ENDOCRINE PROFILING IN BLACK SOUTH AFRICAN FANCONI ANAEMIA PATIENTS, HOMOZYGOUS FOR A *FANCG* FOUNDER MUTATION

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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

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Declaration

I, **Bronwyn Dillon**, 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)

8th day of July in 2019 in Johannesburg

Contribution of the candidate to the paper

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Bronwyn Dillon, student number 0300869W, declare that this Research Report is my own work and that I contributed significantly (collection and analysis of all data) towards the research findings presented in the paper intended for publication below.

Signature of student:

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Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of her Research Report.

Article title: Endocrine Profiling in Black South African Fanconi Anaemia Patients, Homozygous for a *FANCG* Founder Mutation.

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This Research Report is dedicated to my daughter, Jessica Bezuidenhout.

Presentations arising from this research project

1. Dillon B, Feben C, Poole J, Wainwright R, Segal D, Krause A. Endocrine profiling in Black South African Fanconi anaemia patients, homozygous for a *FANCG* founder mutation – a look at preliminary data. Southern African Society for Human Genetics, Biennial Congress 2017, 13th – 16th August, Durban, KwaZulu-Natal, South Africa (poster presentation).

Abstract

Fanconi anaemia (FA) is an uncommon, phenotypically diverse, hereditary condition associated most commonly with bone marrow failure, multiple physical congenital abnormalities, and an increased susceptibility to the development of haematological and solid tissue malignancies. Less recognised manifestations of FA include endocrine abnormalities. International discourse has highlighted that these abnormalities are widespread among individuals with FA. To date there has been no systematic study that has evaluated the endocrine abnormalities in Black South African patients with FA, particularly those homozygous for a founder mutation (c.637_643delTACCGCC) in *FANCG*. The objectives of this research were to evaluate endocrine gland function in Black South African patients with FA and a single FA genotype, and to determine the frequency and nature of endocrine abnormalities in this group. The study comprised of a cross-sectional, descriptive study of 24 Black South African patients with FA (homozygous for a founder *FANCG* mutation), between the ages of 2 years and 21 years, recruited from South African tertiary academic hospitals. Measured outcomes included: growth, pubertal status, endocrine hormone function (including screening of the growth hormone axis, thyroid gland function, and glucose and insulin metabolism) and bone age. Endocrine dysfunction was present in 70.8% (17 of 24) of the study cohort, including short stature in 45.8% (11 of 24), abnormal IGF-1/IGFBP-3 in 25.0% (6 of 24), insulin resistance in 41.7% (10 of 24), and abnormal thyroid function in 16.7% (4 of 24). No abnormalities of glucose metabolism were identified. Abnormal pubertal status was seen in three males (12.5% of the study cohort). Abnormal bone ages were present in 34.8% (8 of 23) of the cohort, and abnormally fused carpal bones were present in 13.0% (3 of 23). It was concluded that endocrine abnormalities occur at a high frequency in Black South African patients with FA, similar to other FA cohorts. Our data are specific to FA patients with a single genotype, and therefore this provides the first genotype-phenotype information on endocrine abnormalities in Black South African patients, homozygous for a founder *FANCG* mutation. Recommendations regarding endocrine screening in this patient subgroup are made, including, but not limited to, baseline testing of thyroid function, fasted insulin and glucose, and IGF-1 and IGFBP-3.

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Special thanks to my husband, Shawn Bezuidenhout, and my parents, Blythe and Sandy Dillon, for their continuous support and love.

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List of Abbreviations

BA	Bone age
BMI	Body mass index
CHBAH	Chris Hani Baragwanath Academic Hospital
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
FA	Fanconi anaemia
FARF	Fanconi Anemia Research Foundation
FT4	Free thyroxine
GH	Growth hormone
GHD	Growth hormone deficiency
HOMA	Homeostasis model assessment
HOMA-IR	Homeostasis model assessment of insulin resistance
HSCT	Haematopoietic stem cell transplantation
IGF-1	Insulin-like growth factor 1
IGFBP-3	Insulin-like growth factor-binding protein 3
kg	Kilogram
L	Litre
MC	Microcephaly
ml	Millilitre
mm	Millimeter
MPH	Mid-parental height
NA	Not available

NHLS	National Health Laboratory Service
OGTT	Oral glucose tolerance test
OWFA	Overweight-for-age
SBAH	Steve Biko Academic Hospital
SD	Standard deviation
SDS	Standard deviation score
SS	Short stature
TSH	Thyroid stimulating hormone
UAH	Universitas Academic Hospital
UWFA	Underweight-for-age
WHO	World Health Organization

Research Report in the format of a “submissible” paper

CLINICAL RESEARCH ARTICLE

Endocrine Profiling in Black South African Fanconi Anaemia Patients, Homozygous for a *FANCG* Founder Mutation

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Précis: Twenty-four Black South African patients with Fanconi anaemia (FA), homozygous for a founder *FANCG* mutation (c.637_643delTACCGCC), were evaluated for abnormalities of endocrine function. Evaluations included assessment of growth, bone age, pubertal stage, thyroid function, growth hormone axis, and insulin and glucose metabolism. Endocrine abnormalities were present in a high frequency (70.8%) of the study cohort, in keeping with other reported FA cohorts; however, the individual endocrine dysfunctions varied somewhat in frequency when compared to these FA cohorts. Recommendations for endocrine screening in this FA subgroup are made including, but not limited to, baseline testing of thyroid function, fasted insulin and glucose, and IGF-1 and IGFBP-3.

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

Abstract

Context: Fanconi anaemia (FA) is an uncommon, phenotypically diverse, hereditary condition associated most commonly with bone marrow failure, multiple physical congenital abnormalities, and an increased susceptibility to the development of haematological and solid tissue malignancies. Less recognised manifestations of FA include endocrine abnormalities. International discourse has highlighted that these abnormalities are widespread among both children and adults with FA. To date there has been no systematic study that has evaluated the endocrine abnormalities in Black South African patients with FA, particularly those homozygous for a founder seven base-pair deletion mutation (c.637_643delTACCGCC) in *FANCG*.

Objective: To evaluate endocrine gland function in Black South African patients with FA and a single FA genotype, and to determine the frequency and nature of endocrine abnormalities in this group.

Study Design, Settings and Patients: Cross-sectional, descriptive study of 24 Black South African patients with FA (homozygous for a founder *FANCG* mutation), between the ages of 2 years and 21 years, recruited from tertiary academic hospitals within South Africa.

Main Outcomes Measured: Growth, pubertal status, endocrine hormone function (including screening of the growth hormone axis, thyroid gland function, and glucose and insulin metabolism) and bone age.

Results: Endocrine dysfunction was present in 70.8% (17 of 24) of the study cohort, including abnormal IGF-1/IGFBP-3 in 25.0% (6 of 24), insulin resistance in 41.7% (10 of 24), abnormal thyroid function in 16.7% (4 of 24), and short stature in 45.8% (11 of 24). No abnormalities of glucose metabolism were identified. Patients with endocrine abnormalities were not significantly shorter than their counterparts. Abnormal pubertal status was seen in three males (12.5% of the study cohort). Abnormal bone ages were present in 34.8% (8 of 23) of the cohort, and abnormally fused carpal bones in 13.0% (3 of 23).

Conclusions: Endocrine abnormalities occur at a high frequency in Black South African patients with FA, similar to other FA cohorts. Our data are specific to FA patients with a single genotype, and therefore provide the first genotype-phenotype information on endocrine abnormalities in Black South African patients, homozygous for a founder *FANCG* mutation. Recommendations regarding endocrine screening in this patient subgroup are made, including, but not limited to, baseline testing of thyroid function, fasted insulin and glucose, and IGF-1 and IGFBP-3.

Keywords: Endocrine abnormalities, thyroid hormone, growth hormone, insulin, glucose, bone age.

1. Introduction

Fanconi anaemia (FA) is an uncommon, phenotypically diverse, hereditary chromosome breakage disorder characterised by deoxyribonucleic acid (DNA) hypersensitivity to cross-linking agents at a molecular level, with resultant chromosome instability (1). To date, 22 FA-associated genes have been identified, designated FANCA – W, demonstrating the marked genetic heterogeneity that FA exhibits (2). These FANC genes encode FA proteins, which operate together in a shared FA pathway, considered a DNA damage response or DNA repair pathway that regulates the cells' resilience to harmful DNA interstrand cross-linking agents (1,3). If this pathway becomes disrupted, by a pathogenic variant in a FA-related gene, the cellular and clinical abnormalities suggestive of FA manifest (4).The FA subtypes are inherited predominantly in an autosomal recessive manner; however, heterozygous dominant-negative mutations in the *FANCR* gene and hemizygous mutations in the *FANCB* gene result in the less common autosomal dominant and X-linked forms of FA, respectively (1,5,6).

Although FA is thought to be a rare disorder (estimated prevalence 1 – 5 per million), the prevalence in certain South African population groups, such as the Afrikaner and Black populations, has been found to be much higher (7). The term 'Black' has been used to describe individuals deriving from sub-Saharan Bantu-speaking indigenous ancestry groups (8). Morgan *et al.* proposed that the birth incidence of FA in the Black South African population is higher than 1 in 40 000 based on carrier frequency data obtained from gene frequency studies (9). The proposed reason for this higher incidence is a genetic founder mutation in the *FANCG* gene (9). In the Black South African FA population studied, a seven base-pair deletion mutation (c.637_643delTACCGCC) was identified in 82.5% of individuals tested (present in a homozygous state in 77.5%) (9). Black South African patients with FA thus represent a unique patient cohort from a genetic homogeneity perspective. When compared to other FA cohorts, individuals with FA, specifically homozygous for the founder *FANCG* mutation, have been found to have significant growth restriction and a higher incidence of renal abnormalities, abnormal skin pigmentary lesions and present with severe cytopenia (10,11). Given this predominantly genetically homogeneous group,

molecular genetic testing for the founder *FANCG* mutation is now the first line diagnostic test for Black patients suspected to have FA (12).

Clinically, FA is associated most commonly with bone marrow failure, multiple congenital physical abnormalities, and an increased susceptibility to the development of haematological and solid tissue malignancies (13). Less recognized manifestations of FA include a wide range of abnormalities of endocrine gland function, which are influenced to a certain extent by the various treatments used in the management of patients with FA, such as androgen therapy or haematopoietic stem cell transplantation (HSCT) (13). Internationally, more recent discourse by Giri *et al.* and Rose *et al.* has highlighted that endocrine abnormalities are widespread (79%) among both children and adults with FA (13,14). Of the endocrine abnormalities identified, the most notable were short stature and/or growth hormone (GH) deficiency (GHD) (51%), abnormal gonadal function (65%), hypothyroidism (37%), and dysfunctional glucose/insulin metabolism (39%) (13). Strikingly, a more recent study identified at least one endocrine abnormality in 79% of the overall study group (14). As the studies of Giri *et al.* and Rose *et al.* represent the two most definitive papers on endocrine abnormalities in FA, the data in this study have been compared to these (13,14). Under-nutrition, low body mass index (BMI), raised BMI, reduced bone mineral density, and pituitary gland abnormalities are other endocrine abnormalities that have been documented in patients with FA (15).

While numerous previous research studies have documented the major endocrine abnormalities in patients with FA, these studies have assessed individuals with FA of various genotypes to give general frequencies of these disorders. Very little genotype-specific information has yet been documented in the literature. Our study aimed to evaluate the need for routine screening of endocrine status in Black South African patients with FA, with a specific focus on patients homozygous for the founder *FANCG* mutation.

2. Subjects and Methods

A. Subjects

Patients were recruited from the Paediatric Haematology/Oncology Units at four tertiary academic hospitals in South Africa: Chris Hani Baragwanath Academic Hospital (CHBAH) in Johannesburg, Gauteng (five patients (21% of the cohort)); Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg, Gauteng (five patients (21%)); Steve Biko Academic Hospital (SBAH) in Pretoria, Gauteng (six patients (25%)); and Universitas Academic Hospital (UAH) in Bloemfontein, the Free State (eight patients (33%)). They were assessed at their respective units for the research project. Recruitment took place over 19 months, from January 2017 to August 2018.

The present study included 24 Black South African patients, confirmed to be homozygous for the seven base-pair *FANCG* deletion mutation (c.637_643delTACCGCC). Targeted mutation analysis had been performed by the Molecular Genetics Laboratory of the National Health Laboratory Service (NHLS) in Braamfontein, Johannesburg, South Africa. Patients who met the study inclusion criteria and their parent/guardian were required to read the information document, available in English, Afrikaans or Sesothu (Appendices A-F), and read and sign informed consent/assent (Appendices G-I) indicating their wish to participate in the study. These patients consented/assented to a file review, physical examination (including external genitalia examination for Tanner staging), a fasted venous blood draw, and wrist and hand X-rays.

Ethics clearances were obtained from The University of the Witwatersrand Human Research Ethics Committee (certificates M160220 and M1703108), the Faculty of Health Sciences Research Ethics Committee of the University of Pretoria (certificate 547/2017) and the Health Sciences Research Ethics Committee of the University of the Free State (certificate UFS-HSD2017/1406/3107) (Appendix J).

B. Clinical Examination

Patients underwent a clinical examination, which included anthropometric measurements and Tanner pubertal staging. The anthropometric measurements included weight (to the nearest 0.1 kilogram (kg)) using a Seca© electronic scale, height (to the nearest millimeter (mm)) using a floor-standing Seca© stadiometer, and head circumference (to the nearest mm) using a tape measure. Height, weight, and head circumference measurements were obtained in triplicate, from which a final average measurement was obtained. Growth anthropometric measurements were expressed as standard deviation (SD) scores (SDS), based on age- and sex-matched growth charts from the Handbook of Physical Measurements (16). In the evaluation of a child with short stature, calculation of the genetic height potential of a child is often performed. When the child's current height is not in keeping with the projected adult height derived from a mid-parental height (MPH) assessment, other intrinsic or extrinsic factors are likely contributing to the child's short stature. A limitation of our study was the lack of available parental height measurements to calculate the MPH and thereby estimate the genetic height potential of the study patients. Body mass index was calculated for each patient using the standard BMI formula (weight (kg)/height $(m)^2$). Body mass index was expressed as a SDS calculated using the World Health Organization (WHO)-AnthroPlus software (17).

Tanner staging was assessed using a Tanner staging chart and a Prader orchidometer to assess testicular volume in the male patients (18,19). The normal age range for the onset of puberty for females was considered as eight years to 13 years, and for males was between nine years six months and 13 years six months (18-20). These are the ages at which 95% of children attain Tanner stage two pubertal development (18-20). Pubarche is the development of pubic and axillary hair, thelarche the development of breast buds, and gonadarche is testicular volume equal to or greater than four millilitres (ml) (21). A clinical data collection sheet was drafted and completed to record the data obtained during the study visit (Appendix K).

Short stature is defined as height-for-age more than 2 SD below the WHO growth reference mean; underweight-for-age is defined as weight more than 2 SD below the mean on a WHO weight-for-age

growth chart; overweight-for-age is defined as weight-for-height greater than 2 SD above WHO growth reference mean (for children under 5 years) and BMI greater than 1 SD above WHO growth reference mean (for children aged 5 to 19 years) (22); microcephaly is considered as head circumference more than 2 SD below the growth reference mean (23).

C. Endocrine Hormone Testing

Endocrine hormone testing included glucose and insulin metabolism (by measuring fasting plasma glucose and insulin levels); thyroid gland function (by measuring thyroid stimulating hormone (TSH) and free thyroxine (FT4)); and screening of the GH axis (consisting of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor-binding protein 3 (IGFBP-3) measurements). Measurements were assessed from a single, overnight fasted venepuncture sample (drawn between 08h00 and 10h00), and were evaluated in two Lancet Laboratory branches, in Johannesburg and Bloemfontein. Blood measurements were evaluated against the laboratory age and sex matched control reference ranges.

Fasting insulin and glucose levels were used to calculate the homeostasis model assessment of insulin resistance (HOMA-IR) index. This model uses the following formula: fasting insulin concentration (milliunits per litre) multiplied by fasting glucose concentration (millimoles per litre) divided by 22.5 (24). A HOMA-IR value of greater than two was considered as insulin resistant, in order to standardize with the cut off value used by Giri *et al.*'s FA cohort and to allow direct comparison (13).

Subclinical hypothyroidism is defined as serum TSH above the upper limit of normal age-matched ranges with a normal level of FT4, and overt hypothyroidism is characterized by a raised TSH in combination with a lower than normal FT4 (25).

The diagnosis of GHD is not obtained from a single test but rather is a process involving clinical evaluation, radiological assessment (bone age and central nervous system imaging), and biochemical testing (including provocative (stimulation) GH testing and IGF-1 and IGFBP-3 testing) (26).

D. Radiographic Studies

Left wrist and hand X-rays were performed on the day of the study visit and reviewed by Professor David Segal, Paediatric Endocrinologist, to assess bone age (BA) using the published standards of Greulich and Pyle (27). Bone age was expressed as being normal, advanced (more than 2 SD above the mean for age and sex) or delayed (more than 2 SD below the mean for age and sex) (28). In addition to bone age, the X-rays were also examined for bony abnormalities.

It is important to note that the bone age standards published by Greulich and Pyle in 1959 are based on European population data (27). These standards have low accuracy when estimating skeletal age of African individuals, and skeletal age standards specific to African populations still need to be developed (29). For this reason, the reporting of delayed/advanced BA in the present cohort may be inaccurate.

E. Statistical Methodology

Data were captured and analyzed statistically using Microsoft Excel (2013). The frequency of growth disturbances and endocrine abnormalities were documented and comparisons were made, where possible, with the Giri *et al.* and Rose *et al.* cohorts (13,14). These cohorts included individuals with FA with varying genotypes.

Continuous variables (such as weight, height and head circumference) were compared to the Giri *et al.* and Rose *et al.* FA cohorts using an unpaired *t* test. Fisher's exact test was used to calculate the p value from a 2 x 2 contingency table of categorical variables (such as number of individuals with an endocrine abnormality). Differences in means were considered statistically significant with p values <0.05. Statistical data were verified by staff of the Epidemiological Data Centre of The University of the Witwatersrand.

3. Results

A. Demographic Data

The total study group consisted of 24 Black South African patients, homozygous for the seven base-pair *FANCG* founder mutation (c.637_643delTACCGCC). Thirteen (54%) of the patients were male and 11 (46%) female. The median age was 9.5 years (range 3 – 19 years), with the majority of patients (23 (96%)) under 18 years of age at the time of data collection.

Giri *et al.*'s FA cohort consisted of 45 patients (19 males and 26 females) between the ages of two to 49 years (28 were 18 years or younger, and 17 were over 18 years old) (13). Giri *et al.*'s study analyzed retrospective endocrine data, whereas the present study is a cross-sectional study (13). Our study makes comparisons to the entire Giri *et al.* cohort, and where possible to the paediatric cohort specifically (13).

Rose *et al.*'s FA cohort consisted of 78 children (43 females and 35 males) between the ages of 0.3 and 15.9 years, and 42 adults (defined by attainment of adult height) (19 females and 23 males) between the ages of 13.5 and 31 years (14). Twenty-six patients of the *Rose et al.* FA cohort were 18 years or older (14 females and 12 males) (14). Our study makes comparisons to the entire Giri *et al.* cohort, and where possible to the paediatric Giri *et al.* cohort specifically, and to the paediatric Rose *et al.* cohort specifically (13, 14).

B. Clinical Examination

1. Growth measurements

Growth measurements (weight, height and head circumference) are depicted in Figure 1. The growth measurements of the Black South African FA cohort were compared with those of the patients in Giri *et al.*'s cohort (Table 1) (13).

1.1 Weight measurements

The median weight SDS for the study cohort was -1.6 (range 0.6 to < - 3.0; mean -1.7 ± 0.9). Fifty percent (12 of 24) of the cohort was an appropriate weight-for-age. Forty-two percent (10 of 24) were underweight-for-age, compared to 22.0% identified in Giri *et al.*'s cohort (p value = 0.10) (13). The median BMI for the study cohort was -0.7 (range 1.2 to -2.3; mean -0.7 ± 1.0). Only 8.3% (2 of 24) were overweight-for-age, compared to almost 27.0% overweight in Giri *et al.*'s cohort, although this was not statistically significant (p value = 0.12), and 11.0% of children (p value = 1.00) with FA in Rose *et al.*'s cohort (13,14).

1.2 Height measurements

The median height SDS for the study cohort was -1.9 (range 0.32 to < -3; mean -1.8 ± 1.0). Short stature was present in 45.8% (11 of 24) of patients, similar to the 51.1% seen in Giri *et al.*'s cohort (p value = 0.80) (13).

1.3 Head circumference measurements

The median head circumference SDS for the study cohort was -1.7 (range 0.0 to <-3; mean -1.7 ± 0.8). Microcephaly was present in 33.3% (8 of 24) of patients. Of the eight patients with microcephaly, three had a normal weight and height, one was underweight, one was short, and three were both underweight and short.

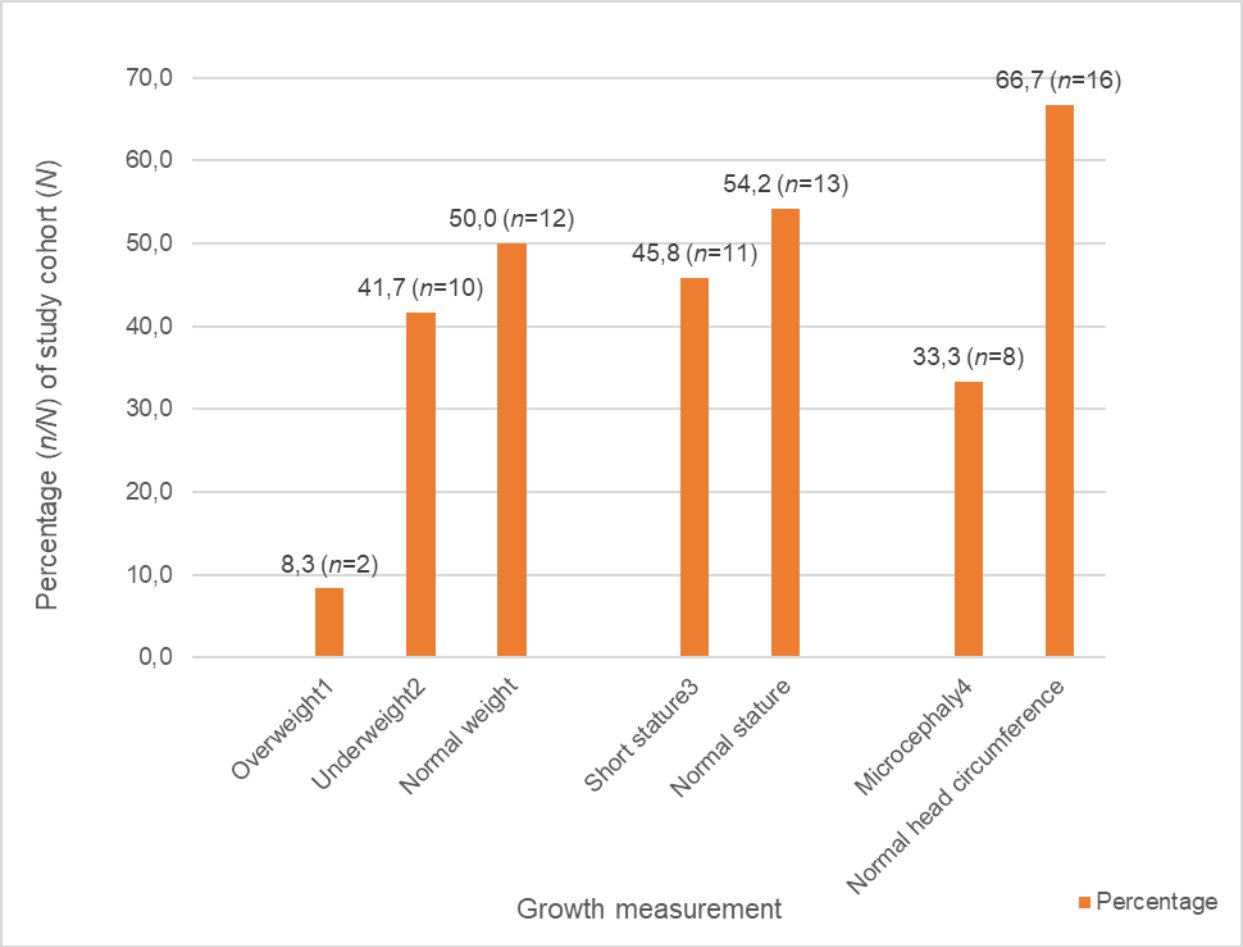

Figure 1 Growth measurement distribution of Black South African patients with FA (*N*=24)

Notes: [1]Overweight-for-age is defined as weight-for-height greater than 2 SD above WHO growth reference mean (for children under 5 years) and BMI greater than 1 SD above WHO growth reference mean (for children aged 5 to 19 years); [2]underweight-for-age is defined as weight more than 2 SD below the mean on a WHO weight-for-age growth chart; [3]short stature is defined as height-for-age more than 2 SD below the WHO growth reference mean (22); [4]microcephaly is defined as head circumference more than 2 SD below the growth reference mean (23).

Abbreviations: FA, Fanconi anaemia; SD, standard deviation; WHO, World Health Organization; BMI, body mass index.

Table 1 Comparison of the growth measurements between the present Black South African FA cohort and Giri *et al.*'s FA cohort (13)

Growth measurement	**Black South African FA cohort (*N*=24), *n/N* (%)**	**Giri *et al.*'s FA cohort (13) (*N*=45), *n/N* (%)**	**p value**
Underweight[1]	10/24 (41.7)	10/45 (22.2)	0.10
Overweight[2]	2/24 (8.3)	12/45 (26.7)	0.12
Short stature[3]	11/24 (45.8)	23/45 (51.1)	0.80
Microcephaly[4]	8/24 (33.3)	NA	NA

Notes: [1]Underweight-for-age is defined as weight more than 2 SD below the mean on a WHO weight-for-age growth chart; [2]Overweight-for-age is defined as weight-for-height greater than 2 SD above WHO growth reference mean (for children under 5 years) and BMI greater than 1 SD above WHO growth reference mean (for children aged 5 to 19 years); [3]short stature is defined as height-for-age more than 2 SD below the WHO growth reference mean (22); [4]microcephaly is defined as head circumference more than 2 SD below the growth reference mean (23).

Abbreviations: FA, Fanconi anaemia; NA, not available.

1.4 Growth measurements and their relationship to pubertal status and bone age

Eighteen percent (2 of 11) of the FA individuals with short stature had abnormal pubertal development (one precocious and one delayed gonadarche). Of the FA individuals with short stature, 45.5% (5 of 11) had abnormal (delayed) bone ages.

2. Pubertal assessment

As nine of the 11 females in the present study were receiving androgen therapy (which can cause virilization as a side effect) only thelarche, and not pubarche, was used to assess pubertal stage. All 11 females had appropriate pubertal development, according to breast development.

As 11 of the 13 males in the present study were receiving androgen therapy, only gonadarche, and not pubarche, was used to assess pubertal stage. Seventy-seven percent (10 of 13) of males had age-appropriate pubertal development. Two of the males had delayed gonadarche (15.4%) and one had precocious gonadarche (7.7%). All of the males with delayed and precocious gonadarche had normal bone ages.

C. Endocrine Hormone Testing

Fifty-eight percent (14 of 24) of the study cohort had at least one endocrine abnormality (including abnormal screening of the GH axis, insulin resistance and abnormal thyroid functions (hypothyroidism and subclinical hypothyroidism). The majority (37.5% (9 of 24)) of these patients had one endocrine abnormality, 16.7% (4 of 24) had two endocrine abnormalities and one patient (4.2%) had three endocrine abnormalities. Frequency of endocrine abnormalities were compared to those in Giri *et al.*'s FA cohort (Table 2) (13).

1. Growth hormone axis testing

Twenty-five percent (6 of 24) of the study cohort had abnormal IGF-1 and/or IGFBP-3 levels (Figure 2). All of the abnormal results were low IGF-1 and/or IGFBP-3. Screening of the growth hormone axis was normal in 75.0% (18 of 24) of the present study cohort. Of the patients with low IGF-1/IGFBP-3, 33.3% (2 of 6) also had abnormal thyroid function (one subclinical hypothyroidism and one hypothyroidism). Fifty percent (3 of 6) of the patients with low IGF-1/IGFBP-3 had short stature.

2. Thyroid function

The laboratory-provided normal TSH reference range for the individuals in the present study cohort was 0.35 - 4.94 uIU/mL, and the normal FT4 range was 9.0 - 19.0 pmol/L. Abnormal fasting thyroid function levels were identified in 16.7% (4 of 24) of the study cohort (Figure 2). Overt hypothyroidism was seen in one individual (4.2%) in the present study, compared to 37.1% of Giri *et al.*'s entire FA cohort (p value = 0.00) (and 20.0% (5 of 20) of Giri *et al.*'s patients aged 18 years or less (p value = 0.08)), and 61% of Rose *et al*.'s FA cohort (p value = 0.00) (13,14). Three individuals (12.5%) had subclinical hypothyroidism, compared to 14.3% in Giri *et al.*'s FA cohort (p value = 1.00) (13).

3. Insulin and glucose metabolism

No abnormal fasting glucose levels were identified in the present study cohort (Figure 2). High insulin levels were found in 37.5% (9 of 24) of the present study cohort. The number of patients with insulin

resistance in the present study was 41.7% (10 of 24), matching that of Giri *et al*.'s cohort (p value = 1.00) (13). Of the 10 individuals with insulin resistance, two (20.0%) were also overweight-for-age.

Table 2 Comparison of endocrine abnormalities between the present Black South African FA cohort and Giri *et al.*'s FA cohort (13)

Endocrine abnormality	**Black South African FA cohort (*N*=24), *n/N* (%)**	**Giri *et al.*'s FA cohort (13) (*N*=35), *n/N* (%)**	**p value (Fisher's exact test)**
Thyroid function abnormality	4/24 (16.7)	18/35 (51.4)	0.01
Hypothyroidism*	1/24 (4.2)	13/35 (37.1)	0.00
Subclinical hypothyroidism[#]	3/24 (12.5)	5/35 (14.3)	1.00
Impaired fasting glucose	0/24 (0.0)[§]	10/41 (24.4)[‡]	0.01
Insulin resistance[Φ]	10/24 (41.7)	10/24 (41.7)	1.00
Low IGF-1[β]	2/24 (8.3)	6/9 (66.7)	0.00
Low IGFBP-3[β]	5/24 (20.8)	3/9 (33.3)	0.65

Notes: *Increased TSH and decreased FT4 (25); [#]Increased TSH and normal FT4 (25); [§]Laboratory fasting blood glucose reference greater than 6.0 mmol/L; [‡]Laboratory fasting blood glucose range greater than 5.6-6.9mmol/L (13); [Φ]Insulin resistance was determined by a HOMA index greater than two; [β]Age and sex-matched reference ranges; Statistically significant p values are highlighted in blue text.

Abbreviations: FA, Fanconi anaemia; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor-binding protein 3; HOMA, homeostasis model assessment; TSH, thyroid stimulating hormone; FT4, free thyroxine.

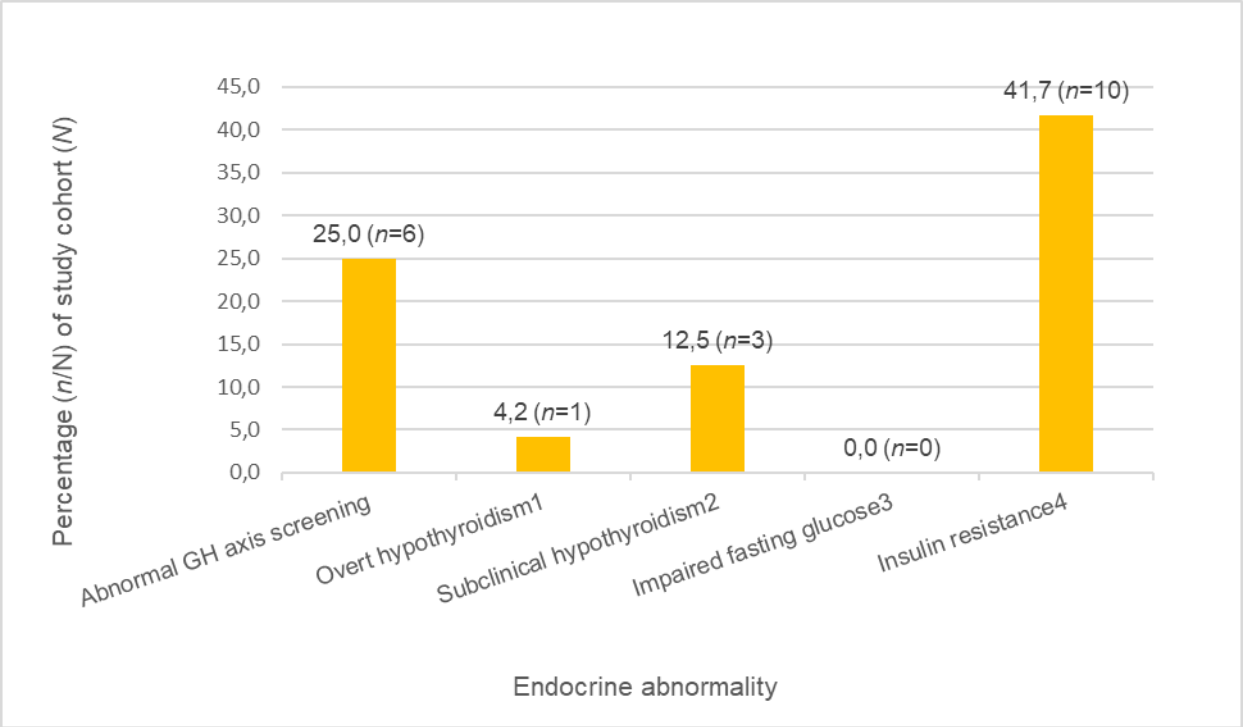

Figure 2 Endocrine abnormalities in Black South African patients with FA (*N*=24)

Notes: [1]Overt hypothyroidism refers to increased TSH and decreased FT4 (25); [2]subclinical hypothyroidism refers to Increased TSH and normal FT4 (25); [3]impaired fasting glucose refers to a laboratory fasting blood glucose reference greater than 6.0 mmol/L; [4]insulin resistance was determined by a HOMA index greater than two.

Abbreviations: FA, Fanconi anaemia; GH, growth hormone; HOMA, homeostasis model assessment; TSH, thyroid stimulating hormone; FT4, free thyroxine.

D. Radiographic Studies

1. Bone age

Hand and wrist X-rays were available in 23 of the 24 patients. Bone age was abnormal in 34.8% (8 of 23) of the present study cohort. Advanced bone age was seen in 8.7% (2 of 23), a similar frequency to that of Giri *et al.*'s cohort (7.7%) (p value = 1.00) (13). Delayed bone age was seen in 26.1% (6 of 23) of the present cohort, a higher, although not statistically significant, frequency compared to Giri *et al.*'s cohort (15.4%) (p value = 0.68) (13). Only one of the patients with advanced bone age was receiving androgen therapy, and all but one of the patients with delayed bone age were receiving androgen therapy. Three male individuals (13.0% of the study cohort) had abnormal fusion of the same two carpal (triquetral and lunate) bones (Figure 3).

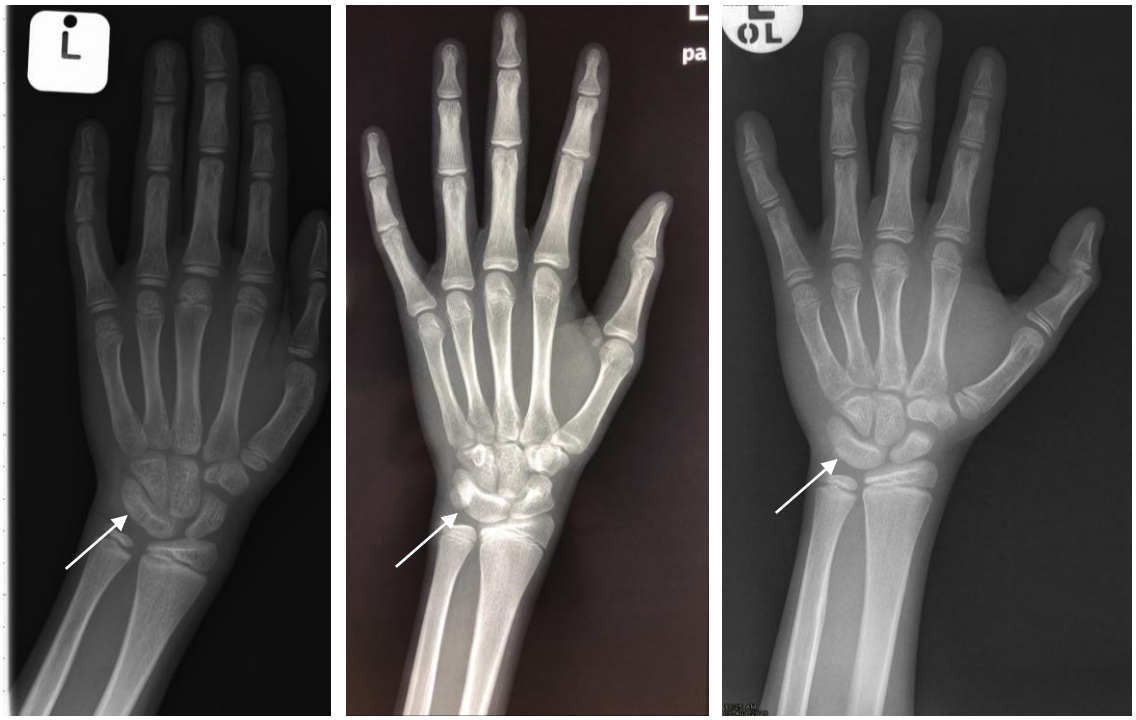

Figure 3 Left hand and wrist anteroposterior X-rays of three male Black South African patients with FA, demonstrating fusion of the triquetral and lunate carpal bones

Notes: Fusion of the carpal bones is indicated by the arrows. **Abbreviations:** FA, Fanconi anaemia.

E. Endocrine abnormalities and their relationship to growth, pubertal status and bone age

1. Growth measurements and endocrine abnormalities

Table 3 details the endocrine abnormalities associated with the various abnormalities in growth measurements. In the present study, 70.0% (7 of 10) of the underweight individuals were also of short stature. Half of the underweight individuals had at least one endocrine abnormality. Of the two overweight individuals, one had short stature. Both overweight patients had endocrine abnormalities.

One or more endocrine abnormality was identified in 72.7% (8 of 11) of the individuals with short stature. Of the individuals with at least one endocrine abnormality (insulin resistance, abnormal thyroid function, abnormal testing of the GH axis), 57.1% (8 of 14) had short stature. Of the individuals with no endocrine abnormality, 30.0% (3 of 10) had short stature. However, patients with one or more endocrine abnormality were not significantly shorter than those with no endocrine abnormality; mean height SDS -2.0 ± 0.9 compared to -1.6 ± 1.1 respectively (p value = 0.35). This differs to Giri *et al.*'s cohort whose patients with one or more endocrine abnormality were statistically significantly shorter than those without (mean height SDS -2.7 ± 2.0 compared to -1.3 ± 1.4, respectively (p value = 0.01) (13).

At least one endocrine abnormality was present in 50.0% (4 of 8) of the patients with microcephaly.

2. Pubertal assessment and endocrine abnormalities

Of the three individuals with abnormal pubertal development, one had insulin resistance and two had abnormal testing of the GH axis (both individuals had delayed gonadarche). Thirty-three percent (2 of 6) of patients with abnormal testing of the GH axis had abnormal (delayed) gonadarche. In view of the small sample size, statistical conclusions about pubertal status and endocrine abnormalities cannot be reliably made.

3. Bone age and endocrine abnormalities

Two thirds (4 of 6) of the individuals with delayed BA had at least one endocrine abnormality (including abnormal thyroid functions, abnormality of GH axis testing and insulin resistance). Fifty percent (2 of 4) had abnormal IGF-1/IGFBP-3 testing, 50.0% (2 of 4) had abnormal thyroid functions (one subclinical hypothyroidism and overt hypothyroidism), and 75.0% (3 of 4) had insulin resistance. Neither of the two individuals with advanced age had an endocrine abnormality. Two of the six patients with abnormal testing of the GH axis had abnormal (delayed) bone ages. Abnormal (delayed) bone age was seen in two of the four individuals with abnormal thyroid function

Table 3 Association of abnormal growth measurements and endocrine abnormalities[§] in Black South African patients with FA (*N*=24)

	Abnormal growth measurement				
	UWFA	**OWFA**	**SS**	**UWFA + SS**	**MC**
Percentage of study cohort (%)(*n/N*)	41.7(10/24)	8.3(2/24)	45.8(11/24)	29.2(7/24)	33.3(8/24)
Percentage with at least one endocrine abnormality[§] (%)(*n/N*)	50.0(5/10)	100.0(2/2)	72.7(8/11)	57.1(4/7)	50.0(4/8)
Endocrine abnormality (%)					
Subclinical hypothyroidism	10.0(1/10)	50.0(1/2)	0.0(0/11)	0.0(0/7)	0.0(0/8)
Hypothyroidism	0.0(0/10)	0.0(0/2)	9.1(1/11)	0.0(0/7)	0.0(0/8)
Insulin resistance	30.0(3/10)	100.0(2/2)	63.6(7/11)	42.9(3/7)	37.5(3/8)
Abnormal IGF-1/IGFBP-3	50.0(2/10)	50.0(1/2)	27.3(3/11)	14.3(1/7)	12.5(1/8)

Notes: [§]Excluding short stature as an endocrine abnormality.

Abbreviations: FA, Fanconi anaemia; UWFA, underweight-for-age; OWFA, overweight-for-age; SS, short stature; MC, microcephaly; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor-binding protein 3

4. Discussion

The aetiology of endocrine abnormalities seen in FA is both multifactorial and complex. Although there is not yet a single agreed-upon cause for all of the endocrine dysfunction observed in FA, the pathogenesis may be related to direct damage to endocrine cells with the impaired DNA damage response and DNA repair mechanisms that are known to be associated with this disease (14).

The Fanconi Anemia Research Foundation (FARF) recommends standard endocrine screening and testing in all patients with FA based on the predicted prevalence of the endocrine dysfunction summarized in a 2015 comprehensive literature review conducted by Petryk *et al.* (15). These screening and testing guidelines include, but are not limited to, GH screening, bone age X-rays, FT4 and TSH measurements, fasting glucose and insulin measurements, and clinical pubertal staging. The authors advocate for multidisciplinary approach to FA patient care, including incorporating the expertise of an endocrinologist into medical surveillance and management (15). Although FA is generally regarded as a progressively fatal disease, this should not negate the need for proper identification and comprehensive management of the full spectrum of FA-associated endocrinopathies (13). Early intervention for these endocrinopathies will allow for opportunities to decrease morbidity and ultimately improve these individuals' quality of life (13).

In South Africa, there are currently no similar guidelines for the investigation and treatment of endocrine disorders in patients with FA. Although patients with FA are managed in tertiary care haematology/oncology units, protocols for endocrine profiling differ between centres. Most patients who attend these haematology/oncology units reside in poor communities and have limited resources and access to healthcare services, apart from their haematology/oncology visits. This, together with the constrained public healthcare sector, prescribes that endocrine screening programs be financially prudent and designed to detect significant endocrine abnormalities, for which treatment is available.

We report for the first time the nature and frequency of endocrine abnormalities in Black South African patients with FA. These observations provide important genotype-phenotype correlations, as our study cohort was comprised solely of FA patients with the same *FANG* founder mutation (c.637_643delTACCGCC), which occurs in >78% of local patients (9). Additionally, these observations may serve as the basis for the development of standardized guidelines for endocrine profiling in Black South African patients with FA.

Nutrition and weight gain of an individual with FA is governed by both endocrine and non-endocrine factors. Poor weight gain can be attributed to numerous factors associated with FA, including gastrointestinal abnormalities, or the increased demand for, and decreased intake of calories associated with chronic illness (15). International studies have found the BMI-for-age to be normal in only approximately 50% of individuals with FA (13,14). The present study found a similarly high frequency of underweight-for-age patients with FA to the study cohorts of Giri *et al.* and Rose *et al.* (13,14). Food security has improved in South Africa over the past decade, however, many households still do not have access to nutritionally valuable foods in sufficient quantities, which may be adding to this high frequency of underweight-for-age patients in the present study (30). Seventy percent of the present cohort found to be underweight-for-age also had short stature. The degree of short stature (mean SDS -1.83 ± 1.0) is proportional to the degree of decreased weight (mean SDS -1.69 ± 0.9).

Two patients in our study cohort were found to be overweight-for-age. Both of these patients had insulin resistance, and one had the additional finding of subclinical hypothyroidism. Increased weight gain can occur with both of these endocrine derangements. The frequencies of overweight or obese patients in previous cohorts is higher than that of the Black South African FA cohort, 26.6% vs 8.3% respectively (13). Future studies could evaluate the socioeconomic and nutritional statuses of the Black South African patients with FA, to determine the extent of food insecurity as a confounding factor when evaluating growth parameters.

Short stature is one of the most well recognized physical abnormalities associated with FA. The aetiology of short stature in patients with FA is manifold, and includes both endocrine and non-endocrine causes, including complications from FA treatment (15). Commonly observed endocrine causes of short stature in FA consist of GHD, hypothyroidism, and hypogonadism. International studies have documented the presence of short stature at high frequencies of between 51.0% and 60.0% (13,14). In the present study, 45.8% of the patients were of short stature. When compared to Giri *et al.*'s FA cohort, these findings are slightly lower and not statistically significant, but nonetheless remain a high frequency (13).

International studies have shown that individuals with FA who have hormone deficiencies are markedly shorter compared to their counterparts without hormone deficiencies, suggesting that superimposed endocrine dysfunction further influences the short stature that is inherently associated with FA (13,14,31). In the 2012 study by Rose *et al.*, short stature was associated with hormone abnormalities in 42.3% of child patients and 30% of short statured adults (14). A higher frequency (72.7%) of patients with short stature (of whom 10 were ≤ 18 years and one was >18 years) had one or more endocrine abnormalities in the present cohort, although this finding was not statistically significant. Interestingly, our study showed that patients with at least one endocrine abnormality were not significantly shorter than their counterparts without endocrine dysfunction.

Puberty, gonadal function, and fertility can all be affected in patients with FA (15). Children with FA can suffer from peripheral precocious puberty or they can have delayed onset of puberty (15). Abnormal pubertal development was present in a minority (12.5%) (3 of 24) of FA patients in the present study, based on clinical Tanner staging assessing only thelarche and gonadarche, all of whom were males. Direct assessment of gonadal function (by biochemical stimulation testing) was not included in our study due to financial constraints. A larger sample size is required to make any meaningful correlations between pubertal stage and endocrine dysfunction. Unfortunately, owing to the lack of HSCT in the state healthcare sector in South Africa (personal communication Dr Rosalind Wainwright, 31 January 2019), the majority of Black South African patients with FA do not survive to reproductive ages, making analyses of fertility status difficult.

Growth hormone deficiency is a recognized abnormality observed in patients with FA, with half of the patients evaluated by Giri *et al.* having documented GHD (13). These patients were noted to be significantly shorter when compared to those individuals with FA who had normal GH levels (13).

Presently, in South Africa the cost of performing a GH stimulation test is approximately two and a half times greater than the cost of IGF-1 and IGFBP-3 combined (personal communication with Lancet Laboratory, 29 January 2019). Funding constraints limited the testing of the GH axis in the present study cohort to IGF-1 and IGFBP-3 testing.

As IGF-1 and IGFBP-3 are screening tests, we are unable to comment on confirmed GHD in the present cohort, and thus unable to directly compare it to other international FA cohorts. Despite this limitation, we observed a statistically significantly lower frequency of patients with low IGF-1 levels compared to Giri *et al.*'s cohort (8.3% *vs* 66.7% respectively), an unexpected finding as IGF-1 is negatively influenced by poor nutrition (13,15). We also observed a lower frequency of patients with low IGFBP-3 levels compared to that of Giri *et al.*'s cohort; 20.8% *vs* 33.3% respectively (13). These findings suggest that GHD may be a less common finding in Black South African patients with FA. Poor growth and growth velocity should be used as a means of screening to guide which patients should undergo GH stimulation testing in the Black South African FA cohort.

International recommendations suggest initiating GH treatment in patients with FA only if GHD has been compellingly documented, as there is currently no consensus on the long-term safety of GH treatment in patients with FA (15). In the state healthcare sector in Johannesburg, South Africa, GH therapy is provided to only a limited number of patients and even those with documented growth hormone deficiency may not be able to access the medication even when a documented clinical need exists (personal communication with Professor David Segal, 29 January 2019). If GHD were to be diagnosed in patients with FA in South Africa, there is concern as to whether the patient would have access to GH therapy.

Thyroid function is affected in individuals with FA; however, the pathophysiological explanation for thyroid disturbances in FA is not well understood. It may be due to the direct death of thyroid cells secondary to unrepaired DNA damage (15). Hypothyroidism was identified in a (statistically significant) lower frequency (4.2%) in our study cohort than in international studies, which may be related to the cross-sectional design of the present study (13,14). The presence of thyroid antibodies, as a potential cause of hypothyroidism, were not tested for.

Various disturbances in glucose metabolism occur frequently (39.0%) in children with FA; including impaired fasting glucose, insulin resistance, and overt type two diabetes mellitus (DM) (13,32). Patients with FA are at a higher risk of developing DM when compared to the general population (33). Previous FA studies have documented an increased frequency of glucose homeostatic abnormalities among individuals with FA; Giri *et al.* documented impaired fasting glucose (fasting blood glucose greater than 5.6 - 6.9 mmol/L) and/or glucose intolerance in 24% and overt DM (fasting blood glucose equal to or greater than 7 mmol/L) in 10% of their study cohort; Rose *et al.* documented higher levels of glucose intolerance with 30% of their adult cohort and 68% of their paediatric cohort affected (13,14). Factors extrinsic to the presence of FA (such as transfusion-related haemochromatosis, androgen treatment, and presence of increased weight/obesity) and factors intrinsic to FA (such as the presence of reactive oxygen species and the evidence that FA heterozygotes are also at increased risk of abnormal glucose metabolism) play a role in an individual's susceptibility to a disturbance in glucose homeostasis (13,33,34).

Interestingly, in the present study, none of the Black South African patients with FA were found to have impaired fasting glucose or overt type two DM, despite the majority of these patients having received long-standing androgen therapy and multiple blood transfusions. Blood ferritin levels (used to assess total body iron stores) were not measured in the present study, thereby comments regarding the influence of multiple blood transfusions on the endocrine status of this cohort cannot be made. Both fasting plasma glucose testing and the two hour plasma glucose value obtained during an oral glucose

tolerance test (OGTT) (performed with 75 grams of oral glucose) are deemed acceptable diagnostic modalities for DM and prediabetes mellitus; however, according to the American Diabetes Association's 2019 position statement, the two hour plasma glucose value identifies more individuals with DM and prediabetes than the fasting plasma glucose testing (35). An OGTT was not performed on the present study patients due to cost limitations, and thus some individuals with impaired glucose homeostasis may not have been detected. Although at present, in South Africa, the cost of an OGTT is approximately two and a half times more expensive than that of a fasting plasma glucose test (personal communication with Lancet Laboratory, 29 January 2019) it would be the preferred diagnostic test for DM.

Insulin resistance was identified in 41.7% of our study population, which corresponded to the incidence of insulin resistance in Giri *et al.*'s cohort and contrasted with the minimal numbers of insulin resistance in Rose *et al.*'s cohort (13,14).

Abnormal BA were found in a moderate (34.8%) frequency in the present study cohort. Twenty-six percent of the cohort had delayed BA, contrasting (not statistically significantly) with the lower frequency (15.4%) of delayed BA identified in Giri *et al.*'s cohort (13). Advanced BA was noted at a lower frequency (8.7%) than delayed BA in the present cohort, and at a similar frequency to that reported by Giri *et al.* (7.7%) (13).

Endocrine abnormalities were not seen in the individuals with advanced bone age, but were noted at a high frequency (66.7%) in the delayed bone age group. Androgen therapy, used to enhance red cell formation and improve platelet counts in patients with FA, accelerates bone maturation and thereby increases BA (36,37). Only one of the two individuals with advanced BA in the present study was taking androgen therapy (danazol, a synthetic orally administered androgen, at a dose of 100mg daily for 17 months). Interestingly, almost all of the individuals with delayed BA in our cohort were also receiving danazol (for a minimum of one year duration, at a dose of 3.5-7.7 mg/kg/day), suggesting the possible presence of a yet unidentified strong intrinsic or extrinsic factor delaying bone age.

Various factors cause a delay in BA, including reduced sex hormones, hypothyroidism, malnutrition, chronic illness, GHD, and treatment with corticosteroids (15,38). Half of the delayed BA group in the present cohort also had abnormal testing of the GH axis, and the same two individuals also had abnormal thyroid functions (subclinical and overt hypothyroidism) suggesting these may be contributing factors to the delay in bone maturation seen in these patients.

Of interest it was noted that three male individuals (13.0% of the study cohort) had abnormal fusion of the same two carpal (triquetral and lunate) bones. Whether or not this lunotriquetral coalition or fusion is merely an incidental finding or a finding associated with FA requires further investigation, as lunotriquetral coalition has been documented in the general population but its true population incidence is unknown (39). No documented association between carpal coalition and FA could be found in the literature.

We considered the cross-sectional study design and small sample size of 24 Black South African patients with FA to be the main identified limitations of our study. A more accurate assessment of the frequency and nature of endocrine abnormalities in Black South African patients with FA could be achieved by assessing a larger study size, in conjunction with a prospective study design; however, this may prove to be difficult due to the rarity of this disease. Despite study limitations, the present study is the first endocrine profiling study on Black South African patients with FA, who are homozygous for a *FANCG* founder mutation (c.637_643delTACCGCC). In addition, the present study provides insight into specific FA genotype-phenotype correlations, which provides useful information tailored to the South African Black population.

5. Conclusion

Our study confirms that endocrine abnormalities (including abnormal IGF-1/IGFBP-3, insulin resistance, abnormal thyroid functions, and short stature) occur in a high frequency (70.8%) of Black South African patients with FA. Short stature (45.8%), abnormal IGF-1/IGFBP-3 (25.0%), insulin resistance (41.7%), and abnormal thyroid functions (hypothyroidism and subclinical hypothyroidism) (16.7%) were all documented. The total frequency of endocrine abnormalities in the present cohort closely resembled the frequency identified in Giri *et al.*'s cohort (73%), although the observed frequencies of the individual endocrine abnormalities differ somewhat, suggesting possible genotype-phenotype correlations (13). Genotype-phenotype correlations for the seven base-pair founder deletion mutation (c.637_643delTACCGCC) in *FANCG* possibly include lower frequencies of GHD, overt hypothyroidism and impaired fasting glucose.

Based on the frequencies of endocrine abnormalities observed in the present cohort, and given the resource limitations in the South Africa state healthcare sector, it would be a pragmatic recommendation that baseline fasted thyroid function (FT4 and TSH), glucose and insulin levels, and IGF-1 and IGFBP-3 levels be performed at the time of diagnosis. Due to the cross-sectional nature of the present study, timing intervals for follow up testing cannot be commented on. Pubertal assessment (through Tanner staging), and growth measurements (including weight, height, head circumference, and growth velocity) should be assessed at baseline and at regular intervals (at least six monthly) thereafter. Bone age should be monitored in response to growth velocity concerns, at an interval advised by a paediatric endocrinologist. Patients shown to have abnormalities would require review by a paediatric endocrinologist such, that appropriate therapy can be instituted.

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Appendix A

Information sheet (adult participant, English)

NATIONAL HEALTH LABORATORY SERVICE
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Information & Consent Document – Adult Participant

Title: **Endocrine Profiling In Black South African Fanconi Anaemia Patients.**

Investigators: **Dr Candice Feben, Dr Bronwyn Dillon, Professor Amanda Krause & Professor David Segal**

Good day,

My name is Dr Candice Feben. I am a medical geneticist working in the Division of Human Genetics at the National Health Laboratory Services. I have a research interest in patients with Fanconi anaemia.

I would like to invite you to participate in my research study. Please read through the information below before agreeing to partake. If any of the details are unclear, or if you require an explanation on any of the information, please do not hesitate to ask me. Participation in this study is entirely optional and will in no way change the treatment you are receiving from the hospital, nor will it be detrimental to your health in any way. Similarly, if you do not wish to participate, your management and treatment will not be affected.

Your confidentiality and privacy will be protected at all times. If you agree to participate and then later change your mind, you may withdraw from the project at any time. There are no consequences to early withdrawal from the project.

What is the aim of this project?

Endocrine gland problems have been widely documented in patients with Fanconi anaemia and the current international recommendations for care include routine endocrine assessments for affected individuals to allow treatment of endocrine gland problems without delay. In South Africa, we do not yet offer this screening routinely to all our patients. The aim of this project is to assess thyroid function, glucose and insulin metabolism, bone age and growth status in a group of patients to assess the frequency and nature of endocrine problems specifically in Black patients with Fanconi anaemia in South Africa. This may help us to make specific recommendations for care of patients in the state healthcare system in South Africa.

What will be required of participants?

If you wish to participate, you will be required to have about 20mls (four tablespoons) of blood drawn. We need the blood sample to be taken in the starved state – in other words, you will not eat on the morning of the testing. We will also need to perform a physical examination on you. This examination will include measurements of height and weight, as well as assessing your stage of sexual development. This part of the examination is called a Tanner staging examination and will involve a non-invasive external examination of your pubic hair growth, breast development (if a girl), and testicular and penile development (if a boy). The testing and examination process should not take longer than one hour in total. We would also like to get an X-ray of your hands to assess the hand bones and their stage of development. You may choose not to have the X-ray done and can still participate in the study.

A second blood sample may need to be taken on a separate occasion should any of the initial blood tests be found to be abnormal.

Lastly, we will also measure the heights of the participant's parents.

Are there any side effects or complications of the procedure?

Venepuncture is an uncomfortable procedure which takes a few seconds to perform. As medical doctors, either myself or my colleague, Dr Bronwyn Dillon, will perform the venepuncture under strict sterile conditions to prevent infections. Other side effects that you may experience include slight pain at the site and bleeding – every effort will be made to prevent or lessen these complications. If you already have a port in situ, we will draw blood from this site.

What will happen to the results of the tests?

If the test results are normal, you will be informed verbally and a copy of the results will be given to your doctor for your hospital file. If any endocrine gland problems are detected, the results will be communicated to your treating haematologist/oncologist so that the correct treatment can be provided.

Are there any other benefits to participants?

There is no payment for participants in this study. If you wish to participate, your travel costs to and from the hospital will be reimbursed, and you will be provided with a light snack at the end of the examination.

Additionally, if you have not received genetic counselling regarding Fanconi anaemia, this service will be available to you, in collaboration with the Genetic Counselling Division of the National Health Laboratory Services, Johannesburg. You are under no obligation to attend a counselling session, but should you wish to attend a session, it will be arranged for you. This session will give you more information about Fanconi anaemia and will explain the risks to other family members. During this session, you may decide to have other family members tested for Fanconi anaemia. This would require a blood sample to be taken from those individuals.

Who can I contact if I have questions or complaints?

If you require any further information on the project, please do not hesitate to contact me on (011) 489-9338 or 078 080 8841. This protocol has been submitted to and approved by the Human Research Ethics Committee of the University of Witwatersrand. If you have any concerns about the nature of the research or feel you/your child were mistreated in any way, please contact Professor Cleaton-Jones on (011) 717-2229. The study has also been approved by the Research Ethics Committee of the University of Pretoria and the University of the Free State.

Appendix B

Information sheet (adult participant, Afrikaans)

NATIONAL HEALTH LABORATORY SERVICE
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Inligting & Instemmings Dokument – Volwasse Deelnemer

Titel: **Endocrine Profiling In Black South African Fanconi Anaemia Patients.**
(Endokriene Profiele In Swart Suid Afrikaanse Fanconi se Anemie Pasiënte)

Navorser: **Dr Candice Feben, Dr Bronwyn Dillon, Professor Amanda Krause & Professor David Segal**

Goeie dag,

My naam is Dr. Candice Feben. Ek is 'n mediese genetikus wat in die Afdeling Menslike Genetika by die National Health Laboratory Services werk. Ek het 'n navorsingsbelangstelling in pasiënte met Fanconi se anemie.

Ek wil u graag uitnooi om aan my navorsingstudie deel te neem. Lees asseblief die onderstaande inligting deur voordat u besluit om deel te neem. As enige van die besonderhede onduidelik is, of as jy 'n verduideliking verlang oor enige van die inligting, moet asseblief nie huiwer om my te vra nie. Deelname aan hierdie studie is heeltemal opsioneel en sal op geen manier die behandeling wat u van die hospitaal ontvang verander nie, en dit sal ook nie nadelig wees vir u gesondheid nie. Net so, as u nie wil deelneem nie, sal u behandeling nie geraak word nie.

U vertroulikheid en privaatheid sal te alle tye beskerm word. As u instem om deel te neem en dan later van plan verander, kan u enige tyd van die projek onttrek. Daar sal geen gevolge wees as u besluit om vroeër uit die projek te onttrek nie.

Wat is die doel van die projek?

Endokriene klierprobleme is wyd gedokumenteer in pasiënte met Fanconi se anemie en die huidige internasionale aanbevelings vir sorg, sluit in roetine-endokrien assesserings vir geaffekteerde individue om die behandeling vir hierdie probleme vroegtydig te ondersoek. In Suid-Afrika bied ons hierdie behandeling nog nie routine aan vir al ons pasiënte nie. Die doel van hierdie projek is om skildklierfunksie, glukose- en insulienmetabolisme, beenouderdom en groeistatus in 'n groep pasiënte te evalueer om die frekwensie en aard van endokrien probleme spesifiek in swart pasiënte met Fanconi se anemie in Suid-Afrika te evalueer. Dit kan ons help om spesifieke aanbevelings te maak vir die versorging van pasiënte in die staatsgesondheidsorgstelsel in Suid-Afrika.

Deelnemer Voorletters:
Deelnemer nommer:
Protokol: FA
Weergawe: March 2018
Navorser: Dr C. Feben
Goedgekeur deur HREC and UFS HSREC

Wat word van deelnemers verwag?

As u wil deelneem, sal ongeveer 20 ml (vier eetlepels) bloed getrek word. Ons benodig ‘n bloedmonster wat in ‘n vastende toestand geneem word - met ander woorde, jy sal nie die oggend van die toets kan eet nie. Ons sal ook 'n fisiese ondersoek op u moet doen. Hierdie ondersoek sluit in metings van lengte en gewig, asook ‘n evaluering van u stadium van seksuele ontwikkeling. Hierdie deel van die ondersoek word 'n Tanner-ondersoek genoem en sal 'n nie-indringende eksterne ondersoek van u pubiese haargroei, borsontwikkeling (indien 'n meisie) en testikulêre en penisontwikkeling (indien 'n seun) insluit. Die toets- en ondersoekproses sal nie langer as een uur duur nie. Ons wil ook graag 'n X-straal van u hande kry om die handbene en hul stadium van ontwikkeling te assesseer. U kan kies om nie die X-straal te doen nie en kan steeds aan die studie deelneem.

Indien enige van die aanvanklike bloedtoetse abnormaal voorkom sal 'n tweede bloedmonster moontlik op 'n aparte geleentheid geneem moet word.

Laastens sal ons ook die hoogte/lengte van die deelnemer se ouers kry.

Is daar enige newe-effekte of komplikasies van die prosedure?

Om bloed te trek is 'n ongemaklike prosedure wat 'n paar sekondes neem om uit te voer. As mediese dokter, sal ekself of my kollega, Dr. Bronwyn Dillon, die bloed trek onder streng steriele toestande uitvoer om infeksies te voorkom. Ander newe-effekte wat u mag ervaar, sluit in pyn en ligte bloeding. Alle pogings sal aangewend word om hierdie komplikasies te voorkom of te verminder. As u reeds 'n port in situ het, sal ons bloed van hierdie port trek.

Wat sal met die resultate van die toets gebeur?

As die toetsuitslae normaal is, sal u mondelings ingelig word en 'n afskrif van die uitslae sal aan u dokter vir u hospitaallêer gegee word. Indien enige endokriene klierprobleme opgetel word, sal die resultate aan u behandelende hematoloog / onkoloog oorgedra word sodat die korrekte behandeling verskaf kan word.

Is daar ander voordele vir deelnemers?

Daar is geen betaling vir deelnemers aan hierdie studie nie. As u wil deelneem, sal u reiskoste na en van die hospitaal vergoed word, en u sal aan die einde van die ondersoek 'n ligte versnapping ontvang.

Verder, as u nog nie genetiese berading rakende Fanconi se anemie ontvang het nie, sal hierdie diens in samewerking met die Genetiese Berading Afdeling van die National Health Laboratory Services, Johannesburg, beskikbaar wees. U is nie verplig om 'n beradingsessie by te woon nie, maar indien u sou wou, sal dit vir u gereël word. Hierdie sessie sal jou meer inligting gee oor Fanconi se anemie en sal die risiko's vir ander familielede verduidelik. Tydens hierdie sessie kan u besluit om ander familielede te toets vir Fanconi se anemie. Dit sal vereis dat 'n bloedmonster van daardie individue geneem word

Wie kan ek kontak as ek vrae of klagtes het?

As u enige verdere inligting oor die projek verlang, moet asseblief nie huiwer om my te kontak by 011 489-9338 of 078 080 8841. Hierdie protokol is aan die Etiekkomitee van die Universiteit van Witwatersrand voorgelê en goedgekeur. As u enige kommer het oor die aard van die navorsing of voel u / u kind is op enige manier onregverdig behandel, kontak asseblief vir Professor Cleaton-Jones by (011) 717-2229. Die studie is ook deur die Navorsingsetiekkomitee van die Universiteit van Pretoria en die Universiteit van die Vrystaat goedgekeur.

Deelnemer Voorletters:
Deelnemer nommer:
Protokol: FA
Weergawe: March 2018
Navorser: Dr C. Feben
Goedgekeur deur HREC and UFS HSREC

Appendix C

Information sheet (adult participant, Sesotho)

NATIONAL HEALTH LABORATORY SERVICE
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Lesedi le Tumello ka kutlwisiso – bakeng sa bana

Title: **Diprofile tsa Endocriene bakuding ba batho ba batsho Afrika Borwa banang le lefu la Fanconi Anemia**

Bafuputsi: **Dr Candice Feben, Dr Bronwyn Dillon, Professor Amanda Krause & Professor David Segal**

Dumela ,

Lebitso laka ke Dr Candice Feben. Ke setsebi sa diphatsa tsa mafu (medical geneticist), ke sebetsa karolong ya National Health Laboratory Services, Human Genetics Gauteng. Kenale thahasello ya thutodipatlisiso ya ho sebetsa le batho ba nang le lefu le bitswang Fanconi Anemia.

Ke rata ho mema ngwana wa hao hore a nke karolo thutopatlisisong ena yaka. Ke kopa hore o bale thlahisolesiding ena peele o ka dumela ho nka karolo thutopatlisisong ena. Haebe ho na seseng seo o hlokang hore re se hlalose hofeta mona, ke kopa hore o phuthullehe ho mpotsa dipotso.

Ho nka karolo thutopatlisisong ena ke ka biokgethelo, se keke sa ama phekolo eo ngwana wa hao a e tholang mona sepetlele, mme e keke ya bea bophelo ba ngwana kotsing. Phekolo ya ngwana e ke ke ya ameha, ho hang.

Dintho tsohle tsa ngwana wa hao ditla tshirelletswa, ka nako yohle mabapi le thuto ena. Haeba o dumela ho nka karolo thutopatlisisong, ena ebe o fetola mohopolo wa hao ka mora nako, o dumelletswe ho tlohela. Ha hona ditla morao tse tlang ho wena hao ka fetola monahano.

Sepheo sa projeke ee ke sefe?

Mathata a di tshwelesa ya endocrine (Endocrine gland) ke ntho e tlalewhang bakuding ba nang le lefu lena la Fanconi Anemia, mme dikgothalletso ho tswa mose kwana bakeng sa thlokomelo ya batho ba nang le lefu lena di akaralletsa tekolo ya tswhelesa e tlwaelehileng ya endocrine (endocrine gland) sena se etsa hore bohle bahlokang kalafo ba thole thuso esale ka nako.

Mona Afrika borwa, ha re eso ka re qala diteko tsena bakuding ba rona. Sepheo sa thutopatlisiso ena ke ho hlahloba hore Thyroid esebetsa ka tshwanelo bakuding ba rona ba nang le lefu la Fanconi Anemia. Re hlahloba le tswekere, dilemo tsa masapo, le kgolo baneng ba nang le lefu la Fanconi Anemia. Sena se tla rethusa ho hlahloba hore ke hangata ha kae, le ho fumana mofuta wa bakudi ba nang le mathata a endocrine, ha holo holo ba kuding ba batho ba batsho mona Afrika borwa, ke ba bakae. Sena seka re thusa ho etsa dikgothalletso tse itseng ho hlokomela bakudi barona haholo ba fumanag kalafo hotswa dipetlele tsa mmuso.

Se hlokahalang ho tswa ho monka karolo thutong ena?

Ha eba ngwana wa hao o nka karolo thutong ena, re tla hloka hanka di mililitara tse mashome a mabedi (20mL) kapa dikgaba tse pedi tsa madi ho tswa ho yena. Retla kopa madi ankuwe peele ngwana a eja hoseng hoo re tla etsa diteko. Re hloka ho hlahloba mmele wa ngwana. Diteko tse ding diakaretsa bo telele le boima ba mmele, le kgolo ya boha. Hlahlobo ena ya boha e bitswa "Tanner staging" ka sekgowa, mme yona e hlahloba (ntle le ho utwisa bohlooko) kgolo ya maboya, le matswele bananeng kapa kgolo ya marete le botona ha ele moshanyana. Diteko tsena le hlahlobo tsena di tla nka hora elengwe feela. Re tla kopa le honka di-xray tsa matsoho, ho hlahloba masapo a matsoho le kgolo ya ngwana. O ka kgona ho tswella pele ka karolo thutong ena le haeba o sa batle ho nka di-xray.

Ka mora diteko, ha eba refumana ho sa tlwaelehang mading a ngwana, re tla kopa ho nnka madi hape ka tsatsi leleng.

Re tla qetella ka honka tekanyo ya botelele ba batswadi ba ngwana.

Ana ho nale ditla morao kapa mathata ha o nka mohato ona?

Honka madi ke ntho a sa tlwaelehang mme e kanna ya utlisana bohloko hanyane, efela e ka pelenyana. Nna, ke le ngaka, le emong was batho bao ke sebetsang le bona, Dr Bronwyn Dillon, re tla nka madi, ka tsela a hlwekileng ho netefatsa hore hahona tshwaetso ya mafu. Ditla morao tsa hlahlobo ena ngwaneng etlaba ho tswa madi hanyane lehlakoreng leo renkang madi ho lona le bohlokonyana bo bonyenyane moo re tla hlaba teng. Re tla nka boiteko bohle ho netefatsa hore teko tsena ha di utlwisi bohloko kapa bohloko bateng ha bo kaalo. Ha eba ngwana o kentse "port in situ" re tla nka madi ho tswa teng.

Ho tla etsahalang ka dipheto tsa dihlahlobo tsena?

Haeba dipheto tsa hlahlobo ele tse tlwaelehileng, o tla jwetswa mme kopi ya di phetho tsa diteko etla fuwa ngaka ya hao, mme e kenywe le faeleng ya ngwana ya sepetlele. Haeba di phetoho tsa diteko di bontsa ho sa tlwaelehang le mathata a tshwelewsa ya endocrine (endocrine gland), ho tla buisanwa le ngaaka e ntseng a hlahloba ngwana mabapi le kankere (oncologist) kapa ngaka ya madi (hematologist), sena se tla ba dumella ho lokisa kalafo ya ngwana mabapi le dipheto tsa diteko tsena.

Ana ho na le molemo wa ho nka karolo thutong ena?

Ba nka karolo thutong ena ha bapatalwe. Haeba ngwana wa hao ke monka karolo, ditshenyeho bakeng sa ho nka leeto ho tla fihla sepetlele kapa tliliniking di tla patalwa. Le ngwana o tla fuwa senekenyana ha dihlahlobo di fela.

Hape, haeba ha o so fumane Genetic couselling mabapi le lefu lena la Fanconi Amemia, ho ka kgona hore o kopane le genetic cousellor ho tla hlalosa lefu lena. Genetc cousellor ena ke motho ya rometsweng ho tswa National Health Laboratory Services, Gauteng. Hao ya tlamelletswa tla kopana le genetic cousellor, empa ha o lakatsa, o kanna wa tla ho kopana le yena. Kopanong eo ya hao le genetic cousellor, o tla fumana tlhahisolesiding ka lefu lena la Fanconi Anemia. Mme genetic cousellor o tla o hlalosetsa ditlamorao tse ka amang babang ba leloko la hao mabapi le lefu lena. Nakong eo ontseng o buwa le genetic cousellor, o kanna wa etsa qeto ya ho kgothallentsa babang ba leloko ho nka diteko tsa ho hlahloba lefu lena le Fanconi Anemia mading. Mme re tla hloka ho nka madi ho tswa ho bohle ba batlang ho hlahlojwa.

Nka ikopanya lemang ha eba ke nale dipotso kapa ditletlebo?

Haeba a hloka thlahisoleseding ka seseng le seseng mabapi le projeke kapa thutopatlisiso ena, founela nna ho 011 489-9338 kapa 078 080 8841. Thutopatlisiso ena e rometswe, ya ba ya amohelwa ke komiti ya dipatlisiso molao univesiting ya Witwatersrand (WITS) (+ univesiting ya Free State). Haeba ona le matshwenyeho ka tlhaho kapa tsela ya thutopatlisiso ena, kapa o ikutlwa ekare wena kapa ngwana wa hao ha le ya tshwarwa hantle, ke kopa o founele Cleaton-Jones ho (011) 717-2229.

Appendix D

Information sheet (child participant, English)

NATIONAL HEALTH LABORATORY SERVICE
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Information & Consent Document – Child Participant

Title: **Endocrine Profiling In Black South African Fanconi Anaemia Patients.**

Investigators: **Dr Candice Feben, Dr Bronwyn Dillon, Professor Amanda Krause & Professor David Segal**

Hello,

My name is Dr Candice. I am doing a project looking at children with Fanconi anaemia, which is the name of the condition which you have been diagnosed with and the reason that you attend the hospital.

I would like to invite you to join my project and help me to collect information about your condition. You may speak to your family before you make a decision to join the project. You may also choose not to join and this will not affect your treatment in any way.

The project is looking at "gland" problems in children with Fanconi anaemia. These "gland" problems include problems with sugar balance, energy levels and growth. These are all problems which might be faced by children with Fanconi anaemia. Treatment is available for these problems if we know about them.

If you decide to join the project, you will be seen by me on a day when you come to the clinic. I will spend about one hour with you, asking you and your family questions about your condition. I will then examine you and take measurements of your weight, your height and your head. I will also take a look at your chest and your genitalia. This examination will not be painful and your family will be with you the whole time. I will also need to read your hospital file. I will need to take some blood from you. I am sure you know that this can be uncomfortable, but I will try to be as gentle as possible and I will try to take the blood at the same time as your routine bloods. If you have a port, I will use this to get the blood sample. You are not allowed to eat before I take the blood so I will ask your parents for their permission for the blood taking before I see you at the clinic. When we have finished you will get a snack to take home with you.

The results of the blood tests will be given to your parents and your doctor and your doctor will decide if any treatment is necessary. If you have any questions or if there is anything you do not understand, please feel free to ask me. I have given your parents/guardians a full copy of the information with all the telephone numbers to reach me if necessary.

Thank you for your help.

Appendix E

Information sheet (child participant, Afrikaans)

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Inligting & Instemmings Dokument – Kind Deelnemer

Titel: **Endocrine Profiling In Black South African Fanconi Anaemia Patients.** (Endokriene Profiele In Swart Suid Afrikaanse Fanconi se Anemie Pasiënte)

Navorser: **Dr Candice Feben, Dr Bronwyn Dillon, Professor Amanda Krause & Professor David Segal**

Goeie dag,

My naam is Dr. Candice. Ek doen 'n projek wat kyk na kinders met Fanconi se anemie, die naam van die kondisie waarmee jy gediagnoseer is en die rede waarom jy hospitaal toe kom.

Ek wil jou graag uitnooi om aan my projek deel te neem en my te help om inligting oor jou toestand in te samel. Jy kan met jou familie praat voordat jy besluit om deel te neem. Jy kan ook kies om nie aan die projek deel te neem nie en dit sal nie jou behandeling op geen manier beïnvloed nie.

Die projek kyk na "klier" probleme wat by kinders met Fanconi se anemie voorkom. Hierdie "klier" probleme sluit in probleme met suikerbalans, energievlakke en groei. Dit is alles probleme wat kinders met Fanconi se anemie affekteer. Behandeling is beskikbaar vir hierdie probleme as ons daarvan weet.

As jy besluit om aan die projek deel te neem, sal jy deur my gesien word op 'n dag wanneer jy die kliniek besoek. Ek sal ongeveer een uur saam met jou spandeer, en jou en jou familie vrae vra oor jou toestand. Ek sal jou dan ondersoek en jou weeg, jou lengte neem en jou kop meet. Ek sal ook na jou bors en jou geslagsdele kyk. Hierdie ondersoek sal nie pynlik wees nie en jou gesin sal die hele tyd by jou wees. Ek sal ook jou hospitaallêer moet lees.

Ek sal ‘n bloedmonster moet neem. Ek is seker jy weet dat dit ongemaklik kan wees, maar ek sal probeer om so sag as moontlik te wees en ek sal probeer om die bloed op dieselfde tyd as jou roetine-bloed te neem. As jy 'n port het, sal ek dit gebruik om die bloedmonster te kry. Jy mag nie eet voordat ek die bloed geneem het nie. Ek sal jou ouers se toestemming vra dat die bloed geneem kan word voordat ek jou by die kliniek sien. As ons klaar is, kry jy 'n verversing om saam met jou huis toe te neem.

Die resultate van die bloedtoetse sal aan jou ouers en jou dokter gegee word en jou dokter sal besluit of enige behandeling nodig is. As jy enige vrae het of as daar iets is wat jy nie verstaan nie, voel asseblief vry om my te vra. Ek het 'n volledige afskrif van die inligting vir jou ouers / voogde gegee met al die telefoonnommers waar julle my in die hande kan kry, indien nodig.

Dankie vir jou hulp.

Appendix F

Information sheet (child participant, Sesotho)

NATIONAL HEALTH LABORATORY SERVICE
School of Pathology, University of the Witwatersrand
Division of Human Genetics

GENETIC COUNSELLING CLINIC

Hospital Street, Johannesburg, 2001 | PO Box 1038, Johannesburg, 2000
[T]: +27 11 489 9223 | [M]: +27 78 080 8841 | [F]: +27 11 489 9226 | [E]: human.genetics@nhls.ac.za

Lesedi le Tumello ka kutlwisiso – bakeng sa bana

Title: **Diprofile tsa Endocriene bakuding ba batho ba batsho Afrika Borwa banang le lefu la Fanconi Anemia**

Difuputsi: **Dr Candice Feben, Dr Bronwyn Dillon, Professor Amanda Krause & Professor David Segal**

Dumela,

Lebitso laka ke nna Dr Candice. Ke etsa projeke a batlisisang bana ba nang le lefu la Fanconi Anemia, eleng boemo boo o fumanweng ona le bona, hape e leng le baka leo o tlileng sepetlele ka lona.

Ke rata ho o mema hore o nke karolo thutopatlisisong ena yaka mme o nthuse ke kgone ho etsa diphuputso ka boemo bona ba hao. O ka kgona hore o buwe le baleloko lahao peele o dumela ho nka karolo projekeng ena. Haeba hao batle ho nka karolo, seo sekeeke sa ama kalafo kapa phekolo ya hao ka letho.

Thutopatlisiso ena e hlahloba mathata a amang ditswelesa (glands) tsa bana ba nang le lefu la Fanconi Anemia. Bothata ba ditswelesa bo akaretsa, ho leka- lekana ha tswekere, maemo a maatla, le kgolo baneng ba nang le lefu lena la Fanconi Anemia. Phekolo e teng bakeng sa mathata ana, ha feela re tseba ka ona.

Haeba o tla rata ho nka karolo thutopatlisisong ena, ke tla rata ho kopana le wena tsatsing leo otlang tliliniking ka lona. Ke tla qeta nako enkang hora le wena, ke tla botsa wena le baleloko la hao dipotso mabapi le boemo ba hao. Ke tla o hlahloba, mme ke nke ditekanyo tsa hao tsa mmele (weight) le botelele (height), le tekanyo ya hlooho. Ke nke hape le ditekanyo tsa sefuba sa hao le ho hlahloba tsa botona le botsehadi. Dihlalhobiso tsena di keke tsa o utlwisa bo hloko, mme ba leloko la hao dumelletswe ho ba teng nako yohle ha ke ntse ke etsa di hlahlobo tsena. Hape, ke tlo kopa ho re o ntumelle ke bale faele ya hao ya sepetlele.

Ke tla hloka honka madi hotswa ho wena. Seo seka o utlwisa bohloko ha nyane, empa, ke tla leka ho ba bonolo ho wena, hore ke seke ka o utlwisa bohloko. Ke tla nka madi ka nako e tlwalelehileng ya ho nka madi. Haeba ona le port, ke tla sebedisa yona ho nka madi. O keeke wa kgona nka se jewang peele ke nka madi. Ke tla kopa batswadi ba hao tumelo ya ho nka madi pele ke kopana le wena tliliniking. Ha re qeta, o tla fumana senekenyana seo o ka senkang ho ya hae le sona.

Do phetoho tsa madi di tla fuwa batswadi ba hao, mme ngaaka ya hao etla o eletsa haeba hotla hlokahala kalafo.

Haeba o na le dipotso kapa o hloka thlalosetso, ke kopa o phuthullehe ho mpotsa dipotso tsohle. Ke file batswadi bahao kapa mohlokomdi wa hao leqephe le nang le thlahisoleseding le dinomoro tsaka tsa mohala hore le ngfounele ha o ka hlokahala.

Ke lebohela thuso ya hao.

Participant Initials:
Participant Number:
Protocol: FA
Version: March2018
Investigator: Dr C. Feben
Approved by HREC and UFS HSREC

Appendix G

Informed consent/assent forms (English)

NATIONAL HEALTH LABORATORY SERVICE
School of Pathology, University of the Witwatersrand
DIVISION OF HUMAN GENETICS
Hospital Street, Johannesburg, 2001 | PO Box 1038, Johannesburg, 2000
[T]: +27 11 489 9211 | [F]: +27 11 489 9226 | [E]: human.genetics@nhls.ac.za

INFORMED CONSENT FOR PARTICIPANTS 18 YEARS OR OLDER

I _____ hereby declare that I have read and understood the information document for the research report entitled **Endocrine Profiling In Black South African Fanconi Anaemia Patients.**

I declare that I have had sufficient opportunity to ask questions about the research and that I have decided to participate in the research study without coercion. I understand that participation or non-participation in this study, will not affect my medical care.

I _____ hereby give consent to:

- Physical examination (including Tanner staging examination) ☐
- Venepuncture ☐
- X-rays of the hands ☐

I HAVE / HAVE NOT attended a genetic counselling session previously.

If not, I WOULD / WOULD NOT be interested in attending a genetic counselling session.

I WOULD / WOULD NOT like to receive feedback on the overall results of this study.

Signed on _____ day of _____ 20____ at _____

Patient	Name: _____	Signature_____
Witness	Name: _____	Signature_____
Translator	Name: _____	Signature_____
Researcher	Name: Dr B. Dillon _____	Signature_____

Participant Initials:
Participant Number:
Protocol: FA
Version: March 2018
Investigator: Dr C. Feben
Approved by HSREC and UFS HSREC

NATIONAL HEALTH LABORATORY SERVICE
School of Pathology, University of the Witwatersrand

DIVISION OF HUMAN GENETICS

Hospital Street, Johannesburg, 2001 | PO Box 1038, Johannesburg, 2000
[T]: +27 11 489 9211 | [F]: +27 11 489 9226 | [E]: human.genetics@nhls.ac.za

INFORMED CONSENT FOR PARTICIPATION UNDER 18 YEARS

I _____ parent/guardian of _____ hereby declare that I have read and understood the information document for the research report entitled **Endocrine Profiling In Black South African Fanconi Anaemia Patients.**

I declare that I have had sufficient opportunity to ask questions about the research and that I have decided to allow my child to participate in the research study without coercion. I understand that participation or non-participation in this study, will not affect my child's medical care.

I_____ parent/guardian of _____ hereby give consent to:

- Physical examination (including Tanner staging examination) ☐
- Venepuncture ☐
- X-rays of the hands ☐

I HAVE / HAVE NOT attended a genetic counselling session previously.

If not, I WOULD / WOULD NOT be interested in attending a genetic counselling session.

I WOULD / WOULD NOT like to receive feedback on the overall results of this study.

Signed on _____ day of _____ 20_____ at _____

Parent/Guardian	Name:_____	Signature_____
Witness	Name: _____	Signature_____
Translator	Name: _____	Signature_____
Researcher	Name: Dr B. Dillon_____	Signature_____

Participant Initials:
Participant Number:
Protocol: FA
Version: March 2018
Investigator: Dr C. Feben
Approved by HSREC and UFS HSREC

NATIONAL HEALTH LABORATORY SERVICE
School of Pathology, University of the Witwatersrand

DIVISION OF HUMAN GENETICS

Hospital Street, Johannesburg, 2001 | PO Box 1038, Johannesburg, 2000
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ASSENT FOR CHILDREN UNDER 18 YEARS

To be completed with Informed Consent for Participation Under 18 years

I _____ agree that I would like to participate in the project called **Endocrine Profiling In Black South African Fanconi Anaemia Patients.**

I understand why the project is important and what is expected of me. I have had my questions answered and explained. I have not been forced to take part in this project.

I agree to:

- Physical examination (including examination of breasts and pubic area) ☐
- Blood taking ☐
- X-rays of the hands ☐

I WOULD/WOULD not like to receive information about the project when it is completed.

Signed on _____ day of _____ 20_____ at _____

Patient	Name:_____	Signature_____
Witness	Name: _____	Signature_____
Translator	Name: _____	Signature_____
Researcher	Name: Dr B. Dillon_____	Signature_____

Participant Initials:
Participant Number:
Protocol: FA
Version: March 2018
Investigator: Dr C. Feben
Approved by HSREC and UFS HSREC

Appendix H

Informed consent/assent forms (Afrikaans)

NATIONAL HEALTH LABORATORY SERVICE
School of Pathology, University of the Witwatersrand
Division of Human Genetics

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Hospital Street, Johannesburg, 2001 | PO Box 1038, Johannesburg, 2000
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INGELIGTE TOESTEMMING VIR DEELNAME ONDER 18 JAAR
(Moet voltooi word met 'n kinderinstemmings dokument)

Ek _____ ouer/voog van _____ verklaar hiermee dat ek die inligtingsdokument vir die navorsingsverslag getiteld '**Endocrine Profiling In Black South African Fanconi Anaemia Patients**' gelees en verstaan het.

Ek verklaar dat ek genoeg geleentheid gehad het om vrae oor die navorsing te vra en dat ek besluit het om my kind toe te laat om sonder dwang aan die navorsingstudie deel te neem. Ek verstaan dat my besluit om deel te neem, of nie deel te neem aan hierdie studie, nie my kind se mediese sorg sal beïnvloed nie.

Ek_____ ouer/voog van _____ gee hiermee toestemming vir:

- Fisiese ondersoek (insluitend 'n 'Tanner staging' ondersoek) ☐
- Bloedtrek ☐
- X-strale van die hande ☐

Ek HET / HET NOG NIE 'n genetiese beradingsessie bygewoon nie.

Indien nie, SAL EK/ SAL EK NIE belang stel om 'n genetiese beradingsessie by te woon.

Ek SAL / SAL NIE graag terugvoer ontvang oor die algehele resultate van die studie.

Geteken op die _____ dag van _____ (maand) 20_____ by _____

Ouer/Voog Naam:_____ Handtekening _____

Getuie Naam:_____ Handtekening _____

Vertaler Naam:_____ Handtekening _____

Navorser Naam: Dr C. Feben_____ Handtekening _____

Deelnemer Voorletters:
Deelnemer nommer:
Protokol: FA
Weergawe: March 2018
Navorser: Dr C. Feben
Goedgekeur deur HREC and UFS HSREC

NATIONAL HEALTH LABORATORY SERVICE
School of Pathology, University of the Witwatersrand
Division of Human Genetics

GENETIC COUNSELLING CLINIC

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INGELIGTE TOESTEMMING VIR DEELNAME 18 JAAR OF OUER

Ek _____ verklaar hiermee dat ek die inligtingsdokument vir die navorsingsverslag getiteld '**Endocrine Profiling In Black South African Fanconi Anaemia Patients**' gelees en verstaan het.

Ek verklaar dat ek genoeg geleentheid gehad het om vrae oor die navorsing te vra en dat ek besluit het om sonder dwang aan die navorsingstudie deel te neem. Ek verstaan dat my besluit om deel te neem, of nie deel te neem aan hierdie studie, nie my mediese sorg sal beïnvloed nie.

Ek_____ gee hiermee toestemming vir:

- Fisiese ondersoek (insluitend 'n 'Tanner staging' ondersoek) ☐
- Bloedtrek ☐
- X-strale van die hande ☐

Ek HET/HET NOG NIE 'n genetiese beradingsessie bygewoon nie.

Indien nie, SAL EK/SAL EK NIE belang stel om 'n genetiese beradingsessie by te woon.

Ek SAL/SAL NIE graag terugvoer ontvang oor die algehele resultate van die studie.

Geteken op die _____ dag van _____ (maand) 20_____ by _____

Pasiënt Naam:_____ Handtekening _____

Getuie Naam:_____ Handtekening _____

Vertaler Naam:_____ Handtekening _____

Navorser Naam: Dr C. Feben_____ Handtekening _____

Deelnemer Voorletters:
Deelnemer nommer:
Protokol: FA
Weergawe: March 2018
Navorser: Dr C. Feben
Goedgekeur deur HREC and UFS HSREC

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INSTEMMING VIR KINDERS ONDER 18 JAAR

Moet voltooi word vir ingeligte toestemming vir deelname onder 18 jaar

Ek _____ stem saam dat ek graag wil deelneem aan die projek '**Endocrine Profiling In Black South African Fanconi Anaemia Patients**'.

Ek verstaan hoekom die projek belangrik is en wat van my verwag word. Alle vrae wat ek gehad het is beantwoord en verduidelik. Ek is nie gedwing om deel te neem aan hierdie projek nie.

Ek stem in tot:

- Fisiese ondersoek (insluitend 'n 'Tanner staging' ondersoek) ☐
- Bloedtrek ☐
- X-strale van die hande ☐

Ek SAL/SAL NIE graag terugvoer ontvang oor die algehele resultate van die studie.

Geteken op die _____ dag van _____ (maand) 20_____ by _____

Pasiënt Naam:_____ Handtekening _____

Getuie Naam:_____ Handtekening _____

Vertaler Naam:_____ Handtekening _____

Navorser Naam: Dr C. Feben_____ Handtekening _____

Deelnemer Voorletters:
Deelnemer nommer:
Protokol: FA
Weergawe: March 2018
Navorser: Dr C. Feben
Goedgekeur deur HREC and UFS HSREC

Appendix I

Informed consent/assent forms (Sesotho)

NATIONAL HEALTH LABORATORY SERVICE
School of Pathology, University of the Witwatersrand
Division of Human Genetics

GENETIC COUNSELLING CLINIC

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[T]: +27 11 489 9223 | [M]: +27 78 080 8841 | [F]: +27 11 489 9226 | [E]: human.genetics@nhls.ac.za

FOROMO YA TUMELLANO BA KENG SA NGWANA YA TLASA DILEMO TSE 18
(foromo ena e tshwanelwa ho tlatswa le tokomane ya tumello ya ngwana)

Nna _____motswadi/ mohlokomedi wa_____ ke dumela hore ke badile ebile ke utlwisisa thlahisoleseding ka thuto ena e bitswang "**Diprofile tsa Endocriene bakuding ba batho ba batsho Afrika Borwa banang le lefu la Fanconi Anemia**

Ke dumela hore ke fumane monyetla wa ho botsa dipotso ka thuto ena, le ho dumella ngwana waka ho nka karolo thutong ena ntle le kgang. Ke utlwisisa hore ho nka karolo kapa ho senke karolo thutong ena ho keeke wha ama kalafo ya ngwana waka.

Nna_____ motswadi/ mohlokomedi wa _____
ke dumella:

- Hlahlobo ya mmele (mmoho le hlahlobo ya Tanner staging) ☐
- Ho nkwe madi (Venepuncture) ☐
- Di-xray tsa matsoho ☐

Nkile ka / ha ke so ka ke kopana ke genetic couesellor

Ha ho le jwalo, NKA THABELA / NKEKE KE THABELA ho kopana le genetic cousellor

NKA THABELA / NKEKE KA THABELA ho fumana diphetho tsa thutopatlisiso ena

Ho saena mohlokomedi ka _____ letsatsi la _____ 20_____ kae_____

motswadi / mohlokomedi lebitso :_____ Saena: _____

Paki lebitso: _____ Saena: _____

Mofetoledi (Translaitara) lebitso: _____ Saena: _____

Mosebeletsi wa difuputso lebitso: : Dr C. Feben_____ Saena: _____

Participant Initials:
Participant Number:
Protocol: FA
Version: March 2018
Investigator: Dr C. Feben
Approved by HREC and UFS HSREC

NATIONAL HEALTH LABORATORY SERVICE
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FOROMO YA TUMELLANO BA KENG SA MOTHO WA DILEMO TSE 18 HO YA HODIMO

Nna _____ ke dumela hore ke badile ebile ke utlwisisa thlahisoleseding ka thutopatlisiso ena e bitswang "**Diprofile tsa Endocriene bakuding ba batho ba batsho Afrika Borwa banang le lefu la Fanconi Anemia"**

Ke dumela hore ke fumane monyetla wa ho botsa dipotso ke thuto ena le ho dumella ngwana waka ho nka karolo thutong ena ntle le kgang. Ke utlwisisa hore ho nka karolo kapa ho senke karolo thutong ena ho keeke wha ama kalafo ya ngwana waka.

Nna _____ ke dumella:

- Hlahlobo ya mmele (mmoho le hlahlobo ya Tanner staging) ☐
- Ho nkwe madi (Venepuncture) ☐
- Di-xray tsa matsoho ☐

Nkile ka / ha ke so ka ke kopana ke genetic couesellor

Ha ho le jwalo, NKA THABELA / NKEKE KE THABELA ho kopana le genetic cousellor

NKA THABELA / NKEKE KA THABELA ho fumana diphetho tsa thutopatlisiso ena

Saena ka _____ letsatsi la _____ 20____ ka _____

Paki lebitso: _____ saena: _____

Mofetoledi (Translaitara) lebitso:_____ saena: _____

Participant Initials:
Participant Number:
Protocol: FA
Version: March 2018
Investigator: Dr C. Feben
Approved by HREC and UFS HSREC

NATIONAL HEALTH LABORATORY SERVICE
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Tokomane ya tumello ya ngwana a tlasa dilemo tse 18
(Tokomane ena a tshwanelwa ho tlatswa le foromo ya tumellano bakeng sa bana ba ka tlasa dilemo tse 18)

Nna _____ke dumela ho nka karolo thutopatlisisong ena e bitswang "**Diprofile tsa Endocriene bakuding ba batho ba batsho Afrika Borwa banang le lefu la Fanconi Anemia"**

Ke utlwisisa bo hlokwa ba thuto ena le se lebelletsweng ho tswa ho nna. Dipotso tsohle tseo ke nang le tsona di arabilwe mme ke hlaloseditswe tsohle. Ha ke ya hatellwa ho nka karolo thutopatlisisong ena.

Ke dumela ho:

- Hlahlobo ya mmele (mmoho le hlahlobo ya Tanner staging) ☐
- Ho nkwe madi (Venepuncture) ☐
- Di-xray tsa matsoho ☐

NKA THABELA / NKEKE KA THABELA ho fumana diphetho tsa thutopatlisiso ena

Saena: ka _____ letsatsi la _____ 20_____ ka _____

Paki Lebitso: _____ saena: _____

Mofetoledi (Translaitara) lebitso:_____ saena: _____

Mosebeletsi wa difuputso lebitso:_____ saena: _____

Participant Initials:
Participant Number:
Protocol: FA
Version: March 2018
Investigator: Dr C. Feben
Approved by HREC and UFS HSREC

Appendix J

Ethics clearance certificates

R14/49 Dr Candice Feben et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160220

NAME: Dr Candice Feben et al
(Principal Investigator)
DEPARTMENT: School of Pathology
Chris Hani Baragwanath Academic Hospital
National Health Laboratory Services

PROJECT TITLE: Endocrine Profiling in Black South African Patients with Fanconi Anaemia

DATE CONSIDERED: 26/02/2016

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR:

APPROVED BY:
Professor P. Cleaton-Jones,Chairperson, HREC (Medical)

DATE OF APPROVAL: 27/05/2016

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 in Room 10004,10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised 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 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 review in February and will therefore be due in the month of February each year.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Dr Bronwyn Dillon et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M1703108

NAME: (Principal Investigator)	Dr Bronwyn Dillon et al
DEPARTMENT:	Human Genetics Chris Hani Baragwanath Academic Hospital Charlotte Maxeke Johannesburg Academic Hospital National Health Laboratory Services
PROJECT TITLE:	Endocorine Profiling in Black South African Fanconi Anaemia Patients, Homozygous for FANCG Founder Mutation
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-Study (M160220)
SUPERVISOR:	Dr Candice Feben and Prof Amanda Krause
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	24/04/2017

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 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised 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 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 review in March and will therefore be due in the month of March 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

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

23/11/2017

Approval Certificate
New Application

Ethics Reference No: 547/2017

Title: Endocrine profiling in Black South African Fanconi anaemia patients, homozygous for a FANCG founder mutation

Dear Dr Bronwyn S Dillon

The **New Application** as supported by documents specified in your cover letter dated 14/11/2017 for your research received on the 14/11/2017, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 22/11/2017.

Please note the following about your ethics approval:
- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**547/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

*** Kindly collect your original signed approval certificate from our offices, Faculty of Health Sciences, Research Ethics Committee, Tswelopele Building, Room 4.59 / 4.60.*

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

28-Jun-2018

Dear **Mr Bronwyn Dillon**

Ethics Clearance: **Endocrine Profiling in Black South African Fanconi Anaemia Patients, Homozygous for a FANCG Founder Mutation**

Principal Investigator: **Mr Bronwyn Dillon**

Department: **Genetics (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2017/1406/3107**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Appendix K

Data collection sheet

NATIONAL HEALTH LABORATORY SERVICE
School of Pathology, University of the Witwatersrand
DIVISION OF HUMAN GENETICS
Hospital Street, Johannesburg, 2001 | PO Box 1038, Johannesburg, 2000
[T]: +27 11 489 9211 | [F]: +27 11 489 9226 | [E]: human.genetics@nhls.ac.za

Data Collection Sheet

Endocrine Profiling in Black South African Fanconi Anaemia Patients, Homozygous for a *FANCG* Founder Mutation

Name:					
Participant number:					
Hospital number:					
Date of birth:			**Sex (F/M):**		
Clinical history:	Transfused: Yes / No, if yes: Date and type of last transfusion: Hearing assessment: Genetic counselling received?: Other:				
Date of examination:			**Age at examination:**		
Growth assessment:					
Weight (kg)	/ /		centile		SD
Height (cm)	/ /		centile		SD
OFC (cm)	/ /		centile		SD
BMI (kg/m^2)					
Father's height (cm)	/ /		centile		SD
Mother's height (cm)	/ /		centile		SD
Midparental height (cm) (sex adjusted)					
Pubertal assessment:					
Male	Testes:	*Tanner 1*	*Tanner 2*	*Tanner 3*	*Tanner 4*	*Tanner 5*
	Pubic hair:	*Tanner 1*	*Tanner 2*	*Tanner 3*	*Tanner 4*	*Tanner 5*
Female	Breast :	*Tanner 1*	*Tanner 2*	*Tanner 3*	*Tanner 4*	*Tanner 5*
	Pubic hair:	*Tanner 1*	*Tanner 2*	*Tanner 3*	*Tanner 4*	*Tanner 5*

X-ray assessment:				
	Bone age:			
Blood results:				
	Glucose (fasting)		Insulin (fasting)	
	T4 (free thyroxine)		TSH	
	IGF-1		IGFBP3	
Androgen therapy:				
	Yes / No			
If yes:	Name of therapy:		Dose:	
	Initiation date and age:			
	Duration of therapy:			

Appendix L

Approved research protocol

CANDIDATE'S SURNAME: **Dillon**	FIRST NAME/S: **Bronwyn**	STUDENT NUMBER: **0300869W**

CURRENT QUALIFICATIONS: **BSc (The University of KwaZulu-Natal)**
BSc (Honours) (The University of KwaZulu-Natal)
MBBCh (The University of the Witwatersrand)

TEL: **011-489-9227** | CELL: **073-245-8803** | E-MAIL: **bronwyn.dillon@nhls.ac.za**

DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: **Master of Medicine (MMed)**

PART-TIME OR FULL-TIME: **Full-time**

FIRST REGISTERED FOR THIS DEGREE: | TERM : **1** | YEAR: **2015**

DEPARTMENT:
Division of Human Genetics, National Health Laboratory Service (NHLS) and The University of the Witwatersrand

TITLE OF PROPOSED RESEARCH:
Endocrine Profiling in Black South African Fanconi Anaemia Patients, Homozygous for a *FANCG* Founder Mutation

CANDIDATE'S SIGNATURE:  DATE: 01/02/2016

SUPERVISOR #1'S NAME: **Dr Candice Feben**

% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #1: **40%**

SUPERVISOR #1'S QUALIFICATIONS: **MBBCh, DCH, MMed, FCMG(SA)**

SUPERVISOR #1'S DEPARTMENT:
Division of Human Genetics, NHLS and The University of the Witwatersrand

SUPERVISOR #1'S ADDRESS / TEL / E-MAIL:
Jack Metz Building, NHLS Braamfontein, Cnr Hospital and de Korte Street, Braamfontein, 2017
Tel: 011-489-9338, Email: candice.feben@nhls.ac.za

SUPERVISOR #2'S NAME: **Professor Amanda Krause**

% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #2: **40%**

SUPERVISOR #2'S QUALIFICATIONS: **MBBCh, PhD**

SUPERVISOR #2'S DEPARTMENT:
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SUPERVISOR #2'S ADDRESS / TEL / E-MAIL:
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Tel: 011-489-9219, Email: amanda.krause@nhls.ac.za

SUPERVISOR #3'S NAME: **Dr David Segal**

% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #3: **20%**

SUPERVISOR #3'S QUALIFICATIONS: **MBBCh, FAAP (USA)**

SUPERVISOR #3'S DEPARTMENT:
The University of the Witwatersrand and The Wits University Donald Gordon Medical Centre

SUPERVISOR #2'S ADDRESS / TEL / E-MAIL:
Wits Donald Gordan Medical Centre, 18 Eton Road, Parktown, Johannesburg, 2193
Tel: 011-726-0016, Email: david@endo.co.za

UNIVERSITY OF THE WITWATERSRAND
BOWEKWA
02 FEB 2016
FACULTY OF HEALTH SCIENCE
YORK ROAD, PARKTOWN 2193
JOHANNESBURG

SYNOPSIS OF RESEARCH: [Use ANOTHER page if More space is required]: **See next page**

ETHICS PENDING: / ETHICS APPROVED: (circle appropriate symbol) **Pending**	IF Y SUPPLY ETHICS CLEARANCE No:

SIGNATURE OF SUPERVISOR/S:			
	Dillon		Supervisor #3's Signature on next page.

CANDIDAT: SURNAME: **Dillon**	FIRST NAME/S: **Bronwyn**	STUDENT NUMBER: **0300869W**
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DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: **Master of Medicine (MMed)**		
PART-TIME OR FULL-TIME: **Full-time**		
FIRST REGISTERED FOR THIS DEGREE:	TERM : **1**	YEAR: **2015**
DEPARTMENT: **Division of Human Genetics, National Health Laboratory Service (NHLS) and The University of the Witwatersrand**		
TITLE OF PROPOSED RESEARCH: **Endocrine Profiling in Black South African Fanconi Anaemia Patients, Homozygous for a *FANCG* Founder Mutation**		
CANDIDATE'S SIGNATURE:		DATE: 01/02/2016
SUPERVISOR #1'S NAME: **Dr Candice Feben**		
% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #1: **40%**		
SUPERVISOR #1'S QUALIFICATIONS: **MBBCh, DCH, MMed, FCMG(SA)**		
SUPERVISOR #1'S DEPARTMENT: **Division of Human Genetics, NHLS and The University of the Witwatersrand**		
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SUPERVISOR #2'S NAME: **Professor Amanda Krause**		
% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #2: **40%**		
SUPERVISOR #2'S QUALIFICATIONS: **MBBCh, PhD**		
SUPERVISOR #2'S DEPARTMENT: **Division of Human Genetics, NHLS and The University of the Witwaters**		
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SUPERVISOR #3'S NAME: **Dr David Segal**		
% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #3: **20%**		
SUPERVISOR #3'S QUALIFICATIONS: **MBBCh, FAAP (USA)**		
SUPERVISOR #3'S DEPARTMENT: **The University of the Witwatersrand and The Wits University Donald Gordon Medical Centre**		
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SYNOPSIS OF RESEARCH: [Use ANOTHER page if More space is required]: **See next page**		
ETHICS PENDING: ETHICS APPROVED: **Pending** (circle appropriate symbol)		IF Y SUPPLY ETHICS CLEARANCE No:
SIGNATURE OF SUPERVISOR/S:		

Endocrine Profiling in Black South African Fanconi Anaemia Patients, Homozygous for a *FANCG* Founder Mutation

Research Proposal

Master of Medicine (MMed) in Medical Genetics

Dr Bronwyn Dillon

HPCSA Registration Number: MP0732400

Wits Student Number: 0300869W

Training Number: W-SH-01-02

September 2017

(revised version)

Supervisors:

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Professor Amanda Krause

MBBCh, PhD

Medical Geneticist/Associate Professor and Head of Department, Division of Human Genetics, National Health Laboratory Service and The University of the Witwatersrand

Professor David Segal

MBBCh, FAAP (USA)

Paediatric Endocrinologist

The University of the Witwatersrand and the Wits University Donald Gordon Medical Centre

1. INTRODUCTION AND BACKGROUND

Fanconi anaemia (FA) is an uncommon, phenotypically diverse hereditary condition associated with bone marrow failure, multiple congenital abnormalities and an increased susceptibility to malignancy [Giri *et al.*, 2007]. At a molecular level FA is considered a chromosome breakage disorder characterised by DNA hypersensitivity to cross-linking agents, with resultant chromosomal instability [Alter and Kupfer, 2013].

1.1 Fanconi anaemia-related genes

At present, 19 FA-associated genes (*FANCA, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P, Q, R, S* and *T*) have been identified, demonstrating the marked genetic heterogeneity that FA exhibits [Alter and Kupfer, 2013; Bogliolo *et al.*, 2013; Hira *et al.*, 2015]. A variety of mutations within these genes give rise to the FA complementation groups (or subtypes) which are inherited predominantly in an autosomal recessive manner [Alter and Kupfer, 2013]. The majority of FA causative mutations are found in the *FANCA* gene (± 60 – 70% of all cases), followed by *FANCC* (± 14% of all cases) and *FANCG* (± 10% of all cases) [Tischkowitz and Hodgson, 2003; Shimamura and Alter, 2010]. Most patients are compound heterozygotes and few common mutations exist [Tischkowitz and Hodgson, 2003].

1.2 The Fanconi anaemia pathway

The FA-related genes code for FA proteins, which are believed to operate together in a shared "FA pathway". This is considered a DNA damage response or DNA repair pathway that controls the cells' resistance to harmful DNA interstrand cross-linking agents [Taniguchi and D'Andrea, 2006; Alter and Kupfer, 2013]. If this pathway becomes disrupted, by a mutation in a FA-related gene for example, the cellular and clinical abnormalities suggestive of FA manifest [Garcia-Higuera *et al.*, 2001].

1.3 Fanconi anaemia in South Africa

Although FA is thought to be a rare genetic disorder (estimated prevalence 1-5 per million), the prevalence in certain South African population groups has been found to be much higher [Tipping *et al.*, 2001; Morgan *et al.*, 2005; Taniguchi and D'Andrea, 2006]. Morgan *et al.* (2005) proposed that the birth incidence of FA in the Black South African population is approximately 1 in 40 000 based on

carrier frequency data obtained from gene frequency studies. The proposed reason for this higher incidence is a genetic founder mutation in the *FANCG* gene. In the Black South African FA population studied, a homozygous seven base-pair deletion mutation (c.637_643delTACCGCC) has been identified in 77.5% [Morgan *et al.*, 2005; Wainstein *et al.*, 2013]. In a further 5% of this population, the common deletion mutation was identified in the heterozygous state [Morgan *et al.*, 2005; Wainstein *et al.*, 2013]. Black South African patients with FA thus represent a unique patient cohort from a genetic homogeneity perspective. Given this predominantly genetically homogeneous group, molecular genetic testing for the founder *FANCG* mutation is now the first line diagnostic test for Black patients suspected to have FA [Wainstein *et al.*, 2013]. This is in contrast to international testing standards where a chromosome breakage or fragility test would be used to confirm the clinical diagnosis [Alter and Kupfer, 2013].

1.4 The clinical features

Fanconi anaemia shows marked clinical heterogeneity in addition to genetic heterogeneity. The hallmark of disease presentation is progressive bone marrow failure, usually during the first decade of life and has an estimated cumulative incidence of 90% by the age of 40-50 years [Taniguchi and D'Andrea, 2006; Giri *et al.*, 2007; Alter and Kupfer, 2013]. In addition to bone marrow failure affected individuals are also susceptible to haematological malignancies, most commonly acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS), as well as an assortment of solid tissue tumours [Alter, 2003; Alter and Kupfer, 2013]. An international study published in 2003, by Alter, suggested that the cumulative probability of developing leukaemia by 30 years of age was 40% and the cumulative probability of developing solid tumours was 76% by the age of 45 years [Alter, 2003]. In South Africa, Black patients with FA have been shown to have severe aplastic anaemia at the time of diagnosis of FA; most demise prior to leukaemic conversion or the development of solid tumours [Feben *et al.*, 2015]. This is predominantly owing to the poor availability of haematopoietic stem cell transplantation in the state healthcare system in South Africa [Feben *et al.*, 2015].

1.4.1 Physical congenital abnormalities

Fanconi anaemia is also characterised by a variable spectrum of physical congenital abnormalities, which are present in 60 – 75% of individuals affected by the condition [Alter and Kupfer, 2013]. These

abnormalities mainly include skin pigmentation abnormalities, growth restriction and malformations of the skeletal system. The range of skeletal system malformations are diverse and include abnormalities of the upper limbs (particularly the thumbs), lower limbs, spine and craniofacial structures [Shimamura and Alter, 2010; Alter and Kupfer, 2013]. Other widely recognised physical abnormalities include abnormalities of the eyes, ears, renal system and genitalia. Abnormalities of the cardiopulmonary, gastrointestinal and central nervous systems are also seen, however they occur at a lower frequency [Tischkowitz and Hodgson, 2003; Shimamura and Alter, 2010; Alter and Kupfer, 2013]. Feben *et al.* (2014) showed skin pigmentary abnormalities to occur at a frequency of 97% in the Black South African cohort studied; ear, eye and hand anomalies in ≥70%; and renal anomalies in 37%. In addition to these features, Black South African patients with FA were found to be both stunted for age (z-score <-2) and underweight for age (z-score <-2) [Feben *et al.*, 2014].

1.4.2 Endocrine abnormalities

Less recognised manifestations of FA include a wide range of endocrine abnormalities. Internationally, more recent discourse has highlighted that endocrine abnormalities are widespread (73%) among both children and adults with FA [Giri *et al.*, 2007]. Of the endocrine abnormalities identified by Giri *et al.* (2007), the most notable were short stature and/or growth hormone (GH) deficiency (51%), abnormal gonadal function (65%), hypothyroidism (37%), and dysfunctional glucose/insulin metabolism (39%) [Giri *et al.*, 2007]. Under nutrition, low body mass index (BMI), raised BMI, reduced bone mineral density (BMD) and pituitary gland abnormalities are other endocrine abnormalities that have been documented in patients with FA [Petryk *et al.*, 2015].

While numerous international research studies have documented the major endocrine abnormalities in patients with FA, these studies have assessed individuals with FA of various genotype to give general frequencies of these disorders. Very little genotype-specific information has yet been documented in the literature.

Short stature is one of the well recognised physical abnormalities associated with FA. In a study by Rose *et al.* (2012) short stature was present in 60% of children with FA and in 58% of adults with FA.

The aetiology of short stature in individuals with FA is manifold and includes both endocrine and non-endocrine causes (including complications from FA treatment) [Petryk *et al.*, 2015]. Observed endocrine causes of short stature in FA consist of GH deficiency, hypothyroidism and hypogonadism. Individuals with FA who have hormone deficiencies are markedly shorter (mean height SD -2.7 ± 2.0) compared to their counterparts without hormone deficiencies (mean height SD -1.3 ± 1.4), suggesting that superimposed endocrine dysfunction further influences the short stature inherently associated with FA [Wajnrajch *et al.*, 2001; Giri *et al.*, 2007]. Growth hormone deficiency is another recognised endocrine abnormality noted in FA. Giri *et al.* (2007) showed that 50% of the patients evaluated had GH deficiency. These patients were noted to be significantly shorter when compared to those individuals with FA who had normal GH levels [Giri *et al.*, 2007]. Forlenza *et al.* (2014) concluded, in their retrospective case review study of patients with FA (that had undergone haematopoietic cell transplantation) that the administration of recombinant human GH positively affected growth, with an overall height increase in 75% of the patients reviewed.

Thyroid function is also affected in individuals with FA, however the exact pathophysiology of hypothyroidism in FA remains unclear [Petryk *et al.*, 2015]. It has been proposed that it is potentially the unrepaired damage to DNA that may lead to the direct death of thyroid cells [Petryk *et al.*, 2015]. Of 70 FA children that were tested, Rose *et al.* (2012) showed 61% had low thyroid levels. Hypothyroidism was also noted in 37% percent of 27 adult individuals with FA [Rose *et al.*, 2012]. It is recommended that thyroid function in patients with FA be evaluated annually and appropriate treatment be initiated without delay when indicated [Petryk *et al.*, 2015].

Disturbances in glucose and insulin metabolism have been identified in patients with FA. It has been shown that abnormalities in glucose metabolism are frequent in children with FA; these include hyperglycaemia and/or impaired glucose tolerance, insulin resistance, and overt diabetes mellitus [Giri *et al.*, 2007; Elder *et al.*, 2008]. Petryk *et al.* (2015) recommend that all patients should be tested for glucose and insulin abnormalities upon diagnosis of FA, in addition to blood pressure monitoring and counselling on lifestyle modification where appropriate, as the cardiovascular disease risk in patients with FA has not yet been established [Petryk *et al.*, 2015].

Puberty, gonadal function and fertility can all be affected in patients with FA [Petryk *et al.*, 2015]. Children with FA can suffer from precocious puberty or they can have delayed onset of puberty [Petryk *et al.*, 2015]. Low-dose oestrogen and testosterone therapies can be administered, to females and males respectively, who show a delayed pubertal onset [Petryk *et al.*, 2015]. Abnormalities of the reproductive system in male patients with FA include cryptorchidism, hypospadias, small testes and phallus for age and infertility (partly due to decreased spermatogenesis) [Giri *et al.*, 2007; Petryk *et al.*, 2015]. Although rare cases of fertile females with FA have been documented, females with FA often enter premature menopause and may exhibit structural genital tract abnormalities, such as a unicornuate uterus [Petryk *et al.*, 2015].

The aetiology of endocrine abnormalities seen in FA is both multifactorial and complex. Although there is not yet a single agreed-upon cause for all of the endocrine dysfunction observed in FA, the pathogenesis may be related to direct damage to endocrine cells with the impaired DNA damage response and DNA repair mechanisms that are known to be associated with this disease [Rose *et al.*, 2012].

1.5 Motivation for research

Although, FA is generally regarded as a progressively fatal disease this should not negate the need for the proper identification and comprehensive management of the full spectrum of FA-associated endocrinopathies [Giri *et al.*, 2007]. Early intervention for these endocrinopathies will allow for opportunities to decrease morbidity and ultimately improve these individuals' quality of life [Giri *et al.*, 2007]. To date there is no previous comprehensive study that has evaluated the endocrine abnormalities in Black South African patients with FA. As such there are currently no standard protocols for the investigation and management of endocrine abnormalities in this patient group. The Black South African population is a unique cohort from a genetic perspective. Such a study would allow for further genotype-phenotype correlations and will hopefully show the benefit of endocrine screening to guide standards of care for these patients in South Africa.

2. STUDY AIM AND OBJECTIVES

The *aim* of the proposed research report is to evaluate the need for routine screening of endocrine status in Black South African patients with FA based on the described frequency of the endocrine disorders in this cross-sectional descriptive study.

The *objectives* of this research report are:

1. To evaluate thyroid function (using thyroid-stimulating hormone (TSH) and free thyroxine (fT4) measurements), glucose and insulin metabolism (by assessing glucose and insulin levels), growth hormone (GH) status (using insulin-like growth factor 1 (IGF-1) and insulin-like growth factor-binding protein 3 (IGFBP-3) levels as screening tests), growth (by assessing bone age and anthropometric measurements) and pubertal status (by Tanner staging) in Black South African patients with FA.
2. To determine the frequency and nature of endocrine abnormalities (abnormal thyroid function; impaired glucose and insulin metabolism; impaired growth and bone maturation; GH deficiency; and abnormal pubertal development) in Black South African patients with FA; specifically those homozygous for the *FANCG* founder mutation.

3. RESEARCH METHODOLOGY

3.1 Sample group

The target study population will be Black South African patients with FA who have a homozygous founder mutation (c.637_643delTACCGCC) in the *FANCG* gene. The researchers' aim is to collect a study sample of approximately 25 - 30 patients with FA, between the ages of 2 years and 21 years, confirmed homozygous for the *FANCG* deletion mutation.

3.2 Study location

This research project is to be conducted in collaboration with the Paediatric Haematology Units at Chris Hani Baragwanath Academic Hospital, a tertiary level hospital in Soweto, South Africa; Charlotte Maxeke Johannesburg Academic Hospital, a quaternary level hospital in Johannesburg, South Africa; Steve Biko Academic Hospital, in Pretoria; and Universitas Academic Hospital, a tertiary

level hospital in Bloemfontein. Patients with FA are managed by these units and will be assessed at these unit for the research project.

3.3 Collaborators

The Clinical Heads of the Paediatric Haematology Units have been consulted with and will act as collaborators for this study - Dr Rosalind Wainwright (Chris Hani Baragwanath Academic Hospital); Professor Janet Poole (Charlotte Maxeke Johannesburg Academic Hospital); Professor David Reynders (Steve Biko Academic Hospital); and Dr Johannes du Plessis (Universitas Academic Hospital). The collaborators' roles will be to assist with coordinating patient appointments to coincide with the researchers' visit to the unit, and to provide clinical advice and expertise in the field of Paediatric Haematology. Professor David Segal, a Paediatric Endocrinologist working at the Wits University Donald Gordon Medical Centre, will be a supervisor on the project. He will interpret and analyse endocrine data collected during the study and will assist in determining the bone age of the study participants.

3.4 Patient ascertainment

The Paediatric Haematology Units at Chris Hani Baragwanath Academic Hospital, Charlotte Maxeke Johannesburg Academic Hospital, Steve Biko Academic Hospital and Universitas Academic Hospital have been approached to collaborate on this research project and permission for collaboration has been obtained in writing from all collaborators. Patients who meet the inclusion criteria for this research project and who consent to participation will be assessed against a FA database that has been compiled by the Division of Human Genetics at the National Health Laboratory Service (NHLS) in Johannesburg, South Africa. This FA database contains information regarding the patients' clinical information, genetic testing (molecular and cytogenetic) performed, and whether the patient has received genetic counselling previously. From this information, patients who have tested positive for a homozygous *FANCG* deletion mutation (c.637_643delTACCGCC) will be identified and will be invited to participate in the research project. Molecular genetic testing will be offered to patients who have not yet had molecular characterisation of their FA (as part of their routine investigations and work-up). Should they test homozygous for the *FANCG* deletion mutation, they will also be invited to participate in the research project.

3.5 Inclusion criteria

Patients (between the ages of 2 years and 21 years) who are currently being managed by the Paediatric Haematology Unit at Chris Hani Baragwanath Academic Hospital, Charlotte Maxeke Johannesburg Academic Hospital, Steve Biko Academic Hospital and Universitas Academic Hospital, who have been confirmed homozygous for the *FANCG* deletion mutation and who consent to a physical and genital examination and a fasted venous blood draw will be included in the study. Patients meeting the study inclusion criteria and their parents/guardians will be required to read an information document and sign informed consent should they wish to participate in the study. The drafted consent form includes three options: consent for a physical examination (including Tanner pubertal staging and anthropometric measurements); fasted venepuncture; and X-rays of the hands to determine bone age.

3.6 Clinical examination

Participants will undergo a clinical examination, which will include anthropometric measurements (weight, height and head circumference) and Tanner pubertal staging. Tanner staging assesses pubertal development based on primary sexual features (external genitalia and breast development) and secondary sexual features (pubic hair growth). Mid-parental heights will also be obtained, to determine the genetic height potential of the patient. A clinical tick-sheet has been drafted to document the data obtained (see Appendix attached).

3.7 File review

A retrospective file review will take place during the consultation. The aim of the file review is to supplement any information clinically obtained (including presence of thyroid hormone, insulin, glucose and GH abnormalities; and abnormalities of pubertal development), birth anthropometrics and whether the patient has received any treatment, specifically androgen therapy.

3.8 Blood tests and other investigations

Following consent, venepuncture will be performed to measure the following:

- Glucose and insulin homeostasis (by measuring fasting plasma glucose and insulin levels);
- Thyroid gland function (by measuring TSH and free T4); and
- Growth hormone status (using a screening approach consisting of IGF-1 and IGFBP-3 measurements).

These endocrinological measurements will be assessed from a single, overnight fasted venepuncture sample and will be evaluated under identical conditions in one laboratory. Should any of the initial test results be abnormal, a second venepuncture may be required. This further testing will be discussed with the patient and caregiver, and referral will be made to the Endocrine Clinic at Chris Hani Baragwanath Academic Hospital, Charlotte Maxeke Johannesburg Academic Hospital, Steve Biko Academic Hospital or Universitas Academic Hospital for the further testing to be carried out. Individuals found to have abnormal GH screening tests will also be referred to the Endocrine Clinic for a full GH stimulation test. Blood measurements will be evaluated against the laboratory age and sex matched control reference ranges. Hand X-rays will be reviewed in order to assess bone age using the published standards of Greulich and Pyle. If there are no prior X-rays available, X-rays of the hands will be requested if consent has been provided.

3.9 Timing of the study

Retrospective file review, physical examination, X-rays and venepuncture of the study population aim to commence in April 2016 and continue until December 2017. Each full clinical history, examination and venepuncture is estimated to take one hour in total. The clinical file review and documentation of additional necessary information is envisaged to take an additional 30 minutes. Data analysis and the write up of findings will extend to August 2018.

	2016			**2017**		**2018**
	Feb	Mar	Apr - Dec	Jan - Jul	Aug - Dec	Jan - Aug
Protocol submission						
Protocol assessment						
Ethics application						
Participant recruitment						
Data collection						
Data analysis						
Writing of research report						

4. LIMITATIONS OF THE STUDY

4.1 Study group and ascertainment of sample

Due to the time allocated for this research project, long term follow-up of the endocrine function of this cohort of patients will not be possible. This is an observational, descriptive cross-sectional study. Only patients who are currently attending the Paediatric Haematology Units at Chris Hani Baragwanath Academic Hospital, Charlotte Maxeke Johannesburg Academic Hospital, Steve Biko Academic Hospital and Universitas Academic Hospital will be considered for the study, for this reason ascertainment of the study sample is another limitation of this research. Due to the nature of the disease being studied patients with very severe disease (for example, early onset of bone marrow failure) may demise before being referred to the Paediatric Haematology Unit, and would thus be missed.

4.2 Bias

Inter-observer bias will be avoided by permitting only one researcher to perform the clinical examination. In addition, only Professor David Segal will be assessing the bone ages, to avoid inter-observer bias. Standardised growth charts and measuring equipment will be used in an attempt to minimize intra-observer bias. Importantly, the growth and measurement charts used routinely in South African clinical care setting at present are not derived from the South African population and so are unfortunately not specific to our patients.

Due to time limitations it will not be possible to evaluate a control group of healthy Black South African children – however, clinical experience would suggest that the endocrine abnormalities likely to be found in patients with FA do not occur at significant frequencies in the healthy population.

5. DATA ANALYSIS

The aim is to obtain a sample size of approximately 25 - 30 participants, which would be acceptable for statistical analysis but may not be reached due to the rarity of FA. Analysis of the collected endocrine data will be reviewed by the researchers. Results of the physical examination, bone ages and endocrine testing will be expressed as median, range, or mean ± 2 standard deviations (SD) and

will be compared to laboratory standards, in addition to a descriptive analysis of the data. If required, the data will be discussed with statisticians from The University of The Witwatersrand Epidemiological Data Centre.

6. ETHICAL CONSIDERATIONS

This research project requires a physical examination, which includes assessment of Tanner pubertal stage. The study also requires a fasted venous blood draw. For these reasons, full informed consent is required. As information and consent documents are drafted in English, efforts will be made to thoroughly explain the contents of these documents in the patients' home language if necessary, to ensure patient understanding. Patient participation in the study will be completely voluntary and non-coerced. There will be no cost to the patient or parents/guardian, or to Chris Hani Baragwanath Academic Hospital, Charlotte Maxeke Johannesburg Academic Hospital, Steve Biko Academic Hospital or Universitas Academic Hospital as the cost of investigations will be covered by grant funding (unless required as part of the Paediatric Haematology Unit's routine care). Parents/guardians will be fully reimbursed for their travel costs to and from Chris Hani Baragwanath Academic Hospital, Charlotte Maxeke Johannesburg Academic Hospital, Steve Biko Academic Hospital or Universitas Academic Hospital. Patients and their parents/guardians will be provided with refreshments (a light snack and beverage) on the day of examination. No additional remuneration will be offered. Genetic counselling will be offered to the participants who have not received prior genetic counselling for their condition. This will be done in collaboration with the genetic counsellors of the Division of Human Genetics of the National Health Laboratory Service, Johannesburg, South Africa. Genetic counselling is advised, however it is voluntary and non-obligatory. In the event that the family chooses to attend a counselling session, FA molecular testing will be offered to the probands' siblings. This will allow pre-clinical identification of new patients with FA who may benefit from timely referral to the Paediatric Haematology Unit.

Fanconi anaemia molecular genetic test results will be conveyed to the family via the clinical head of the Paediatric Haematology Unit at Chris Hani Baragwanath Academic Hospital, Charlotte Maxeke Johannesburg Academic Hospital, Steve Biko Academic Hospital and Universitas Academic Hospital.

Results of the individual endocrinological measurements will be fedback to the patients' attending doctor (Dr Wainwright, Professor Poole, Professor Reynders, or Dr du Plessis) in an effort to preserve continuity of care and ensure appropriate clinical intervention where necessary. For this reason, patients will initially be identified until after the results have been fedback to the patients and their attending doctor, after which the patient data will be de-identified and analysed anonymously for the study. Should patients wish to know the overall results of the research project, this information will be communicated to them in writing on completion of the study. An application for ethics clearance has been submitted to the Health Sciences Research Ethics Committee of the Universities of Pretoria and the Free State, in August 2017. Approval is pending. The Human Research Ethics Committee (HREC) of the University of the Witwatersrand has approved the study at Chris Hani Baragwanath Academic Hospital and Charlotte Maxeke Johannesburg Academic Hospital (clearance certificate number M160220 and M160220).

7. FUNDING AND BUDGET

The anticipated main source of funding for this research project will be from the Phyllis Knocker Bradlow grant. An application for this grant has been made by Dr C. Feben (project co-supervisor). I have also made an application for a Medical Faculty Research Endowment Fund (MFREF) grant and will be applying for a NHLS Research Trust grant. It is envisaged that approximately R65 000 will be required for completion of the entire project.

The proposed budget is as follows:

Item	**Description**	**Total (R)**
Stationery	Paper, printing, photocopying (growth charts, tick sheets, information and consent documentation)	1300
Travel reimbursement (patients)	Estimated 30 patients x R50 per trip to and from Chris Hani Baragwanath Academic Hospital	1500
Travel expenses (researchers)	Estimated 8 trips to Chris Hani Baragwanath Academic Hospital at 34km per round trip @ R4.50 per km Flights for 2 researchers JHB – Bloemfontein (return) Car hire (Bloemfontein) One night's accommodation in Bloemfontein for 2	1224 6000 2000 1600

	researchers	
Blood tests	TSH R223 x 30 = R6990 Free Thyroxine (T4) R199 x 30 = R5970 Fasting glucose R41.10 x 30 = R1233 Fasting insulin R141.40 x 30 = R4242 IGF1 R398.40 x 30 = R11952 IGFBP3 R539.80 x 30 = R16194	46581
Patient refreshments	R40 x 30 patients x 2 (includes parent/ guardian)	2400
Sundry expenses	Miscellaneous	2000
	Overall total	**64605**

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Appendix

FA Endocrine Profiling: Data Collection Sheet

Name

DOB (Age)

Date of examination

Growth assessment

Weight		centile	SD
Height		centile	SD
OFC		centile	SD
BMI		centile	SD
Father height			
Mother height			
Mid-parental height (sex adjusted)			

Pubertal assessment

Male	testes	Tanner1	Tanner2	Tanner3	Tanner4	Tanner5
	pubic hair	Tanner 1	Tanner2	Tanner3	Tanner4	Tanner5
Female	pubic hair	Tanner 1	Tanner2	Tanner3	Tanner4	Tanner5
	breast	Tanner 1	Tanner2	Tanner3	Tanner4	Tanner5

X-ray assessment

Bone age

Blood results

TSH
T4
Fasting glucose
Fasting insulin
IGF1
IGBP3

Appendix M

Journal of Clinical Endocrinology and Metabolism Author Guidelines

JCEM Author Guidelines

Please submit manuscripts through http://www.editorialmanager.com/jcem.

Purpose and Scope

The Journal of Clinical Endocrinology & Metabolism is the world's leading peer-reviewed journal for endocrine clinical research and clinical practice information. Each issue provides up-to-date coverage of new developments that enhance our understanding of pathophysiology, diagnosis and treatment of endocrine and metabolic disorders. Regular features of special interest include original reports of important advances in patient-oriented endocrine and metabolic research, personal perspectives on endocrinologic topics, clinical trials, clinical reviews, clinical practice guidelines, and case reports.

More about the journal

Editor-in-Chief, Associate Editors, and Editorial Board

Article Types

The following types of articles, including preprints from recognized repositories, will be considered for publication:

Original Articles

• **Clinical Research Articles** are original, investigative, clinical studies based on previously unpublished data. There are no upper or lower word/figure/table limits. All figures and tables must be original.

Manuscript Preparation Guidelines and Checklist

Below is a checklist of the basic format requirements. For more information, see Editorial Policies. If you have any questions, contact the editorial staff at publications@endocrine.org.

Guidelines on General Preparation of Initial Submissions

• Read the Editorial Policies. NOTE: Endocrine Society journals allow submissions from preprints: see Preprint Repositories and Prior Publication.

• For initial submissions ONLY, authors may upload a single PDF of all submission files. However, if authors choose to upload a .doc version of their manuscript file, the system will be able to extract much of the metadata automatically, which may speed moving through the online submission form. Please note that regardless of which option authors choose, text, figures, and tables must be uploaded as separate files at revision.

• Submit paper in English through the Editorial Manager system at www.editorialmanager.com/jcem/.

• Use a double-spaced, single-column format with 1-inch margins.

• Number all text lines throughout the manuscript.

• Paginate the entire document.

• Place all tables and figures after the references and clearly label each.

• Gather needed information prior to starting the submission process in Editorial Manager:

• Full names, institutions, and email addresses for each author.

• Submitting authors are required to provide an ORCiD when uploading a manuscript.

• Appropriate funding information for each author.

• Disclosure information for each author.

• Names and email addresses for three recommended reviewers.

• Original manuscript number if manuscript being submitted was previously rejected by the journal to which it is being resubmitted.

• No cover letter is needed. A text block is provided during the submission process for special requests.

• Appropriate figure file specifications as detailed in the Figure Guidelines have been followed.

Units of Measure and Standard Abbreviations

• Use the international system of units (SI) where possible, or other metric units. If non-metric units are mentioned, please give their SI equivalent in parentheses.

• Temperature should be expressed in degrees Celsius (*e.g.*, 28°C) and time of day using the 24-hour clock (*e.g.*, 0800 h, 1500 h).

• Do not express molecular weight in daltons. Molecular weight is considered to be the relative molecular mass of a substance, *i.e.*, the ratio of the mass of one molecule of the substance to 1/12 of the mass of one atom of carbon 12. Therefore, molecular weight is dimensionless. The dalton is a unit of mass equivalent to 1/12 of the mass of one atom of p1 carbon 12.

• All nonstandard abbreviations in the text must be defined immediately after the first use of the abbreviation.

Checklist and Guidelines

Title Page

___ Full title of 120 characters or fewer that provides a concise statement of the article's major contents.

___ Authors' full names and institutions.

___ Short title of 50 or fewer characters for page headings.

___ No more than six keywords.

___ Corresponding author's contact information.

___ Name and address of person to whom reprint requests should be addressed.

___ Any grants or fellowships supporting the writing of the paper.

___ Disclosure summary.

Abstract

___ No longer than 250 words and prepared as a structured abstract.

___ Does not refer directly to the text or references.

___ Describes in complete sentences the purpose, methods, results, and main conclusions.

___ Aimed to a general audience with specialized terminology kept to a minimum.

Précis

___ Submit a brief description no longer than 200 characters, including spaces, that serves to buttress the content of the title by simply stating what was done and what was found.

___ Should not be a reformat of the abstract.

Introduction

___ An introductory statement that places the work in historical perspective, explaining its intent and significance.

The following two sections are expected in a research article:

Materials and Methods

___ Describes in sufficient detail for other investigators to repeat the work.

___ Make all appropriate resource deposits. See Resource Deposits for full instructions.

Results

___ Results should briefly present the experimental data in text, tables, or figures.

Discussion

___ Focus Discussion on the interpretation and significance of the findings or information reviewed with concise objective comments that describe their relation to other work in that area.

Acknowledgments

___ Include names of people who contributed to the study but did not meet the requirements for authorship.

Data Availability

___ Options described in the Data Availability section.

References

___ Use the AMA (American Medical Association) Style Guide for references. List all authors for the initial submission. See examples of correctly formatted references below.

___ List references in consecutive numerical order (in parentheses) in the text, figures, and tables and list in the same numerical order at the end of the manuscript. References in tables and figures should be cited in sequence with those in the text. The numbering should shift to the table or figure after the table or figure is first mentioned in the text. All references in the table or figure should be cited in sequence. The numbering of citations should then return to the text and continue for subsequent citations. NOTE: Provided sequence is preserved, it is acceptable for a reference to appear only in a figure or table. EXAMPLE: If Table 1 contains five references and the first citation of the table occurs immediately after Ref. 10 in the text, then the references numbered within the Table 1 must be Refs. 11-15. Within the text, after the first citation for Table 1, reference sequencing resumes with Ref. 16, and so on.

___ Supplemental data must be submitted to a repository and cited in the manuscript bibliography. For more information see Extended Data Sets and Supplemental Materials.

___ Do not cite the following in the reference list:

• Unpublished observations

• Personal communications

• Submitted manuscripts

• Manuscripts in preparation

___ “In press” manuscripts can be included in the reference list if they meet the following criteria:

• Accepted for publication but not yet in final published form

• Can be cited with a DOI (Digital Object Identifier)

• The journal name is provided

___ *Abstracts:* If it is necessary to cite an abstract because it contains data not published elsewhere, it must be designated as such in the text and in the reference list.

___ Examples of references. Note that when citing RRIDs, the URL must follow the citation format as shown below:

• JOURNAL CITATION: Binoux M, Hossenlopp P. Insulin-like growth factor (IGF) and IGF-binding proteins: comparison of human serum and lymph. *J Clin Endocrinol Metab.* 1988;67(3):509–514.

• ABSTRACT CITATION: MacLaughlin DT, Cigarros F, Donahoe PK. Mechanism of action of Mullerian inhibiting substance. Program of the 70th Annual Meeting of the Endocrine Society, New Orleans, LA, 1988, p 19 (Abstract P1-21).

• BOOK CITATION: Bonneville F, Cattin F, Dietemann J-L. Computed tomography of the pituitary gland. Heidelberg: Springer-Verlag; 1986; 15–16.

• BOOK CHAPTER CITATION: Burrow GN The Thyroid: nodules and neoplasia. In: Felig P, Baxter JD, Broadus AE, Frohman LA, eds. Endocrinology and metabolism. 2nd ed. New York: McGraw-Hill; 1987:473–507.

• REPOSITORY CITATION: Brown C, Jones M, Cohen M. Data from: Medical device-regulation process: review of safety notices and alerts. Dryad Digital Repository 2017. Deposited 2 January 2018. http://doi.org/10.9561/dryad.585t4

• ANTIBODY CITATION: RRID:AB_2629219, https://scicrunch.org/resolver/AB_2629219

• CELL LINE CITATION: RRID:CVCL_1H60, https://scicrunch.org/resolver/CVCL_1H60

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Legends for Figures and Tables

___ Provide all legends separately after the references.

___ Mini-reviews only: if figure or table is reproduced or adapted from a previously published source, include an acknowledgment line at the end of the legend.

___ Clearly and completely describe the content of the figure or table so it can be understood without reference to the text.

___ Explain any symbols or the significance of any color that is important for understanding the content.

___ Use color descriptors as necessary.

___ Figures and tables must be numbered to appear sequentially. Figure 1 must be followed by Figure 2 and any subsequent figures in numerical order, and Table 1 must be followed by Table 2 and any subsequent tables in numerical order.

Tables

___ Construct tables simply and design them to be clear without reference to the text.

___ Provide a concise heading and footnotes if needed.

___ Generally, submitted tables should not consist of more than four or eight manuscript pages (for portrait or landscape presentation, respectively). This is so that the composed, typeset tables are limited to two journal pages (if in standard, portrait orientation) or four journal pages (if in landscape orientation) with normal font size. If, when composed, tables exceed the specified limits, production and ultimate publication will be delayed until the requirements are met.

Figure Guidelines

Image Integrity Guidelines

___ The Endocrine Society uses an image forensic screening process to determine if manipulation has occurred.

___ No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.

___ Adjustments of brightness, contrast, or color balance are acceptable only if they are applied to the whole image and do not obscure, eliminate, or misrepresent any information present in the original.

___ The grouping of images from different parts of the same gel, field or exposure, or from different sources, must be made explicit by the arrangement of the figure (e.g., dividing lines) and in the text of the figure legend.

___ The author agrees to provide the editorial office with the original data used to produce the figure if requested.

General

___ Review the detailed Digital Art Guidelines.

___ If using color, present information so as to minimize difficulty for readers with color vision deficiency, *e.g.*, by using symbols and an optimized color palette. Please consult Figure Preparation Guidelines for Color Vision Deficiency.

___ Titles should be clear and informative. Use minimal wording on figures and confine explanation of figures to their legends.

___ Legends should clearly and completely describe the appropriate content.

___ Color charges will apply to all submitted color figures and cannot be replaced with black and white versions after acceptance.

___ Figures must be numbered to appear sequentially. Figure 1 must be followed by Figure 2 and any subsequent figures in numerical order.

Specifications

___ Resolution:

• Low-resolution figures are not acceptable for production.

• Line art (monochrome): 600–1200 DPI

• Halftone (grayscale only): 300 DPI

• Combination (halftone with type or lines) or color: 600 DPI

___ File Format:

• Submit one file per figure.

• Preferred format is EPS, TiF, PPT, PDF, and Word. (JPEG, although not preferred, will be considered on a case-by-case basis. PNG, BMP, and GIF files should not be submitted.)

• Fonts should be embedded in the file.

___ File Name:

• Use the following naming convention for original submission: Author Last Name, figure number, and file format extension (*e.g.*, Smith_fig1.eps).

• Use the following naming convention for revised figures: Author Last Name, manuscript number, figure number, and file format extension (*e.g.*, Smith_jc.2016-1234_fig1.eps).

___ Color Mode:

• Preferred color mode is RGB.

• Color density should be no more than 300%.

• All color art reproduction in print will result in author charges.

• Color figures are not converted to black and white after acceptance.

___ Shading:

• Make differing shades vary by at least 20%, *i.e.*, 25%, 45%, 65%.

___ Graphs:

• Graphs with axis measures containing very large or small numbers should convert to easily readable notations. Example: For an ordinate range of “counts per minute” values from 1,000 to 20,000, the true value may be multiplied by 10-3 (scale would read from 1 to 20) and the ordinate axis display “cpm (×10-3).” Similarly, for a Scatchard plot with values ranging from 0.1 to 2 femtomolar (10–15 M), the scale may run from 0.1 to 2 with the abscissa labeled “M(×1015).

• Three-dimensional bar graphs will not be published if the information they refer to is only two-dimensional.

Supplemental Data

___ Supplemental data are no longer allowed as uploads submitted with a manuscript. Supplemental data must instead be submitted to a repository and the accession number provided in the Reference section and cited in the manuscript. For more information see Extended Data Sets and Supplemental Materials.

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- Heading levels and text layout have been prepared for you.
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CLINICAL RESEARCH ARTICLE

Manuscript Title in Initial Caps

Author name [Please list all authors here in the order in which you would like them to appear; do not include qualifications][1] Author name[2]

[1]Author affiliations [list all author affiliations in the form of department, institution, city, state, country]; [2]Author affiliations

Precis: State what was done and what was found in up to 200 characters

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Disclosure. **The author reports no conflicts of interest in this work.** [Each manuscript needs to include a disclosure of financial interest or other conflict of interest statement. This is where these statements go].

Abstract: Structured abstracts are commonly used for clinical research; see recommendations of the International Committee of Medical Journal Editors. For submissions to the Journal of Clinical Endocrinology

and Metabolism that report research and for Clinical Research Article submissions to the Journal of Endocrinology, structured abstracts are required. The structured abstract should be no longer than 250 words. Research Articles generally have an abstract with the following headings: Context; Objective, Design, Setting; Patients or Other Participants; Intervention(s); Main Outcome Measure(s); Results; Conclusions. Reviews generally have an abstract with headings: Context; Evidence Acquisition; Evidence Synthesis; Conclusions. If meta-analysis is used, the abstract should have headings: Context; Objective; Data Sources; Study Selection; Data Extraction; Data Synthesis; Conclusions. Case Reports generally have an abstract with headings: Context; Case description; Conclusions. Position and Consensus Statements generally have an abstract with headings: Objective; Participants; Evidence; Consensus Process; Conclusions.

Keywords: [Choose four to six keywords. These should not be repetition of words given in the title.]

1. Introduction

[This is an example of text formatting. Please note that citations are in bracketed, baseline numerals following the punctuation. Abbreviations are defined in full at their first instance.]

Subdermal contraceptive implants have been studied and used in humans for over twenty years. [1,2] Contraceptive implants provide long-acting, highly effective reversible contraception. The most recently introduced subdermal implant, Implanon® (N.V. Organon, Oss, the Netherlands), also referred to as the etonogestrel (ENG) implant, is a single rod implant that offers three years of contraceptive efficacy. [3–6] The ENG implant has been used in more than 30 countries, including Australia, Indonesia, and the Netherlands, and was approved by the United States Food and Drug Administration (FDA) in 2006. The ENG implant is an excellent option for women with contraindications to estrogen in addition to any woman who desires long-acting reversible contraception.

The ENG implant is a single rod implant measuring 40 mm long and 2 mm in diameter with a solid core of ethylene vinyl acetate (EVA) impregnated with 68 mg of etonogestrel, the biologically active metabolite of desogestrel. [7,8] The EVA copolymer allows controlled release of hormone over three years of use.[9] Each implant is provided in a disposable sterile inserter for subdermal application.

[Generally each major section of your manuscript should have a heading. The most common breakdown of a paper is given below, with some subheadings related to the above example text. Please delete or include as needed.]

2. Material and Methods *or* Subjects and Methods

A. Etonogestrel Implant [This is an example of a level 2 heading.]

1. Efficacy measures in this study [This is an example of a level 3 heading.]

3. Results

4. Discussion

5. Conclusion [This section, which is optional, may state principal conclusions if these are not included in the discussion]

6. Appendix

7. Acknowledgments

[Use a level 4 head]*Author contributions.*

8. References

[The following are examples of our reference style]

1. Burnier M, Fricker AF, Hayoz D, all authors. Pharmacokinetic and pharmacodynamic effects of YM087, a combined V1/V2 vasopressin receptor antagonist in normal subjects. *Eur J Clin Pharmacol*. 1999;55:633–637.
2. Decaux G. Long-term treatment of patients with inappropriate secretion of antidiuretic hormone by the vasopressin receptor antagonist conivaptan, urea, or furosemide. *Am J Med*. 2001;110:582–584.
3. Fried LF, Palevsky PM. Hyponatremia and hypernatremia. *Med Clin North Am*. 1997;81:585–609.
4. Gheorghiade M, Konstam MA, Burnett JC Jr, all authors; Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study With Tolvaptan (EVEREST) Investigators. Short term clinical effects of tolvaptan, an oral vasopressin antagonist, in patients hospitalized for heart failure: the EVEREST Clinical Status Trials. *JAMA*. 2007;297:1332–1343.
5. Vaprisol (conivaptan HCl injection) [package insert]. Deerfield IL: Astellas Tokai Co.; February 2006.

Table 1 [Table titles are in sentence case and do not end with a full-stop.]

Notes:

Abbreviations: AUC, area under the curve; LS, least squares; NE, not estimable. [These are examples of format.]

Figure 1 [Title of figure is in sentence case and ends in a full stop. It should be clear and informative]

[Figure legend should completely describe the content]

[The figure should be provided as a separate file in TIF or EPS format with a name that identifies which figure it is]

Appendix N

Turn-it-in report

0300869w:Fanconi_anaemia_MMED_Submissable_Format_p…

ORIGINALITY REPORT

16%	12%	14%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	C Feben, T Haw, D Stones, C Jacobs, C Sutton, J Kromberg, A Krause. "Fanconi Anaemia in South African Patients with Afrikaner Ancestry", South African Journal of Child Health, 2017 Publication	1%
2	jcem.endojournals.org Internet Source	1%
3	Submitted to London School of Hygiene and Tropical Medicine Student Paper	1%
4	Textbook of Clinical Pediatrics, 2012. Publication	1%
5	Candice Feben, Jennifer Kromberg, Rosalind Wainwright, David Stones, Janet Poole, Tabitha Haw, Amanda Krause. "Hematological consequences of a FANCG founder mutation in Black South African patients with Fanconi anemia", Blood Cells, Molecules, and Diseases, 2015	1%

	Publication	
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