 new cases. In addition, the same report with regards to the African continent, estimated that 36 000 children are diagnosed with cancer annually in Africa, but that the population-based registry covers only 2% of the total African population. The low population cover alone demonstrates the significant underreporting of childhood cancer on the African continent. Despite the work done in developing the registry, it remains unclear what proportion of cancers occur as part of a CPS.

1.1.3. Profiling of childhood cancer

Profiling of paediatric solid tumours can be done in various ways. Sharma *et al.* (2017), in their regional cancer centre (Kashmir — India), profiled paediatric solid tumours according to histology and body region. They identified the most common among these to be CNS (central nervous system) tumours (26%), germ cell tumours (14.5%) and Ewing's sarcoma (14%). In a similar study in France, Desandes *et al.* (2016) profiled solid neonatal tumours, and their results showed a different pattern – with neuroblastoma being the most typical (47%), followed by germ cell tumours (29%) and lastly, CNS tumours (10%). It would seem unlikely that environmental exposures are the only role player in the development of cancer in childhood, considering the short life span that a child might have had. More likely, genetic predisposition plays a prominent role in childhood carcinogenesis.

1.1.4 Identification of patients likely to have a CPS

The Goudie *et al.* (2017) group worked on the McGill Interactive Paediatric Oncogenetic Guidelines (MIPOGG) to develop an approach to identify paediatric oncology patients most likely to benefit from a genetic evaluation. That report expressed the lack of guidelines necessary to identify children at high risk of having germline cancer-predisposing mutations and planned to incorporate clinical data (such as tumour characteristics, personal, and family histories) into tumour-specific algorithms to answer the difficult question of whether a child should be referred to a genetic service. These criteria have been used in this study and are shown in **Table 2.1**. In a subsequent report, Goudie *et al*. (2021) concluded that MIPOGG exhibited a favorable accuracy profile for CPS screening and reduced time to CPS recognition.

Jongmans *et al.* (2016) developed and used a referral "selection tool" that included family and personal history, type of tumour, and clinical examination findings to

clinically determine whether to refer a child to Clinical Genetics services. Jongmans' selection tool is comprehensive and in synergy with the proposed criteria for use by Goudie *et al*.'s group (MIPOGG) tool. The key difference between the two criteria, being that Jongmans *et al*. (2016) refers to "a child with excessive treatment toxicity" as one of the referral indications, whereas Goudie *et al*. (2017) do not include this criterion.

1.2 Study rationale

In Gauteng, profiling of paediatric cancer patients for genetic assessment is virtually non-existent. There are also no formal clinical guidelines to help paediatricians and paediatric oncologists determine which patients need to be referred for a genetics assessment.

The present research project made use of family history and tumour histology or clinical characteristics in the patient, such as dysmorphic features, congenital anomalies or benign tumours, to ascertain whether the affected child had any signs indicative of a CPS. It was envisioned that the data from this project would assist in the development of relevant clinical guidelines to ascertain patients that would benefit from being prioritized for a genetic assessment.

2. Subjects & Methods

The study recruited children diagnosed with solid tumours. Participant selection was by convenience sampling.The research project was linked (nested) into a larger research project aimed at studying the genetic landscape of CPS mutations in children. Ethics clearances were obtained from the University of the Witwatersrand Human Research Ethics Committee (certificates M180855 (larger study) and M190558 (this study)) **(Appendix A).**

2.1 Subjects

Participants were recruited from two academic hospitals in Gauteng (namely Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital) linked to the Faculty of Health Sciences, University of the Witwatersrand. Recruited participants were aged between 0 – 18 years and diagnosed with a solid tumour, and were out-patients in the above-mentioned paediatric oncology units from January 2019 to December 2019. Patients with haematological cancers and lymphomas were excluded from the study to narrow the scope of the research project and, based on the understanding that these cancers (haematological and lymphomas) have a different aetiology and are more commonly studied.

Children were recruited from all ancestry groups (African, Mixed, Asian, and Caucasians). Patients of African ancestry were of particular interest, because they are an understudied population. Patients who were attending the clinic as outpatients at the participating recruitment site for their routine care by a paediatric oncologist were invited to participate.

Each patient, with the parent attending with them, was seen in a private consulting room. The study was explained to the prospective participants. Patients who met the study inclusion criteria (diagnosed solid tumours) and their parent/guardian were required to read the information document, available in English. Verbal translation of the documents was available for those who required it. Informed consent was obtained from the parents/guardians of the participants. The obtained consent included permission to access the patients' medical records, document family history, and perform a comprehensive clinical examination (including photographs). Each participant was allocated a unique study number at this point. The consent form and information sheet is in **Appendix B**.

2.2 Methods

Relevant personal clinical history (including demographic information) and family history (by drawing a family pedigree) were obtained. The participant was then examined clinically for any signs of a genetic syndrome and relevant dysmorphic features (or congenital abnormalities). A file review was also performed to obtain relevant data, including special investigations and histology.

The criteria in **Table 2.1** below (adapted from Goudie *et al.* 2017) were then used to determine whether a child would be classified as having a likely CPS or not, based on personal history, family history and characteristics of the tumour as ascertained by the researcher (including histological presentation). A 3-generation family pedigree was also obtained and used to ascertain whether an individual's family was a "likely CPS family" or "unlikely CPS family". Patients needed to meet a minimum of one of the universal criteria or to have specific tumours to be classified as likely CPS. This approach is follows those of Goudie *et al.* (2017) and Jongmans *et al.* (2016) in their recommendations on classifying or categorizing patients that should be referred for genetic services

Table 2.1: Universal criteria and tumours that should be referred to determine the likelihood of a CPS. Adapted from: Goudie *et al.* (2017)

Criterion	Subcategory	Description
Universal Criteria	**Personal History Criteria** (PHC)	> 1 primary tumour (asynchronous or synchronous) – PHC1 (personal history criteria subtype 1)
		Bilateral or multifocal primary tumours – PHC2 (personal history criteria subtype 2)
		Dysmorphic feature(s) or congenital abnormality(ies) that, according to the clinician's judgement, may be related to the cancer – PHC3 (personal history criteria subtype 3)
	Family History Criteria (FHC)	Known cancer predisposition syndrome in the family
		Close relative with cancer at 18 years OR a parent /sibling/half-sibling with cancer at 50 years
		Close relative with same cancer type or same organ affected by cancer at any age
		Close relative with multiple primary tumours
		∗ close relative: parent, sibling/half-sibling, aunt/uncle, cousin, grandparent
Histology Criteria (Tumours with high risk of a germline mutation –for direct referral) (Hist.)	**Ocular/ CNS**	Atypical Teratoid Rhabdoid Tumour; Endolymphatic sac tumour; Choroid plexus carcinoma; Pineoblastoma; Subependymal giant cell tumour; Hemangioblastoma; Pituitary blastoma; Vestibular Schwannoma; Dysplastic cerebellar gangliocytoma; Optic pathway glioma; Retinoblastoma
	Renal/ Neuroblastic	Cystic Nephroma; Renal Angiomyolipoma; Renal cell carcinoma; Renal rhabdoid tumour
	Bone / Soft Tissue	Desmoid tumour; Gardner Fibroma; Malignant peripheral nerve sheath tumour; Nasal chondromesenchymal hamartoma
	Other	Adrenocortical carcinoma; Hepatoblastoma; Pheochromocytoma; Small Cell Carcinoma of the Ovary, Hypercalcaemic Type; Cardiac rhabdomyoma; Medullary thyroid cancer; Paraganglioma; Carcinoma: of the breast, lung, cervix, uterus, bladder; Colorectal carcinoma; Ovarian Sertoli-Leydig cell tumour; Pleuropulmonary blastoma; Gastrointestinal stromal tumour; Parathyroid tumour; Trichilemmoma

2.2.1 Personal history

Patients and care-givers were asked about the clinical presentation of cancer and the presenting symptoms. Of particular interest were the age of cancer diagnosis, progression of the disease, and reaction to chemotherapy or any form of severe toxicity. Patients were asked (or information obtained from file) about the details of the cancer presentation, the number of tumours (PHC1), whether tumours were bilateral/synchronous (and whether the cancer was multifocal) or unilateral/asynchronous (PHC2) . Patients were further asked about developmental milestones (gross motor, fine motor, and speech), as developmental delay/intellectual/behavioural abnormalities and birth defects were important in determining whether associated syndromic features were present. This information was used to assess PHC3 (outlined in Table 2.1 above). All the information collected in this manner was captured in the clinical tick sheet, **see Appendix C.**

2.2.2 Family history

Family history was taken by means of drawing a family pedigree about the index case (presenting with cancer), siblings, parents, and other second-degree relatives. Particular note was taken of individuals with similar or related cancers based on known genetic cancer syndromes. Relevant family history included questions in keeping with the family history criteria (FHC) , as outlined in Table 2.1 above.

2.2.3 Clinical Examination

Patients underwent a clinical examination (anthropometric measurements, head and neck examination, systems examination, and limbs, back, and skin examination). The anthropometric measurements included weight (to the nearest 0.1 kilogram (kg)) using an electronic scale, height (to the nearest millimetre (mm)) using a floor-standing Seca™ stadiometer, and a head circumference (to the nearest mm) using a tape measure. Height, weight, and head circumference were expressed as percentile scores, based on age- and sex-matched growth charts from the Handbook of Physical Measurements (Hall *et al*. 2007). The head and neck examination included a detailed description of facial dysmorphology features, which were recorded on a tick sheet (**see Appendix C**). A standard systems exam of the chest, abdomen and the CNS (central nervous system) was recorded on a tick sheet. Limb, back and skin exams were also

recorded on the clinical tick sheet. Clinical characteristics in the patient such as dysmorphic features and/or congenital anomalies, and/or benign tumours were noted, for example, umbilical hernia in BWS or neurofibromas in NF1. Patients were considered syndromic if they fulfilled a diagnostic criteria for a specific syndrome or group of syndromes (e.g. neurofibromatosis type 1, overgrowth syndromes, etc.) and also, if they had more than 3 dysmorphic features (associated with other anomalies – e.g. macrocephaly, pigmentary abnormalities, birth defects, etc.). Photographs of the face , hands, or other body part were taken of patients, particularly of unusual physical features (e.g. benign tumours, up slanting palpebral fissures, low set ears, flat nasal bridge, transverse palmar crease, etc.) if consent for clinical photography was obtained.

2.2.4 File Review

A brief hospital file review was conducted to look at the important clinical, radiological (including important sonographic reports, e.g. renal ultrasound and cardiac echo, etc. – to exclude congenital structural abnormalities), and histology reports. Information about tumour histology was obtained and recorded on the tick sheet (**see Appendix C**). Histopathological classification of the tumour in the majority of the cases had been done by the Anatomical Pathology Department of the National Health Laboratory Service (NHLS) in Johannesburg, South Africa. This was in turn evaluated for its significance on informing how likely a condition was a CPS and would thus aid in patient classification (criterion Hist in Table 2.1).

2.2.5 Statistical Methodology

The data collected on the patients was captured on an Excel spreadsheet designed by the researcher for this study to summarize the data. In the Excel spreadsheet, the data were grouped into different categories, namely: demographic data, age of diagnosis, personal history criteria, family history criteria, histology criteria and features detected on clinical examination eg macrocephaly, syndromic, and skin pigmentary abnormalities.

The criteria applied were as described by Goudi *et al*. (2017), referring to personal history criteria (designated PHC1, PHC2 or PHC3), family history (FH) criteria, or tumour histology (Hist.) criteria (see Table 2.1 above). These parameters were evaluated and analyzed to determine if they were contributory to the CPS

classification. Each case was individually assessed to determine the likelihood of the CPS, particularly from a clinical genetic perspective. Cases were divided into those suggestive of CPS and those not suggestive.

Data were captured, and quantitative data were analyzed statistically using SPSS software (statistic version 22). Descriptive statistical analysis of percentages and frequencies was used to summarize the data. Graphs were prepared using Excel and Graph Pad Prism software. The p values were calculated using the chi-square test, and statistical significance was fixed at $p < 0.05$.

3. Results

The research project intended to assess personal history and clinical examination, family history, and tumour histology, and to determine their importance in identifying whether a child with a paediatric tumour and/or their family had features indicative of a CPS. Each of the factors is presented individually before they are combined.

3.1 The demographic characteristics of the study participants

Sex distribution of participants showed that (of the 62 participants) there were slightly more females (n = 35) [56.5%] than males (n = 27) [43.5%]. The difference was not significant (x^2 = 1.03; p= 0.310).

Ethnic distribution of the study participants showed that 88.8% (n = 55) were Africans, 4.8% (n = 3) were Mixed ancestry, 3.2% (n = 2) were Asian, and 3.2% (n = 2) were Caucasians. This follows the demographics of the wider South African population.

Of the 62 participants, at recruitment, the majority (43.5%) were aged between 6 – 10 years (n = 27), 32.3% were aged between 11 – 18 years (n = 20), and 24.2% were aged between 1 – 5 years (n = 15). There were no participants that were enrolled, whilst they were aged between 0 – 11 months.

In the cohort, 63% (n = 39) of participants were diagnosed with cancer below the age of 5 years. The majority of children (56.5%) were diagnosed with cancer between 1 and 5 years, 22.6% between 6 and 10 years of age , 14.5% in the 11 – 18 years group, and (6.5%) under 1 year (Table 3.1). There was a significant difference observed in the age group distribution based on the age of diagnosis (or the age of presentation) with cancer amongst participants (x^2 = 1.48 ; p < 0.001) (see Table 3.1).

Table 3.1 The age of cancer diagnosis in paediatric tumour study participants**.**

Age group	**Number**	**Percentage**
0 – 11 months	4	6.5
1 – 5 years	35	56.5
6 – 10 years	14	22.6
11 – 18 years	9	14.5
Total	62	100

3.2 Assessment of universal criteria in the participants

Overall, the number of patients classified as 'likely CPS' (n=44) was significantly higher than those classified as 'unlikely CPS (n=18) ($x^2 = 10.90$; p < 0.001).

The analysis of the probands' personal history and examination criteria are presented first, followed by the family history, and then the histological classification. Each criterion is presented individually. The overall summary analysis of how many participants were classified as likely CPS based on the individual criteria is shown in Table 3.8.

3.2.1 Assessment of Personal History Criteria in probands.

When the three personal history criteria (PHC) were assessed, 45/62 of the participants would have been suspected of having a CPS overall. Amongst the 'likely CPS' group the majority (86.7%; n= 39) would have been detected by applying PHC3 (Dysmorphic feature(s) or congenital abnormality(ies) that, according to the clinician's judgement, may be related to the cancer), while the lowest percentage (2.2%; n = 1) would have been detected applying PHC1(> 1 primary tumour (asynchronous or synchronous). Five (11.1 %; n = 5) participants would have been detected by applying PHC2 (bilateral or multifocal primary tumours) alone. Four (8.9%; n = 4) participants that met PHC2 criteria, would have also met PHC3 (i.e. according to clinical examination findings, even if personal history of bilateral tumours were excluded).

Table 3.2 Distribution of personal history criteria (PHC) amongst likely CPS probands

Characteristic	Number	Percentage
PHC1	1	2.2
PHC2	5	11.1
PHC3	39	86.7

3.2.1.1 Assesment of PHC1

There was one participant that met PHC1 criteria. This was a 9 year old male patient (PC0039.1.1) of mixed ancestry with two primary tumours (a rhabdomyosarcoma and an adrenocortical carcinoma, diagnosed at the age of 2 years and 8 years respectively.

In addition, the adrenocortical carcinoma is a tumour considered 'for direct referral' for genetic testing, therefore, on considering histology this patient would also have been classified as likely CPS. This patient had subtle dysmorphic features – including, malar hypoplasia, bitemporal narrowing, epicanthus inversus, high arched palate, and short 5th hand digits with clinodactyly; and so would have fulfilled PHC3 criteria. There was also a significant family history, that raised a concern for a Li Fraumeni syndrome in this proband's family. Multiple family members had cancers at young age (< 50 years). The proband's mother had died from breast cancer, the maternal half-sister had died from brain cancer, the maternal uncle had died from stomach cancer, the maternal uncle had a brain cancer and a maternal cousin's son had eye cancer. The pedigree is shown in **Figure 3.1**

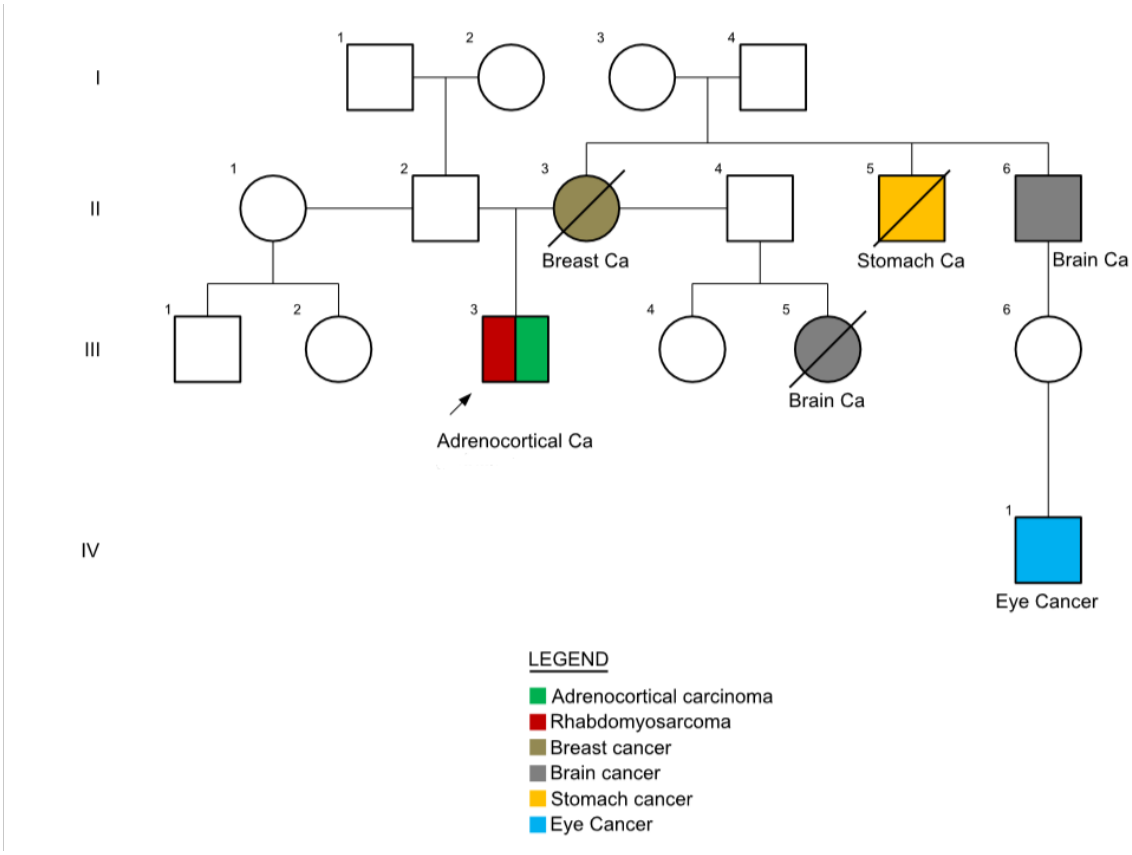

Figure 3.1 Family pedigree of PC0039.1.1. This figure illustrates the family pedigree of proband PC0039.1.1, which is in keeping with Li Fraumeni syndrome.

3.2.1.2 Assessment of PHC2

The probands that met PHC2 criteria are shown in Table 3.3. Four of the five cases also met PHC3.

Table 3.3 Distribution of personal history criteria type 2 (PHC2) amongst the likely CPS probands

Case identified	**Clinical presentation**
PC0016.1.1	Choriocarcinoma of the left and right ovary
PC0047.1.1	Bilateral Wilms' tumour
PC0056.1.1	Bilateral retinoblastoma
PC0063.1.1	Bilateral Wilms' tumour
PC0064.1.1	Bilateral Wilms' tumour

The case (PC0016.1.1) met PHC2. She was a 12 year old girl with bilateral ovarian choriocarcinoma (diagnosed at the age of 6 years), she also had overgrowth (weight and height >97th centile), macrocephaly and subtle dysmorphic features (broad nasal bridge, epicanthic folds) and breast Tanner stage 5 development. This patient also had a paternal half-sister with a leukemia, however, this was not considered significant enough as there is no known genetic correlation between leukemia and a choriocarcinoma.

The other 4 cases (3 with bilateral Wilms' tumour, and 1 with bilateral retinoblastoma) had dysmorphic features which according to the researcher were related to the cancer, and therefore they would have been considered likely CPS based on the clinical examination (PHC3), irrespective of them having bilateral tumours (PHC2). (**See Appendix D**)

3.2.1.3 Assessment of PHC3

There were 39 cases that according to clinical assessment had dysmorphic features that could be related to a CPS diagnosis (PHC3). (see PHC3 table in **Appendix D**) . Thirty five (n = 35) of these probands were considered syndromic, seven (n = 7) with recognisable syndromes and 28 with unrecognized syndromes, but with features such as macrocephaly, skin pigmentary abnormalities, congenital heart defects, failure to thrive, short stature or alternatively, in keeping with a group of known syndromes (e.g. RASopathies). (see Table 3.5). Four (n = 4) of the 39 cases were not considered syndromic, because they lacked other hard features, despite being dysmorphic.

Amongst the probands that were syndromic, seven (n = 7) had recognizable syndromes (see table 3.4 below). These cases fulfilled a diagnostic criteria (**See Appendix E**).

Table 3.4 Distribution of personal history criteria type 3 (PHC3) amongst the recognized syndrome probands

Number	Case Identified	Tumour Type	Syndromes Considered/Suspected
1.	PC0007.1.1	Pilocytic astrocytoma	NF1
2.	PC0023.1.1	Pilocytic astrocytoma	NF1
3.	PC0013.1.1	Nasopharyngeal carcinoma	Proteus syndrome
4.	PC0020.1.1	Nephroblastoma	BWS
5.	PC0021.1.1	Nephroblastoma	BWS
6.	PC0035.1.1	Nephroblastoma	BWS
7.	PC0059.1.1	Nephroblastoma	BWS

** NF1 – neurofibromatosis type 1, BWS – Beckwith Wiedmann syndrome

The table below shows the probands who met PHC3 criteria, amongst the unrecognized syndrome probands.

Table 3.5 Distribution of personal history criteria type 3 (PHC3) amongst the unrecognized syndrome probands

Number	Case Identified	Tumour Type	Syndrome group Considered/Suspected
1.	PC0024.1.1	Ovarian CA	Overgrowth
2.	PC0026.1.1	Retinoblastoma	Overgrowth
3	PC0015.1.1	Brainsterm glioma	RASopathy
4.	PC0038.1.1	Craniopharyngioma	RASopathy
5.	PC0060.1.1	Nephroblastoma	RASopathy
6.	PC0041.1.1	Nephroblastoma	MCA
7.	PC0042.1.1	Nephroblastoma	MCA
1.	PC0043.1.1	Retinoblastoma	Rb mutation spectrum other than point mutations
2.	PC0056.1.1	Bilateral Retinoblastoma	Rb mutation spectrum other than point mutations
3.	PC0047.1.1	Bilateral nephroblastoma	Suspected nephroblastoma associated syndrome
11.	PC0063.1.1	Bilateral nephroblastoma	Suspected nephroblastoma associated syndrome
12	PC0064.1.1	Bilateral nephroblastoma	Suspected nephroblastoma associated syndrome
13.	PC0048.1.1	Osteosarcoma	Short stature syndrome
14.	PC0001.1.1	Nephroblastoma	Short stature syndrome
15.	PC0004.1.1	Nephroblastoma	Short stature syndrome
16.	PC0022.1.1	Nephroblastoma	Tall stature syndrome
17.	PC0003.1.1	nephroblastoma	Macrocephaly
18.	PC0012.1.1	Nephroblastoma	Macrocephaly
19.	PC0018.1.1	Nephroblastoma	Macrocephaly
20.	PC0053.1.1	Ovarian cancer	Macrocephaly

Table 3.5 Distribution of personal history criteria type 3 (PHC3) amongst the unrecognized syndrome probands (continued)			
Number	**Case Identified**	**Tumour Type**	**Syndrome group Considered/Suspected**
22.	PC0054.1.1	Nephroblastoma	Macrocephaly
23.	PC0057.1.1	Phaeochromocytoma	Macrocephaly
24.	PC0058.1.1	Osteosarcoma	Macrocephaly
25.	PC0011.1.1	Rhabdomyosarcoma	Skin pigment abnormalities & GR
26.	PC0019.1.1	Nephroblastoma	Skin pigment abnormalities
27	PC0027.1.1	Hepatoblastoma	Skin pigment abnormalities
28.	PC0031.1.1	Brain astrocytoma	Skin pigment abnormalities & FTT

** MCA – multiple congenital abnormalities, Rb – retinoblastoma gene mutations (various types) FTT – failure to thrive, GR - growth restriction

The 35 probands that were considered syndromic, would have needed to be referred to a medical geneticist for assessment, recognition of their features, further evaluation, and appropriate genetic testing. Apart from dysmorphic features, other features that were important in recognising syndromes included macrocephaly (>3 SD) in 19 probands, and skin pigmentary abnormalities in 33 probands (and often these would occur in combination in a proband). If macrocephaly would occur together with skin pigmentary abnormalities in a proband, a suspicion of a PTEN related disorder was considered. These cases included some of those that had met PHC2 criteria (in table 3.3 above).

3.2.2. Assessment of Family History Criteria (FHC) in probands

Out of 62 families, five (n = 5) were classified as likely CPS families, and 57 were classified as unlikely CPS families, based on the family pedigree analysis. The number of participants was significantly higher from the 'unlikely CPS' family group compared with the 'likely CPS' family group ($x^2 = 10.90$, p < 0.001).

The table below shows the probands that met FHC criteria.

Table 3.6 Distribution of family history criteria amongst the likely CPS probands

Number	Case identified	Clinical presentation
1.	PC0023.1.1	Pilocytic astrocytoma, with NF1 and has a father with NF1.
2.	PC0051.1.1	Rhabdomyosarcoma, brother has a retinoblastoma. Likely familial RB1 gene mutation.
3.	PC0039.1.1	2 primary tumours (rhabdomyosarcoma, adrenocortical CA), mother had breast CA, maternal half-sister had brain CA. maternal uncle had stomach cancer, another maternal uncle has brain cancer, and a maternal cousin's son has an eye cancer. Li Fraumeni syndrome considered.
4.	PC0028.1.1	Osteosarcoma, maternal cousin had a sarcoma and maternal aunt had a breast CA. Li Fraumeni syndrome considered.
5.	PC0032.1.1	Medullary thyroid CA, paternal grand mother had a breast CA and uterine CA, and paternal grandfather had leukemia and thyroid abnormalities. There was already a confirmed MEN2A diagnosis in the family with a known mutation. Some of the cancers are not associated with this condition.

In two (n = 2) probands (PC0051.1.1 & PC0028.1.1), the probands' tumours would not immediately have suggested a CPS, but once the family history was considered, a likely CPS status could be assigned. PC0051.1.1 had a brother with a retinoblastoma, whereas he had a rhabdomyosarcoma, which is a tumour that can be associated with Rb1 gene mutations. PC0028.1.1 had close relatives with a sarcoma and a breast cancer, raising a suspicion of a Li Fraumeni syndrome in this family.

Proband PC0023.1.1 had clinical features of NF1, which on the basis of PHC3, he would have been considered likely CPS with the tumour he presented with. His father had NF1, and hence family history of NF1 was present.

Proband PC0039.1.1 had 2 primary tumours (rhabdomyosarcoma and adrenocortical carcinoma), which would have been sufficient to classify him as likely CPS on the basis of PHC2. In addition to this, he would have been classified as likely CPS on the grounds of histological consideration; because, adrenocortical carcinoma is a tumour for consideration for direct referral to genetic testing. This proband, also had a family history raising a suspicion of likely Li Fraumeni syndrome. See family pedigree, figure 3.1 above.

Proband PC0032.1.1 had a family history in keeping with MEN2A, and was diagnosed with a medullary thyroid carcinoma (a tumour for direct referral to genetic testing). This proband was later noted to have had a positive germline mutation in the *RET* proto-oncogene, tested through routine clinical care.

3.2.3. Assessment of Histology Criteria in probands

Nine (n = 9) participants would have been considered likely CPS, by looking at their histology reports alone. (See Table 3.7 below). These tumours are considered sufficient reason for further genetic analysis (i.e. for direct referral, as proposed by Goudie *et al.* 2017) despite some of them having fulfilled other criteria for likely CPS classification.

Table 3.7 Distribution of histology criteria amongst the likely CPS probands

Number	Case identified	Tumour histology
1.	PC0039.1.1	2 primary tumours – Rhabdomyosarcoma and adrenocortical carcinoma.
2.	PC0056.1.1	Bilateral retinoblastoma
3.	PC0005.1.1	Malignant peripheral nerve sheeth tumour
4.	PC0026.1.1	Retinoblastoma
5.	PC0027.1.1	Hepatoblastoma
6.	PC0043.1.1	Retinoblastoma
7.	PC0057.1.1	Phaeochromocytoma
8.	PC0032.1.1	Medullary thyroid CA
9.	PC0050.1.1	Retinoblastoma

The table below (Table 3.8) summarises the universal criteria & histology criteria combinations in assigning a likely CPS classification.

Table 3.8 Percentage distribution based on reasons for likely CPS classification

Characteristics in proband or family	Number	Percentage (%)
PH only	32	72.7
PH + Hist	6	13.6
PH + FH	2	4.5
PH + Hist + FH	1	2.2
Hist	1	2.2
FH	1	2.2
Hist + FH	1	2.2
Total	44	100

** PH – personal history, Hist – Histology, FH – family history

3.3 Tumour distribution amongst participants

Nephroblastoma was the most common tumour identified, in 37.1% of all cases. Phaeochromocytoma and hepatoblastoma were the least common tumours (yet are 'likely CPS' based on histology criteria); (see **Table 3.9**).

Table 3.9. Percentage of most common tumours amongst participants

Characteristic	Number	Percentage
Nephroblastoma	23	37.1
Brain tumour	9	14.5
Osteosarcoma	5	8.1
Ovarian CA	5	8.1
Rhabdomyosarcoma	5	8.1
Retinoblastoma	4	6.5
Neuroblastoma	3	4.8
Nasopharyngeal CA	2	3.2
Thyroid CA	2	3.2
Dermatofibrosarcoma	1	1.6
Infantile Hepatic Hemangioma	1	1.6
Phaeochromocytoma	1	1.6
Hepatoblastoma	1	1.6

Amongst the 'likely CPS' group, nephroblastoma was found to be the most common tumour in comparison with the other types of tumours (47.7%). Whereas neuroblastoma, nasopharyngeal CA, thyroid CA, phaeochromocytoma and hepatoblastoma showed the lowest frequency with a percentage of 2.3% each, a significant difference was observed between common tumours (p = 0.001) (see **Table 3.10**).

Table 3.10 Percentage of most common tumours amongst the 'likely CPS' group.

Characteristic	Frequency	Percentage
Nephroblastoma	21	47.7
Brain tumour	5	11.4
Retinoblastoma	4	9.1
Osteosarcoma	3	6.8
Rhabdomyosarcoma	3	6.8
Ovarian CA	2	4.5
Neuroblastoma	1	2.3
Nasopharyngeal CA	1	2.3
Thyroid CA	1	2.3
Phaeochromocytoma	1	2.3
Hepatoblastoma	1	2.3
Infantile hepatic hemangioma	1	2.3
Total	44	100

3.4 Overall assignment of how participants were assigned a likely CPS classification

The researcher assigned a likely CPS status based on the criteria that was more cost effective to implement, for example, histology was considered first because it is a primary test done in childhood cancer, then family history which can be done by the oncology clinician or a genetic counsellor, and then lastly a dysmorphology assessment which should be done by the medical geneticist, upon referral by the oncology clinician. See **Figure 3.2 below.** This summarises the overall assignment of how participants were given a likely CPS classification, as the researcher was trying to develop a guideline for genetic testing referral.

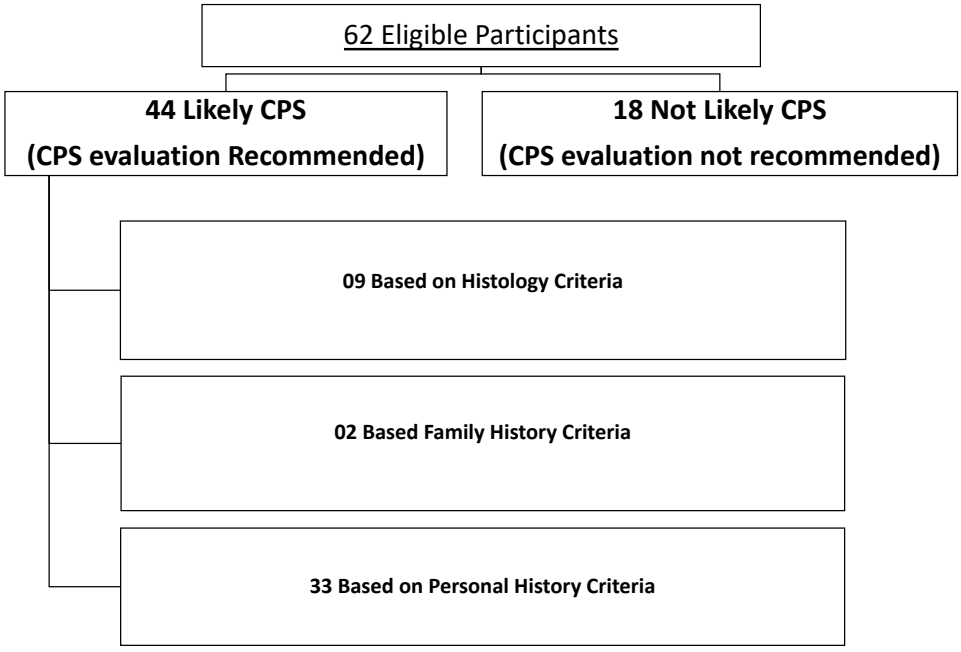

Figure 3.2. Overall assignment of how participants were given a likely CPS classification. This figure illustrate the basis upon which a likely CPS classification was assigned to probands.

Amongst the 'likely CPS' group, 9 were based on histology criteria, 2 were based on family history criteria, and 33 patients were assigned based on personal history criteria. This assignment is placed in order of priority (histology criteria first, followed by family history criteria, and lastly personal history criteria), as outlined in figure 4.1, in the following section. There were a number of cases that could have been classified

on differential combinations of these criteria, as presented in **Table 3.8 above**. Personal history criteria were more likely to classify probands as CPS, compared to histology and family history, but it requires a medical geneticist to assess these cases **(see Table 3.8 above).**

4. Discussion

In this study, we investigated the likelihood of a cancer predisposition syndrome (CPS) in a group of children (age 1 – 18 years) diagnosed with solid tumours. In the paediatric population, CPSs are a prominent cause of cancer-related morbidity and mortality (Cortes-Santiago & Patel, 2021). In addition, CPSs may harbour a high level of morbidity apart from cancer related complications, if there are other congenital anomalies (e.g. omphalocoele, requiring surgical repair in BWS). The present study was carried out to investigate clinical factors in paediatric patients in Gauteng, South Africa, with solid tumours. We assessed each participant's personal history, family history, clinical examination and tumour histology to determine their value in suggesting a CPS classification in the individual. Further to this, we evaluated the likelihood of whether their families could have a CPS or not, by analyzing the family pedigrees. Clinically determining the possibility of a CPS in an individual or their family will help practitioners (paediatricians or paediatric oncologists) understand which of their patients (and their patient's families) will benefit from clinical genetics services and genetic testing. The services of a clinical geneticist would be to carefully evaluate an individual (or their family) for important clinical clues that will guide genetic testing. Prompt recognition of a child with CPS has implications for cancer management, surveillance, genetic counselling, and cascade testing of relatives; and diagnosis of CPS requires practitioner expertise and access to genetic testing as suggested by Goudie *et al*. (2021). Whenever there is a high suspicion of a CPS or where a positive genetic test result is found, heightened screening of the individual and their family (often referred to as 'at risk relatives') would be implemented to improve their general health outcomes — meaning early surveillance for tumours (i.e. aim to reduce mortality and morbidity from the familial cancer syndrome) and assessment for birth defects that may cause morbidity. Furthermore, genetic counselling on recurrence risk should be considered for the families affected with testing of 'at risk relatives'.

We used the "universal criteria and tumours that should be referred to cancer genetics services (direct referral)", as proposed by Goudie *et al*. (2017), to determine whether an individual or their family were 'likely CPS' or not. We further analyzed the components of these criteria that were most valuable in helping reach a conclusion about the 'likely CPS' status of an individual/their family. Utilizing these criteria was

more practical for our setting, as opposed to a screening tool applied by Postema *et al*. (2021), where they used a checklist, 2D and 3D photographic series and digital assessment of these by a clinical geneticist in determining the likelihood of a CPS in an individual, because telemedicine diagnostic tools are not well entrenched in our routine patient service. This approach however could be considered in nationwide multicenter projects. Understanding, which components of the criteria were important in reaching this conclusion, would help us suggest locally plausible guidelines for clinicians, as envisaged in our objectives.

4.1 Demographic characteristics of the study participants.

In terms of our study demographics, 88.8% (n = 55) of our participants were of African descent (ancestry). We had particular interest in understanding cancer trends in the African paediatric population, because cancer is an under-prioritized issue in Africa, partly due to the overwhelming burden of communicable diseases, as argued by Parkin *et al* (2008). The South African Children's Cancer Study Group (SACCSG) tumor registry, established in 1987, is the only existing dedicated national paediatric tumour registry in Africa. Diagnosis can be very challenging in Africa due to the inadequacy of diagnostic facilities and lack of advanced equipment and staff (especially pathologists), as outlined by Kruger et al (2013). Africans were of particular interest, because they are an understudied population.

A Norwegian study by Kollerud et al (2018), that analyzed the risk of childhood solid tumours and family history of cancer less than 0.4% representation was from immigrants from Africa and the Middle East combined. Our results revealed a significant difference (p = 0.001) in the age of diagnosis with cancer amongst participants of different age groups. Sixty three percent (63%; n = 39) were diagnosed with cancer below the age of 5 years. This is of primary interest to us, because we would like to understand why children develop cancer when they have had minimal exposure to the environment. Whether single germline variants or high risk polygenic variants account for such early development of cancer in children, remains to be fully understood. In Gröbner, et al (2018), there was no observed age of onset bias, and in our knowledge no studies comment on age of onset as a bias for a likely CPS. Plon and Nathanson (2005) concluded that, the percentage of childhood cancers that are caused by a clearly inherited predisposition varies significantly from only a few

percentage to more than 50% with individual tumor types. Sweet-Cordero and Biegel (2019) suggested that paediatric cancers have contributed to a better knowledge of tumorigenic pathways in individuals of all ages.

4.2 Application of the universal criteria amongst participants to determine the likely CPS status

The diagnosis of CPS has essential consequences for the children and their families. In the present study, the number of patients classified as 'likely CPS' was significantly higher (71%) than 'unlikely CPS' cases. In our observation, family history is a low predictor (8% pick up rate) of the likely CPS status in an individual. In a Norwegian cohort, where the risk of childhood solid tumours was analysed amongst probands with a family history of cancer, it was concluded that other genetic mechanisms or environmental factors were involved in tumorigenesis, because the associations remained high despite controlling for hereditary cancer syndromes. Kollerud *et al*. (2018). In two separate cohorts that were followed up by Schwermer *et al*. 2021, (in Germany) to identify CPS in children with cancer employing a questionnaire, only 4% amongst the positive cases were identified as a result of a family history being suggestive.

Our results show that there are important clinical cues that clinicians can look for on clinical assessment, suggesting a 'likely CPS' in a child presenting with a solid tumour. As expected, our results do not comment on genomic sequencing or its correlation with clinical CPS assignment in paediatric solid tumour patients, because in this paper we are not reporting molecular germline result variants.

In a paper published by Goudie *et al*. (2021), reporting the performance of the McGill Interactive Pediatric OncoGenetic Guidelines (MIPOGG) for identifying CPSs; 99.5% (in phase 1) & 43.9% (in phase 2) of patients were recommended for CPS evaluation as contrasted to the 70.9% we recommended for CPS evaluation. In the limitations discussion, Goudie *et al* (2021), acknowlwdged that in phase 1 most children had phenotypic features, striking family histories, or tumour types suggestive of CPS, creating a population bias and rendering patients more easily identifiable by MIPOGG. We await to see in our cohort, how many likely CPS cases will be identified to carry a germline pathogenic variant (GPV). Goudie *et al*. (2021) identified 17.6% (in phase 2) amongst their likely CPS, to have had a GPV in a pediatric-onset CPS gene.

Other models or methods used by different researchers with interest in CPS evaluation, have shown different results. In Postema *et al* (2021), using a virtual screening tool, 57% (208/363) were assessed as likely CPS and amongst these, only 1% were shown to carry a pathogenic variant in a cancer predisposition gene (there were many other confounders / limitations with this study). In Schwermer *et al* (2021), using a questionnaire, 30% (86/287) were assessed as likely CPS and 9.4% were confirmed to have a pathogenic variant in a cancer predisposition gene. Sweet-Cordero and Biegel (2019) reported that the distinctions between paediatric and adult malignancies had been highlighted by genomic sequencing research. While many adult cancers have several somatic mutations, paediatric malignancies usually have fewer somatic mutations but a higher prevalence of germline changes in cancer predisposition genes.

4.3 Role (and use) of tumour histology.

Walsh *et al*. (2020) documented that the diagnosis of CPS can typically be confirmed with the help of molecular genetic testing of patients based on medical, histological, and family history markers.

Tumour histology is important for the early assignment of patients to 'likely CPS' in those who have tumours that appear on the recommended list for a direct referral to genetics services. On its own, tumour histology is not an adequate screening method, as the tumours for direct referral to genetics services had a low prevalence in this study (12.9 %) amongst the spectrum of solid cancers. Amongst the 'likely CPS' group, 15% of patients will be detected as 'likely CPS' (using the direct for referral histology criteria), meaning more than 85% of potential CPS cases would be missed if we were to follow the histology criteria alone. Tumour histology, though remains a key or influential guide to directing genetic testing, because it is the first line objective test perfomed in defining the presenting tumour. Nguyen *et al* (2021) investigated cancer type (histology) irrespective of other features (family history and physical examination) as a predictor for 'likely CPS'. They argued that certain cancer types, always suggest the presence of an underlying CPS (e.g. pleuropulmonary blastoma being suggestive of *DICER1* germline variant or bilateral retinoblastoma being suggestive of *RB1* mutations). In Nguyen *et al* (2021), 14% of children had cancer types that fall into the category for "direct referral", and they commented that this list is evolving. Recently,

Brecht *et al*. (2021) documented that many people with a predisposing germline variation have diverse characteristics in terms of family history, tumour type, age of onset, and therapeutic toxicity.

4.4 Clinical evaluation and management

Paediatricians are likely to encounter children with significant personal or **family cancer histories** in the course of their work (Coury *et al*., 2018). However, Chan *et al*. (2018) suggested that due to variable identifiable clinical presentations and family histories, assessing CPS in paediatric tumours is difficult for paediatric oncologists, particularly in groups with unknown germline frequencies. Our experience demonstrates this point, as in our cohort only one proband with a solid tumour had been offered diagnostic germline genetic testing. A higher risk of acquiring cancer may be linked to a family history of specific cancers (Murff *et al*., 2004). Our results, however, showed that a family history is important but it does not have a major effect on predicting a CPS, amongst a wider group of participants with solid tumours.

Amongst 'likely CPS' cases, most (72%) patients were identified based on **clinical examination** (personal history criteria (PH)). Largely, PHC3, defined as personal history criteria 3 (i.e., dysmorphic feature(s) or congenital abnormality(ies) that, according to the clinician's judgement, may be related to the cancer) had more weighting (85%) than PHC1 or PHC2, as a contributing factor within the PH criteria in assigning an individuals' likelihood for CPS status in our cohort.

Amongst cases that were confirmed to have CPS, in two cohorts that were followed up by Schwermer *et al* (2021) – 70.4% (19/27) in one cohort; and 75% (18/24) in another cohort, had been recommended for genetic evaluation based on suggestive "physical features", that is PHC3 criteria in our context. In this paper, some tumours were heamatological and the "physical features", were mostly in patients with Down syndrome or NF1. Paediatric oncologists and paediatricians may need to be specifically guided around the PHC3 criterion and key physical features as this seems to be the biggest source of referral for CPS diagnosis.

In addition to these cues (histology, family history and detailed clinical examination), we observed that certain clinical features should not be ignored in assessing an individual's eligibility for referral for clinical genetics evaluation for a possible CPS.

These features include, in our findings, skin pigmentary abnormalities (e.g. café au lait macules), macrocephaly, and syndrome specific features (e.g. pre-auricular pits, macroglossia, hemihypertrophy in case with a nephroblastoma as this may point towards Beckwith-Wiedermann syndrome(BWS), which has a rather different testing approach to conventional NGS (next generation sequencing) methods), because when viewed in combination are significant enough, and they should alert a clinician to refer a patient for genetic evaluation. These features, considered in combination with personal history criteria (supportive of CPS), had more weight than when considered alone. There are no studies to our knowledge, that have highlighted skin pigmentary abnormalities and macrocephaly as subsets that ought to be given attention in determining the likely CPS status on a proband. Another study elucidated that macrocephaly is a relatively common clinical feature in the paediatric population and is often syndromic (Jones & Samanta, 2021). The positive predictive value of these features remain to be determined with ongoing genetic studies.

Features specific to a genetic syndrome ('syndromic') were statistically significant (p-value = 0.016) in determining individuals' classification into CPS or not CPS category. Thirty five (n = 35/ 79.5%) patients of the 'likely CPS' group, were considered syndromic (recognizable (n = 7) or non – recognizable (n = 28)). Amongst the unrecognized syndromic cases, there were commonly associated features that triggered a suspicion of a group of conditions. For example, patients presenting with short stature, relative macrocephaly, hypertelorism, ptosis, short neck and pectus carinatum raised a suspicion of a Rasopathy. Patients who presented with failure to thrive, developmental delay, congenital heart defects and other dysmorphic features (including, up slanted palpebral fissures, thin vermilion border, hypertelorism) were thought to have multiple congenital abnormalities (MCA). Those that had a weight and height above the 97th centile, with other features (e.g. café au lait macules, joint laxity) were raising a concern for overgrowth syndromes. The inability to ascribe a significant value though for this feature, could be attributed to multiple factors; e.g., meeting a clinical criterion for most genetic syndromes requires additional investigations such as x-rays, ultrasounds, etc. In addition, recognition of specific syndromes is often dependent on the experience of the clinical geneticist, who might be able to pick up critical clinical signs that can be assimilated together to formulate a diagnosis. Some other studies reported neurofibromatosis type 1, Beckwith-Wiedemann syndrome,

multiple endocrine neoplasia, Li-Fraumeni syndrome, von Hippel–Lindau syndrome, and familial adenomatous polyposis all as common CPSs in children (Monsalve *et al*., 2011). Therefore, detecting clinical signs related to a known CPS is a critical task for a diagnosing pathologist (or clinician). Some of our participants met criteria for these syndromic conditions (e.g. BWS, Proteus syndrome, NF1, etc.), but these were not detected during their tumour management. See **Appendix E** for diagnostic criteria for these conditions.

4.5 Tumour distribution in the cohort

Our findings revealed that the frequencies of the common tumours were significantly different from one another among the 62 participants. Nephroblastoma is the most common tumour in the 'likely CPS' and 'unlikely CPS' groups, and this makes it unhelpful (on histological grounds) as a guiding clue to determining the likely CPS status of an individual. Hence, it will be recommended that all cases with nephroblastoma are carefully assessed, as 50% of them will be 'likely CPS' based on scrutinization by personal history (PH) criteria.

In contrast, phaeochromocytoma and hepatoblastoma were the least common tumours according to prevalence, but nonetheless, are important diagnoses indicative of CPSs by histology. Similarly, in the 'likely CPS' group, nephroblastoma was the most common tumour than other types of tumours, while neuroblastoma, nasopharyngeal CA, thyroid CA, phaeochromocytoma, and hepatoblastoma were the least common tumours. Our results are inconsistent with the findings of Desandes *et al.* (2016) that neuroblastomas are more common in children, followed by central nervous system tumours, leukaemias, and retinoblastomas. These differences may be accounted for by the fact that Africans are often under-represented in international studies, and that in our cohort patients were not selected systematically based on locally known tumour frequencies. They were ascertained based on outpatient attendance and convenience sampling.

4.5 Recommendations

Assigning an individual into a 'likely CPS' status, should be considered carefully in our setting – considering the limited resources. An individual assigned 'likely CPS status' requires genetic testing to clarify their status. We assigned a likely CPS status based on the criteria that were more cost effective to implement, for example, histology was considered first because it is a primary test done in childhood cancer as part of the initial clinical work-up. Family history should be assessed next as this can be done by the oncology clinician or a genetic counsellor, and then lastly a dysmorphology assessment which should be done by a medical geneticist, upon referral by the paediatric oncology clinician (who would have established that a child has dysmorphic features). We therefore recommend that histology, followed by family history and then clinical examination should be considered in that order, in determining the likely CPS status of an individual. As a local guideline for CPS evaluation, we recommend that a diagnosing paediatric oncologist should consider:

1) Histology, and refer for genetic testing if tumour is identified as a direct referral, as proposed by Goudie *et al.* (2017)
2) Family history of cancer, in close relatives (parents, siblings or half-siblings, grand parents and first degree cousins, etc.) as proposed by Goudie *et al.* (2017)
3) Clinical examination and tumour characteristics as proposed by Goudie *et al.* (2017)

The "universal criteria & tumours for direct referral" as suggested by Goudie *et al* (2017), is believed to be a good starting point to guide our referral for genetic evaluation and testing, although this will require verification after molecular testing of the current cohort has been performed on the larger study.

A flow chart is recommended, as a quick reference point in clinic for a diagnosing paediatric oncologist (see Figure 4.1). This research could substantially improve patient management techniques in providing care for children with solid tumours and their families. Children identified to be likely CPS should be referred for CPS evaluation, that is, appropriate genetic assessment and/or testing.

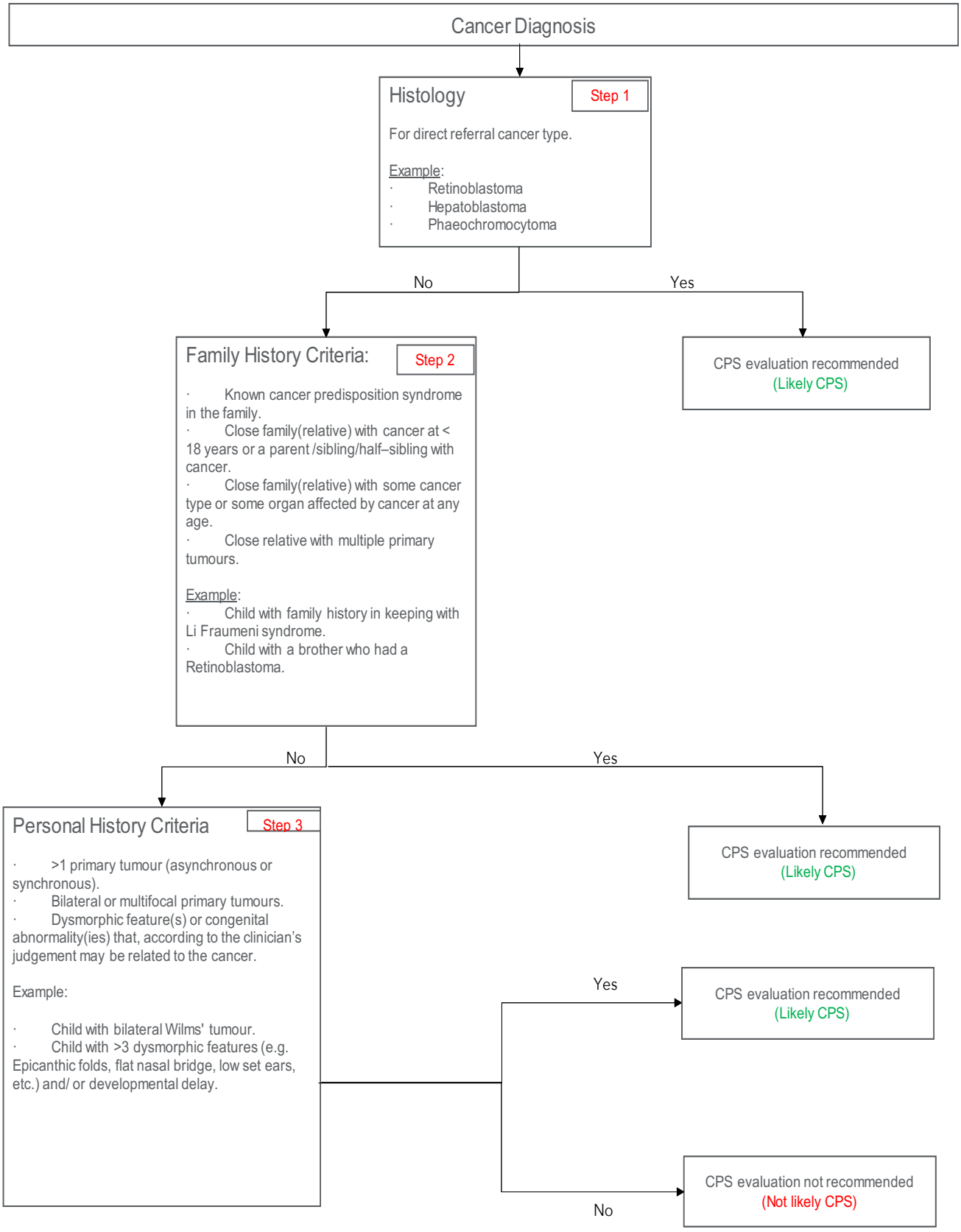

Figure 4.1 CPS evaluation flow chart. This figure illustrate a recommended flow chart for referring patients for genetic assessment and testing.

4.6 Limitations

Our study has a number of limitations; 1) We had a small sample size, and participants were randomly selected from an oncology outpatient clinic; therefore, it would be harder to extrapolate our findings particularly, the tumour distribution. 2) Validation of clinical findings by an experienced geneticist, would be preferable to a trainee geneticist in our instance (some syndromes may not have been recognized); and 3) Sequencing data is required to support the findings and validate the proposed guideline. Therefore more research is needed in this area.

5. Conclusions

The number of patients diagnosed as ‘likely CPS’ was significantly higher than ‘unlikely CPS’ cases. Amongst ‘likely CPS’ categorized participants, most patients were identified based on personal history criteria (PHC). Nephroblastoma was the most common solid tumour in our cohort. Careful evaluation of the presenting histology, family history and clinical examination are key, to guiding every clinician to ascertain whether an individual (and their relatives) would benefit from a medical genetics consultation and genetic testing. This study may have substantial implications for cancer prevention in children and the clinical management of probands and family members. Further studies are needed to find molecular insights into paediatric patients with solid tumours in Gauteng, South Africa, and some of these are ongoing.

6. Acknowledgements

I would like to thank my supervisors, Professor Amanda Krause, Dr Candice Feben and Dr Lindiwe Lamola, for their academic support and guidance throughout this research report. To my primary supervisor, Amanda, thank you for the continued mentorship that you have offered me over the past couple of years. Your dedication to your work and research is truly inspiring. Thank you for your continuous support in developing my research career.

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Special thanks to my partner, Fikile Mpofana and my parents Nhlanhla and Nozinto Mabaso for their support, prayers and love. Lastly, I would like to thank the patients with solid tumours and their families for their willingness to participate, without whom this research would not have been possible.

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Appendices

Appendix A

Ethics clearance certificates

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R14/49 «Tit init name»

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190558

NAME: (Principal Investigator)	Dr Njabulo Christian Mabaso
DEPARTMENT:	Human Genetics Charlotte Maxeke Johannesburg Academic Hospital Chris Hani Baragwanath Academic Hospital Donald Gordon Medical Centre
PROJECT TITLE:	Cancer predisposition syndromes and their genetic contribution to paediatric solid tumours in patients in Gauteng, South Africa
DATE CONSIDERED:	31/05/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Lindiwe Lamola, Candice Feben and Amanda Krause
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/10/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **May** and will therefore be due in the month of **May** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Dr L Lamola, et al
School of Pathology
Department of Human Genetics
National Health Laboratory Service

E-mail: Lindie.Lamola@wits.ac.za

CC: Supervisor: Not applicable <>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 26/02/2019

REF: *R14/49*

PROTOCOL NO: **M180855** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Inherited cancer predisposing mutations in a cohort of childhood cancer patients*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

Appendix B

Consent forms and information sheet

Inherited cancer predisposing mutations in a cohort of childhood cancer patients

CONSENT FORM – PARENTAL CONSENT FOR CHILD PARTICIPANTS

PARTICIPANT STUDY ID:

The purpose of this study, procedures to be followed, risks and benefits, have been explained to me and my child. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have questions, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I have read the attached information sheet this and agree to allow my child to participate in this research study with the understanding that I may withdraw my child at any time. To the best of my ability, I have discussed the study with my child, who agrees to be in the study. I have explained to my child that he/she may approach the researchers to be re-consented when he/she turns 18 if he/she wishes and that he/she may withdraw from the study at any time. I have been told that I will be given a signed and dated copy of this consent form if I would like one.

	Please initial
I understand that the purpose of this consent form is for me to inform the study what they can or cannot do with my child's samples, clinical information and photos.	
I understand that all procedures/tests on the samples have been or will be approved by the Human Research Ethics Committee of the University of the Witwatersrand. I understand that every time a new study is done on my child's sample, permission will be obtained from this Ethics Committee for the study to make sure that it is used only for the purposes stated above.	
I understand that if I have any questions at any time, they will be answered.	
I understand that confidentiality will be maintained at all times through the use of a code and numbering system.	
I understand that I may withdraw my child from the study at any time.	
I have discussed the study with my child.	

I am in agreement that my child's samples may be stored and used for the purposes described in the information sheet.		
I understand that I can choose to have my child's sample destroyed at any time.		
I understand that my child may not benefit directly from the research done on their sample.		
If this research does identify information that is relevant to my child's cancer, I would like to be contacted to receive the information.	Yes ☐	No ☐
If this research does identify genetic information that would be important or of health benefit to my family, or me, I would like to be contacted to receive the information.	Yes ☐	No ☐

1 Modderfontein Road, Sandringham, Johannesburg, South Africa
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CONSENT FORM – PARENTAL CONSENT FOR CHILD PARTICIPANTS

PERMISSION FOR STORAGE		
I agree to have my samples stored in the research facility for future childhood cancer studies only without any identifiers.	Yes ☐	No ☐
I agree to have the data generated from my samples stored without any identifiers and be reanalysed if need be.	Yes ☐	No ☐

PERMISSION FOR REUSE				
	Samples		**Data**	
I agree that my child's samples & data (including clinical data) can be shared with other investigators for research *related to childhood cancers* once the ethics committee has approved the new study. My child's samples & data will be made available without any identifiers.	Yes ☐	No ☐	Yes ☐	No ☐

PERMISSION FOR PHOTOGRAPHS		
I agree to the use of my child's photographs for medical records ONLY.	Yes ☐	No ☐
I agree for my child's photographs to be used for teaching purposes AND to be used for medical records but NOT for medical publication.	Yes ☐	No ☐
I agree for my child's photographs to be used in medical publications, including medical journals, textbooks and electronic publications AND to be used for teaching purposes AND to be used for my child's medical records. I understand that the images may be seen by members of the general public, in addition to scientists and medical researchers that use these publications in their professional education. Although these photographs will be used without identifying information, such as my child's name, I understand that it is possible that someone	Yes ☐	No ☐

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may recognize my child.	☐ Understood

Researcher	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)
Parent Participant	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)

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ASSENT FORM FOR CHILD PARTICIPANTS

PARTICIPANT STUDY ID:

The purpose of this study, procedures to be followed, risks and benefits, have been explained to me. I have been allowed to ask the questions I have, and my questions have been answered to my satisfaction. I have been told whom to contact if I have questions, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I have read this assent form (or had it read to me) and agree to participate in this research study with the understanding that I may withdraw at any time. I understand that that I may approach the researchers to be re-consented when I turn 18 if I want to and that I may withdraw from the study at any time. I have been told that I will be given a signed and dated copy of this consent form if I would like one.

Researcher	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)
Parent or Legal Guardian	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)
Child Participant	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)
Translator (if applicable)	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Printed Name & Surname _____ ☐☐/☐☐/☐☐☐☐ Signature/Thumbprint Date (dd/mm/yyyy)

This report is intended solely to record the observations and/or opinion of the writer. It does not constitute a medico-legal report.

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Inherited cancer predisposing mutations in a cohort of childhood cancer patients

Dear Potential Participant,

Hallo, we are Dr. Lindiwe Lamola, Dr. Candice Feben and Prof. Amanda Krause from the Division of Human Genetics at the University of the Witwatersrand and National Health Laboratory Service. We are doing research on childhood cancers. We work with other members of the Division of Human Genetics to study the genetic factors that cause childhood cancers in South Africans.

As we are trying to understand the genetic causes of cancer, we would like to invite your child to take part in this study, because your child has cancer. Participation in the study will mean answering some questions about the health and background of your child and giving a blood and/or saliva sample for genetic studies. We will also examine your affected child, review their clinical file and investigations, and take a blood and/ or saliva sample from them.

What are childhood cancers?

Childhood cancers are the cancers that develop in children from birth to 18 years of age. Childhood cancer is not just one disease, but a group of several major types of disease. There are many types of childhood cancers; these may include; neuroblastoma, retinoblastoma, Wilms tumor, and cancers of the brain, bone, and soft tissue. The childhood cancers are not common and differ from adult cancers in the way they grow, spread and how they are treated.

What is cancer?

Cancer is an abnormal growth of cells. Cells are small basic building blocks of all living things. All parts of the body are made up of cells. Cancer develops when damaged cells grow and spread faster than normal healthy cells.

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What causes childhood cancers?

Childhood cancers are complex diseases and doctors sometimes aren't sure why some children get cancer and others don't. However, about 10% (1 in 10) of all cancers in children are caused by inherited changes in your genes – inherited means these changes can be passed from parents to children. For this study, we are interested in studying the genetic causes of childhood cancers in order to understand their role in the development of cancer.

What will the study involve?

In this study, we would like to understand the factors which may have played a part in your child developing cancer. To do this, we want to learn more about your family, specifically whether other family members have had cancer, and to learn more about your child, specifically related to his/her type of cancer, how old the child was when the cancer was diagnosed and whether there are any other problems which your child may have been born with or may have developed over time. We would like to take a full family history from you and then perform a clinical examination of your child, looking at his/her weight and growth, skin, chest, abdomen, limbs and spine. The family history and examination will take approximately half an hour. We would like to look at the records in your child's hospital file to make sure that we collect all the relevant information

In order to study the genes, we will take a sample of blood and/or saliva from your child. Our blood and saliva contains cells. All the cells in our bodies contain a chemical called DNA that we inherit from our parents. DNA carries the instructions that your body must follow in order to exist and function. It contains all your genes. Your cells also contain a chemical called RNA. RNA is formed from DNA and helps DNA to do its job.

We can take the DNA and RNA out of the cells that are in the blood or the saliva sample that we take from your child. Once we have isolated the DNA and/or RNA we can study the genes. In this study, we may need to examine all of the genes to try and find the gene that may be the cause of your child's cancer.

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What can you expect from the study?

Once you understand what the study is about and are happy to take part, we will ask you to sign a consent form on behalf of your child to join the study. If your child is old enough and able to understand the study, they may sign an assent form. A copy of these forms will be given to you to keep.

Once you have signed the consent form and your child has signed the assent form we will ask you some questions about your child's background and health. A complete clinical examination will be performed on your affected child. We may ask to access data relevant to your child's diagnosis from his/her clinical file or test results. This information will help us to understand your child's condition better and to interpret the data generated by our research.

A nursing sister or doctor will take up to three teaspoons (15ml) of blood from your child. Venupunctures (drawing blood) are normally done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site. Your protection is that experienced staff perform the procedures under sterile conditions.

We will also ask for a saliva (spit) sample from your affected child. A nursing sister or doctor will use a small, soft sponge to collect the saliva sample. The sponge is placed inside your child's mouth against the cheek and gently moved along the gums and cheek to soak up as much saliva as possible. He/she will then place the saliva in a special container and might repeat the process if we do not get enough saliva the first time. This procedure is painless and harmless.

Long-term storing of samples and data

Once we have done the research that we are planning for this project, we would like to store your child's DNA, genomic information and data on their health history and laboratory test results for future research on childhood cancer, and we will ask you to sign a separate consent form to give us permission to do this. The sample will be stored together with other participants' samples. The samples will be used in future childhood cancer studies.

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Confidentiality

Your child's samples will be stored in a locked fridge and the personal information will be stored on a secure computer in a locked office. Only the investigators of this study will have access your child's name and personal details. Each participant will be given a code and number for the study so that no one knows who you are. The blood, saliva, RNA and DNA samples and the associated data will not be attached to your names – they will only have their code and number on them.

What are my rights as a participant?

You and your child do not have to join this study. Whether he/she decides to join this study or not will not affect your treatment in our clinic. You can also decide to leave the study at any time. You may choose to have your child's biological samples (blood, DNA, RNA and saliva) destroyed at any time. If you do not want your child to be part of this study or decide to leave the study, it will not affect your child's regular treatments or medical care in any way. We invite your child to approach the researchers to be re-consented when he/she turns 18 if he/she wishes. He/she can also decide to leave the study at any time.

You may not get any benefit directly from this study, but we hope it may help others who are affected by childhood cancers in the future. We may contact you in future if we believe we have identified the cause of your child's condition if you give your permission for this. Please understand that what we are trying to do is very difficult and could take a long time.

Contact details

If you have any problems you can contact us at any time on this telephone number: Dr Lindiwe Lamola, Dr Candice Feben or Prof Amanda Krause – 011 489 9223.

PLEASE NOTE!

Some patients may experience psychological distress should a complication be detected. You can contact the Genetic Counselling Unit at the Division of Human Genetics, NHLS Braamfontein to arrange a session with an HPCSA-registered genetic counsellor to discuss these issues. They are experienced and qualified counsellors who are able to offer distress counselling, decision support, address concerns associated with genetic disorders and testing and explain medical terminology. Appointments can be arranged by contacting Ms Janet Robbie on (011) 489 9223 or Ms Shelley Macaulay on (011) 489 9243.

If you have any complaints or problems with this research you may report them to the Chair of the Human Research Ethics Committee at the University of the Witwatersrand on this telephone number – (011) 717 1234.

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Appendix C

Data Collection Sheet

Solid Tumor - Clinical Record Phenotype Evaluation Tick Sheet

Demographic Data

Number _____

Date of Birth _____

Current Age _____

Age at presentation _____

Clinic Attending _____

Reason for Initial attendance at clinic _____

Tumor Type _____

Tumor Histology _____

Previous Tumours _____

Age at Diagnosis _____

Close relatives	Affected	Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased

***Close relative** parent, sibling/half-sibling, aunt/uncle, cousin, grandparennt

Contact Details

Postal Adress _____

Mother Cell _____

Father Cell _____

Home Tel _____

Email Adress _____

Pedigree Completed Suggestive family history Not suggestive family history

Not Completed

Growth & Stature						
Birth Weight (kg)	>4	2.5-4	1.5 - 2.5	1.0 - 1.5	<1.0	
Current Weight	>97th	50th - 97th	10th - 50th	3rd - 10th	<3rd	
Current Height	>97th	50th - 97th	10th - 50th	3rd - 10th	<3rd	
Current Head Circumference	>97th	50th - 97th	10th - 50th	3rd - 10th	<3rd	
BMI	<17	17-25	25-30	>31		
BP	_____	specify	_____	Normal Range		
Hemihypertrophy	_____	specify				
Facial Features						
Courseness	No	Yes				
Macroglossia	No	Yes				
Prominent forehead	No	Yes				
Malar Hypoplasia	No	Yes				
Flat nasal bridge	No	Yes				
Supernumerary teeth/ abnormal	No	Yes				

Beaked Nose	No	Yes				
Small Chin/ Micrognathia	No	Yes				
Long philthrum	No	Yes				
Short Columella	No	Yes				
Mucosal neuromas (Lips/tongue)	No	Yes				
Other	No	Yes				
Central Nervous System Abnormalities						
Nerual tube defects	No	Yes	Specify	_____	Treatment	
Hypotonia	No	Yes	Specify			
Cerebellar signs (e.g. unsteady gait)	No	Yes	specify			
Other central nervous system abnormality	No	Yes	Specify	_____		
Eye Variation						
Palpebral fissure length	>+2SD	>+1SD; <+2SD	Mean -+1SD	Mean >-1SD	<-1SD; >-2SD	<-2SD
Intercantal distannce	>+2SD	>+1SD; <+2SD	Mean -+1SD	Mean >-1SD	<-1SD; >-2SD	<-2SD
Strabismus	No	Left	Right			

Squint	No	Left	Right			
Epicanthic folds	No	Left	Right			
Palpebral fissure position	N/A	Upslanting	Downslanting			
Cataracts	No	Left	Right			
Congenital hypertrophy of the pigment epithelium (	No	left	right			
Nystagmus	No	Left	Right			
Ptosis	No	Left	Right			
Other eye abnormalities	No	Left	Right	Specify	_____	
Ear Variation						
Hearing tested	No	Yes			Method	_____
Hearing loss	No	Left	Right		Mild	Moderate
		Sensorineural	Conductive			
Low set ears	No	Left	Right			
Anterior ear lobe creases	No	Left	Right			
Posterior helical pits	No	Left	Right			
Other ear abnormalities						

Cardiovascular Abnormaities						
Cardiovascular evaluation	No	ECG Yes	ECHO Yes	Cardiologist	Yes	
		No	No	No		
PS/VSD/ASD	No	Yes		Specify	_____	
Other Structural defects	No	Yes		Specify	_____	
Other cardiac defects	_____					
Gastrointestinal Malformations						
Duodenal/Jejunal atresia	No	Yes				
Tracheo-oesophageal fistula	No	Yes				
Omphalocoele	No	Yes				
Diastasis recti	No	Yes				
Umbilical hernia	No	Yes				
Imperforate anus	No	Yes				
Organomegaly	No	Yes				
Other gastrointestinal anormalies						
Renal Malformations						

Renal sonar done	No	Yes				
Kidney malformation						
*Horseshoe	No	Left	Right	Diagnosed on:	_____	
*Pelvic	No	Left	Right	Diagnosed on:	_____	
*Dysplastic	No	Left	Right	Diagnosed on:	_____	
*Hypoplastic	No	Left	Right	Diagnosed on:	_____	
*Agenesis	No	Left	Right	Diagnosed on:	_____	
Other	Specify	_____		Diagnosed on:	_____	
Collecting system malformation						
*Hydronephrosis	No	Left	Right	Diagnosed on:	_____	
*Hydroureter	No	Left	Right	Diagnosed on:	_____	
*Reflux Nephrophathy	No	Left	Right	Diagnosed on:	_____	
Other	Specify	_____		Diagnosed on:	_____	
Renal artery malformation	No	Left	Right	Diagnosed on:	_____	
Genital Malformations						
MALES						
Undescended testes	No	Left	Right			
Hypospadias	No	Yes				
Ambigous Genitalia	No	Yes				
Other male genital abnormality						
	_____					

FEMALES						
Hypoplastic vulva	No	Yes				
Upper Limb Malformations						
Polydactyly (pre- & post axial)	No	Left	Right			
Macrodactyly	No	Left	Right			
Camptodactyly	No	Left	Right			
Other Upper limb malformations	No	Left	Right	Specify	_____	
Lower Limb Malformations	No	Left	Right			
Polydactyly (pre- & post axial)	No	Left	Right			
Macrodactyly	No	Left	Right			
Camptodactyly	No	Left	Right			
Other Upper limb malformations	No	Left	Right	Specify		
Vertebral abnormalities						
Scolosis	No	Yes				
Kyphosis	No	Yes				
Other Vertebral abnomalities	_____					

Skin Changes						
Café au lait spots	No	Yes	Specify	_____		
(>2 patches, each >5mm)						
Hyperpigmentation	No	Yes	Specify	_____		
Hypopigmentation	No	Yes	Specify	_____		
Lentigines	No	Yes	Specify	_____		
Neurofibromas	No	Yes	specify	_____		
				_____		
Hyperkeratosis	No	Yes	Specify	_____		
Sparse hair	No	Yes	Specify	_____		
Papillomas	No	Yes	Specify			
Poikiloderma	No	Yes	Specify			
Dark melanin spots	No	Yes	Specify			
Thickened skin: soles of feet	No	Yes	Specify			
Coarse hair	No	Yes	Specify	_____		
Hirsutism	No	Yes	Specify			

Deep-set nails	No	Yes	Specify	_____		
Dystrophic nails	No	Yes	Specify	_____		
Facial trichilemmomas	No	Yes	Specify	_____		
Acral keratosis	No	Yes	Specify	_____		
Other pigmentary changes	_____					
Developemntal delay						
Age at sitting	<8 months	8-12 months	>12 months			
Age at walking	<18 months	18 - 24 months	> 24 months			
Speech	Appropriate		Delayed			
School performance	Appropriate		Delayed			

NOTES

Genetic counselling recommended/requested	No	Yes	Previously Counselled	
Close relatives tested	No	Yes	Name:	Lab Ref:
			Name:	Lab Ref:
			Name:	Lab Ref:
			Name:	Lab Ref:
			Name:	Lab Ref:

Appendix D

Table PHC3 Distribution of personal history criteria type 3 (PHC3) amongst the likely CPS probands

Number	Case Identified	Tumour type	Clinical Features	Age of Diagnosis	Syndromic	Recognizable Syndrome
8.	PC0001.1.1	Nephroblastoma	SS (-3.1 SD), Short PFL, USPFs, Skin Hyperpigmentation R thigh	3 years	Yes	No
9.	PC0003.1.1	Nephroblastoma	USPFs, long PFL, Mc (+2.04 SD)	5 years	Yes	No
10.	PC0004.1.1	Nephroblastoma	SS (-1.8SD), Rt Mc, bulbous nose, SC, SFDwCd	6 years	Yes	No
11.	PC0005.1.1	Neuroblastoma	EF, BNR, SC	1 year	Yes	No
12.	PC0007.1.1	Pilocytic astrocytoma	Multiple CALs, axillary freckling, Mc (+1.81 SD)	8 years	Yes	NF1
13.	PC0011.1.1	Rhabdomyosarcoma	GR,LAHL,MH,HAP,Mg, CAL R chest, R 5th digit clinodactyly, R hand TPC	3 years	Yes	No
14.	PC0012.1.1	Nephroblastoma	PF, SE, Rt Mc (+1.61 SD), SFDwCd, brachydactyly of the toes & hypopigmented lesion on the forehead	3 years	Yes	No
15.	PC0013.1.1	Nasopharyngeal carcinoma	MH, HAP, dental crowding, hyperpigmented nevi on R side of face & chest, CALs, CT Nevi on sole of both feet, SFDwCd, scoliosis (L curvature)	12 years	Yes	Proteus Syndrome
16.	PC0015.1.1	Brainstem glioma	SE,Dc, deficient ear lobes, soft upturned helices, LSEs, MH, HAP with dental crowding, short neck. WSNs. SS (-3.03 SD), Rt Mc (+0.73 SD). Ataxia and signs of UMN lesion.	3 years	Yes	No
17.	PC0017.1.1	Nephroblastoma	Triangular face,MH, USPFs, LSEs, crumpled with overfolded helices, L hand single palmar crease and extra creases on digits	13 years	Yes	No

18.	PC0018.1.1	Nephroblastoma	MH, USPFs, HAP, full philtrum, LSEs, hypopigmented macules on the R & L thigh, Rt Mc (+0.56 SD)	06 months	Yes	No
19.	PC0019.1.1	Nephroblastoma	MH, HAP, short neck, SFDwCd,large CALs on the back & R leg	3 years	Yes	No
20.	PC0020.1.1	Nephroblastoma	Bitemporal narrowing, macroglossia, FNB, MH, Ht, USPFs, deficient antitragus, umbilical hernia & diastesis recti; ataxia, overgrowth; Had a large BW = 4.4kg, Rt Mc	1 year	Yes	BWS
21.	PC0021.1.1	Nephroblastoma	SE, USPFs, short PFL, Ht, MH, ear lobe creases, Pigmented nevi L finger, thigh, & knee, Mc (+2.67 SD)	2 years	Yes	BWS
22.	PC0022.1.1	Nephroblastoma	SE, bulbous nose, ataxia, LL hypotonia, Kyphoscoliosis, Tall stature & jnt contracture,Pigmented nevi (R index finger, neck and shoulder).	5 years	No	N/A
23.	PC0023.1.1	Pilocytic astrocytoma	Bitemporal narrowing, large ears, toungue protrusion, EF, USPFs, Lisch Nodules, CALs, axillary freckling	9 years	Yes	NF1
24.	PC0024.1.1	Ovarian cancer	Brachycephaly, overgrowth, SE, EF, USPFs, Ht; CAL R breast, back, skin striae on legs, hyperpigmented macule L thigh, hypertrophic scars.	12 years	Yes	No
25.	PC0026.1.1	Retinoblastoma	Cupped ears with overfolded helices, MH, WSNs, SFDwCd	1 year	Yes	No
26.	PC0027.1.1	Hepatoblastoma	large ears, SE, bulbous nose, Mg, SFDwCd, elbow joint restrictions, CALs: left inguinal and left cubital fossa	1 year	Yes	No

27.	PC0028.1.1	Osteosarcoma	High squared forehead, MH, SFDwCd, pigmented nevus (x 1) on the R side of abdomen	16 years	No	N/A
28.	PC0031.1.1	Brain astrocytoma	SE, LSEs, triangular face, SFDwCd, hypoplastic scrotum, large CAL on the right upper thigh (4x2 cm), hyperpigmented lesion on the medial aspect of R UL. No freckling. FTT (Wt and Ht < 3rd)	2 years	Yes	No
29.	PC0033.1.1	Nephroblastoma	MH, anteverted nares, LSEs with overfolded helices, HAP, SFDwCd	7 years	No	N/A
30.	PC0035.1.1	Nephroblastoma	PF, bitemporal narrowing, SE, MH, FNT, HAN, DSPF, EF, abnormal dentition on the lower jaw, LSE, Overfolded ear helices with creases, post auricular crease, short neck, WSNs, Shortchest, brachydactyly, Hypoplastic toe nails, Mild thoraco-Lumbar scoliosis, CALs, Pigmented nevus, multiple pigmented nevi, Rt Mc (on the background of FTT = Wt and Ht below 3rd centile)	3 years	Yes	BWS
31.	PC0038.1.1	Craniopharyngioma	SS (-6,2 SD), long eyelashes, left eye ptosis, small nose, HAN, MH, tendency to protrude tongue, Mg, short neck, SFDwCd, joint laxity at PIP, Reduced elbow extension	6 years	Yes	No
32.	PC0039.1.1	Rhabdomyosarcoma & adrenocortical carcinoma	Bitemporal narrowing, epicanthus Inversus, MH, HAP, SFDwCd	2 years	No	N/A
33.	PC0041.1.1	Nephroblastoma	Bitemporal prominances, pointed forehead, SE, USPFs, MH, BNR,HAN, SC, thin vermilion boarder of upperlip, shortneck, WSNs, hypoplastic toe and fingernails, SFDwCd, bilateral single palmar creases, CHD, DD ,Mc (+1,81 SD)	6 years	Yes	No

34.	PC0042.1.1	Nephroblastoma	Epicanthus inversus, Ht, Bulbous nose, USPFs, MH, open mouth, HAP, WSN, SFDwCd, CALsx3, DD, FTT	2 years	Yes	No
35.	PC0043.1.1	Retinoblastoma	Sparse and soft hair, laterally splayed eyebrows, microcephaly (-2,26 SD), LSEs, cupped, depressed nasal bridge, FNT, tented upperlip, SFDwCd, multiple hyperpigmented skin lesions	1 year	Yes	No
36.	PC0044.1.1	Nephroblastoma	MH, deficient earlobes, Ht, bulbous nose with SC, pointed chin, broad nasal root, SFDwCd, Pigmented nevus right hand	1 month	Yes	No
37.	PC0047.1.1	Bilateral nephroblastoma	laterally splayed eyebrows, MH, USPFs, BNR, FNT, deficient ear helices, flat chest, SFDwCd, CALs x 2	3 years	Yes	No
38.	PC0048.1.1	Osteosarcoma	FNB,BNR, EF, smooth philtrum,SFDwCd, thoracic scoliosis	17 years	No	N/A
39.	PC0053.1.1	Ovarian cancer	MH, proptotic eyes, prognathism, HAP, SFDwCd, Lumbar region gibbus, mild thoraco-lumbar scoliosis, pigmented streaky skin lesions, Mc (+3,45 SD)	3 years	Yes	No
40.	PC0054.1.1	Nephroblastoma	PF, SE, MH, Mg, HAP, open mouth, WSNs, SFDwCd, CALs x 3 – abdomen, chest, back), Mc (+2,44 SD)	4 years	Yes	No
41.	PC0056.1.1	Bilateral retinoblastoma	SE, MH, broad nose, thin vermilion border, ear lobe creases, WSNs, SFDwCd, hyperpigmented macule on the R UL	8 months	Yes	No
42.	PC0057.1.1	Phaeochromocytoma	EF, EFSPs, Flat nasal tip, broad nose, HAP, LSEs, shortneck , WSNs, inverted nipples,	8 years	Yes	No

			SFDwCd,Mc (+1,81 SD), sympomatic (excessive sweating, raised BP)			
43.	PC0058.1.1	Osteosarcoma	Short neck, MH, LSEs, SFDwCd, hypopigmented macules R leg & thigh, Mc (+4,18 SD)	6 years	Yes	No
44.	PC0059.1.1	Nephroblastoma	laterally splayed eyebrows, deep infra obital crease, USPFs, short PFL, BNR, tubular nose, SC, deep chin crease, retro Mg, LSEs, Left posterior ear crease, short neck, no macro glossia, umbilical hernia (post repair), SFDwCd, overlapping fourth and fifth toes, capillary hemangiomas	1 year	Yes	BWS
45.	PC0060.1.1	Nephroblastoma	PF, SE, prominent eyes,Ht,MH, BNR, FNT, HAN, deep philthrum groove, pectus carinatum, SFDwCd, restriction on elbow extension	1 year	Yes	No
46.	PC0063.1.1	Bilateral nephroblastoma	Large birth weight, PF, MH, anteverted nares, beaked nose, Mg, LSEs, preauricular pit, mild valvular PS, trivial TR, small testes, SFDwCd, proximally inserted thumbs, deep set nails, proximally inserted toes, broad 5th toes, left 2nd and 3rd toe overlap; Delayed speech	1 year	Yes	No
47.	PC0064.1.1	Bilateral nephroblastoma	Bushy eyebrows, peri-orbital puffiness, BNR, FNT, prominent upper lip, Mc, over folded ear helices, mild hypotonia, hepatomegaly, SFDwCd, CALs (chest & suprapubic)		Yes	No

*SS – short stature, PFL – palpebral fissure length, USPFs – Up slanted palpebral fissures, EF – epicanthic folds, Rt Mc – relative macrocephaly, Mc – macrocephaly, SC – short collumela, BNR – broad nasal root, FNT – flat nasal tip, SFDwCd – short 5th digits with clinodactyly, HAN – hypoplastic alae nasi, Ht – hypertelorism, Mg – micrognathia, SD – standard deviation, CAL – café au laits macules, GR – growth restriction, LAHL – low anterior hairline, MH – malar hypoplasia, HAP – high arched palate, R – right, L – left, TPC – transverse palmar crease, PF – prominent forehead, SE – sparse eyebrows, CT – connective tissue, Dc – dolicocephaly, LSEs – low set ears, WSNs – widely spaced nipples, FNB – flat nasal bridge, LL – lower limbs, CHD – congenital heart disease, DD – developmental delay, FTT – failure to thrive, BWS – Beckwith-Wiederman syndrome, NF1 – neurofibromatosis type 1, UMN – upper motor neuron,

Appendix E

Diagnostic criteria of syndromes recognized through clinical examination or analysis of family pedigrees.

Diagnosis	Diagnostic Criteria
NF1	• Six or more CALs • Two or more neurofibromata of any type or one or more plexiform neurofibromata • Freckling in the axilla, neck, or groin • Optic glioma (tumour in the optic pathway) • Two or more Lisch nodules • Distinctive bony lesion: dysplasia of the sphenoid bone / thinning or dysplasia of the long bone cortex • First - degree relative with NF1

BWS	• Cardinal features
	  ○ Macroglossia
	  ○ Exomphalos
	  ○ Lateralized overgrowth
	  ○ Multicofal or bilateral Wilm's tumour
	  ○ Hyperinsulinism (lasting >1 week and requiring escalated treatment)
	  ○ Pathology findings: adrenal cortex cytomegaly, placental mesenchymal dysplasia or pancreatic adenomatosis.
	• Suggestive features
	  ○ Birth weight > 2 SDS above the mean
	  ○ Facial naevus simplex
	  ○ Polyhydramnios and / or placentomegaly
	  ○ Ear creases and / or pits
	  ○ Transient hypoglycaemia (lasting < 1 week)
	  ○ Typical BWSp tumours (neuroblastoma, rhabdomyosarcoma, unilateral Wilm's tumour, hepatoblastoma, adrenocortical carcinoma or phaeochromocytoma)

	○ Nephromegaly and / or hepatomegaly ○ Umbilical hernia and / or diastasis recti
Proteus Syndrome	Mosaic distribution of lesions + progressive course + sporadic + one from A, or two from B or three from C • A - Connective tissue naevus (fibrous connective tissue with gyral pattern occurring most frequently on the soles, but also found on the palms and abdomen) • B – i)Epidermal naevus (linear, whorled, or verrucous and usually found on the neck, trunk, or extremities). Appear like areas of leathery **discoloured skin and** are present from infancy. ii) Disproportionate overgrowth of the limbs (arms/legs/hands/feet/digits), skull, external auditory meatus, vertebrae, or viscera (spleen / thymus). iii) Specific tumours < 20 years (bilateral cystadenomas, parotid monomorphic adenoma) • C – i) Dysregulated adipose tissue (lipomas, or regional absence of fat). ii) Vascular malformations (capillary malformation, venous malformation, lymphatic malformation). iii) Facial phenotype (dolicocephaly, long face, minor downslanting palpebral fissures and / or minor ptosis, wide or anteverted nares, open mouth appearance)
Li Fraumeni syndrome	• Proband with sarcoma diagnosed at <45 years of age, and • At least one first – degree relative with any cancer diagnosed at < 45 years of age, and • Another first – or second – degree relative in the lineage with any cancer at <45 years of age or sarcoma at any age.

Appendix F

Definitions of key data categories/entries

Data Entry	Definition
Likely CPS family	referred to a family that on a pedigree clearly demonstrated a Mendelian pattern of inheritance suggestive of a CPS. This pattern would either follow an autosomal dominant, autosomal recessive or X-linked inheritance and/or fit one of the known CPSs.
Unlikely CPS family	a family does not demonstrate any form of Mendelian inheritance of cancer, based on the pedigree.
Pigmentary abnormalities	refers to skin pigment changes that were significant for noting on an individual case, e.g. CALs
Macrocephaly	refers to "true" macrocephaly (OFC >97th centile) or relative macrocephaly (in relation to other growth parameters; particularly weight and height).
Syndromic	refers to an individual that, had an identifiable syndrome (based on phenotypic presentation – e.g. NF1, BWS, etc.). This also included information that may have been obtained from the family history data, e.g. MEN2A case. It also referred to a group of identifiable disorders, e.g. overgrowth syndrome, PIK3CA/AKT pathway disorders, etc.

Appendix G

Approved Research Protocol

CANCER PREDISPOSITION SYNDROMES AND THEIR GENETIC CONTRIBUTION TO PAEDIATRIC SOLID TUMOURS IN PATIENTS IN GAUTENG, SOUTH AFRICA

INTRODUCTION

Malignant solid tumours are relatively uncommon in children, when compared to adult cancer. Development of cancer in children poses a challenge and provides an opportunity for understanding basic cancer pathogenesis on the background of minimal exposure to environmental carcinogens in a child's life.

Environmental factors that predispose adults to development of cancers are, to a fair extent, understood, however when it comes to tumorigenesis in childhood, little is known about the predisposing factors. In this study, genetic predisposition to the development of cancer in children will be evaluated, with a particular focus on solid tumours.

There is consensus internationally, amongst a number of studies that about 8% of children with cancer harbour a hereditary (familial) predisposition. There is a growing interest in understanding childhood cancers, so as to potentially develop novel therapies for treatment.

Besides providing valuable information on potential treatment solutions, the limited knowledge on childhood cancer profiles limits physicians and scientists alike in developing new approaches to diagnosing and assessing the prognostic features of childhood cancers. Understanding genetic contribution to childhood cancer profiles would help mitigate risks to other at risk-family members, and help manage other predicted health risks in the proband.

Additionally, there is limited knowledge of childhood cancer profiles on the African continent. In the present study, the researchers are aiming to determine or quantify the significance of genetic predisposition to childhood cancers in Gauteng, South Africa, as determined clinically, and contribute towards the development of clinical guidelines that can be utilized by paediatricians in identifying patients that would benefit from a clinical genetics consultation. This study is nested in a larger study where detailed molecular genetic analysis will also be assessed.

BACKGROUND LITERATURE ANALYSIS AND CRITIQUE

Basics of cancer genetics

Advances in cell biology and molecular genetics have revolutionized our understanding of cancer in recent years. All cancers are caused by genetic mutations in somatic cells leading to aberrant cell division or loss of normal programmed cell death. A small proportion of cancer is strongly linked to inherited germline mutations behaving as Mendelian traits. As a general principle, it is believed that cancers arise as the end result of an accumulation of both inherited and somatic mutations in proto-oncogenes and tumour suppressor genes (Turnpenny & Ellard, 2012). If altered or mutated, tumour suppressor genes and DNA mismatch repair (MMR) genes significantly increase the likelihood of cancer development either through an environmentally stimulated pathway or by progression from an inherited germline aberration (Turnpenny & Ellard, 2012). These germline aberrations give rise to cancer predisposition syndromes (CPS).

For the purposes of the present research project, a cancer predisposition syndrome (CPS) can be defined as a genetic condition (caused by an inherited or de novo genetic alteration), that places an individual at a significantly higher risk of developing cancer than individuals in the general population. Features, that could be suggestive of a CPS in an individual, based on the personal and family history would include : (1) several close relatives (1st or 2nd degree) with a common cancer, (2) several close relatives with related cancers (e.g. breast, ovarian, bowel and endometrial), (3) two family members with the same rare cancer, (4) an unusually early age of onset of cancer, (4) bilateral tumours in paired organs, (5) synchronous or successive tumours in an individual, and (6) tumours in two different organ systems in one individual (Turnpenny & Ellard, 2012).

In addition to the personal and family history, according to Turnpenny & Ellard (2012) clinicians should also be aware of the physical signs that might be indicative of a specific CPS diagnosis in a child presenting with a tumour (see Table 1).

Table 1: Examples of CPS and some of their related features

CPS Diagnosis	**Clinical Features**
Peutz-Jegher syndrome	Melanin spots around the mouth and lips
Cowden disease	Macrocephaly, harmatomas, papillomatosis
Birt-Hogg-Dube syndrome	Dome shaped skin papules or trichodiscomas over the face and neck
Fanconi anaemia	Radial ray defects, postnatal growth failure, skin discolouration
Neurofibromatosis type 1 (NF1)	Café au lait macules, inguinal or axillary freckling, cutaneous neurofibromas
Familial adenomatous polyposis (FAP)	Presence of areas of congenital hypertrophy of the retinal pigment epithelium (CHRPEs)

Adapted from Turnpenny, P. D. and Ellard, S. (2012) *Emery's Elements of Medical Genetics.* Philadelphia, PA: Elsevier/Churchill Livingstone.

By combining a thorough personal and family history and a focused clinical examination, the identification of a CPS in a child may be helpful to guide care and surveillance. The clinical benefits for the patient, when a CPS is timeously diagnosed (even prior to any molecular confirmation) include the immediate commencement of key surveillance initiatives for the presenting tumour and for the associated clinical and oncologic complications, which, may be part of the particular CPS. Many cancer predisposing syndromes are inherited as autosomal dominant traits that are highly penetrant. This level of risk means that more invasive means of screening with more frequent and earlier initiation of screening protocols are justified than would be acceptable for the population in general (Turnpenny & Ellard, 2012). Identifying a CPS in a child, might assist the clinician, affected child and the family in optimizing care and the overall management plan. Jongmans *et al.*, (2016) and Postema *et al.*, (2017) highlighted the key benefits to identifying patients that have a CPS, including: (1) modified treatment strategies, (2) improved surveillance measures, (3) knowledge for family members who are at increased risk for themselves (4) opportunities for reproductive counselling and prenatal diagnosis, and (5) providing an answer for many parent's key question: "why did my child get cancer?"

Prevalence and incidence of childhood cancer

South African perspectives

Stefan *et al.*, (2015), published the first report from the South African Children Tumour Registry (SACTR), describing the incidence of childhood cancer over a 21- year period between 1987 and 2007. The study estimated the average annual incidence of childhood cancer at approximately 2340 new cases. In addition, the report estimated that 36 000 children are diagnosed with cancer annually in Africa, but that only 2% of the total African population is covered by a population-based registry. This alone demonstrates the significant underreporting of childhood cancer on the African continent. Despite the work done, in developing the registry – it remains unclear as to what proportion of cancers occurred as part of a CPS with an inherited cancer predisposition.

International perspectives

Ferrari *et al.*, (2007) published a study in the "Italian TREP project" – which was a national cooperative project on rare paediatric tumours. The study estimated that there are approximately 1800 new cases of childhood cancer a year in Italy. Further, the international agency for research on cancer, estimated that in poor economies the number of children who die from cancer represent 60% of those diagnosed; the equivalent figure being 20% or less in developed countries of Europe and North America (Stefan *et al.*, 2015). Late diagnosis and poor surveillance of cancer syndromes could be important contributory factors to low cancer survival rates amongst children in Africa. Hence, the need to properly triage children with cancer predisposition syndromes (CPS) to medical genetics services for confirming a diagnosis, surveillance and provision of adequate care and treatment.

The abovementioned studies concentrated mainly on the registries of paediatric cancer at a population level and did not specifically delineate cancers on the basis of inheritance exposure risk or any other form of cancer acquisition risk. Zhang *et al.*, (2015), sought to define the prevalence and spectrum of heritable mutations that could potentially explain the

index cancers in children. They concluded that germline mutations in cancer-predisposing genes were found in 8.5% of the children and adolescents with cancer. Family history did not predict the presence of an underlying CPS in most patients (Zhang *et al.*, 2015). Another group estimated that approximately 10% of children with cancer are affected by CPS, although they indicated likely under-reporting of cancer incidence in children (Goudie *et al.*, 2017). Grobner *et al.,* (2018) in a German study investigated the genomic alterations across childhood cancers, and identified 7-8% of children in their cohort who had a predisposing germline variant. There are no comparable studies in South Africa or Africa.

Profiling of childhood cancer

Profiling of paediatric solid tumours can be done in various ways. Sharma *et al.,* (2017) profiled paediatric solid tumours in their regional cancer centre (Kashmir – India) according to histology and body region. They identified the commonest among these to be CNS (central nervous system) tumours (26%), germ cell tumours (14.5%) and Ewing's sarcoma (14%). In a similar study in France, Desandes *et al.*, (2016) profiled neonatal solid tumours, and their results showed a different pattern – with neuroblastoma being the commonest (47%), followed by germ cell tumours (29%) and lastly CNS tumours (10%). It would be inexplicable to assume environmental exposures to be the only role player in the development of cancer in childhood, considering the short life span that a child might have had. Most likely, genetic predisposition plays a prominent role in childhood carcinogenesis.

Goudie *et al.*, (2017) are working on the McGill Interactive Paediatric Oncogenic Guidelines (MIPOGG) that are aimed at developing an approach to identify paediatric oncology patients most likely to benefit from a genetic evaluation. The report expresses the lack of guidelines necessary to identify children at high risk of having germline cancer predisposing mutations and plans to incorporate clinical data (such as tumour characteristics, personal and family histories) into tumour-specific algorithms to answer the difficult question of whether a child should be referred to a genetic service.

Jongmans *et al.,* (2017) developed and used a referral "tool" which included family history, personal history, type of tumour, and clinical examination findings to determine clinically whether to refer a child to Clinical Genetics services. Jongmans' tool is comprehensive and in synergy with the proposed criteria for use by Goudie *et al.*'s group (MIPOGG) as shown in Table 2 below. The key difference between the two criteria, being that Jongmans *et al.*, (2017) makes reference to "a child with excessive treatment toxicity" as one of the referral indications, whereas Goudie *et al.*, (2017) do not include this criterion.

In Gauteng, profiling of paediatric cancer patients for genetic assessment is virtually non-existent. There are also no formal clinical guidelines that exist to help paediatricians and paediatric oncologists to determine which patients need to be referred for a genetics assessment. The proposed research project intends to make use of family history, and additional tumour or clinical characteristics in the patient such as dysmorphic features and/or congenital anomalies and/or benign tumours to ascertain whether the affected child has any signs indicative of a CPS. It is envisioned that the data from this project will assist in the development of relevant clinical guidelines to ascertain patients that will benefit from being prioritized for a genetic assessment. The project will be linked to a larger molecular research project that is aimed at studying the genetic landscape of CPS mutations in children.

Table 2: Universal criteria and tumours that should be referred to cancer genetics services (direct referral)

UNIVERSAL CRITERIA

Personal History Criteria

> 1 primary tumour (asynchronous or synchronous)
Bilateral or multifocal primary tumours
Dysmorphic feature(s) or congenital abnormality(ies) that, according to the clinician's judgement, may be related to the cancer

Family History Criteria

Known cancer predisposition syndrome in the family
Close relative* with cancer at ≤ 18 years OR a parent /sibling/half-sibling with cancer at ≤ 50 years
Close relative* with same cancer type or same organ affected by cancer at any age
Close relative* with multiple prima tumours

* close relative: parent, sibling/half-sibling, aunt/uncle, cousin, grandparent

TUMOURS (with high risk of a germline mutation) FOR DIRECT REFERRAL

Atypical Teratoid Rhabdoid Tumour	Endolymphatic sac tumour	Pineoblastoma	Subependymal giant cell tumour
Choroid plexus carcinoma	Hemangioblastoma	Pituitary blastoma	Vestibular Schwannoma
Dysplastic cerebellar gangliocytoma	Optic pathway glioma	Retinoblastoma	
Renal/ Neuroblastic			
Cystic Nephroma	Renal Angiomyolipoma	Renal cell carcinoma	Renal rhabdoid tumour
Bone/Soft tissue			
Desmoid tumour	Gardner Fibroma	Malignant peripheral nerve sheath tumour	Nasal chondromesenchymal hamartoma
Extrarenal rhabdoid tumour			
Other			
Adrenocortical carcinoma	Hepatoblastoma	Pheochromocytoma	Small Cell Carcinoma of the Ovary, Hypercalcaemic Type
Cardiac rhabdomyoma	Medullary thyroid cancer	Paraganglioma	Carcinoma: of the breast, lung, cervix, uterus, bladder
Colorectal carcinoma	Ovarian Sertoli-Leydig cell tumour	Pleuropulmonary blastoma	
Gastrointestinal stromal tumour	Parathyroid tumour	Trichilemmoma	

Adapted from: Goudie *et al.,* (2017)

STUDY AIMS and OBJECTIVES

The aims of the study are to:

a) Assess whether children with particular types of solid tumours have features that would suggest a CPS and would thus benefit from referral to genetic services
b) Develop novel bedside approaches and guidelines to diagnosing childhood CPS

The aims of the project will be reached by addressing the following objectives:

1. Obtain three-generation family histories from paediatric patients with solid tumours (non-haematological).
2. Utilise a clinical tick sheet designed by the researcher to identify clinical features relevant to inherited cancer syndromes, including dysmorphic features, birth defects and benign tumours
3. Assess whether there are criteria identifiable through family history or clinical examination that identify CPS
4. Develop clinical guidelines that will aid clinicians in identifying patients who need to be referred for further genetic assessment.

This clinical study is nested in a larger study which will ultimately correlate clinical and molecular genetic analysis, and validate the clinical data.

METHODS

Study Participants: The study will recruit children diagnosed with solid tumours (malignant or benign) in the academic hospitals (i.e. Charlotte Maxeke Johannesburg Academic Hospital, Donald Gordon Medical Centre, Chris Hani Baragwanath Academic Hospital) linked to the Faculty of Health Sciences, University of the Witwatersrand. Recruited participants would be children (from age 0 – to – 18 years) diagnosed with a solid tumour, who are/were patients in the above-mentioned hospitals from January 2019 to December 2019. Informed consent will be obtained from the parents/ guardians of the participants. The obtained consent will include permission to access medical records of the patients, to document family history and to perform a comprehensive clinical examination.

A minimum of 60 - 100 patients will be recruited based on time available to the researcher and available patient numbers known to receive care in the study sites. Patients with haematological cancers and lymphomas will be excluded from the study to narrow the scope of the research project.

Study Sites: Paediatric oncology units based in academic hospitals in Gauteng

Study Design: This will be a descriptive study, assessing newly and previously diagnosed patients with childhood solid tumours. Our collaborators, Dr Jennifer Geel and Dr Gita Naidu, from Charlotte Maxeke Academic Hospital, Donald Gordon Medical Centre, and Chris Hani Baragwanath Academic Hospital, will assist in the identification and referral of potential participants for the study.

Upon referral of a patient with a diagnosis of a solid tumour the following will be done:

1. Obtaining of personal history to ascertain:
   - Existence of > 1 primary tumour (asynchronous or synchronous)
   - Presence or absence of bilateral or multifocal primary tumours
   - Presence or absence of any known congenital abnormalities or benign tumours

2. Obtaining a family history, including a 3 – generation family pedigree from the accompanying adult (parent/ guardian). Specific details which will be documented include:
   - Known cancer occurrences in the family
   - Close relative with cancer at ≤ 18 years OR a parent/ sibling/ half-sibling with cancer at ≤ 50 years
   - Close relative same cancer type or same organ affected by cancer at any age
   - Close relative with multiple primary tumours
   - Close relative with clinical features suggestive of a CPS

3. Performing clinical examination to assess for:
   - Dysmorphic features
   - Anthropometric measurements
   - Congenital abnormalities
   - Systematic (dermatological, neurological, cardiovascular, respiratory, etc.) examination
   - Clinical record/ file review to access additional medical information, special investigations and histolopathological reports

The information will be recorded on a clinical record sheet (see Appendix A), and kept in a file, to be stored securely at Division of Human Genetics, NHLS Braamfontein and the University of the Witwatersrand offices. The pedigrees will be formally recorded using the Invitae software pedigree program (www.invitae.com/en/family-history).

If a clinical diagnosis is identified by the researchers, the patient or family will be offered genetic counselling.

The flow chart below summarises the steps to be taken in patient recruitment and data collection (Figure 1).

Following individual analysis of the documented information; participants will be classified, as stated in Table 3 below.

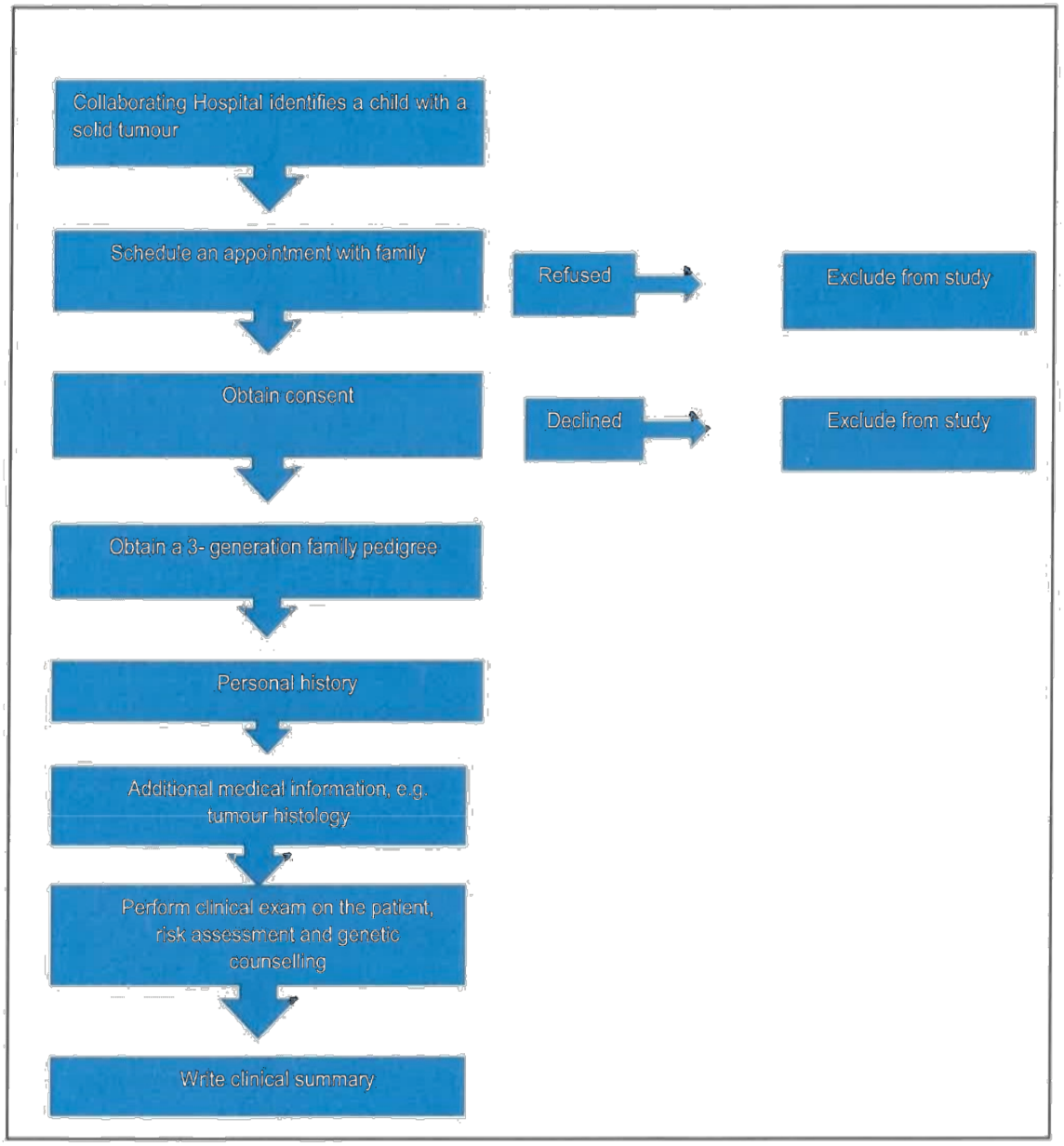

Figure 1: Work-flow for patient recruitment and data collection

Table 3: Individual classification of proband with a solid tumour

Classification	Criteria
Solid tumours likely to have a CPS	Meeting Universal Criteria and Tumours to be sent for direct referral as per Goudie *et al.*, (2017)
Solid tumours not likely to have a CPS	Not meeting Universal Criteria and Tumours to be sent for direct referral as per Goudie *et al.*, (2017)
Uncertain category	Unable to classify.

DATA ANALYSIS

The data will be entered into a RedCap database. The data analysis will be two-fold and will be tabulated using an Excel spread sheet. Most data will be descriptive.

The initial class of data to be analysed will be index case data.

The index case analysis and classification of the tumour will be based on the history, clinical examination and relevant investigations. The data from each case will be assessed in detail, to determine whether it fits the diagnostic criteria for a CPS or not. An index case would thus be classified as:

1. Likely to have CPS
2. Unlikely to have a CPS
3. Uncertain

The next class of data to be analysed, will be the family case data.

Family pedigree data analysis will be classified into:

1. Likely CPS family
2. Unlikely CPS family
3. Uncertain

Definitions of pedigree data categories:

Likely CPS family: This would be a family that clearly demonstrates a Mendelian pattern of inheritance suggestive of a particular CPS. This pattern would either follow an autosomal dominant, autosomal recessive or X-linked inheritance and/or fit one of the known CPSs. The classification could be based on a single individual, should the CPS be likely de novo.

Unlikely CPS family: This would be a family which, on the basis of the pedigree, does not demonstrate any form of Mendelian inheritance of cancer.

A descriptive statistical analysis of percentages of probands and families that meet the criteria for a CPS will be performed using statistical software programmes that are freely available (Excel; Graphpad statistical calculator). Additional information to be analysed will include the number of affected first and second degree relatives, average age of cancer diagnoses per tumour type and/or per CPS type. If sufficient data are obtained, distinguishing clinical features will be assessed and analysed.

ETHICS

Application for ethical clearance has been submitted to the Human Research Ethics Committee at the University of the Witwatersrand.

Consent forms (for study participation by parents and guardians of minors) and assent forms (for minors to participate in the study) are available, and they address, key ethical concerns of – (1) Inclusion of minors for research, and (2) Reimbursement for patients and families.

TIMING

	2018						2019	
	May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct - Dec**	**Jan-Jun**	**Jun-Dec**
Protocol								
Ethics application								
Data collection completion								
Data analysis								

FUNDING

The present project is nested within a larger project. An NHLS (National Health Laboratory Service) Research Grant, NRF Thuthuka Grant, CANSA grant have been applied for. Dr Lamola has also applied for FRC funding from the University of the Witwatersrand. I will also

apply for FRC funding in 2019. The key costs associated with this project specifically, are indicated in the budget table below:

Item	Estimated Costs
Participant transport costs reimbursement	R50-00 per patient and family member (60 clients) = R6000.00
Researcher travel costs	R650-00 per trip (8 trips)= R5200.00
Printing/Photocopying of consents and questionnaires	R1000-00
Dissertation binding	R200-00
Total Costs	R 12 400 -00

PROBLEMS/ LIMITATIONS

The potential limitations of the project are:

Children with cancer and their families are already under significant stress and pressure due to routine clinical service and therefore, they may not want to participate in a research study which does not offer tangible benefit to them / their child.

A diverse group of CPSs may be identified – this may complicate data analysis. However, the researcher would want to develop broad guidelines and thus a broad array of patients need to be sampled

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B] *Books*

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APPENDIX A

Solid Tumor - Clinical Record Phenotype Evaluation Tick Sheet

Demographic Data

Number _____
Date of Birth _____
Current Age _____
Age at presentation _____
Clinic Attending _____

Reason for Initial attendance at clinic _____

Tumor Type _____

Tumor Histology _____

Previous Tumours _____

Age at Diagnosis _____

Close relatives	Affected	Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
***Close relative**	parent, sibling/half-sibling, aunt/uncle, cousin, grandparennt						

Contact Details	Postal Adress	_____
	Mother Cell	_____
	Father Cell	_____
	Home Tel	_____
	Email Adress	_____

Pedigree	Completed	Suggestive family history	Not suggestive family history
	Not Completed		

Growth & Stature						
Birth Weight (kg)	>4	2.5 - 4	1.5 - 2.5	1.0 - 1.5	<1.0	
Current Weight	>97th	50th - 97th	10th - 50th	3rd - 10th	<3rd	
Current Height	>97th	50th - 97th	10th - 50th	3rd - 10th	<3rd	
Current Head Circumference	>97th	50th - 97th	10th - 50th	3rd - 10th	<3rd	
BMI	<17	17 - 25	25 - 30	>31		
BP		specify		Normal Range		
Hemihypertrophy		specify				
Facial Features						
Courseness	No	Yes				
Macroglossia	No	Yes				
Prominent forehead	No	Yes				
Malar Hypoplasia	No	Yes				
Flat nasal bridge	No	Yes				
Supernumerary teeth/ abnormal	No	Yes				

Beaked Nose	No	Yes				
Small Chin/ Micrognathia	No	Yes				
Long philthrum	No	Yes				
Short Columella	No	Yes				
Mucosal neuromas (Lips/tongue)	No	Yes				
Other	No	Yes				
Central Nervous System Abnormalities						
Nerual tube defects	No	Yes	Specify		Treatment	
Hypotonia	No	Yes	Specify			
Cerebellar signs (e.g. unsteady gait)	No	Yes	specify			
Other central nervous system abnormality	No	Yes	Specify			
Eye Variation						
Palpebral fissure length	>+2SD	>+1SD; <+2SD	Mean -+1SD	Mean >-1SD	<-1SD; >-2SD	<-2SD
Intercantal distannce	>+2SD	>+1SD; <+2SD	Mean -+1SD	Mean >-1SD	<-1SD; >-2SD	<-2SD
Strabismus	No	Left	Right			

Squint	No	Left	Right			
Epicanthic folds	No	Left	Right			
Palpebral fissure position	N/A	Upslanting	Downslanting			
Cataracts	No	Left	Right			
Congenital hypertrophy of the pigment epithelium	No	left	right			
Nystagmus	No	Left	Right			
Ptosis	No	Left	Right			
Other eye abnormalities	No	Left	Right	Specify		
Ear Variation						
Hearing tested	No	Yes			Method	
Hearing loss	No	Left	Right		Mild	Moderate
		Sensorineural	Conductive			
Low set ears	No	Left	Right			
Anterior ear lobe creases	No	Left	Right			
Posterior helical pits	No	Left	Right			
Other ear abnormalities						

Cardiovascular Abnormaities						
Cardiovascular evaluation	No	ECG Yes	ECHO Yes	Cardiologist	Yes	
		No	No	No		
PS/VSD/ASD	No	Yes		Specify		
Other Structural defects	No	Yes		Specify		
Other cardiac defects						
Gastrointestinal Malformations						
Duodenal/Jejunal atresia	No	Yes				
Tracheo-oesophageal fistula	No	Yes				
Omphalocoele	No	Yes				
Diastasis recti	No	Yes				
Umbilical hernia	No	Yes				
Imperforate anus	No	Yes				
Organomegaly	No	Yes				
Other gastrointestinal anormalies						
Renal Malformations						

Renal sonar done	No	Yes				
Kidney malformation						
*Horseshoe	No	Left	Right	Diagnosed on:	_____	
*Pelvic	No	Left	Right	Diagnosed on:	_____	
*Dysplastic	No	Left	Right	Diagnosed on:	_____	
*Hypoplastic	No	Left	Right	Diagnosed on:	_____	
*Agenesis	No	Left	Right	Diagnosed on:	_____	
Other	Specify	_____		Diagnosed on:	_____	
Collecting system malformation						
*Hydronephrosis	No	Left	Right	Diagnosed on:	_____	
*Hydroureter	No	Left	Right	Diagnosed on:	_____	
*Reflux Nephrophathy	No	Left	Right	Diagnosed on:	_____	
Other	Specify	_____		Diagnosed on:	_____	
Renal artery malformation	No	Left	Right	Diagnosed on:	_____	
Genital Malformations						
MALES						
Undescended testes	No	Left	Right			
Hypospadias	No	Yes				
Ambigous Genitalia	No	Yes				
Other male genital abnormality	_____					

FEMALES						
Hypoplastic vulva	No	Yes				
Upper Limb Malformations						
Polydactyly (pre- & post axial)	No	Left	Right			
Macrodactyly	No	Left	Right			
Camptodactyly	No	Left	Right			
Other Upper limb malformations	No	Left	Right	Specify		
Lower Limb Malformations	No	Left	Right			
Polydactyly (pre- & post axial)	No	Left	Right			
Macrodactyly	No	Left	Right			
Camptodactyly	No	Left	Right			
Other Upper limb malformations	No	Left	Right	Specify		
Vertebral abnormalities						
Scolosis	No	Yes				
Kyphosis	No	Yes				
Other Vertebral abnomalities						

Skin Changes						
Café au lait spots (>2 patches, each >5mm)	No	Yes	Specify			
Hyperpigmentation	No	Yes	Specify			
Hypopigmentation	No	Yes	Specify			
Lentigines	No	Yes	Specify			
Neurofibromas	No	Yes	specify			
Hyperkeratosis	No	Yes	Specify			
Sparse hair	No	Yes	Specify			
Papillomas	No	Yes	Specify			
Poikiloderma	No	Yes	Specify			
Dark melanin spots	No	Yes	Specify			
Thickened skin: soles of feet	No	Yes	Specify			
Coarse hair	No	Yes	Specify			
Hirsutism	No	Yes	Specify			

Deep-set nails	No	Yes	Specify		
Dystrophic nails	No	Yes	Specify		
Facial trichilemmomas	No	Yes	Specify		
Acral keratosis	No	Yes	Specify		
Other pigmentary changes					
Developemntal delay					
Age at sitting	<8 months	8-12 months	>12 months		
Age at walking	<18 months	18 - 24 months	> 24 months		
Speech	Appropriate		Delayed		
School performance	Appropriate		Delayed		

NOTES

Genetic counselling recommended/requested	No	Yes	Previously Counselled	
Close relatives tested	No	Yes	Name:	Lab Ref:
			Name:	Lab Ref:
			Name:	Lab Ref:
			Name:	Lab Ref:
			Name:	Lab Ref:

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