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Phenotypic consequences in black South African Fanconi anaemia patients homozygous for a FANCG 637-643 deletion mutation

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2012
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PHENOTYPIC CONSEQUENCES IN BLACK SOUTH AFRICAN FANCONI ANAEMIA PATIENTS HOMOZYGOUS FOR A *FANCG 637-643* DELETION MUTATION

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

Johannesburg, South Africa, 2012

DECLARATION

I, Candice Feben, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Medical Genetics at the University of the Witwatersrand, Johannesburg. It has not been submitted at this University previously or at any other University.



Candice Feben

7 June 2012

ABSTRACT

Fanconi anaemia (FA) is a genotypically and phenotypically heterogeneous genetic condition, characterized microscopically by chromosomal breakage and instability and usually inherited in an autosomal recessive manner. Affected individuals often present with a diverse variety of physical congenital abnormalities and most progress to haematological disease including bone marrow aplasia and myelodysplasia in early childhood.

In South Africa, Black individuals with FA share a common causative founder deletion mutation in the Fanconi G gene (*FANCG del*) in 82% of cases. They are thus an ideal patient cohort for a genotype-phenotype correlation study. Thirty Black patients, homozygous for *FANCG del*, were ascertained from haematology/oncology clinics in Johannesburg and Bloemfontein. They were subjected to a comprehensive clinical examination to document their physical features. A concurrent review of each participant's hospital file allowed data to be collected regarding disease presentation and haematological progression.

Significant growth abnormalities and a high frequency of skin pigmentary anomalies were found in the research cohort. Although subtle, anomalies of the eyes, ears and hands were noted in a high frequency. The overall physical phenotype does not appear to be appreciably different from that described in other Fanconi anaemia cohorts; however, affected Black individuals may present with more severe haematological indices and have poorer outcome than FA individuals of heterogeneous genotype. Further, it would appear that haematological disease progression cannot be predicted by the presence of physical abnormalities.

ACKNOWLEDGEMENTS

I would like to thank the following people for their assistance in completing this project:

My supervisors, Professor Amanda Krause and Ms Tabitha Haw, for their continuous support, unlimited patience, guidance and encouragement.

Professor Jennifer Kromberg, for her innovative ideas, editing and mentorship.

Dr Linda Wainwright, Professor David Stones and Professor Janet Poole, for collaborating with the project, helping me to find appropriate patients and for providing valuable advice and haematological expertise.

Professor Elena Libhaber for her generous assistance and advice regarding the statistical analysis of the project.

The Medical Research Council of South Africa for financial assistance.

Clinical and support staff at Chris Hani Baragwanath Hospital, Charlotte Maxeke Johannesburg Academic Hospital and Universitas Hospital for their help in coordinating patient visits, collecting blood samples and aiding with translation when necessary.

Clinical, counselling, laboratory and support staff in the Department of Human Genetics for their ongoing encouragement and for enduring endless research questions.

The Fanconi patients and their families who agreed to participate in the project and who allowed me to learn so much from them and be inspired by their courage and bravery.

My family and friends for believing in me and giving me the space and strength to pursue my dreams.

TABLE OF CONTENTS

DECLARATION ii

ABSTRACT iii

ACKNOWLEDGEMENTS iv

TABLE OF CONTENTS v

LIST OF FIGURES ix

LIST OF TABLES x

NOMENCLATURE xii

1. INTRODUCTION & LITERATURE REVIEW 1

1.1 Preface 1
1.2 Introduction 2
1.2.1 Clinical phenotype of FA 2
1.2.2 Molecular pathogenesis of FA 4
1.2.3 Diagnostic modalities 8
1.2.4 Management strategies 10
1.3 Genotype-phenotype correlations in FA – literature review 11
1.3.1 International FA research 11
1.3.2 South African FA research 14
1.4 Aims of the present study 16

2. PATIENTS & METHODS 18

2.1 Introduction 18
2.2 Patient ascertainment 18
2.2.1 Patient selection and inclusion criteria 18
2.2.2 Sample size 19
2.2.3 Collaboration 19
2.2.4 Informed consent 20
2.3 Ethics 20
2.4 Haematological assessment 20
2.5 Clinical examination & file review 21
2.5.1 Growth measurements 22
2.5.2 Central nervous system examination 22
2.5.3 Eye evaluation 23
2.5.4 Ear evaluation 23
2.5.5 Cardiovascular, renal and gastrointestinal evaluation 23
2.5.6 Genital evaluation 24
2.5.7 Upper and lower limb evaluation 24
2.5.8 Vertebral evaluation 25
2.5.9 Pigmentary changes 25
2.5.10 Endocrine assessment 26
2.5.11 Developmental assessment 26
2.6 Molecular genetic testing 27
2.7 Data analysis & statistics 27
2.7.1 Analysis of the demographic data and presenting complaints 28
2.7.2 Analysis of the haematological data 28
2.7.3 Analysis of the clinical phenotypic data 30
2.8 Summary 31

3. RESULTS 32

3.1 Introduction 32
3.2 Demographic data 32
3.3 Presenting complaints 33
3.4 Age at presentation and age at bone marrow failure 34
3.5 Haematological indices at presentation 36
3.6 Transfusion dependence 37
3.7 Clinical features 38
3.7.1 Growth measurements 38
3.7.2 Central nervous system anomalies 40
3.7.3 Eye anomalies 40
3.7.4 Ear anomalies 43
3.7.5 Cardiac anomalies 45

3.7.6 Gastrointestinal anomalies 45
3.7.7 Renal anomalies 45
3.7.8 Genital anomalies 46
3.7.9 Upper limb anomalies 46
3.7.10 Lower limb anomalies 48
3.7.11 Vertebral anomalies 49
3.7.12 Pigmentary anomalies 49
3.7.13 Endocrine anomalies 51
3.7.14 Developmental assessment 52
3.8 Comparison of physical anomalies in *FANCG del* cohort with physical anomalies previously reported in other FA cohorts 53
3.9 Screening tools to assess Black patients with a possible diagnosis of FA 55
3.9.1 A clinical screening system based on significant parameters identified in the *FANCG del* cohort 56
3.9.2 The IFAR Score as a screening tool in the South African context 57
3.10 Physical abnormalities as predictors of haematological outcome 57
3.11 Summary 59

4. DISCUSSION 60

4.1 Introduction 60
4.2 Summary of the aims of the project 60
4.3 Genotype-phenotype correlation in *FANCG del* patients 61
4.3.1 Haematological phenotype in *FANCG del* homozygotes 61
4.3.2 Physical phenotype in *FANCG del* homozygotes 64
4.3.2.1 Growth measurements 64
4.3.2.2 Facial features 65
4.3.2.3 Skeletal abnormalities 67
4.3.2.4 Skin pigmentation anomalies 68
4.3.2.5 Renal malformations 68
4.3.2.6 Central nervous system, cardiac, gastrointestinal and genital malformations 69
4.3.2.7 Endocrine evaluation 69
4.3.2.8 Developmental progression 70
4.4 Screening tools when considering the diagnosis of FA in Black patients in South Africa 72
4.5 Physical abnormalities as predictors of haematological outcome 73
4.6 Spectrum of phenotypic variability in FA – impact on diagnosis? 74
4.7 Summary 76

5. CONCLUSION 77

5.1 Introduction 77
5.2 Recommendations for future research 77
5.3 Limitations of the study 78
5.4 Concluding comments 79

APPENDIX A: Consent documentation 82

APPENDIX B: Ethics approval 89

APPENDIX C: Clinical Tick Sheet 91

APPENDIX D: Growth & physical parameter charts 95

REFERENCES 99

LIST OF FIGURES

FIGURE 3.1 -Age of patients with FA attending haematology/oncology clinics in Johannesburg and Bloemfontein 33

FIGURE 3.2 - Median age at presentation with symptoms suggestive of FA 35

FIGURE 3.3 - Current growth measurements by centile – weight, height and head circumference for age in 30 patients with FA 39

FIGURE 3.4 – Ocular anomalies in a patient with FA (up-slanting palpebral fissures, epicanthic folds and short palpebral fissure length for age) 41

FIGURE 3.5 - Ear length represented by centile for age in 30 patients with FA 43

FIGURE 3.6 (a and b) - Low set ears with overfolded helices in two patients with FA 44

FIGURE 3.7 - Structural renal anomalies detected on ultrasound evaluation in 29 patients with FA 46

FIGURE 3.8 - Most commonly observed upper limb abnormalities in 30 patients with FA 47

FIGURE 3.9 – Large hyperpigmented axillary macule (arrow) in a patient with FA 49

FIGURE 3.10 (a and b) – Hypopigmented macules (arrows) observed on clinical examination of the skin in patients with FA 50

LIST OF TABLES

TABLE 1.1 - Overview of the 13 FA genes 6

TABLE 1.2 - The International Fanconi Anaemia Registry Score (IFAR) 15

TABLE 3.1 - Frequency of presenting complaints in 30 patients with FA 34

TABLE 3.2 - Average haematological indices at the time of diagnosis with FA 37

TABLE 3.3 - Frequency of underweight for age and stunting in FA children and healthy South African children, stratified by age 40

TABLE 3.4 – Ocular manifestations in two FA cohorts 42

TABLE 3.5 - Ear anomalies, other than small ear length, observed in 30 patients with FA 44

TABLE 3.6 - Upper limb anomalies observed rarely in 30 patients with FA 48

TABLE 3.7 - Pigmentary anomalies observed in 30 patients with FA 50

TABLE 3.8 - Endocrine dysfunction in two groups of patients with FA 52

TABLE 3.9 - Comparison of physical anomalies in *FANCG del* cohort with four other FA cohorts **54**

TABLE 3.10 – Comparison of average scores between patients with transfusion dependence before 8 years of age and non-transfusion dependent patients older than 8 years of age using two screening systems **59**

NOMENCLATURE

ALL	acute lymphocytic leukaemia
AML	acute myeloid leukaemia
BMI	body mass index
CDC	Centre for Disease Control and Prevention
DEB	diepoxybutane
DNA	deoxyribose nucleic acid
EP	epicanthic folds
FA	Fanconi anaemia
FANC A,B, C, D1, D2 E, F, G, I, J, L, M, N, O, P	Fanconi anaemia complementation groups; used interchangeably with the gene associated with each group
FANCG del	637_643 deletion mutation (637_643delTACCGCC) in Fanconi G gene; used to designate the present research cohort
fl	femtolitre
g/dl	grams per decilitre
HAZ	height for age z-score
Hb	haemoglobin
IFAR	International Fanconi Anaemia Registry
IQ	intelligence quotient
l	litre
MCV	mean corpuscular volume
MDS	myelodysplastic syndrome
MMC	mitomycin C
NHLS	National Health Laboratory Service
PCR	polymerase chain reaction
Plt	platelet count
SD	standard deviation
SPF	palpebral fissure length shorter than -2 standard deviations of the mean for age
T4	thyroxine
TFD	transfusion dependent
TSH	thyroid stimulating hormone
UPS	up-slanting palpebral fissures
VACTERL	vertebral defects, anal atresia, tracheo-oesophageal fistula, renal dysplasia, limb defects
WAZ	weight for age z-score
WCC	white cell count
WHO	World Health Organization
95% CI	ninety five percent confidence interval of the mean

1. INTRODUCTION & LITERATURE REVIEW

1.1 PREFACE

Fanconi anaemia (FA) is a rare condition of chromosomal instability, inherited in either an autosomal recessive or an X-linked manner (Alter & Kupfer, 2011; Meetei, Levitus, Xue *et al.,* 2004). It is both genotypically and phenotypically heterogeneous and is a disease which affects individuals from diverse ethnic origins.

In South Africa, affected individuals are seen from all ethnic and social backgrounds. In particular, the Black, Afrikaans and Ashkenazi Jewish population groups have been closely studied from a molecular perspective and causative genetic founder mutations have been characterized in these groups (Whitney, Saito, Jakobs *et al.,* 1993; Tipping, Pearson, Morgan *et al.*, 2001; Morgan, Essop, Demuth *et al.,* 2005). Although much is known about the general FA phenotype, little research has been conducted on direct genotype-phenotype correlations particularly for the founder mutations in these populations.

Patients diagnosed with FA in South Africa usually attend haematology/oncology clinics for management of their most serious disease manifestation, bone marrow failure. Based on gene frequency values calculated in our Black population, we would expect a birth incidence of FA of at least 1/40000 (Morgan *et al.,* 2005). This expected patient number differs markedly from the actual number seen in tertiary haematology/oncology clinics across the country.

It is thought that one of the reasons for the discrepancy between the expected number of patients and the number actually receiving treatment in haematology/oncology clinics in South Africa is under-recognition of the phenotypic characteristics of the condition, with many affected individuals dying from their bone marrow disease before a confirmed diagnosis of FA is made (Macdougall, Greeff, Rosendorff *et al.,* 1990; Morgan *et al.*, 2005).

The present research project aimed to assess if a specific recognizable phenotype could be delineated for Black patients with FA, homozygous for a founder FA complementation group G mutation. This would possibly facilitate earlier recognition of this devastating condition and earlier referral of patients to haematology/oncology clinics which may lead to improved outcomes.

This chapter contains a detailed account of the clinical, molecular, diagnostic and management aspects of FA, as well as a literature review of the pertinent international and local studies which precede this report.

1.2 INTRODUCTION

1.2.1 Clinical phenotype of FA

Clinically, FA is characterized by a diverse spectrum of congenital abnormalities, principally involving skin pigmentation, growth, stature and development. A wide variety of abnormalities of the skeletal system, cardiovascular system, genitourinary system, gastrointestinal system and central nervous system are well documented (De Kerviler, Guermazi, Zagdanski *et al.*, 2000), as is endocrine dysregulation, involving glucose homeostasis, growth hormone and thyroid function (Giri, Batista, Alter *et al.,* 2007).

Major congenital abnormalities are reported to occur in up to two thirds of patients, with one third having either minor abnormalities or no congenital malformations (Auerbach, 2009). Composite frequency figures for the physical anomalies observed in a genetically heterogeneous FA cohort were recently updated and published by Shimamura and Alter (2010). Of the reported physical anomalies, pigmentary abnormalities, including café au lait macules, hyperpigmentation and hypopigmentation are the most common, purported to occur in up to 40% of affected individuals. Short stature is a common anomaly, also occurring in 40% of affected individuals. Upper limb abnormalities (found in 35% of individuals) include radial and ulnar ray anomalies, as well as digital anomalies such as clinodactyly, polydactyly and brachydactyly. Lower limb abnormalities, in contrast, occur in approximately 5% of affected individuals and are usually minor anomalies such as pes planus and toe syndactyly. Anomalies of the eyes (found in 20% of individuals) include microphthalmia, epicanthic folds, abnormalities of palpebral fissure position, cataracts, ptosis, nystagmus and strabismus. Renal anomalies, involving the structure of the kidneys, collecting systems and renal vasculature are reported to occur in 20% of cases. Male genital abnormalities (found in 25% of males), including hypogenitalism, cryptorchidism and azoospermia, occur far more frequently than female genital abnormalities. Female genital anomalies may be structural uterine, ovarian or vulval abnormalities or functional problems such as delayed menarche, irregular menses and early menopause (found in 2% of females) (Shimamura & Alter, 2010).

Bone marrow failure remains the hallmark of the disease, with initial pancytopaenia and progression to acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) or aplastic anaemia (Tischkowitz & Hodgson, 2003). Although initial haematologic findings are diverse, thrombocytopaenia and macrocytosis (raised mean corpuscular volume) usually precede the onset of more severe haematologic anomalies (Auerbach, 2009). Based on data collected by

the International Fanconi Anaemia Registry (IFAR), haematologic abnormalities present in childhood at a median age of seven years, with progression to bone marrow failure by the age of 40 years in more than 90% of patients. Evaluation of the bone marrow of affected individuals at first detection of a haematologic abnormality may show increased or decreased cellularity or features in keeping with MDS or AML. Further, cytogenetic evaluation of the bone marrow may reveal clonal changes in a significant proportion of cases (Butturini, Gale, Verlander *et al.,* 1994; Auerbach, 2009).

FA is also recognized as a cancer susceptibility syndrome (Alter, 1996; Auerbach, 2009). A predisposition to solid tumours of the head and neck, liver, oesophagus and female genital tract are well described and have become more evident in first world countries, as the treatment for the haematological complications of the disease has improved (Rosenberg, Greene & Alter, 2003). In developed nations, the availability of haemopoetic stem cell transplantation as treatment for bone marrow aplasia has prolonged life and is therefore predicted to result in a higher prevalence of solid tumours as a common clinical manifestation in later life (Alter, 1996; Auerbach, 2009). In South Africa, solid tumours are rarely seen in patients with FA as most are thought to still demise in childhood and adolescence from bone marrow disease. Haemopoetic stem cell transplantation is available to a minority of patients and usually only in the private healthcare sector (personal communication, Dr R. Wainwright, University of the Witwatersrand, April 2011).

1.2.2 Molecular pathogenesis of FA

As of 2009, 13 complementation groups or genetic subtypes of FA had been described, each associated with numerous causative mutations within a specific gene. The complementation groups have been designated *FANC A, B, C, D1, D2, E, F, G, I, J, L, M,* and *N.* Two further

putative genes, *RAD51C* (putative complementation group O) and *SLX4* (putative complementation group P) are also thought to be involved in the molecular pathogenesis of the condition (Alter & Kupfer, 2011). Worldwide, as documented in the IFAR, complementation group *A*, *C* and *G* mutations are the most prevalent, accounting for 60.5%, 16% and 10% respectively (Auerbach, 2009).

Each of the causative FA genes has been localized and its specific protein product identified (Table 1.1). Much research has been conducted over many years to elucidate the mechanisms in which the protein products interact with each other and to define their functions. At the present time, it is thought that the FA gene products are involved in a highly complex, multi-step, S-phase specific cellular response to damaged deoxyribose nucleic acid (DNA). This pathway is activated when DNA replication is stalled (de Winter & Joenje, 2009).

The gene products are divided into three groups – those in the core nuclear complex (*FANC A, B, C, E, F, G & L*), those in the ubiquinated gene complex (*FANC D2 & I*) and those in the downstream gene complex (*FANC D1, J & N*) (Auerbach, 2009). *FANCM* appears to orchestrate the localization of the core complex to damaged DNA, once DNA replication has stalled at a damaged DNA site (it may be classified as part of the nuclear core complex) (de Winter & Joenje, 2009).

As these proteins function along the same cellular pathway and within many sub-complexes, defects in any single sub-unit affect the overall functioning of the pathway, resulting in the clinical phenotype recognized as FA (de Winter & Joenje, 2009). A diagrammatic representation of the FA pathway can be obtained from Kee and D'Andrea (2010).

TABLE 1.1 Overview of the 13 FA genes (adapted from de Winter & Joenje, 2009)

Subtype	Gene	Location	Functions
A	*FANCA*	16q24.3	Partner of FANCG, contains nuclear localization signal
B	*FANCB*	Xp22.2	Partner of FANCL, contains nuclear localization signal
C	*FANCC*	9q22.3	Partner of FANCE
D1	*FANCD1/BRCA2*	13q12.3	Supports RAD51 filament formation, contains BRC repeats and OB-fold
D2	*FANCD2*	3p26	Monoubiquinated and phosphorylated following DNA damage
E	*FANCE*	6p21.3	Partner of FANCC and FANCD2, Chk1 target, contains nuclear localization signal
F	*FANCF*	11p15	Adaptor protein, stabilizing A/G and E/C interaction
G	*FANCG*	9p13	Partner of FANCA, contains TPR motifs
I	*FANCI*	15q26.1	Partner of FANCD2, monoubiquinated and phosphorylated following DNA damage
J	*FANCJ/BRIP1*	17q22-24	5'-3'DEAH helicase, unwinds G-quadruplex DNA structures
L	*FANCL*	2p16.1	E3 ubiquitin ligase, contains RING-finger and WD40 domains
M	*FANCM*	14q21.3	Translocase, contains DEAH helicase and ERCC4/XPF like nuclease domain
N	*FANCN/PALB2*	16p12.1	Partner of BRCA2, essential for stability and localization of BRCA2

Recent research conducted by Vaz, Hanenberg, Schuster *et al.* (2010), elucidated the possible role of a further gene, *RAD51C*, which is also thought to function in the FA pathway. Biallelic mutations in this gene produce a clinical phenotype very similar to FA. Further, mutations in the *SLX4* gene have now also been reported in individuals diagnosed with FA, previously unclassified in terms of complementation (Kim, Lach, Desetty *et al.,* 2011; Stoepker, Hain, Schuster *et al.,* 2011).

All of the FA genes exhibit mutational heterogeneity, which is evidenced by the large number of sequence variants and mutations that have been described, particularly in the *FANCA* and *FANCG* genes (Wijker, Morgan, Herterich *et al.,* 1999; Demuth, Wlodarski, Tipping *et al.,* 2000; Auerbach, Greenbaum, Pujara *et al.,* 2003).

Causative FA founder mutations have been described in the Ashkenazi Jewish population (Whitney *et al.,* 1993) and in both the Afrikaans (Tipping *et al.*, 2001) and Black populations of South Africa (Morgan *et al.,* 2005).

In the South African Black population, a seven base-pair deletion mutation (c.637_643delTACCGCC) in the *FANCG* gene is reported to be present in 82% of patients with FA. The mutation is thought to produce a truncated FANCG protein (Morgan *et al.*, 2005). Previous studies by Demuth *et al.* in 2000 and Auerbach *et al.* in 2003, in which the spectrum of mutations in the *FANCG* gene in various ethnic groups was investigated, showed that the *637-643* deletion mutation was not present in their study cohorts. Haplotype analysis suggests that this mutation is an ancient one, originating in Bantu-speaking populations of sub-Saharan Africa (Morgan *et al.*, 2005).

In the Afrikaans population of South Africa, three mutations in the *FANCA* gene were found to account for more than 80% of the mutations causing FA. The most common of these is a deletion mutation involving exons 12 to 31 (Del E12-31), accounting for approximately 60% of cases. The second most common mutation is an overlapping deletion mutation involving exons 11 to 17 (Del E11-17). The third is a point mutation (3398delA) (Tipping *et al.,* 2001).

In the Ashkenazi Jewish community, a single splice site mutation in the *FANCC* gene (IVS4+4A>T), accounts for most cases of FA (Whitney *et al.,* 1993).

1.2.3 Diagnostic modalities

Traditionally the diagnosis of FA is confirmed in the laboratory by examining the response of cells exposed to diepoxybutane (DEB) or mitomycin C (MMC), two commonly used clastogenic DNA cross-linking agents. FA cells show hypersensitivity to these agents, with increased chromosome breakage (Tischkowitz & Hodgson, 2003). This phenomenon, which is not unique to FA and has no correlation with disease severity or with the clinical presentation, does not elucidate the molecular abnormality but rather provides cytogenetic evidence of the underlying disease process (Taniguchi, 2008).

Microscopically, increased spontaneous and induced chromosome breakages and chromosome interchanges (multi-radials) are visualized on a metaphase spread (Neveling, Endt, Hoehn *et al.*, 2009). The results are compared to normal cells and positive control cells run in parallel and should be reported as number of breaks per cell and the number of cells with radial forms (Taniguichi, 2008). Breakage testing is used in most cases where the underlying molecular abnormality is unknown, to confirm the diagnosis of FA. It has proven reproducibility and sensitivity when performed in centres with significant experience. The testing distinguishes between affected and unaffected individuals, with little overlap in either breaks per cell or number of breaks per aberrant cell, despite variability in sensitivity between FA patients. It is also useful for prenatal diagnosis as it can be performed on both chorionic villus and amniocyte cells (Auerbach, 2009).

Internationally, diagnostic molecular testing is available by sequencing and in some cases, by deletion/duplication analysis, for mutations in the *FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM* and *FANCN* genes, and by targeted mutation analysis for the founder mutation in the Ashkenazi Jewish

population. Mutation analysis and sequencing of *FANCO* and *FANCP* is currently offered at specific overseas laboratories on a research basis only (Alter & Kupfer, 2011).

Molecular testing may be considered subsequent to a positive chromosome breakage result or in place thereof in certain instances where a specific founder mutation has a high incidence in a defined population. In the absence of a founder mutation, directed testing subsequent to a positive chromosome breakage test ideally requires that the complementation group or genetic subtype of FA be identified in the individual (by complementation analysis) to allow targeted sequencing of the affected gene (Alter & Kupfer, 2011).

In South Africa, as founder mutations exist in a number of population groups, diagnostic mutation testing is offered by the Molecular Genetics Laboratory, of the Division of Human Genetics, National Health Laboratory Services (NHLS) and School of Pathology (University of Witwatersrand) in Johannesburg. Testing is offered for the common founder Ashkenazi Jewish mutation in the *FANCC* gene, for three founder mutations in the *FANCA* gene in the Afrikaans population and for the seven base-pair deletion founder mutation in the *FANCG* gene in Black South African patients (Standard Operating Procedure, NHLS Division of Human Genetics, 2008). The testing is performed, with high detection rates, in these defined population groups, when the diagnosis of FA is suspected on clinical and haematological grounds (personal communication, Professor A. Krause, University of the Witwatersrand, January 2011).

1.2.4 Management strategies

Androgen administration is the mainstay of initial treatment for FA once marrow aplasia is evident. This therapy is effective in up to 50% of individuals within one month of commencement. It acts to increase the red cell count and haemoglobin level. Platelet and white cell response to this therapy are variable. The complications of the therapy include liver toxicity and a risk for the development of hepatic tumours (Taniguchi, 2008). Other observed side effects include hirsutism, acne, hyperactivity and restricted growth leading to short stature (Tischkowitz & Dokal, 2004). Overall survival is thought to be better in androgen treated versus non-treated patients (Dufour & Svahn, 2008).

Prednisone can be used in combination with androgen therapy to reduce the androgen dose required and the risk of liver toxicity. Alone, prednisone therapy does not have a place in the treatment of marrow failure in FA (Dufour & Svahn, 2008).

Androgen therapy is not advocated in the pre-anaemic phase of the condition owing to the side effect profile of the medication. In the event that an individual presents before features of bone marrow aplasia develop, multivitamin therapy and advice on avoidance of bone marrow toxic agents may help prolong the pre-anaemic phase (personal communication, Dr R. Wainwright, University of the Witwatersrand, November 2011).

Transfusion of platelets and packed cells is used as supportive therapy during crisis periods and at later stages of the disease for palliation (personal communication, Dr R. Wainwright, University of the Witwatersrand, April 2011).

Haemopoetic stem cell transplantation is the only available cure for the haematologic complications of FA, including MDS and AML. However, even after successful transplantation, affected individuals remain at risk for the development of solid tumours. Management of these tumours includes regular surveillance to ensure early detection. Treatment is challenging owing to the increased toxicity of chemotherapy and radiation in individuals with FA (Taniguchi, 2008). Stem cell transplantation is not routinely available to patients with FA receiving treatment in the state health care system in South Africa (personal communication, Dr R. Wainwright, University of the Witwatersrand, April 2011).

1.3 GENOTYPE-PHENOTYPE CORRELATIONS IN FA - LITERATURE REVIEW

Given the gamut of mutations responsible for causing FA, and the diverse spectrum of clinical manifestations, several previous research projects have attempted to elucidate genotype-phenotype correlations, both within complementation groups and for specific mutations, and to use the data obtained to aid with prognostication and management.

1.3.1 International FA research

Internationally, researchers have investigated the phenotypic consequences of different FA complementation groups and some have looked at specific genotype-phenotype comparisons (including Gillio, Verlander, Batish *et al.*, 1997; Faivre, Guardiola, Lewis *et al.*, 2000; Rosenberg, Huang & Alter, 2004). Many of these studies concentrated mainly on the haematological phenotype of affected patients and their progression to bone marrow disease or malignancy, rather than on physical phenotypic characteristics as related to specific genotypes and as predictors of haematological disease progression.

In 1997, in a study conducted by Gillio *et al.*, the phenotypic consequences of mutations in the *FANCC* gene were investigated. The study patients were divided into groups depending on their molecular characterization. It was found that those who carried the IVS4+4A>T mutation or with at least one exon 14 (R548X, L554P) mutation had more major congenital abnormalities, developed early onset haematological disease and had a poorer outcome than those with exon 1 mutations (Gillio *et al.,* 1997). Subsequently, in 2000, Futaki, Yamatshita, Yagasaki *et al.* described the splice site mutation (IVS4+4A>T) in a Japanese patient cohort. These patients did not have severe haematological disease, even when they were homozygous for the mutation, possibly reflecting the influence of an individual's genetic background on the phenotypic presentation (Futaki *et al.,* 2000).

In 2000, Faivre *et al.* compared individuals with FA assigned to different complementation groups with regard to incidence of severe cytopaenia, AML or MDS and physical abnormalities. The researchers found that patients in the *FANCG* complementation group were at greater risk of developing severe cytopaenia and had a higher incidence of AML or MDS than individuals assigned to other non-G complementation groups. Individuals in the *FANCA* and *FANCG* groups exhibited more somatic abnormalities than those in the *FANCC* group, including anomalies of the skin and skeletal system. *FANCC* individuals also tended to have the best prognosis in terms of haematologic outcome (Faivre *et al.,* 2000). In this study, the genotype-phenotype correlation was undertaken predominantly using complementation groups and not specific mutations.

In 2003, data analyzed from the IFAR collected over a 20 year period were used by Kutler, Singh, Satagopan *et al.* to evaluate: the overall survival time, time to bone marrow failure and time to malignancy, both haematologic and solid tumours, in individuals within different

FA complementation groups. These data contradicted those of Faivre *et al.* (2000) and showed that patients in *FANCC* group had a higher incidence of bone marrow failure as well as a significantly poorer survival. Supporting the earlier data published by Gillio *et al.* (1997) from the IFAR, patients in the *FANCC* group with at least one intron 4 (IVS4+4A>T) or at least one exon 14 (R548X, L554P) mutation were again found to fare worse in terms of incidence of bone marrow failure and survival. No information was given regarding more specific genotype-phenotype correlations (apart from those mentioned above) with regard to prognosis and outcome (Kutler *et al.*, 2003).

The above two studies were reviewed by Neveling *et al.* (2009) who pointed out the futility in attempting genotype-phenotype correlations using complementation groups rather than specific mutations.

In 2004, Rosenberg *et al.* studied the association between specific easily diagnosed phenotypic characteristics in 144 North American patients with FA, and their risk for developing bone marrow failure, AML or solid tumours. The researchers assessed the cumulative risk of developing one of these complications with age if particular physical and developmental abnormalities were present. Abnormalities of the radius were found to be predictive in their cohort for early onset of aplastic anaemia (Rosenberg *et al.*, 2004). The researchers did not however draw any specific comparisons with patients' genotypes and as it was conducted retrospectively, many abnormalities including those of the skin, eyes, gastrointestinal tract, urogenital system and central nervous system were excluded.

None of above research elucidated direct genotype-phenotype correlations with regard to precise physical abnormalities detectable in individuals carrying specific mutations.

1.3.2 South African FA research

In the South African context, previous research has endeavoured to draw genotype-phenotype correlations in patients with FA of different ethnic origins.

Two studies conducted many years ago considered FA in people of different race groups. The first, published in 1990 by Macdougall *et al.*, looked at Black South African children diagnosed with FA on the basis of chromosomal breakage studies. The authors reported similar phenotypic characteristics, both clinical and haematological, when Black patients were compared to patients within other ethnic groups. It was noted that FA in the Black population was likely to be under-diagnosed as some features including pigment variation, thumb abnormalities and facial anomalies were easily missed (Macdougall *et al.,* 1990). At that stage, however, no conclusions could be drawn regarding a genotype-phenotype correlation, as neither the complementation group nor the specific causative mutations had been elucidated.

The second study published in 1994, by Macdougall, Rosendorff, Poole *et al.*, also compared FA in different ethnic groups in SA. The children in the study were evaluated using the IFAR score. The IFAR score (Table 1.2) is recognized for its role in the diagnosis of FA; it is however, a non-specific score which identifies clusters of abnormalities, rather than specific phenotypic variations (Auerbach, Rogatko & Shroeder-Kurth, 1989). Using this scoring system, no clinical differences were noted between White and Black patients. Although a difference was found in the response rates to androgen and steroid therapy (with White patients showing a better response), further investigations and analysis were required to confirm the findings (Macdougall *et al.*, 1994). Again, this study was done before specific

mutation analysis was available, and thus parallels could not be drawn from genotype to phenotype.

TABLE 1.2 - The International Fanconi Anaemia Registry Score (IFAR) – Adapted from Auerbach, Rogatko & Shroeder-Kurth (1989).

Variable	Score
1. Growth retardation	1
2. Birthmarks	1
3. Kidney & urinary anomalies	1
4. Microphthalmia	1
5. Learning disabilities	-1
6. Low platelets	1
7. Thumb & radius anomalies	1
8. Other skeletal anomalies	-1

Probability of having FA based on score

Score (sum of variables)	Probability
-1	.00
0	.20
1	.31
2	.75
3	.92
4+	.98

In 2004, the clinical phenotype of 25 Black South African patients, homozygous for the founder *FANCG* mutation was evaluated. In this project, eleven phenotypic parameters as defined by the European FA Registry were investigated retrospectively, including head, heart and kidney abnormalities, hearing deficit and developmental delay, growth retardation, lower limb and skeletal abnormalities and anomalies of the thumb, radius and radial ray. The incidence of these anomalies in Black patients with FA was found to be similar to other *FANCG* cohorts published. It was also found that individuals homozygous for the founder mutation in the Black South African population had a high risk of early onset aplastic anaemia (Haw, 2004). Although the researcher investigated a cohort of patients with a homogeneous genotype, a specific phenotype was not elucidated, possibly owing to the small sample size and the limited clinical data available for each patient. Data on specific phenotypic criteria were not collected, and in the evaluation of the data, many abnormalities

were grouped into "anatomical" categories. For example, persons with a lower limb abnormality, ranging from toe syndactyly or pes planus, to developmental dysplasia of the hip, would have been considered a single positive finding. The researcher therefore, was not able to fully delineate a phenotype for these patients as specific anomalies were not investigated in detail (Haw, 2004).

Morgan *et al.*, (2005) as part of their research which elucidated the *FANCG* 637_643 deletion mutation in South African Black patients, retrospectively evaluated the clinical phenotype of 20 Black individuals homozygous for the deletion mutation. They reported a significantly higher frequency of distal radial ray abnormalities, growth abnormalities and eye abnormalities in comparison to a cohort of European patients with FA, as well as a significantly higher number of severe haematological indices. The study did not however categorize any of these abnormalities specifically to delineate a recognizable phenotype and relied on retrospectively documented clinical information.

1.4 AIMS OF THE PRESENT STUDY

The existence of known cohorts of patients with specific common genotypes in the South African population provides unique opportunities to describe particular phenotypes and to assess whether direct genotype-phenotype correlations exist. Of note, in the South African Black population, 82% of patients affected with FA have been shown to carry a founder mutation in the *FANCG* gene (Morgan *et al.*, 2005), thus providing an ideal patient cohort for a genotype-phenotype correlation study.

It is postulated that if defined phenotypic parameters are examined and documented in detail, one may be able to establish whether a specific phenotype exists for patients who are

homozygous for the *FANCG* 637_643 deletion mutation *(FANGC del)*. Should such a phenotype be delineated, its use in the clinical setting, particularly in areas not serviced by tertiary oncology departments, may contribute to earlier diagnosis and referral of patients with FA, thus improving management and possibly outcome.

It is also postulated that one may be able to correlate phenotypic features with disease progression and outcome, specifically with regard to age at presentation and progression to bone marrow disease. This too, may direct management decisions and treatment options with the aim of improved haematological outcome.

Thus the specific aims of the present study were to establish:

1. if a distinct, clinically recognizable phenotype exists for Black patients with FA who are homozygous for the founder seven base-pair deletion mutation in the *FANCG* gene.
2. if particular physical anomalies and phenotypic features are associated with haematological disease progression and outcome in these patients.
3. possible diagnostic, prognostic and management indicators, based on the described phenotype.

2. PATIENTS & METHODS

2.1 INTRODUCTION

The patients and methods chapter details how the present study was performed, including aspects related to patient ascertainment, sample size, ethics approval and data analysis. The clinical examination of the patients is discussed in detail and reference made to the relevant appendices which include copies of the reference materials used.

2.2 PATIENT ASCERTAINMENT

2.2.1 Patient selection and inclusion criteria

Tertiary haematology/oncology departments, as well as secondary level facilities, where patients with FA are being managed, were approached to collaborate for the research project. Patients were recruited from the Chris Hani Baragwanath Hospital and the Charlotte Maxeke Johannesburg Academic Hospital in Johannesburg and the Universitas Hospital in Bloemfontein. Black individuals with FA, identified in these clinics for possible participation in the project, were cross referenced with a FA database within the Division of Human Genetics at the NHLS in Johannesburg, South Africa. This database characterizes patients in terms of molecular diagnosis, cytogenetic testing done, counselling received and clinical data collected. Patients who had molecular characterization of their FA and who were homozygous for *FANCG del* were immediately eligible to participate in the study. Those who had not had molecular testing were offered testing, as part of the project, in association with the Molecular Genetics Division of the Department of Human Genetics at the NHLS in Johannesburg. If found to be homozygous for *FANCG del* they were also included in the

study. All patients needed to consent to a comprehensive clinical examination (see informed consent – section 2.2.4).

2.2.2 Sample size

The final study sample comprised 30 Black patients with FA who were homozygous for *FANCG del*. The patients were recruited from:

- Chris Hani Baragwanath Hospital in Gauteng - 17 patients
- Charlotte Maxeke Johannesburg Academic Hospital in Gauteng - 7 patients
- Universitas Hospital in the Free State – 6 patients.

2.2.3 Collaboration

Clinical heads, Dr Rosalind Wainwright and Professor Janet Poole, from the Paediatric Haematology/Oncology Departments at Chris Hani Baragwanath Hospital and the Charlotte Maxeke Johannesburg Academic Hospital respectively, were consulted prior to the commencement of the project, and acted as primary collaborators. Their role was in coordinating patient follow up appointments with the researcher's visits to their respective units and in providing clinical and haematological advice and expertise. Professor David Stones of the Haematology/Oncology Department at Universitas Hospital, Bloemfontein, Free State, was also consulted and aided in coordinating the inclusion of patients attending haematology/oncology clinics in the Free State. Patients who regularly travel from Lesotho to the Universitas Hospital for management of their FA were also included in the present study.

2.2.4 Informed consent

Patients who were eligible to participate in the study and their parents or guardians were required to read an information document and sign informed consent if they decided to participate (see Appendix A). The consent form was drafted to include four options –

- clinical assessment and file review only
- clinical assessment, file review and taking of photographs
- clinical assessment, file review and venepuncture if required
- clinical assessment, file review, venepuncture if required, and taking of photographs.

Further consent to radiological procedures was provided as a separate category. Although drafted in English, the consent documentation was translated verbally into the home language of the participant if required, with the help of a translator.

2.3 ETHICS

Submissions were made to the Human Research Ethics Committee of the University of the Witwatersrand and the Ethics Committee of the University of the Free State. Both committees approved the research project (see Appendix B).

Ethics clearance certificate numbers:

- University of the Witwatersrand: M090681
- University of the Free State: ETOVS NR 52/2010.

2.4 HAEMATOLOGICAL ASSESSMENT

Information regarding haematological abnormalities including initial bone marrow biopsy results, initial full blood count results, age of development of aplastic anaemia, treatment received (androgens, corticosteroids), age of transfusion dependence and age of progression to bone marrow malignancy or myelodysplasia was documented from a review of the

medical file. The presence of non-haematological malignancies, including squamous cell carcinomas of the head, neck and oesophagus and hepatocellular carcinomas, was also assessed.

2.5 CLINICAL EXAMINATION & FILE REVIEW

Patients were subjected to a comprehensive clinical examination, using a pre-designed clinical tick sheet to document phenotypic variation (see Appendix C). This tick sheet was used to document growth and stature, facial variation involving the eyes and ears, upper and lower limb and skin abnormalities, as well as renal, urogenital, cardiovascular, skeletal and central nervous system malformations. The tick sheet also included data on neurodevelopment, radiological examinations and surgical procedures which required direct history from the patient/guardian, as well as information obtained from a retrospective review of each patient's clinical records, which took place concurrently with the clinical examination. The file review also provided additional information including, the age of presentation to the haematology/oncology department and the reason for the initial presentation. The patient's hospital files were all kept by the treating paediatric oncologists.

If consent was obtained, photographs, documenting phenotypic variation and radiological findings were taken.

Copies of all the growth charts used during the data collection and analysis can be found in Appendix D.

2.5.1 Growth measurements

Measurement of each patient's current weight, head circumference and height was performed using standard equipment. All weight measurements were taken using the same digital scale. Height and head circumference were measured using a tape measure. The mean of two readings was recorded. Weight for age and height for age data were plotted on Centre for Disease Control and Prevention (CDC, 2000) charts for children aged two to 20 years, as these are the charts currently in use in the Clinical Section of the Department of Human Genetics at the NHLS. However, for comparison with normal paediatric reference values, the growth measurements were also plotted on the 2007 World Health Organization (WHO) Reference growth charts for males and females between birth and 19 years of age (WHO Child Growth Standards, 2011) and converted to z-scores. Head circumference measurements were plotted on charts in "Handbook of Physical Measurements" (Hall, Allanson, Gripp *et al.*, 2007). The body mass index (BMI) was calculated using the formula: weight (kilograms) /height (metres)^2. The patients' birthweights were obtained by direct questioning of their parent or guardian or from the Road to Health Card where available.

2.5.2 Central nervous system examination

Information regarding hydrocephalus, neural tube defects and other structural central nervous system anomalies was obtained on history from the patient's parent or guardian and confirmed by a review of the medical file. The biceps, knee and ankle reflexes were assessed with a patellar hammer using the conventional technique, as hyper-reflexia has previously been documented in patients with FA (Macdougall *et al.*, 1994).

2.5.3 Eye evaluation

Palpebral fissure length and inner canthal distance were measured using a clear ruler, based on the technique described in "Handbook of Physical Measurements" (Hall *et al.*, 2007). Measurements were plotted on standard growth charts found in the same manual. Epicanthic folds, palpebral fissure position, nystagmus, strabismus and ptosis were assessed clinically. Cataracts were assessed using an ophthalmoscope by evaluation of the presence or absence of a red reflex.

2.5.4 Ear evaluation

Ear size was measured using a clear ruler, based on the technique described in "Handbook of Physical Measurements" (Hall *et al.*, 2007) and plotted on a standard growth chart found in the same manual. Ear position was ascertained using the technique described in the same manual. An evaluation of the medical file and direct questioning was used to assess hearing acuity and to determine whether a formal audiometry evaluation had been performed. If audiometry had been performed, the results were recorded.

2.5.5 Cardiovascular, renal and gastrointestinal evaluation

Cardiovascular, renal and gastrointestinal evaluations required information from the patient's parent or guardian and a review of the medical records, as well as a clinical examination. If an abnormality was clinically suspected, and not documented or previously investigated, the patient was referred to the treating clinician for further assessment and evaluation. If clinically normal, no further investigations were performed. At the Chris Hani Baragwanath Hospital, the management protocol for children with FA includes a cardiovascular evaluation by a paediatric cardiologist, irrespective of a clinically detectable abnormality. The results of these patients' electrocardiographs and echocardiograph evaluations were noted and

recorded. Treating paediatric oncologists at the Charlotte Maxeke Johannesburg Academic Hospital and the Universitas Hospital rely on the clinical findings before referring patients for a formal cardiology assessment. If any echocardiograph or electrocardiograph results were present in the file, these were noted and recorded.

Renal ultrasound examinations are considered part of the core standard of care in investigating individuals with suspected or confirmed FA, even in resource restricted settings (personal communication, Dr R. Wainwright, University of the Witwatersrand, March 2011). All units in the various hospitals referred their patients for a renal and abdominal ultrasound to document anomalies. The results of these scans were recorded.

2.5.6 Genital evaluation

The genital examination was performed by inspection and palpation. A goniometer was not used. A Tanner score was assigned for pubic hair pattern and penis development in males and for breast development and pubic hair pattern in females. Graphical representation and explanation of the Tanner score was obtained from "Handbook of Physical Measurements" (Hall *et al.,* 2007). Female patients were questioned directly regarding menarche and menses.

2.5.7 Upper and lower limb evaluation

The upper and lower limbs were examined by inspection and palpation. Specific attention was placed on evaluation of the thumb, including thumb size, position of insertion, number of phalanges and movement. Clinodactyly, transverse palmar creases and polydactyly were evaluated clinically. The radial pulse was palpated using conventional technique. The middle finger length was measured using a clear ruler based on the technique described in "Handbook of Physical Measurements" (Hall *et al.,* 2007) and plotted on standard growth

charts found in the same manual. The fifth digit length was assessed using normal anatomical landmarks as described in "Diagnostic Dysmorphology" (Aase, 1990).

If consent was obtained for an X-ray evaluation, an antero-posterior view of the hands and wrists was requested. This allowed assessment of the distal ends of the radius and ulna as well as the first metacarpal and allowed for a crude assessment of bone age, based on the ossification of the carpal bones. Twenty four patients consented to radiological investigations; four of these X-rays had been examined and reported on by diagnostic radiologists at the respective hospitals. Those that were not reported, were later evaluated by Dr Surtee and Dr Tshvase, diagnostic radiologists at the Charlotte Maxeke Johannesburg Academic Hospital, to confirm or supplement the assessment made by the researcher.

2.5.8 Vertebral evaluation

The spine was assessed clinically for scoliosis and kyphosis and the shoulders for Sprengel's deformity. The length of the neck was assessed clinically. If an abnormality was suspected, an X-ray of the involved area of the spine was requested, provided consent for radiological procedures was obtained.

2.5.9 Pigmentary changes

Designated pigment changes included café au lait spots (>2 spots, each >5millimetres), hyperpigmentation and hypopigmentation. These anomalies were chosen because they were likely to be detected by inspection with the naked eye alone and do not require dermatological expertise or specialized equipment. The skin was evaluated clinically and any dermatological changes, other than those specified, were recorded.

2.5.10 Endocrine evaluation

If consent was obtained for venepuncture, blood was taken for random glucose, thyroid stimulating hormone (TSH), thyroxine (T4) and growth hormone levels. Consent was obtained for venepuncture in 24 patients. All blood samples were processed through the NHLS Chemistry Laboratory at the Charlotte Maxeke Johannesburg Academic Hospital to ensure standardization of results. Samples collected outside of Johannesburg were transported, on ice, to the laboratory at the Charlotte Maxeke Johannesburg Academic Hospital for processing. The transportation procedure was discussed with technologists at the NHLS Chemistry Laboratory at the Charlotte Maxeke Johannesburg Academic Hospital. The procedure was also verified by published data for specimen handling and storage so that the results would not be altered by the transportation and storage of the blood samples (Rehak & Chiang, 1988; Zhang, Elswick, Miller *et al.*, 1998). In the case of the blood for glucose analysis, storage on ice may impact the final result, usually resulting in a falsely low reading (Rehak & Chiang, 1998).

Although blood samples were initially collected for growth hormone analysis; on further literature review it was noted that random growth hormone assessments would not provide sufficient data on the growth hormone status of the patients (Refer: Section 3.7.13). This inclusion of random growth hormone assessment was thus an oversight during the planning stage of the project and was discontinued without further analysis of the data.

2.5.11 Developmental assessment

The developmental assessment was a very brief screening assessment, based on four criteria:

- age at sitting
- age at walking
- age at first words and current speech proficiency
- school performance in terms of appropriate grade for age.

The criteria for appropriate milestones for age are as documented in "Child Health for All" (Westwood, Kibel & Saloojee, 2007). This assessment was not comprehensive, but was used as a guide to detect significant developmental delay in basic motor and intellectual milestones. Confounding variables, including lack of school attendance owing to prolonged hospitalization, were recorded and taken into account when assessing development.

2.6 MOLECULAR GENETIC TESTING

Molecular testing for the *FANCG del* mutation was performed by the Molecular Laboratory at the Division of Human Genetics, NHLS. Testing for *FANCG del* involves a polymerase chain reaction (PCR), using oligonucleotide primers flanking the critical region on chromosome 9p13. The seven base-pair deletion is visualized on gel electrophoresis as a shorter band (Standard operating procedure, NHLS Division of Human Genetics, 2008).

2.7 DATA ANALYSIS & STATISTICS

An Excel spreadsheet was created detailing all the data obtained from the tick sheets, allowing a descriptive evaluation and statistical analysis. The Microsoft Excel (Windows 2003) programme as well as Graphpad statistical calculator were used for statistical analysis of the data. Calculated p values were deemed significant when less than 0.05. The data

analysis was discussed with and verified by Professor Elena Libhaber, Associate Professor at the University of the Witwatersrand, Johannesburg.

2.7.1 Analysis of the demographic data and presenting complaints

The median current age and the age range of the children with FA attending the haematology/oncology clinics was calculated at the date of the researcher's visit. The male to female ratio was calculated and the value compared to the expected 1:1 ratio in autosomal recessive conditions using Fisher's exact test. The reasons for initial presentation to the paediatric haematology/oncology unit were recorded and their relative frequencies calculated.

2.7.2 Analysis of the haematological data

The median age and range of presentation with symptoms suggestive of FA and the median age of bone marrow failure were calculated. The average values for the presenting haematological indices (haemoglobin (Hb), white cell count (WCC), platelet count (Plt) and mean corpuscular volume (MCV)) were calculated. Even though the haematological values in the *FANCG del* research group were obtained from different laboratories in different health care centres, the values were averaged for the purpose of comparison to reference values. It was thought that as the patient values deviated substantially from normal, small inter-laboratory differences in analysis would not adversely affect the interpretation of the results. The standard deviation (SD) and 95% confidence interval (95% CI) were calculated for all average values. After confirming the normal distribution of the *FANCG del* cohort values, the *FANCG del* haematological values were compared to haematological reference values using a one sample t-test. As reference ranges change with the age and sex of the child, the reference values used were the lower limit values for all age groups for Hb, WCC

and Plt and the upper limit value for all age groups for MCV as published by Lo (2011) in Nelson Textbook of Pediatrics (19th ed).

The median age of presentation and onset of haematological abnormalities, rates of MDS and conversion rates to acute lymphocytic leukemia (ALL) and AML in the *FANCG del* cohort were compared with previously published data in other research groups. Statistical analysis was not possible in all cases owing to the lack of data in other research publications, required to perform the analysis. Where possible, frequency data from the *FANCG del* group were compared to frequency data in other FA groups using Fisher's exact test.

Transfusion dependence was defined as requiring more than five transfusions of blood or blood products in one year (personal communication, Dr R. Wainwright, University of the Witwatersrand, March 2011). The number of transfusion dependent patients and the median age of transfusion dependence were calculated.

Transfusion dependent (TFD) and non-transfusion dependent (non-TFD) patients were compared in terms of their median current age and their median age of bone marrow aplasia (using a Wilcoxon two-sample test). An IFAR score (refer Table 1.2) was calculated for each group and the average scores of the groups compared using an unpaired t-test (after the sample variance was calculated as equal using the F-test). Similarly, each group was scored using the specific screening tool developed for the research project (as discussed below – section 2.7.3) and the average scores compared, again using an unpaired t-test (with equal variance). Further, the study sample was stratified into those children who were already TFD before the age of 8 years and those older than 8 years who were not yet TFD (using 8 years of age as an arbitrary cut off based on the average age of TFD in the total sample). The

average IFAR score and screening tool score were calculated in each group as well as the standard deviation and 95% CI of the mean. The purpose of these comparisons was to ascertain if TFD patients presented with more physical anomalies than those who were non-TFD.

2.7.3 Analysis of the clinical phenotypic data

The frequency of each described physical phenotypic parameter was calculated. Where possible the frequency calculated in the *FANCG del* cohort was compared to frequency data published in other FA groups using the Fisher's exact test. If statistical comparison was not possible owing to insufficient data in the literature, the frequency values were compared descriptively to those previously published.

Growth measurements were converted to z-scores utilizing WHO Anthro and WHO Anthro-plus software. Z-scores were calculated in order to standardize the growth measurements as the research patients were of different ages and gender. The z-scores were then used to compare the growth measurements of the *FANCG del* cohort with a cohort of healthy Black South African children as published by Kimani-Murage, Kahn, Pettifor *et al.* (2010). The frequency of individuals with weight for age and height for age z-scores less than -2 in the two groups was compared using the Fisher's exact test (weight for age z-scores could only be calculated for children under the age of 10 as the calculators used do not allow for weight for age z-scores to be calculated in individuals older than 10).

The frequency of endocrine dysfunction in the *FANCG del* cohort was calculated and compared to frequency figures reported by other researchers using the Fisher's exact test.

An IFAR score (refer Table 1.2) was calculated for each *FANCG del* patient. The average score for the cohort was calculated, as well as the SD and 95% CI of the mean.

Physical parameters found to occur in at least half of the research cohort were chosen and used in an attempt to develop a screening tool for health care practitioners to aid in recognizing the FA phenotype (elaborated in Chapter 3.9.1). Each parameter was allocated a score of +1 if present and 0 if absent, giving each patient a total score out of 5. Each of the *FANCG del* patients was scored using this system. The average score for the *FANCG del* cohort, SD and 95% CI of the mean were calculated.

2.8 SUMMARY

Thirty Black patients with FA, homozygous for *FANCG del,* were comprehensively examined to evaluate specific phenotypic features in order to elucidate a genotype-phenotype correlation in this cohort. An inclusive file review augmented the clinical data, but also allowed collection of information regarding disease presentation and hematological progression. Descriptive statistical analyses were performed on the collected data.

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3. RESULTS

3.1 INTRODUCTION

This chapter details the results obtained after the analysis of the data collected in the present *FANCG del* cohort. Demographic data, haematological data and physical phenotypic information are described. Included in this chapter, are tabulated comparative analyses of the results in the research cohort with results published in the literature for other FA cohorts. In some instances comparisons are also made to expected normal values in a reference population. The use of screening tools to identify patients with FA based on their physical and haematological parameters is introduced here and then discussed further in Chapter 4.

3.2 DEMOGRAPHIC DATA

The total sample comprised 30 Black patients homozygous for the *FANCG del* mutation. Of these, 14 patients were female (46.6%) and 16 were male (53.4%). The male to female ratio was 1.14:1, not diverging significantly from the expected 1:1 ratio (p=1 (Fisher's exact test)).

Although all patients were attending haematology/oncology clinics in South Africa at the time of data collection, four affected individuals were resident outside South African borders (two in Lesotho, one in Zimbabwe and one in Mozambique).

The median current age of the 30 *FANCG del* patients attending the haematology/oncology clinics, as calculated at the date of the researcher's visit to the clinic, was 9 years, 3 months

(Range: 3 years – 17 years, 5 months). The current ages of the research patients are represented in Figure 3.1.

Although two affected sibling pairs were receiving treatment in the haematology/ oncology clinics, only the elder sibling was considered eligible to participate. The younger siblings were excluded to prevent confounding results due to possible shared familial physical features.

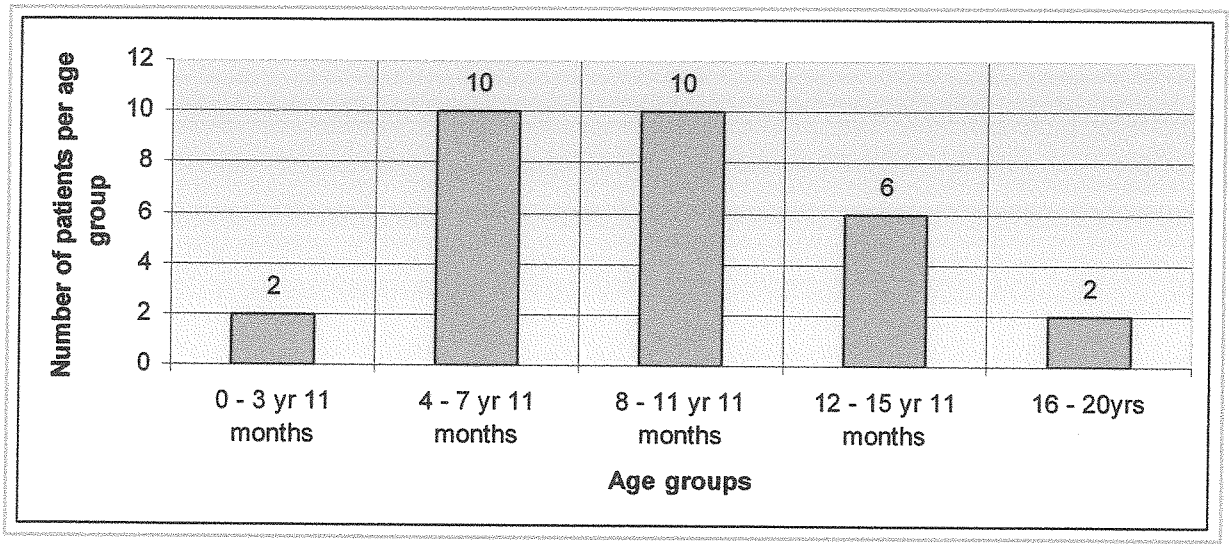


FIGURE 3.1 – Age of patients with FA attending haematology/oncology clinics in Johannesburg and Bloemfontein

3.3 PRESENTING COMPLAINTS

The presenting complaint was defined as the initial reason for presentation at either the referral hospital or clinic or at the treating haematology/oncology clinic. The presenting complaints and their frequencies are presented in Table 3.1. Two individuals had more than one initial complaint. Epistaxis was the most frequent complaint. In the total sample of 30

patients, none presented or were referred to haematology/oncology clinics on the basis of identified congenital malformations or dysmorphic features alone.

TABLE 3.1- Frequency of presenting complaints in 30 patients with FA

Complaint	Number of individuals	Frequency
Epistaxis	16	53.3%
Weakness/fatigue	6	20.0%
Infections	5	16.7%
Haematemesis	2	6.7%
Pallor	2	6.7%
Pigment abnormalities	1	3.4%

3.4 AGE AT PRESENTATION AND AGE AT BONE MARROW FAILURE

The median age at presentation with the above noted symptoms was 7 years, 1 month of age (Figure 3.2). The youngest individual, who presented with epistaxis and was diagnosed with bone marrow failure at the time of diagnosis of FA, was 2 years, 11 months old. The oldest individual, who also presented with epistaxis, and was diagnosed with aplastic anaemia concurrently, was 11 years, 9 months of age.

Bone marrow aspirate and trephine reports were available for review in 27 of the 30 patients (90%). The median age of bone marrow aplasia was calculated as 7 years, 1 month (Range: 2 years, 11 months – 12 years; N=27). It was not possible to differentiate the age at presentation from the age of bone marrow aplasia in the majority of cases (24/27; 88.9%) because these children were confirmed to have bone marrow aplasia at the time of their initial presentation to the haematology/oncology clinic.

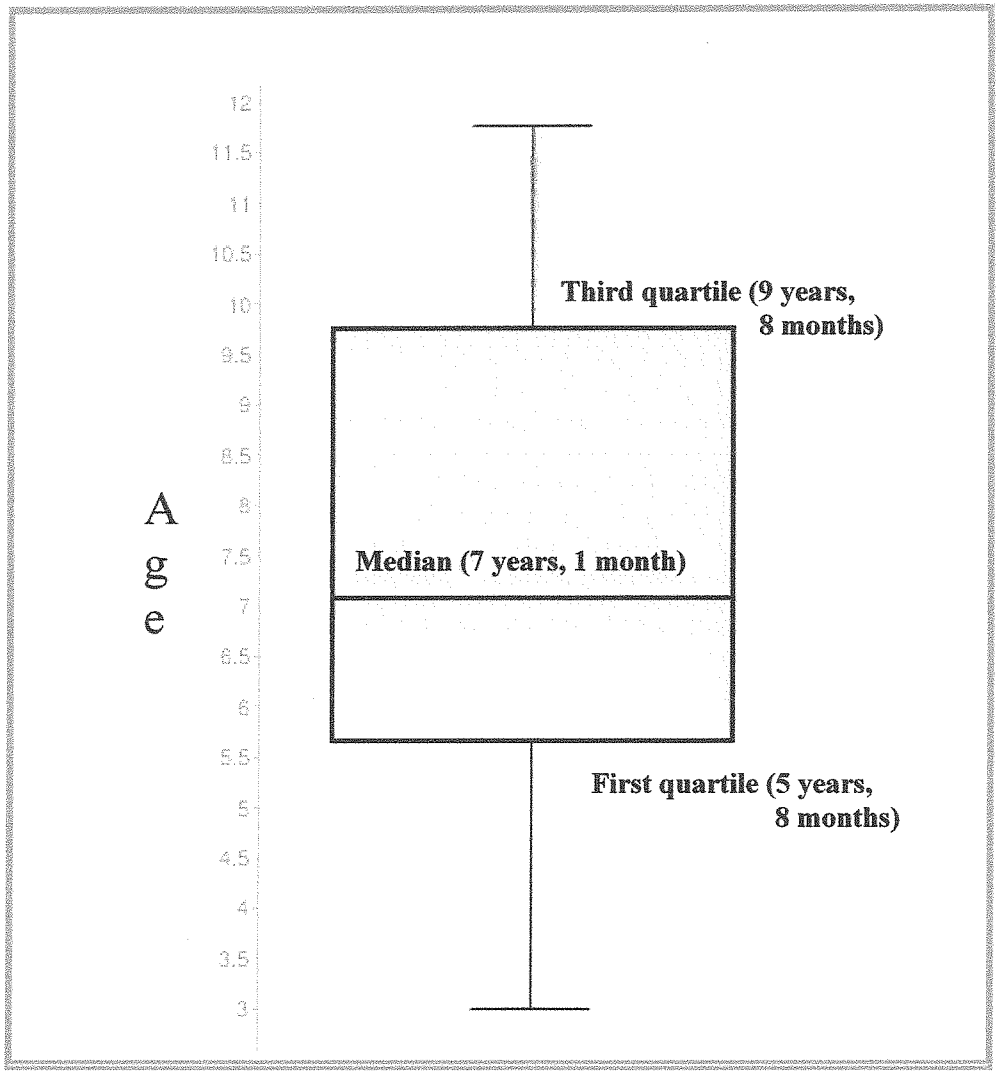


FIGURE 3.2 - Median age at presentation with symptoms suggestive of FA

In three of the 27 cases (11.1%), the age of initial presenting complaint and the age of bone marrow failure could be distinguished. In the first, the disease course progressed over six months from the initial presentation of fatigue, to bone marrow aplasia diagnosed on bone marrow aspirate and trephine biopsy. The second progressed to bone marrow aplasia over 24 months from the initial presentation with epistaxis. The third also presented with epistaxis and progressed over six months to bone marrow aplasia.

Thus in the majority of the *FANCG del* research patients the initial presentation was concurrent with overt bone marrow disease. Owing to the small number of cases that presented to haematology/oncology clinics before bone marrow failure developed, no conclusions could be drawn regarding the disease progression or the time interval from initial symptoms to bone marrow failure.

None of the affected individuals in the *FANCG del* cohort were found to have changes in keeping with AML or ALL on their full blood count parameters or on their bone marrow aspirate and trephine reports. Two of the 27 patients (7.4%) for whom bone marrow biopsy reports were available for review, were found to have features of MDS, at the time of their FA diagnosis. These patients are monitored by regular full blood count analysis for changes in keeping with a marrow conversion to AML.

None of the *FANCG del* patients were known or suspected to have developed any solid tumours or non-haematological malignancies.

3.5 HAEMATOLOGICAL INDICES AT PRESENTATION

The haematological indices recorded at the time of initial presentation are summarized and compared to paediatric reference values in Table 3.2 below. Standard deviations (SD) and 95% confidence intervals (95% CI) are indicated in parentheses in the Table. All values diverge significantly ($p<0.0001$) from the expected values in the normal paediatric population.

TABLE 3.2 – Average haematological indices at the time of diagnosis with FA

Haematological parameter	Average value in FA patient	Reference value	Significance (one sample t test)
Haemoglobin (g/dl) (N=30)	4.77 (SD= 2.05; 95% CI: 4.0 – 5.5)	11.5*	p<0.0001
White cell count (x 10^9/l) (N=30)	2.61 (SD= 1.11; 95% CI: 2.2 – 3.0)	4.0*	p<0.0001
Platelet count (x 10^9/l) (N=30)	23.50 (SD= 13.29; 95% CI: 18.5 - 28.5)	150.0*	p<0.0001
Mean corpuscular volume (fl) (N=26)	106.46 (SD= 7.94; 95% CI: 103.3 -109.7)	95.0**	p<0.0001

* Lower limit reference values for haematological indices in a normal paediatric population as found in Nelson Textbook of Pediatrics (19th ed, 2011).
** Upper limit reference value for mean corpuscular volume in a normal paediatric population as found in Nelson Textbook of Pediatrics (19th ed, 2011).

3.6 TRANSFUSION DEPENDENCE

Transfusion dependent (TFD) patients are those who require frequent and long term transfusion support to maintain life. In practical terms, patients attending the Haematology/Oncology Clinic at the Chris Hani Baragwanath Hospital who require more than five transfusions of blood products per year would be considered TFD (personal communication, Dr R. Wainwright, University of the Witwatersrand, March 2011). Using the above definition, 11 of the 30 patients (36.7%) in the study cohort were TFD at the end of the data collection period (July 2011). The median age of TFD was 7 years, 10 months of age (N=11; Range: 4 years, 8 months – 13 years, 4 months). A comparison between individuals who were TFD and those who were non-TFD in terms of physical and haematological phenotype is detailed in section 3.9.

3.7 CLINICAL FEATURES

3.7.1 Growth measurements

In 15 of the 30 cases (50.0%), the parent or guardian of the affected child was not able to recall their child's birth measurements. In cases where the measurements were known (N=15), the birthweight fell within the normal range (2.5 – 4kg) in 11 cases (73.3%). The remaining four cases (26.7%) had low birthweight (defined as weight between 1.5kg and 2.5kg at term).

The current weight, height and head circumference of each child was plotted on standard growth charts. As is represented in Figure 3.3, most children fell below the 10^{th} centile weight for age (18/30; 60.0%), height for age (22/30; 73.3%) and head circumference for age (23/30; 76.6%). Altogether, 30% (10/30) of patients were found to have both weight and height below the third centile for age. Microcephaly (head circumference less than the 3^{rd} centile for age) was a frequent finding (16/30; 53.3%), even occurring in children whose weight and height for age were within the normal age related range.

The weight for age values in the *FANCG del* patients younger than 10 years of age and the height for age values in all the patients were converted to z-scores (weight for age z-score (WAZ) and height for age z-score (HAZ) respectively) using the WHO Anthro and WHO Anthro-plus anthropometrical calculators. The calculators do not calculate WAZ scores in children older than 10 years. Individuals who are underweight for age have calculated WAZ scores below -2. Similarly an HAZ score less than -2 indicates stunting. The z-scores of the *FANCG del* cohort were compared to WAZ and HAZ scores calculated in a healthy South African paediatric population, as published by Kimani-Murage *et al.* in 2010. These data

indicate that the weight and height for age measurements of the *FANCG del* cohort differ significantly from those of the healthy South African group (Table 3.3).

The WHO Anthro and WHO Anthro-plus calculators were also used to calculate a BMI z-score for each of the *FANCG del* patients. Only two of the 30 (6.7%) patients were found to have BMI z-scores less than -2, indicating that the patients' weight and height measurements were proportionate in most cases (28/30; 93.3%).

A comparative tabulation of the growth measurements in the *FANCG del* cohort with measurements reported in four other FA cohorts is presented in section 3.8.

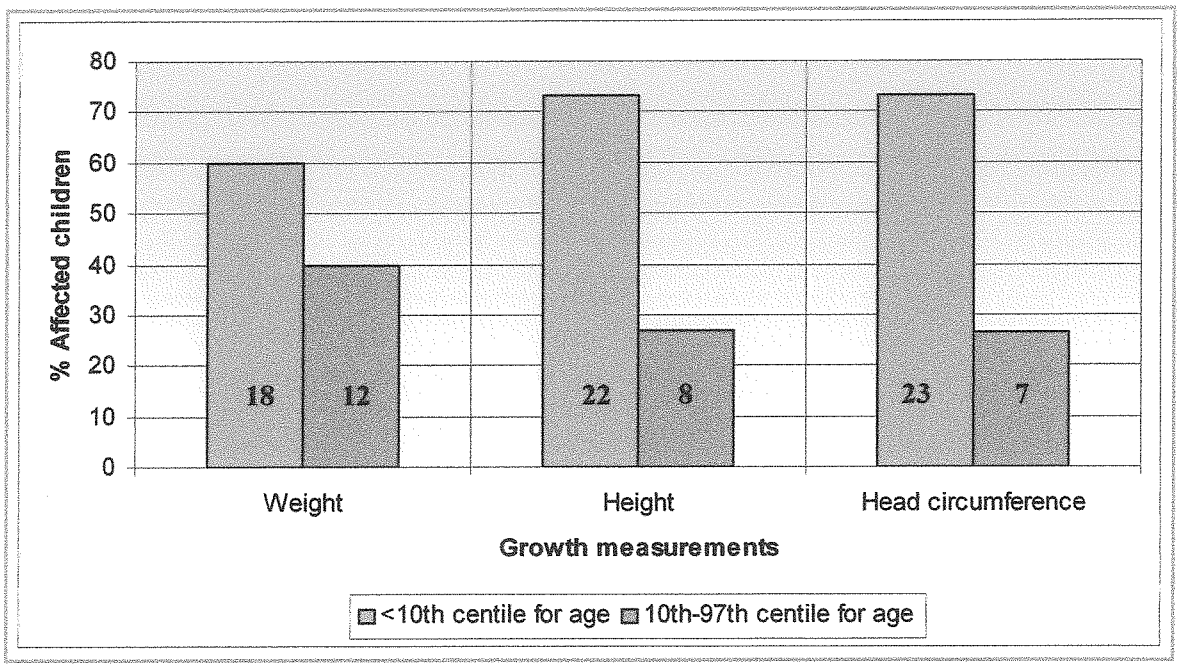


FIGURE 3.3 - Current growth measurements by centile – weight, height and head circumference for age in 30 patients with FA

TABLE 3.3 – Frequency of underweight for age and stunting in FA children and healthy South African children, stratified by age

Weight for age z-score < -2			Height for age z-score <-2					
Age <10 years			Age < 10 years			Age > 10 years		
FA*	Healthy**	p#	FA*	Healthy**	p#	FA*	Healthy**	p#
5/17	118/1641	0.00062	7/17	170/1641	0.0011	8/13	120/1848	0.0001

* *FANCG del* cohort
** Healthy South African cohort – data as published by Kimani-Murage *et al.* (2010)
# p value calculated using the Fisher's exact test. p value considered significant if <0.05.

3.7.2 Central nervous system anomalies

No significant abnormalities of the central nervous system, including neural tube defects, hydrocephalus or congenital structural central nervous system anomalies, were reported by patients or their guardians or were documented in the hospital files. Hyper-reflexia at the knee and ankle joints was noted in 40.0% (12/30) and 33.3% (10/30) of patients respectively.

3.7.3 Eye anomalies

The most frequent eye anomalies present in the research cohort were:

- Up-slanting palpebral fissures (UPF): 24/30 (80.0%)
- Epicanthic folds (EF): 14/30 (46.7%)
- Palpebral fissure length shorter than -2SD of the mean for age (SPF): 13/30 (43.3%)
- Ptosis: 5/30 (16.7%).

These anomalies often occurred in combination:

- All four anomalies: 2/30 (6.7%)
- Three of the four anomalies: 7/30 (23.3%). Of these, the following combinations of anomalies were observed:
    - SPF, UPF and EF: 3/7 (42.9%) (Figure 3.4)
    - SPF, EF and ptosis: 2/7 (28.6%)
    - SPF, UPF and ptosis: 1/7 (14.3%)
    - UPF, EF and ptosis: 1/7 (14.3%)
- Two of the four anomalies: 11/30 (36.7%). Of these, the following combinations of anomalies was observed:
    - SPF and UPF: 6/11 (54.5%)
    - UPF and EF: 4/11 (36.3%)
    - SPF and ptosis: 1/11 (9.1%)
    - The combinations of SPF with EF only, EF with ptosis only and UPF with ptosis only were not observed.

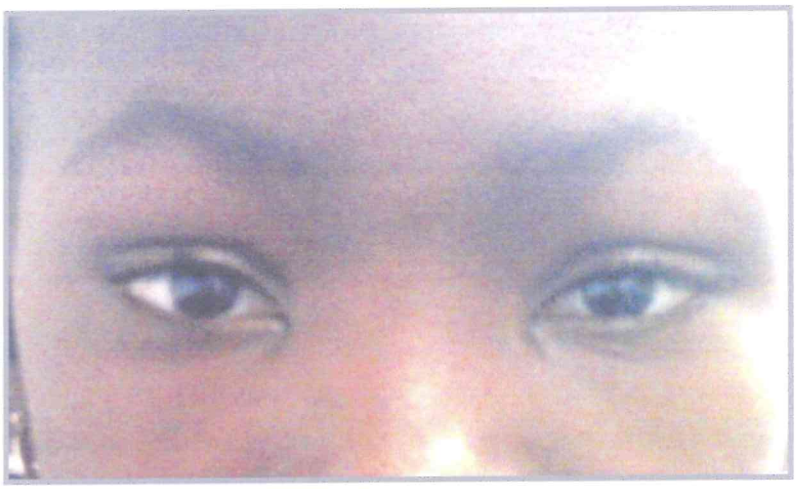


FIGURE 3. 4 – Ocular anomalies in a patient with FA (up-slanting palpebral fissures, epicanthic folds and short palpebral fissure length for age)

One patient was found to have UPF in association with nystagmus. Ten of the 30 (33.3%) patients were noted to have only one clinically observable eye anomaly. None of the 30 patients had current or treated strabismus or cataracts. Most patients (29/30; 96.7%) had inner-canthal distances within the normal range (> (-2SD)).

Although no combination of eye anomalies can be deemed to be specific to the research group, it is worthwhile noting that all of the *FANCG del* patients had at least one anomaly visible on clinical eye examination and that two thirds had more than one.

The frequency of the eye anomalies in the research group was compared to the ocular anomaly frequency figures recently published by Tsilou, Giri, Weinstein *et al.* (2011). The combined frequency of ocular anomalies is not statistically different between the two groups (p=0.4231, Fisher's exact test), although the frequency of individual anomalies differs (Table 3.4). Further comparison of the combined frequency of eye anomalies in the *FANCG del* cohort with frequency figures reported in four other studies is tabulated in section 3.8.

TABLE 3. 4 – Ocular manifestations in two FA cohorts

Ocular abnormality	*FANCG del* Cohort (N=30)	FA Cohort of unspecified genotype* (N=22)	p value ** (Fisher's exact test)
Palpebral fissure length < (-2SD) of the mean for age	13 (43.3%)	18 (81.1%)	0.0093
Epicanthic folds	14 (46.7%)	1 (4.5%)	0.0014
Ptosis	5 (16.7%)	6 (27.3%)	0.3109

*Tsilou, Giri, Weinstein *et al.*, 2010

** Significant p values (<0.05) underlined

3.7.4 Ear anomalies

The most striking ear abnormality in the *FANCG del* patient cohort was short pinna length for age. As depicted in Figure 3.5 below, 80.0% (24/30) of the research patients had ear lengths which measured shorter than -1SD of the mean for age; 36.7% (11/30) had ear lengths shorter than -2SD of the mean for age.

Other ear abnormalities, noted in lower frequencies, are catalogued in Table 3.5. Of these ear anomalies, the most noteworthy is low set ears. The combination of low set ears and short ear length (less than -2SD of mean) was observed in eight of the 30 patients (26.7%). Photographs of two patients with low set ears with overfolded helices are shown below in Figure 3.6 a and b.

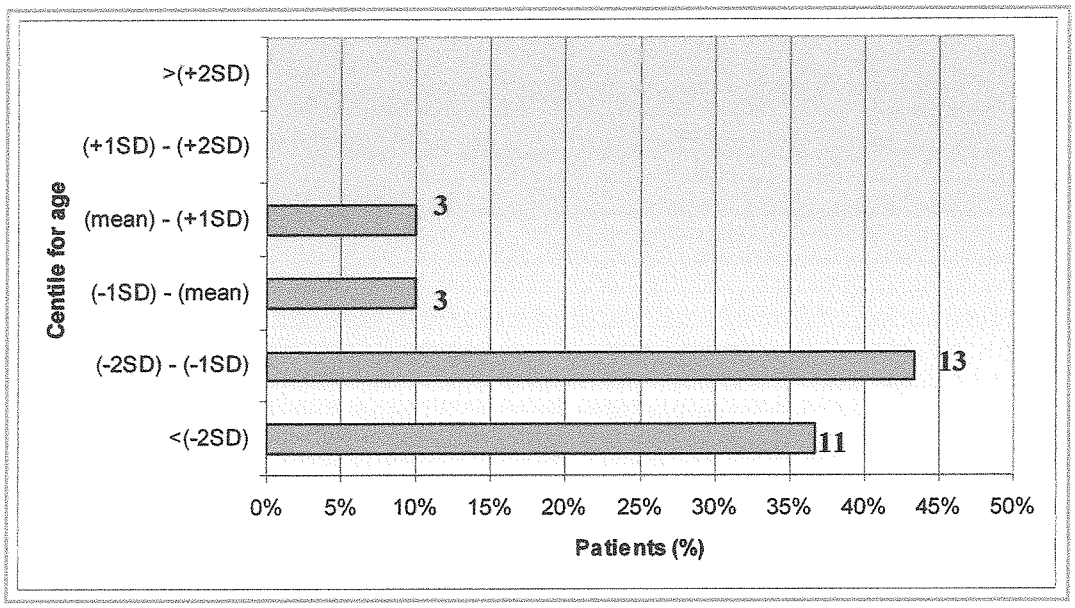


FIGURE 3.5 – Ear length represented by centile for age in 30 patients with FA

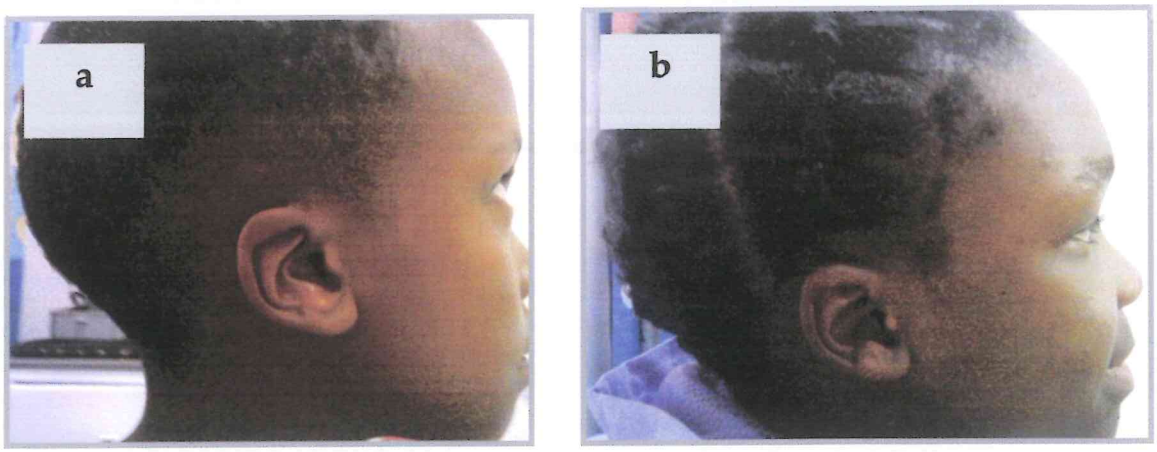


FIGURE 3. 6 (a and b) – Low set ears with overfolded helices in two patients with FA

TABLE 3.5 - Ear anomalies, other than small ear length, observed in 30 patients with FA

Ear anomaly	Number of individuals	Frequency
Low set ears	15	50.0%
Over-folded ear helices	8	26.7%
Pointed helices	2	6.7%

Nine of the 30 (30.0%) patients had undergone formal audiometry assessments; one of whom was diagnosed with hearing loss (1/9: 11.1%). The hearing loss was characterized as left sided, mild sensorineural dysfunction in the ultra high frequency range. No other patients reported subjective hearing problems.

Comparisons of the frequency of ear anomalies in the *FANCG del* cohort with other published figures are tabulated in section 3.8.

3.7.5 Cardiac anomalies

Of the 30 patients included in the present study, 16 had previously been comprehensively assessed by a paediatric cardiologist. Of these 16 patients, only one (6.25%) was shown to have a cardiac abnormality on echocardiography (mild mitral incompetence). The remaining 14 patients were clinically assessed by their treating paediatric oncologist and were re-examined by the researcher during data collection. No structural cardiac abnormalities were suspected in these patients. Section 3.8 tabulates the frequency of congenital cardiac anomalies reported in four other FA cohorts.

3.7.6 Gastrointestinal anomalies

On direct questioning of the patient or their guardian and from the review of the hospital records, three of the 30 patients (10.0%) were found to have gastrointestinal abnormalities. One patient was surgically treated for a tracheo-oesophageal fistula, one had operative repair of an imperforate anus and one had an umbilical hernia (which resolved spontaneously).

3.7.7 Renal anomalies

In total, 29 of the 30 patients (96.7%) included in the present study had undergone renal ultrasound examinations at the time of the researcher's visit to the haematology/oncology units. Of these, 12 (41.4%) were diagnosed with structural renal abnormalities. The renal anomalies detected in the research cohort are detailed in Figure 3.7. The most commonly detected renal anomaly was a pelvic kidney (6/29; 20.7%). A comparison of the frequency of renal and urinary tract anomalies between the *FANCG del* cohort and other FA cohorts is tabulated in section 3.8.

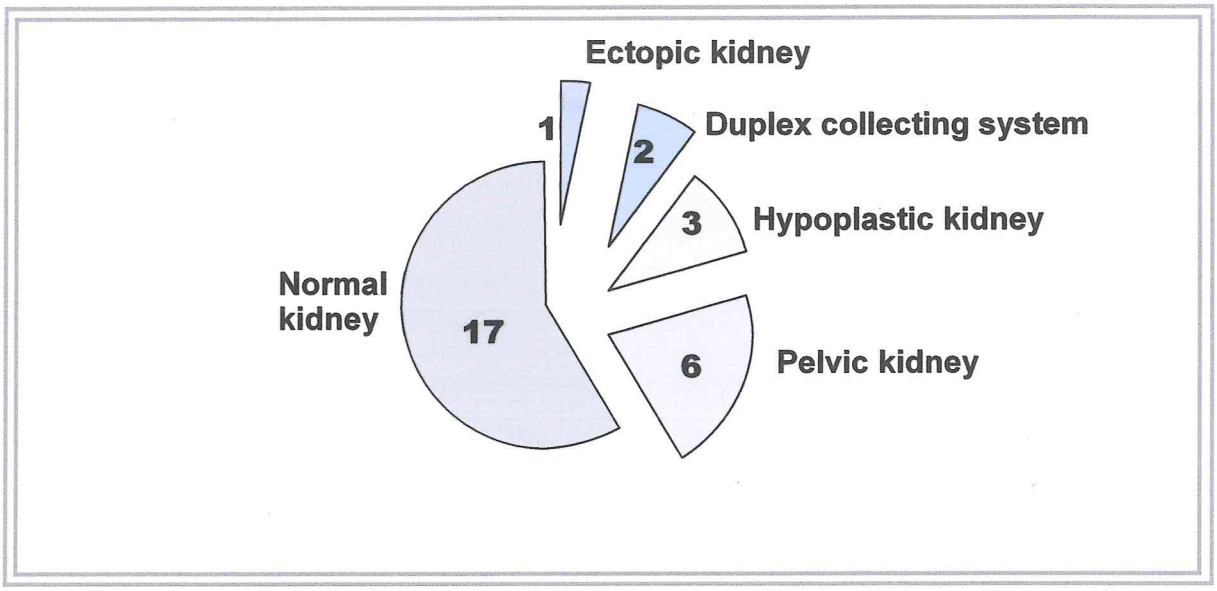


FIGURE 3.7 – Structural renal anomalies detected on ultrasound evaluation in 29 patients with FA

3.7.8 Genital anomalies

A genital examination was performed on all participants who consented to this (N=27). As all participants were on androgen therapy at the time of the examination, the results for the Tanner score were confounded. It was decided not to analyze these data further. No structural genital abnormalities were noted or reported in the research cohort.

3.7.9 Upper limb anomalies

The six most commonly observed upper limb abnormalities and their frequencies are depicted in Figure 3.8. The most common combination of anomalies, occurring in 19 out of 30 *FANCG del* patients (63.3%), was short fifth digits and clinodactyly. Further, although radial ray anomalies were detected in a significant proportion of individuals (combined incidence: 21/30; 70%), these were usually subtle and subjectively determined and may be

difficult to detect on routine clinical examination. Of particular note, none of the research participants were found to have abnormalities of the radius, including hypoplasia or aplasia. Comparison of the combined frequency of thumb and radial ray anomalies in the present *FANCG del* cohort with four other FA cohorts is shown in Section 3.8.

Table 3.6 describes the upper limb anomalies which were observed in a low frequency (less than 5%) in the *FANCG del* cohort. The features described were looked for in the *FANCG del* cohort as they have been reported with variable frequency in other FA groups.

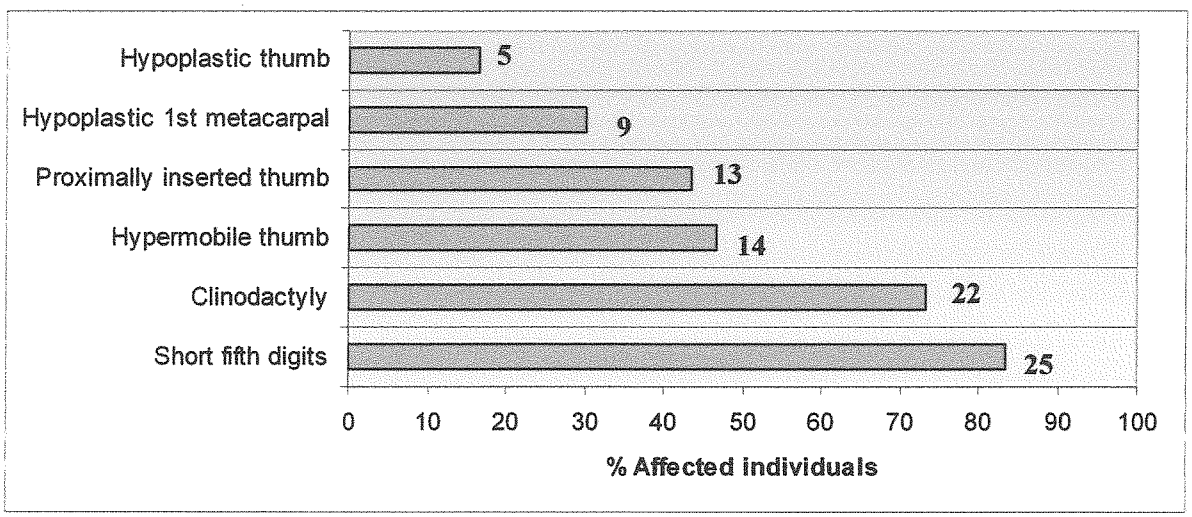


FIGURE 3.8– Most commonly observed upper limb abnormalities in 30 patients with FA

TABLE 3.6 – Upper limb anomalies observed rarely in 30 patients with FA

Upper limb abnormalities previously described in patients with FA globally	Number of *FANCG del* individuals with previously described anomaly (N=30)
Aplastic/hypoplastic ulna	0
Aplastic/hypoplastic radius	0
Polydactyly (unilateral or bilateral)	1 (3.3%)
Absent thumb	0
Stiff thumb	1 (3.3%)
Triphalangeal thumb	0
Transverse palmar crease	0
Undetectable radial pulse (unilateral or bilateral)	1 (3.3%)

3.7.10 Lower limb anomalies

Lower limb anomalies were found to be infrequent in the present *FANCG del* cohort (combined frequency: 2/30; 6.6%). One patient (N=30) was reported to have bilateral developmental dysplasia of the hip (confirmed on pelvic X-ray films) and one had pes planus. Interestingly, 86.7% (26/30) of patients in this cohort were found to have minor 2-3 interdigital webbing, though none had true syndactyly. As interdigital webbing is not a feature which would be noted on routine clinical examination and which is thought anecdotally to occur commonly in many individuals, it was not considered a clinically relevant finding, despite its high frequency.

3.7.11 Vertebral anomalies

Clinically observable spinal anomalies were infrequent in the research cohort (1/30; 3.3%). One patient showed clinically evident scoliosis. Two patients were found to have vertebral anomalies on X-ray (hemivertebrae and ovoid thoracic vertebral bodies). In these patients, the vertebral anomalies were identified coincidentally on posterior-anterior chest X-ray films, performed for other indications and were not suspected on the clinical examination. Although two other patients were thought to have short necks on clinical evaluation, the cervical spine X-rays were normal in both cases.

3.7.12 Pigmentary anomalies

Pigmentary anomalies, such as café au lait macules, hypopigmented and hyperpigmented streaks and macules, were detected on clinical evaluation. Only one of the 30 patients (3.3%) had no clinically identifiable pigmentary anomalies. Although the pattern and distribution of lesions differed between patients (Figure 3.9, Figure 3.10 a and b) most patients (26/30; 86.7%) were noted to have more than one type of pigmentary anomaly (Table 3.7).

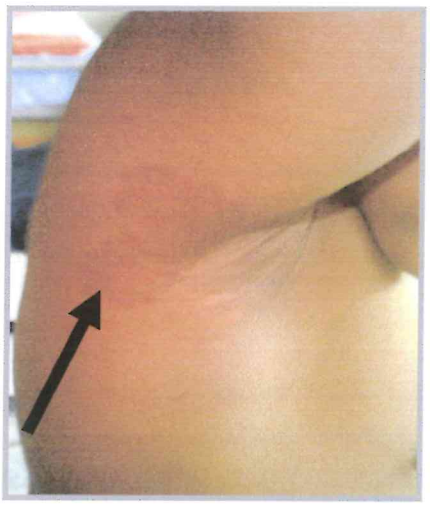


FIGURE 3.9 – Large hyperpigmented axillary macule (arrow) in patient with FA

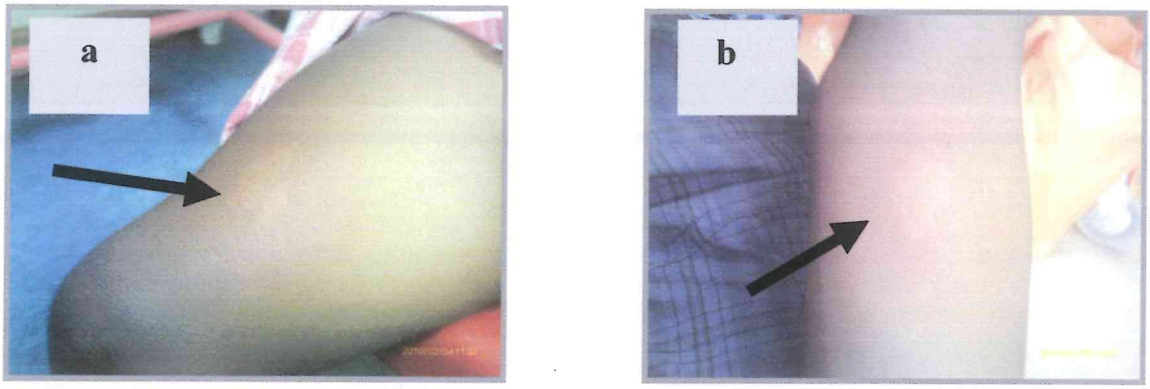


FIGURE 3.10 (a and b) – Hypopigmented macules (arrows) observed on clinical examination of the skin in patients with FA

TABLE 3. 7– Pigmentary anomalies observed in 30 patients with FA

Pigmentary anomalies	Number of *FANCG del* patients	Frequency
No visualized pigmentary anomaly	1	3.3%
Single visualized pigmentary anomaly	3	10.0%
Café au lait macules & hyperpigmented streaks/macules	4	13.3%
Café au lait macules & hypopigmented streaks/macules	4	13.3%
Hypopigmented & hyperpigmented streaks/macules	9	30.0%
Café au lait macules, hypopigmented streaks/macules & hyperpigmented streaks/macules	9	30.0%

Of note, the research cohort was found to have a significantly higher incidence of pigmentary anomalies than other FA cohorts of heterogeneous genotype (tabulated in Section 3.8).

3.7.13 Endocrine anomalies

Consent for venepuncture was obtained from 24 patients. Endocrine dysfunction was detected in four of the 24 patients (16.6%):

- Thyroid dysfunction – two patients were found to have subclinical hypothyroidism and one had overt hypothyroidism (combined frequency: 12.5%).
- Glucose metabolism – one patient was shown to have impaired glucose tolerance (4.2%). This was detected on an oral glucose tolerance test requested by the treating paediatric oncologist and would not have been suspected if only a random glucose sample had been measured (as per the research protocol). The frequency of glucose metabolism abnormalities may also be under-represented, as six of the blood specimens were subjected to storage on ice and transportation, which may have impacted on the chemistry analysis.
- Growth hormone abnormalities – although blood samples were initially taken from patients for random growth hormone levels, it was decided not to include these data in the analysis. The diagnosis of growth hormone deficiency in childhood is a complex process which cannot be made on the basis of a basal serum growth hormone measurement alone. Stimulation tests, although complex to interpret, are likely to be more useful and play a critical role in the diagnosis of growth hormone deficiency (Sizonenko, Clayton, Cohen *et al.,* 2001). It was not appropriate to pursue growth hormone stimulation testing in the research cohort in view of time and cost constraints.

The frequency of thyroid and glucose metabolism dysfunction in the *FANCG del* group was compared to figures reported by Giri *et al.* (2001) in a FA cohort of heterogeneous genotype

(Table 3.8). The significance of this comparison is discussed further in Chapter 4 (section 4.3.2.7).

TABLE 3.8 – Endocrine dysfunction in two groups of patients with FA

Endocrine anomaly	*FANCG del* cohort	Molecularly heterogeneous FA cohort*	Significance# (Fisher's exact test)
Subclinical hypothyroidism (↑ TSH, normal T4)**	2/24 (8.3%)	5/35 (14.0%)	p=0.3970
Overt hypothyroidism (↑ TSH, ↓ T4)**	1/24 (4.2%)	13/35 (37.0%)	p=0.0028
Glucose & insulin metabolism abnormalities***	1/24 (4.2%)	16/41 (39.0%)	p=0.0014

* Based on figures reported by Giri *et al.* (2007). Individuals assessed were assigned to complementation groups A, C, D1, F and J.
** TSH = thyroid stimulating hormone; T4 = thyroxine; ↑ = increased above reference range; ↓ = below minimum reference value.
*** Abnormalities include insulin resistance, glucose intolerance, hyperglycaemia and diabetes mellitus.
# Significant p values ($p<0.05$) are underlined

3.7.14 Developmental assessment

It was not always possible to obtain accurate information on patients' developmental progress. In some cases, the patients were accompanied to the clinic by an older sibling or other family member, such as a grandmother, aunt or uncle. While these individuals took full responsibility for the patient in terms of their medical care and for consent purposes, they were often not accurate historians with regard to developmental milestones and school performance.

The overall perception though, is that children in the *FANCG del* cohort usually have a normal developmental course with age appropriate motor milestones and speech development. Of the patients attending school, most (22/28; 78.6%) were in mainstream schooling and in the appropriate grade for age. School difficulties most often related to non-attendance owing to illness and hospitalization.

3.8 COMPARISON OF PHYSICAL ANOMALIES IN *FANCG DEL* COHORT WITH PHYSICAL ANOMALIES PREVIOUSLY REPORTED IN OTHER FA COHORTS

Despite the wealth of published data detailing the physical anomalies detected in patients with FA, specific anomalies are often not described in detail, but rather grouped into anatomical categories. While the present research report focused on recording physical anomalies more specifically, for comparative purposes the anomalies detected in the *FANCG del* cohort were grouped anatomically. Table 3.9 provides a comparison of the frequency of certain physical anomalies detected in the *FANCG del* cohort with each of four other FA cohorts. The other cohorts were not compared to one another. Absolute numbers and percentage values for each anomaly are provided. Statistical analysis was performed using the Fisher's exact test. Results in blue text are those of the comparison groups which were found to be statistically different from the *FANCG del* cohort ($p<0.05$).

TABLE 3.9 – Comparison of physical anomalies in *FANCG del* cohort with four other FA cohorts

Abnormality	IFAR cohort - heterogeneous genotype [a]	South African Black cohort- unspecified genotype [b]	European FA cohort – complementation group G [c]	FANCG 637-643 deletion mutation in South African Black patients[d]	*FANCG del* cohort [e]
Growth retardation*	139/202 (60.9%)	24/25 (95%)	18/24 (75%)	18/20 (90%)	15/30 (50.0%)
Skin pigmentary anomalies	118/202 (58.4%)	24/25 (95%)	16/24 (66%)	17/20 (85%)	29/30 (96.6%)
Kidney & urogenital anomalies	71/202 (35%)	11/23 (48%)	5/24 (20.8%)	4/20 (20%)	12/29 (41.4%)
Congenital cardiac anomalies	32/202 (15.8%)	2/24 (8%)	1/24 (4.2%)	2/20 (10%)	1/30 (3.3%)
Thumb & radial ray anomalies	103/202 (51%)	18/24 (75%)	13/24 (54%)	17/20 (85%)	21/30 (70%)
Eye anomalies	77/202 (38.1%)	23/25 (92%)	NR**	17/20 (85%)	29/30 (96.6%)
Ear anomalies	44/202 (21.8%)	12/24 (50%)	NR**	NR**	24/30 (80%)

[a] Auerbach *et al.* (1989)
[b] Macdougall *et al.* (1990)
[c] Faivre *et al.* (2000)
[d] Morgan *et al.* (2005)
[e] *FANCG del* cohort – data collected for the present project.
* For the purposes of this table, growth retardation in the *FANCG del* cohort was taken as height or weight below the 3rd centile for age, as definitions for growth retardation were not specified in any of the comparative studies.
** NR = not reported
Blue text: result differs significantly ($p < 0.05$) from *FANCG del* cohort

The *FANCG del* cohort, the Macdougall *et al.* (1994) cohort and the Morgan *et al.* (2005) cohort consisted of Black South African patients with FA. While molecular genetic testing was not available for the Macdougall *et al.* (1994) cohort, it is likely that many of these patients would also have been found to be homozygous for the *FANCG del* mutation, given that the patients were derived from the same ethnic group of Bantu origin as the present *FANCG del* and Morgan *et al.* (2005) cohorts. For the most part, no statistically significant differences were noted between the present *FANCG del* cohort and the above two FA groups, as would be expected given that the patients in each group mostly share the same genotype.

When comparing the *FANCG del* cohort to two international cohorts (IFAR cohort and European cohort), significant differences in the frequency of skin pigmentary anomalies were noted. Although the frequency of eye and ear anomalies in the IFAR cohort was significantly lower than in the *FANCG del* cohort, recently published data supports a higher incidence of ocular anomalies (Tsilou *et al.*, 2010) and otologic anomalies (Shimamura & Alter, 2010), in molecularly heterogeneous FA cohorts, in keeping with the frequency of these anomalies in the *FANCG del* cohort. Thus apart from a higher incidence of pigmentary anomalies, the physical phenotype of the *FANCG del* cohort appears mostly consistent with the phenotype described internationally.

3.9 SCREENING TOOLS TO ASSESS BLACK PATIENTS WITH A POSSIBLE DIAGNOSIS OF FA

Under-recognition of the FA phenotype and poor understanding of the association of FA with congenital abnormalities are the most likely reasons for under diagnosis of FA in South Africa (Macdougall *et al.*, 1994; Morgan *et al.*, 2005). The possibility of developing a

clinical screening tool for assessing individuals with a possible diagnosis of FA, who require further investigations, was evaluated. A screening tool may aid in recognition of the FA phenotype and prompt further investigations to make the diagnosis of FA at an earlier stage. *FANCG del* individuals in the present study were also assessed using the IFAR score (Refer Table 1.2) to evaluate the diagnostic yield of this method.

3.9.1 A clinical screening system based on significant parameters identified in the *FANCG del* cohort

Growth measurements in the *FANCG del* cohort found to differ from expected paediatric reference values and physical anomalies occurring in at least half of the research cohort were identified in an attempt to create a screening tool which could be used in Black patients. Parameters used in the screening tool were based on the findings of the clinical examination only because at primary and secondary care facilities, where the majority of patients with FA initially present, access to specialist equipment and laboratory and radiological services are limited.

The parameters chosen for analysis were:

- Growth measurements: height, weight or head circumference below the third centile for age
- Eye anomalies: any two of the following: SPF, UPF, EF and ptosis
- Ear anomalies: either small ear length for age (less than -2SD of mean) or low set ears (or both)
- Pigmentary skin lesions: more than one of the following: cafe au lait macules, hypopigmented streaks or macules, hyperpigmented streaks or macules

- Hand anomalies: unusual appearing 5th digits (short fifth digits with clinodactyly) or thumbs (any two of hypoplastic thumb, hypoplastic first metacarpal, hypermobile thumb, proximally inserted thumb).

A score of +1 was allocated if a particular parameter was present and a score of 0 if the parameter was absent, giving a total score out of 5.

Using the above method, the average score in the *FANCG del* cohort was 3.5 (SD=1.10; 95% CI: 3.1 – 3.9). Altogether, 26 of the 30 patients (86.7%) with FA had a score of 3 or higher.

3.9.2 The IFAR Score as a screening tool in the South African context

An IFAR score was calculated for each of the patients in the *FANCG del* cohort (refer Table 1.2 (page 15) for the IFAR score). The scores ranged from 0 to 6, with an average score of 3.5 (SD: 1.13, 95% CI: 3.08 – 3.92). Using the IFAR score, the predicted probability of having FA in the research cohort ranged from 92% to 98%. The IFAR score is thus an effective screening tool in the context of FA in Black South African patients.

3.10 PHYSICAL ABNORMALITIES AS PREDICTORS OF HAEMATOLOGICAL OUTCOME

The *FANCG del* cohort was stratified into two groups; those who were TFD and those who were non-TFD at the time of the researcher's visit to the haematology/oncology clinic. Eleven of the 30 patients (36.7%) were TFD and 19 were non-TFD (63.3%). The median current ages of the two groups was not statistically different (9 years, 7 months (Range: 6 years – 16 years, 11 months) in TFD group vs. 8 years, 10 months (Range: 3 years, 6 months – 17 years, 5 months) in non-TFD group, p=0.68 (Wilcoxon two sample test)).

The median age of presentation with haematological abnormalities and bone marrow failure in the TFD group did not differ significantly from the non-TFD group (7 years, 1 month (Range 4 years, 4 months – 11 years) vs. 7 years, 6 months (Range: 3 years – 11 years, 9 months), p=0.6652 (Wilcoxon two sample test). The mean IFAR and *FANCG del* screening tool scores were not significantly different between TFD and non-TFD groups (mean IFAR score: 3.27 in TFD group vs. 3.57 in non-TFD group (p=0.4217, unpaired t-test); mean *FANCG del* tool screening score: 3.73 in TFD group vs. 4.16 in non-TFD group (p=0.4951; unpaired t-test)).

Further, patients were stratified into those children who were TFD before the age of 8 years and those, already older than 8 years of age, who were not TFD. The mean IFAR and *FANCG del* screening tools scores were not significantly different between these groups (Table 3.10). These data suggest that physical anomalies cannot be used to predict haematological outcome in Black South African patients with FA.

The two patients who were shown to have features of MDS on their bone marrow biopsy reports were not TFD at the time of the researcher's visit to the haematology/oncology clinic.

TABLE 3.10 - Comparison of average scores between patients with transfusion dependence before 8 years of age and non-transfusion dependent patients older than 8 years of age using two screening systems

Screening tool	Average score in patients with transfusion dependence before 8 years of age (N=6)	Average score in non - transfusion dependent patients older than 8 years of age (N=11)	p value* (unpaired t-test)
FANCG del screening tool	3.17 (SD=0.408; 95% CI: 2.72- 3.59)	3.45 (SD=1.44; 95% CI: 2.48 – 4.42)	0.6426
IFAR Score	3.83 (SD=0.408; 95% CI: 3.40- 4.26)	3.72 (SD=1.10; 95% CI: 2.98 -4.47)	0.8255

* p values not significant (>0.05)

3.11 SUMMARY

The physical features of the *FANCG del* cohort appear to differ minimally from those previously described in other FA cohorts of heterogeneous genotype, apart from a higher incidence of pigmentary anomalies than has been described in international FA cohorts. From a haematological perspective, individuals homozygous for the *FANCG del* mutation present with severe haematological indices and mostly manifest bone marrow aplasia at the time of presentation with initial symptoms. It does not seem possible to predict haematological disease progression in terms of transfusion dependence and progression to bone marrow aplasia from physical features.

4. DISCUSSION

4.1 INTRODUCTION

FA is an extremely well characterized genetic condition. In South Africa however, the condition is usually still only recognized late in the disease course, at a stage when bone marrow failure is already evident, despite the presence of growth disturbances and congenital malformations in many affected individuals. Under-recognition of the FA phenotype and poor understanding of the phenotypic spectrum of the condition in South Africa are evidenced by the large discrepancy between the expected and observed number of FA cases referred to tertiary haematology/oncology centres in South Africa (Morgan *et al.,* 2005). In this chapter, the physical and haematological phenotype of the research cohort will be summarized and compared to previously published data. Specific focus is placed on how the phenotypic features may be used to facilitate the diagnosis of FA at an earlier stage. The use of screening tools and scoring systems to aid in diagnosing FA will also be addressed.

4.2 SUMMARY OF THE AIMS OF THE PROJECT

Black patients with FA in South Africa are an ideal cohort to assess from a genotype-phenotype perspective as 82% of them carry a common causative deleterious mutation (*FANCG del*) (Morgan *et al.*, 2005) This is a unique situation compared to the marked genetic heterogeneity observed in patients with FA internationally. For the purposes of the present research report it was postulated that if a more precise physical phenotype could be identified, the diagnosis of FA in Black patients could be made more readily with expedient referral to haematology/oncology centres for management to be instituted. The possibility of developing a clinically based screening tool to identify individuals requiring further

investigations and testing for FA was evaluated. It was further postulated that physical phenotypic parameters may be predictive of haematological disease progression in the *FANCG del* cohort. Based on the proposed identifiable phenotype, it was hypothesized that management decisions could be tailored to address the most pertinent complications in individuals found to be homozygous for the *FANCG del* mutation.

4.3 GENOTYPE-PHENOTYPE CORRELATION IN *FANCG DEL* PATIENTS

A comprehensive clinical examination and file review of 30 Black patients with FA allowed a genotype-phenotype correlation, both physical and haematological, to be assessed in detail for individuals with FA, homozygous for *FANCG del.*

4.3.1 Haematological phenotype in *FANCG del* homozygotes

Despite well documented and clinically identifiable physical anomalies in children with FA, aplastic anaemia remains the primary presenting feature for children with FA, usually in the first decade of life (Green & Kupfer, 2009). All individuals in the *FANCG del* research cohort were referred to haematology/oncology clinics already manifesting symptoms of bone marrow disease.

In the *FANCG del* cohort, the median age of presentation with symptoms of FA, usually concurrent with the diagnosis of bone marrow aplasia was 7 years, 1 month of age, consistent with published data for other individuals with FA with the same genotype and with other individuals of heterogeneous genotypes. Morgan *et al.* (2005), found a mean age of diagnosis of 6 years, 6 months of age, in their cohort of 20 South African patients known to be homozygous for the *FANCG del* mutation. The median age of detection of haematologic

abnormalities in the IFAR group (molecularly heterogeneous FA cohort) was reported as seven years (Butturini *et al.*, 1994) and the age of onset of haematological abnormalities in South African patients with FA of unspecified genotype was reported to be 6-7 years (Macdougall *et al.*, 1994). One expects that many of the children in the Macdougall *et al.* (1994) cohort would subsequently have been shown to be homozygous for the *FANCG del* mutation on molecular analysis.

The incidence of MDS and the conversion rates to AML and ALL in the present *FANCG del* group were consistent with frequency figures in patients with FA assigned to complementation group G, as published by Faivre *et al.* (2000). The authors suggested a significantly higher AML/MDS frequency at 10 years of age (6/18; 33%) in *FANCG* individuals compared with individuals with *FANCA* and *FANCC* mutations (Faivre *et al.*, 2000). Thirteen of the patients in the present research group were 10 years of age or older and two of them had features of MDS on their bone marrow biopsy reports (15.4%). There is no significant difference in the incidence of AML/MDS in the present research group to that of the Faivre *et al.* (2000) cohort (p=0.4395, Fisher's exact test). This suggests that individuals with the *FANCG del* genotype may be at similar risk of developing MDS at an earlier age to individuals with FA assigned to other complementation groups.

Faivre *et al.* (2000) also evaluated presenting haematological indices across different FA complementation groups in terms of their severity. They allocated a score of one point for each of three defined severe haematological parameters (Hb of less than or equal to 8g/dl, an absolute neutrophil count of less than or equal to 0.5 x 10^9/l and plt of less than or equal to 20 x 10^9/l), giving each patient in their cohort a score out of three. *FANCG* individuals were found to have a significantly higher scores out of three (mean score: 2.11) than individuals in

complementation groups A and C. The same score could not be calculated for individuals in the present *FANCG del* cohort as absolute neutrophil counts were not recorded. However, using only the haemoglobin and platelet values, 46.7% of the *FANCG del* patients were noted to have levels below those used to define severe cytopaenia. Although the *FANCG del* and Faivre *et al.* (2000) cohorts cannot be compared directly, the low presenting haemoglobin and platelet counts in the *FANCG del* group may indicate that these individuals also present with more severe haematological indices than other patients with FA. The severe haematological indices do not appear to predict an earlier age of bone marrow aplasia however.

None of the present *FANCG del* patients were known or suspected to have developed any solid tumours or non-haematological malignancies. Anecdotally, one Black patient with FA, homozygous for the *FANCG del* mutation, although not a participant in the present project (defaulted from treatment at the Chris Hani Baragwanath Hospital) is suspected to have developed a cervical squamous cell malignancy. This patient is also infected with the human immunodeficiency virus, making it difficult to attribute the malignancy to her FA (personal communication, Dr R. Wainwright, University of the Witwatersrand, March 2011). Apart from this single case, little is know about the progression of Black individuals with FA in terms of non-haematological malignancy and solid tumours.

The most common presenting symptom in the present *FANCG del* cohort was epistaxis, similar to the major presenting complaint documented by Macdougall *et al.* (1990) in a Black African FA cohort. This haemorrhagic symptom relates directly to the pathophysiology of the condition, with thromobocytopaenia often being the first identifiable haematological disturbance, apart from macrocytosis (Auerbach, 2009).

From a diagnostic perspective, the history of epistaxis may provide a useful investigative handle for FA. Promoting awareness around recurrent epistaxis in children and a review of the current investigative strategies for children presenting with same, may result in more expedient referral of affected children to tertiary centres. While the differential diagnosis for epistaxis is large and includes many acquired causes (Haddad, 2011), recurrent epistaxis, particularly in a child with growth restriction or other physical anomalies warrants further evaluation.

4.3.2 Physical phenotype in *FANCG del* homozygotes

Apart from growth disturbances and pigmentary anomalies (discussed below), other physical phenotypic characteristics of the *FANCG del* cohort, including anomalies of the eyes, ears and upper limbs are thought to be subtle and possible to overlook in a general clinical context if not specifically examined for. However, if noted, these features are important clinical markers in considering the diagnosis of FA.

4.3.2.1 Growth measurements

FANCG del homozygotes in the present study were generally found to have low weight, height and head circumference for age. More than one third of *FANCG del* children had weight measurements below the third centile for age and almost half had height measurements below the third centile for age. By comparing weight for age and height for age z-scores in the *FANCG del* cohort with z-scores in a large South African paediatric cohort (Kimani-Murage *et al.,* 2010), a significant degree of underweight for age and stunting in the *FANCG del* research group was found. Furthermore, many of the patients whose growth measurements plotted within the normal range on the growth chart were nevertheless found to have weight, height or both measurements less than the 10^{th} centile for

age. The onset of underweight for age in the research cohort appears to be post-natal in most cases, as the documented birthweights were within the normal range in most cases.

The height for age data in the research cohort may be confounded by androgen therapy (of variable duration) in all the participants. A documented side effect of androgen therapy is reduced final height (Dufour & Svahn, 2008), and as such the short stature observed in the research cohort may in part be related to androgen administration. Given the previously reported high incidence of short stature in individuals with FA of heterogeneous genotype (quoted as 51% by Taniguchi, 2008), the frequency of stunting in the *FANCG del* cohort is still thought to be a significant feature of the condition and a measurement which could be used as part of a screening tool to detect individuals requiring further investigation for FA.

Measurements of child growth should be standard practice in any clinical setting (Westwood *et al.*, 2007). Improved detection of children whose growth measurements fall below the 3rd centile for age is a critical component in the diagnosis of FA. All health care practitioners should be encouraged to measure growth components and plot all children attending clinics for any indication. It is also important to highlight the value of a thorough physical examination in children who are found to have growth restriction, in order to identify the underlying cause for the poor growth. In this way, other physical features which may suggest the diagnosis of FA would be more readily detected.

4.3.2.2 Facial features

While eye and ear anomalies are widely documented in patients with FA (Taniguchi, 2008; Shimamura & Alter, 2010), specific descriptions of the "facial phenotype" defining more specifically the eye and ear conformations and the facial gestalt are not readily available.

In the *FANCG del* cohort, the eye phenotype can be described as an association of two or more of the following features: SPF, EF, UPF and ptosis. The combination of any two of these features was observed in two thirds of the research cohort. The frequency of eye anomalies in the *FANCG del* cohort would appear to be much higher than internationally reported figures which are between 20% and 23% (Taniguchi, 2008; Shimamura & Alter, 2010). However, they are in keeping with recently published data, which suggest a higher frequency of eye anomalies in patients with FA, with 95% in one series reported to have at least one abnormal ocular parameter (Tsilou *et al.*, 2010). While the total frequency of ocular anomalies in the Tsilou *et al.* (2010) series is not statistically different from that in the *FANCG del* cohort, the frequency of specific anomalies differs between the two groups (higher incidence of EF and lower incidence of SPF in the *FANCG del* cohort compared to the Tsilou *et al.* (2010) cohort) . This discrepancy in individual ocular anomalies may be a reflection of the different ethnicity of the two groups, especially as epicanthic folds are thought to be a frequent ethnic variation in Black individuals.

The ear phenotype in the *FANCG del* cohort takes the form of ears which are either small, low set or both. The combined frequency of structural and functional ear abnormalities in the *FANCG del* research cohort was 80%. Shimamura and Alter (2010) quote a 75% frequency of hearing loss and structural otologic anomalies in patients with FA of heterogeneous genotype, which would appear to be in keeping with the frequency noted in the present research cohort and much higher than previously reported frequencies of ear anomalies which approximate 9% (Taniguchi, 2008). Given the high incidence of structural ear anomalies in the research group, it follows that it would be important to screen for functional

abnormalities such as hearing loss. It may be important to recommend formal audiometric assessments in patients with FA attending haematology/oncology centres in South Africa.

It should also be noted, that while many patients were found to have short palpebral fissure length for age and short pinna length for age, when adjusted for head size in those who were microcephalic, these measurements were within the normal range. Bearing this in mind, the facial gestalt, while recognizable to those who have examined many children with FA, is mostly subtle and is easily overlooked in the general clinic setting.

4.3.2.3 Skeletal abnormalities

Upper limb, lower limb and vertebral anomalies were noted and described in the research cohort. While radial ray anomalies are considered more common in patients with FA (Shimamura & Alter, 2010) and form part of the IFAR scoring system, ulnar ray anomalies of the upper limbs were found to occur more commonly in the *FANCG del* research cohort. In Black patients, therefore, radial or ulnar ray anomalies manifesting as unusual thumbs or fifth digits respectively, should be equally weighted when considering the diagnosis of FA. Similarly in a Black child with ulnar ray anomalies in the absence of observable radial ray anomalies, the diagnosis of FA should still be considered in the face of other suggestive clinical features. Of particular importance is the observation that none of the research patients were found to have an abnormality of the radius. In the South African context, aplasia or hypoplasia of the radius cannot be used as a defining feature of FA when considering the diagnosis in a Black child.

Excluding minor cutaneous 2-3 interdigital webbing, lower limb anomalies appear to be infrequent in the present *FANCG del* cohort in keeping with previously reported frequency figures in FA cohorts of heterogeneous genotypes (Taniguchi, 2008; Shimamura & Alter,

2010). Vertebral anomalies also appear to be uncommon in the research cohort. However, the small number of vertebral anomalies detected in the present study may be due to under-ascertainment, as X-rays of the spine were not performed on patients unless a vertebral anomaly was clinically suspected. From a diagnostic perspective, no particular physical anomalies of the lower limbs or vertebrae were noted to be of value in making the diagnosis of FA in Black children.

4.3.2.4 Skin pigmentation anomalies

Skin pigmentary anomalies were found to be a defining clinical feature in the *FANCG del* cohort, with 96.7% of patients noted to have at least one clearly visible pigmentary lesion. While not a discriminating feature of FA, café au lait macules and hypo- and hyperpigmented streaks and macules must raise the suspicion of FA (in the context of the differential diagnosis of pigmentary anomalies), particularly in a child with growth restriction. Of note, the incidence of pigmentary anomalies in the research group was significantly higher than has been reported internationally (Auerbach *et al.*, 1989; Faivre *et al.*, 2000). This may in part be due to the ease with which pigmentary anomalies are noted in darker skin types.

4.3.2.5 Renal malformations

Current standard practice in tertiary haematology/oncology units managing children with FA includes a renal ultrasound investigation in all patients. Based on the high frequency of structural renal anomalies in the *FANCG del* cohort (41.4%, compared to previously published figures of 21% by Taniguchi, 2008), continued renal screening of all Black patients diagnosed with FA is recommended. Further, a renal ultrasound evaluation may prove to be a useful supportive investigation in children found to have one or more other physical features identified on clinical examination, which suggest a diagnosis of FA.

4.3.2.6 Central nervous system, cardiac, gastrointestinal and genital malformations

Apart from renal anomalies, gastrointestinal malformations were the most frequently noted organ malformations in the *FANCG del* cohort. The anomalies detected in the *FANCG del* group (tracheo-oesophageal fistula, imperforate anus, umbilical hernia) have all previously been reported in other patients with FA (Giampietro, Adler-Brecher, Verlander *et al.*, 1993; Taniguchi, 2008). The diagnosis of these anomalies in individuals later diagnosed with FA serves to document further the spectrum of malformations associated with *FANCG del* and to reinforce the importance of a comprehensive physical examination in any child diagnosed with a major malformation, to assess for minor dysmorphic features and other malformations.

Congenital cardiac anomalies were infrequent in the research cohort. As such, in a resource restricted setting, formal echocardiographic evaluations may not be necessary in all FA patients and could be reserved for individuals with cardiac murmurs or features of congestive cardiac failure. In the case of a congenital cardiac anomaly being identified in a child not known to have FA, a full clinical examination is warranted, to document other malformations and physical anomalies. Cardiac malformations have been reported in the FA spectrum of congenital anomalies (Taniguchi, 2008, Shimamura & Alter, 2010).

4.3.2.7 Endocrine evaluation

The endocrine evaluation in the *FANCG del* cohort was not comprehensive. When compared to data published by Giri *et al.* (2007), it would appear that individuals homozygous for *FANCG del* have a significantly lower incidence of overt hypothyroidism and glucose metabolism abnormalities than other individuals with FA of heterogeneous genotype. However, the significance of this comparison may not hold true, given the differences in study design and methodology used. In the Giri *et al.* (2007) study, glucose metabolism

abnormalities were investigated using oral glucose tolerance tests and insulin homeostatic model assessments. The range of abnormalities detectable with these testing methods would be greater that those detected by random glucose measurements as were performed in the present study. Although these data may indicate that individuals with *FANCG del* are at a lower risk for endocrine dysfunction, they more likely reflect the need for improved investigation of South African *FANCG del* patients in terms of their endocrine function. In a small cohort (N=5) of patients with FA assigned to complementation group G in the United States, Wajnrajch, Gertner, Huma *et al.* (2001), found significantly low T4 levels and a high degree of insulin resistance. Although the sample size was small, these data may indicate a trend towards endocrine dysfunction in individuals assigned to complementation group G, possibly including *FANCG del* patients. Further evaluation of the endocrine status of Black patients with FA is warranted.

4.3.2.8 Developmental progression

Given the superficial nature of the developmental assessment performed, no firm conclusions could be drawn regarding the developmental progression of the patients in the research cohort. While it would appear that most of the children with FA function at a developmentally appropriate level for age, subtle learning difficulties, developmental dyspraxias and borderline normal intelligence quotient (IQ) levels could not be detected with the screening assessment used for the purposes of the research. The developmental assessment was also complicated and confounded by poor school attendance owing to repeated hospitalization and by reduced school performance on the basis of fatigue secondary to anaemia in the majority of the patients.

The finding of a relatively normal developmental course in the research cohort is at odds with data published by Macdougall *et al.* (1990) and Macdougall *et al.* (1994) which quote frequency figures for learning difficulties and mental retardation of 44% and 28% respectively in two cohorts of Black South African patients with FA. The difference in the frequency of learning disability between these groups and the research cohort is not readily apparent, although it may reflect the lack of schooling and poor social circumstances of Black South African children at the time of data collection in the two earlier studies. In contrast, learning difficulties form part of the IFAR score and if present act against a diagnosis of FA, suggesting that intellectual disability is uncommon in patients with FA of heterogeneous genotype (Auerbach *et al.*, 1989).

For clarification on the issue of learning disability in Black patients with FA, formal psychometric testing in the *FANCG del* cohort would be necessary to confirm if learning difficulties are truly uncommon in the research group.

4.4 SCREENING TOOLS WHEN CONSIDERING THE DIAGNOSIS OF FA IN BLACK PATIENTS IN SOUTH AFRICA

A screening tool which relies on a clinical examination and readily available equipment and growth charts to assess Black patients suspected of having FA would be a useful tool, especially in resource restricted settings. While not a validated screening method, the *FANCG del* screening tool (detailed in Chapter 3.8) may prove to be an easy means of identifying possible patients with FA. Black children who score 3 or more out of 5 could be referred to tertiary centres for further pediatric or medical genetic evaluation. Investigations

could then be performed to detect other features which support a diagnosis of FA, such as a thromobocytopaenia, a raised MCV or renal anomalies.

In order to validate this system as an effective screening tool in FA, a control group of unaffected, healthy Black children would need to be examined and scored - their scores would need to significantly differ from those of the research cohort. The screening system would also need to be assessed in terms of its specificity for FA and its ability to differentiate FA from other genetic conditions associated with dysmorphic features and poor growth.

In the absence of validation of the screening method described above, the IFAR score remains a valuable tool and its use as a screening method should be promoted when considering the diagnosis of FA. From a practical perspective, the score is easy to administer and calculate, although from a cost and resource perspective performing the full score may be beyond the reach of many South African clinics which do not have access to radiology and laboratory services.

Clearly, in order for either of these scoring systems to be effective, individuals performing the score would need to be trained to recognize the component anomalies of each score. This may prove to be the biggest limitation in the utilization of such systems, particularly in primary health care facilities. However in secondary and tertiary facilities, appropriate staff training may be possible and the cost effectiveness of such an approach could be further evaluated.

4.5 PHYSICAL ABNORMALITIES AS PREDICTORS OF HAEMATOLOGICAL OUTCOME

A comparison between TFD and non-TFD patients within the research cohort found no significant differences in median age of presentation with symptoms suggestive of FA or average scores on the *FANCG del* screening tool and IFAR scoring system. Using both of these scoring systems, no significant differences were found between a subset of patients who were TFD before the age of 8 years and those, already older than 8 years of age, who were not yet TFD. These data would suggest that physical anomalies cannot be used as predictors of haematological outcome in *FANCG del* individuals, nor can prognosis be determined by the presence or absence of congenital malformations.

Rosenberg *et al.* (2004) showed that the presence of radius abnormalities was predictive of bone marrow failure in their cohort of individuals with FA of heterogeneous genotype. Interestingly, abnormalities of the radius were not found in the research group despite the poor haematological outcome, again suggesting that physical features cannot be used reliably to predict prognosis.

4.6 SPECTRUM OF PHENOTYPIC VARIABILITY IN FA - IMPACT ON DIAGNOSIS?

Many reasons for the under-recognition and under diagnosis of FA can be postulated, although the phenotypic variability and poor understanding thereof most likely play an important role.

In South Africa, affected individuals only present or are referred to haematology/oncology centres late in their disease course, most often at a stage where bone marrow aplasia is already evident. This suggests that despite congenital malformations and minor dysmorphic features, the diagnosis of FA was not considered prior to the onset of bone marrow disease. Data published by Giampietro *et al.* (1993) in the United States of America, support the notion of a poor general understanding of the FA phenotype. In their study, the researchers showed that despite congenital malformations being detected in two thirds of patients of unspecified genotype, the diagnosis of FA was made before the onset of haematologic manifestations in only 28% of cases. In the present *FANCG del* cohort, none of the patients were identified on the basis of their congenital anomalies alone. Poor understanding of the association of FA with multiple congenital malformations and minor dysmorphic features may be one of the factors hampering the diagnosis of FA in South Africa.

Under-recognition of the severe FA phenotype in South Africa may be another of the factors which account for the divide between the number of expected and observed cases in our health care system, with many patients dying at a very early age from congenital anomalies or severe bone marrow disease. Anecdotally, reports (unpublished) from Professor G. De Jong, a medical geneticist in Cape Town, South Africa, describe an intrauterine fetal death diagnosed with multiple congenital abnormalities, including bifid thumbs. Molecular genetic testing showed this fetus to be homozygous for the *FANCG del* mutation, suggesting a severe perinatal lethal end of the phenotypic spectrum (personal communication, Professor G. De Jong, University of Stellenbosch, March 2011). A case report published in 2001 by Tercanli, Miny, Siebert *et al.,* details a fetus with increased nuchal translucency on antenatal ultrasound at 12 weeks gestation, later diagnosed with FA after multiple other congenital abnormalities were detected. After birth, this baby developed severe postnatal anaemia and

an embryonal tumour, resulting in death at five months of age. These cases illustrate the severe end of the FA spectrum of congenital anomalies.

Faivre, Portnoi, Pals *et al.* (2005) evaluated the relationship between FA and the VACTERL (vertebral defects, anal atresia, tracheo-oesophageal fistula, renal dysplasia and limb defects) association, emphasizing the overlap in phenotype between the two conditions and the possibility that the diagnosis of FA may be missed in children with multiple congenital anomalies. The authors found that 5% of patients with FA fulfilled criteria for diagnosis of VACTERL association and recommended that FA testing be undertaken in cases of VACTERL association when radial ray anomalies are present or if other features such as pigmentation, growth retardation, microcephaly or dysmorphism are noted. Interestingly, in South Africa, anecdotal evidence suggests that FA is uncommon in cases identified with the VACTERL association (personal communication, Professor A. Krause, University of the Witwatersrand, July 2011). Given the subtle limb anomalies and absence of radial aplasia in the *FANCG del* cohort, it is possible that Black patients with FA do not meet the diagnostic criteria for the VACTERL association. Further research into intrauterine deaths and neonates with multiple congenital anomalies may help to elucidate the severe end of the FA spectrum in Black South African patients.

The mild end of the FA spectrum is also yet to be fully elaborated. In cases where no congenital anomalies are present or where haematological manifestations develop later in life, the diagnosis of FA may never be considered.

4.7 SUMMARY

The phenotype of the present *FANCG del* cohort is not appreciably different from individuals with FA of heterogeneous genotype. However, judicious attention to children presenting to South African clinics with recurrent epistaxis, growth restriction, multiple cutaneous pigmentary anomalies or unusual appearing eyes, ears or hands, may lead to improved referral of these individuals to tertiary centres for further investigation. In this way, while the specific diagnosis of FA may not be suspected, investigations as to the cause of the identified anomalies or unusual features, may lead to an improved diagnostic yield for FA.

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5. CONCLUSION

5.1 INTRODUCTION

The final chapter addresses the recommendations for future research in Black South African patients with FA and elaborates on the limitations of the current research project. Concluding comments on the importance and future use of the information gained in the present project are also made.

5.2 RECOMMENDATIONS FOR FUTURE RESEARCH

In order to improve the diagnosis of FA in the South African Black population, validation of the screening tool detailed in Chapter 3 and Chapter 4 may be important. For validation to be achieved, a sample of healthy Black South African children would need to be examined and scored according to the screening tool. If the scores of the control group and those of the *FANCG del* cohort differ significantly, the screening tool may become a useful system in identifying individuals who warrant further invasive testing or investigations on the basis of their clinical features. Further, a modified version of the IFAR score may need to be considered as this method has already been validated for assessing the likelihood of FA in individuals with certain physical and haematological abnormalities. The feasibility of examining a cohort of healthy South African Black children, as an appropriate control group and for validation of the screening system developed in the present project, is being assessed as part of an ongoing Departmental FA research study.

The endocrine status of the children with FA being managed in haematology/oncology clinics in South Africa remains unclear, despite the testing done for the present research project. Given the previously reported high frequency of hypothyroidism and glucose

metabolism abnormalities in individuals assigned to complementation group G, further research into the endocrine profile of *FANCG del* individuals is recommended. Improved recognition of endocrine dysfunction may lead to improved management and better quality of life for the subset of patients with endocrine abnormalities.

An audit of the absolute neutrophil count in individuals with FA would allow a severe cytopaenia score (as described by Faivre *et al.*, 2000) to be calculated to determine if *FANCG del* individuals do have more severe cytopaenia at the time of presentation with symptoms suggestive of FA than individuals with FA who are assigned to other (non- G) complementation groups.

A project is currently underway in the Division of Human Genetics at the NHLS to describe the phenotype in Afrikaans patients with founder mutations in the *FANCA* gene and then to compare this phenotype with that described in the *FANCG del* research cohort.

5.3 LIMITATIONS OF THE PRESENT STUDY

The *FANCG del* cohort comprised 30 individuals, which is a relatively small sample size. However, given the rarity of FA, a sample size of 30 was deemed adequate for data collection and in view of the central limit theorem, was thought to be sufficient for statistical analysis.

Sample ascertainment was biased as only patients who were attending haematology/oncology departments were considered eligible for the study. It is possible that the patients who were evaluated, may fall within the more severe spectrum of the condition and may thus be more likely to present with certain phenotypic characteristics. Similarly, patients with very severe

disease or early onset bone marrow failure may die before referral to haematology/oncology centres and would thus have been missed. Unfortunately, this limitation could not be overcome, as FA is not being recognized or managed in most primary and secondary level care facilities. A community wide survey of children attending these services and other clinics to evaluate for phenotypic variation, was beyond the scope of this project, given the time and cost implications of such a survey.

A control group was not used in this project, owing to resource and time constraints. As mentioned above, the practicability of examining a control group of healthy children, unaffected by any genetic conditions, is currently being evaluated.

5.4 CONCLUDING COMMENTS

FA in the Black population in South Africa is caused by a founder mutation in the Fanconi G gene in the majority of cases. While a genotype-specific phenotype was postulated in individuals homozygous for the *FANCG del* mutation, it has been shown that for the most part, the physical phenotype in the research cohort is not appreciably different from other individuals with FA irrespective of genotype.

Certain physical anomalies, including disturbances of growth and pigmentary lesions, occur commonly in affected Black individuals and should be readily detected on routine clinical examination. Other characteristics however, such as eye, ear and hand anomalies, are subtle and would most likely be overlooked in an overburdened primary and secondary health care setting. As such, it is difficult to propose guidelines to assist health care practitioners in identifying the FA phenotype more readily. While a screening tool to detect individuals requiring further investigations and evaluation for FA has been proposed, its validity,

sensitivity and specificity needs to be tested in healthy Black children and the challenges with regard to implementation of such a system need to be considered. The IFAR score may be a useful tool for the diagnosis of FA, although given the poor access to clinical, radiological and laboratory services in many areas of South Africa, a modified score may need to be considered.

Based on the current research project, the presence of physical anomalies in Black patients with FA, homozygous for the founder mutation, appears not to be useful in predicting the severity of the haematological course in these individuals. In most cases, those homozygous for the *FANCG del* mutation would be expected to present with severely abnormal haematological indices and to progress to bone marrow aplasia by the age of seven years.

Certain management practices may be influenced by the *FANCG del* phenotype described. All Black individuals with FA should have a renal ultrasound examination as the incidence of structural renal anomalies is high in those homozygous for the *FANCG del* mutation. Formal echocardiographic examinations may be omitted in affected individuals in resource restricted settings as the incidence of congenital cardiac anomalies in the research cohort was low. Further, investigation into the presence of hearing loss in Black patients with FA would be important given the significant incidence of functional otologic anomalies proposed by other research studies.

FA in South Africa is likely to remain an under-recognized and under-diagnosed condition as the full phenotypic spectrum is still to be elucidated. Further, as the physical phenotype is subtle, educating health care practitioners in its recognition remains challenging, particularly

given the vast infectious and non-genetic health burdens in the public health care sector which take priority in terms of care.

APPENDIX A – Consent Documentation

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Information & Consent Document

Title: **Genotype-phenotype correlation in South African Black and Afrikaans individuals with Fanconi anaemia.**

Investigator: **Dr Candice Feben**
Department of Human Genetics, NHLS, Johannesburg, SA

Good day,

My name is Dr Candice Feben. I am currently specializing in Medical Genetics through the Human Genetics Department of the National Health Laboratory Services. As part of my degree, I am required to complete a research project in fulfillment of an MMED qualification.

I would like to invite you/your child 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. If you decide to participate you will be given a copy of this document to take home with you. If you would like to discuss this document with your family, before deciding to participate, you are welcome to do so.

What is the aim of the study?
I have decided to conduct my research on patients diagnosed with Fanconi anaemia. The aim of the study is to characterize specific clinical features in South African Fanconi anaemia patients. It is envisaged that should a phenotype (clinical picture) be identified, this may be used to aid in diagnosis and early referral of affected patients in the future.

Am I obliged to participate in this study?
Participation in this study is entirely optional and will in no way change the treatment you/your child is/are receiving from the hospital. Participation in the study will not be detrimental to your/your child's health in any way. Similarly, your/your child's management at the hospital will be unchanged regardless of whether you choose to participate or not.

What will be required of me/my child if we participate?

If you/your child wish to participate, you/your child will be required to undergo a full clinical examination, where measurements of your/your child's weight, height, head circumference as well as eyes and ears will be made. The examination will also include a chest, heart, abdomen, arm and leg assessment. The examination will not be painful, and will be conducted in a private cubicle. The examination will take place at the haematology/oncology clinic where you receive therapy and should take approximately 90 minutes. I will require you to answer some questions regarding your/your child's past and current health and I will need to look through your hospital file for additional information regarding your/your child's treatment and any investigations you/your child may have had.

I would also like to take a few photographs of your/your child's face, arms and hands, feet and skin. These photographs are exclusively for use in the study and will not be used in any commercial magazines or pamphlets. They will not be made available to members of the public in any form. If they are used in the research report they will be altered to protect your/your child's identity. If you choose not to have photographs taken of you/your child, you may still participate in the study.

If I detect any problems or abnormalities with you/your child during the clinical examination, you will be given the choice of investigating the problem further with the aid of the clinician in charge of your/your child's management.

Will participation in the study cause any pain or physical discomfort?

As part of the study, I will be looking at endocrine abnormalities that may occur in patients with Fanconi anaemia, such as thyroid problems and glucose abnormalities. To evaluate these parameters, a blood sample may be required from you/your child. Venepuncture is an uncomfortable procedure which takes a few seconds to perform. Approximately 10-15mls (2-3 teaspoons) of blood would be drawn by me, under strict sterile conditions to prevent infections. Other side effects you may experience include slight pain at the site and bleeding – every effort will be made to prevent or lessen these complications. Should you choose not to have the blood sample taken, you will still be able to participate in the study.

What other procedures may be necessary?

Some patients with Fanconi anaemia have changes involving the bones of the upper and lower limbs. If I suspect that you/your child have/has any of these changes, you will be given the choice to have X-rays done at the hospital to investigate for these changes. An X-ray is not painful or uncomfortable, but would expose you/your child to a low dose of radiation. Complications are not expected to arise following an X-ray evaluation.

How will participation in this study benefit me/my child?

There will be no direct health benefit for you/your child. However, participation in the study may benefit other patients diagnosed with Fanconi anaemia as it would help to collect information that may enable doctors to improve the management of patients with Fanconi anaemia in the future.

Participation may also provide information that will be useful to you and to other members of your family. If you/your child have not received genetic counselling regarding Fanconi anaemia, this service will be made available to you, in collaboration with the Genetic Counselling Division of the National Health Laboratory Services, Johannesbur

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.

Will I receive payment for participation?
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/your child will be provided with a light snack at the end of the examination.

What will happen to the information collected during the study?
The information collected will be analysed and used to collate a research report which will document my findings and conclusions. All information collected in this study will be strictly confidential. It will be made available to the doctor who is in charge of the oncology department which you/your child is/are attending. Any data documented in my research report or in any scientific journals will not include information that may identify you/your child as a participant.
If you wish to receive feedback on the results of the study, this will be mailed to you at the completion of the study, which is likely to be in 2012.

Who should I contact if I have any questions or comments?
If you require any further information about the study, please do not hesitate to contact me on (011) 489-9338. If you have any questions regarding your rights as a research participant or any complaints regarding the research, you may contact Professor Cleaton-Jones, Chairperson of the Human Research Ethics Committee at the University of the Witwatersrand on (011)717-2229.

This protocol has been submitted to and approved by the Human Research Ethics Committee of the University of Witwatersrand and the Ethics Committee of the University of the Free State.

Child Information Sheet

Title: **Genotype-phenotype correlation in South African Black and Afrikaans individuals with Fanconi anaemia.**

Investigator: **Dr Candice Feben**
Department of Human Genetics, NHLS, Johannesburg, SA

Hello,

My name is Dr Candice. As part of my studies I am compiling a project looking at children with Fanconi anaemia.

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.

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, your head and your face. I will also listen to your heart and lungs, feel your abdomen and look at your skin, arms and legs. 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 would also like to take some photos of you. The photos are only for your file and are not for magazines or newspapers. If you do not want to have photos taken, you can still join the project.

I might 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 do not want your blood drawn, you will still be able to join the project.

When we have finished you will get a snack to take home with you.

If you would like to know the results of the project, these will be sent to your parents/guardians when the project is finished.

If you have any questions or if there is anything you do not understand, please feel free to ask me.

Thank you for your help.

CHILD ASSENT 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 **Genotype-phenotype correlation in South African Black and Afrikaans individuals with Fanconi anaemia.**

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:

Clinical Examination & file review only ☐

Clinical Examination, file review & venepuncture only ☐

Clinical Examination, file review & photographs only ☐

Clinical Examination, file review, venepuncture & Photographs ☐

If required, I DO / DO NOT give consent for X-Rays to be taken of MY CHILD

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 results of this study.

Signed on _____ day of _____ 20__ at _____

Parent/Guardian Name: _____ Signature_____

Patient Name: _____ Signature_____

Witness Name:_____ Signature_____

Translator Name:_____ Signature_____

Researcher Name: Dr C. Feben_____ Signature_____

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 **Genotype-phenotype correlation in South African Black and Afrikaans individuals with Fanconi anaemia.**

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:

Clinical Examination & file review only ☐

Clinical Examination, file review & venepuncture only ☐

Clinical Examination, file review & photographs only ☐

Clinical Examination, file review, venepuncture & photographs ☐

If required, I DO / DO NOT give consent for X-Rays to be taken of ME

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 results of this study.

Signed on _____ day of _____ 20__ at _____

Patient Name: _____ Signature_____

Witness Name:_____ Signature_____

Translator Name:_____ Signature_____

Researcher Name: Dr C. Feben_____ Signature_____

APPENDIX B – Ethics Approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Amanda Krause

CLEARANCE CERTIFICATE	**M090681**
PROJECT	Genotype-Phenotype Correlation in South African Black and Afrikaans Individuals with Fanconi Anaemia
INVESTIGATORS	Dr Amanda Krause.
DEPARTMENT	Division of Human Genetics
DATE CONSIDERED	09.06.26
DECISION OF THE COMMITTEE*	Approved subject to obtaining ethics approval from the other universities

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

DATE 09.07.26 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof A Krause

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

.....

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

Direkteur: Fakulteitsadministrasie / Director: Faculty Administration
Fakulteit Gesondheidswetenskappe / Faculty of Health Sciences
Internal Post Box G40
☎(051) 4052812
Fax nr (051) 4444359
E-mail address: StraussHS.md@ufs.ac.za

Ms H Strauss

2010-04-16

PROF A KRAUSE
NATIONAL HEALTH LABORATORY SERVICE
P O BOX 1038
JOHANNESBURG
2000

Dear Prof Krause

ETOVS NR 52/2010
PROF A KRAUSE — **DEPT OF HUMAN GENETICS, NHLS**
PROF DK STONES — **DEPT OF PAEDIATRICS AND CHILD HEALTH, UFS**

PROJECT TITLE: GENOTYPE-PHENOTYPE CORRELATION IN SOUTH AFRICAN BLACK AND AFRIKAANS INDIVIDUALS WITH FANCONI ANAEMIA.

- You are hereby informed that The Ethics Committee approved the above protocol at the meeting on 13 April 2010.

  [Prof Stones did not take part in the discussion of this study]

- Committee guidance documents: Declaration of Helsinki, ICH, GCP and MRC Guidelines on Bio Medical Research. Clinical Trial Guidelines 2000 Department of Health RSA; Ethics in Health Research: Principles Structure and Processes Department of Health RSA 2004; Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa, Second Edition (2006); the Constitution of the Ethics Committee of the Faculty of Health Sciences and the Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines.

- Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

- The Committee must be informed of any serious adverse event and/or termination of the study.

- A progress report should be submitted within one year of approval of long term studies and a final report at completion of both short term and long term studies.

- Kindly refer to the ETOVS reference number in correspondence to the Ethics Committee secretariat.

Yours faithfully

.....
CHAIR: ETHICS COMMITTEE

339, Bloemfontein 9300,RSA ☎ (051) 405 2812 StraussHS.md@ufs.ac.za
Republiek van Suid-Afrika / Republic of South Africa

APPENDIX C – Clinical Tick Sheet (compiled for the purposes of the research project)

Fanconi Anaemia- Clinical Phenotype Evaluation

Demographic Data

Number _____

Date of Birth _____

Current Age _____

Age at presentation _____

Clinic Attending _____

Reason for initial attendance at clinic _____

Siblings	Affected	Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased
		Yes	No	Unknown	Age	Alive	Deceased

Contact Details

Postal Address _____
Mother cell _____
Father cell _____
Home number _____

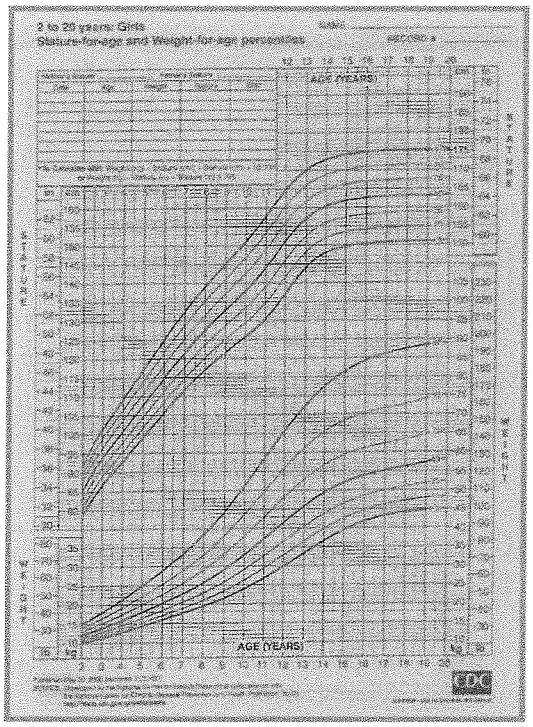
Other cardiac defects							
Gastrointestinal Malformations							
Duodenal/jejenal atresia	No	Yes					
Tracheo-oesophageal fistula	No	Yes					
Imperforate anus	No	Yes					
Umbilical hernia	No	Yes		Treated:	No	Yes	
Liver adenomas	No	Pre androgens	Post androgens				
Other gastrointestinal anomalies							
Renal malformations							
Renal sonar done	No	Yes					
Kidney malformation							
* Horseshoe	No	Left	Right	Diagnosed on:			
* Pelvic	No	Left	Right	Diagnosed on:			
* Dysplastic	No	Left	Right	Diagnosed on:			
* Hypoplastic	No	Left	Right	Diagnosed on:			
* Agesesis	No	Left	Right	Diagnosed on:			
* Other	Specify			Diagnosed on:			
Collecting system malformation							
* Hydronephrosis	No	Left	Right	Diagnosed on:			
* Hydroureter	No	Left	Right	Diagnosed on:			
* Reflux nephropathy	No	left	Right	Diagnosed on:			
* Other	Specify			Diagnosed on:			

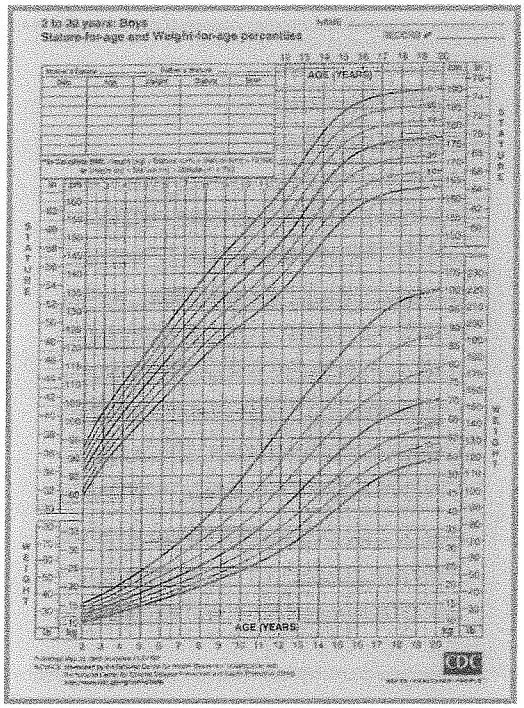
Renal artery malformation	No	Left	Right	Diagnosed on:			
Genital malformations:							
MALES							
Undescended testes	No	Left	Right				
Other male genital abnormality							
Tanner score	1	2	3	4	5		
FEMALES							
Hypoplastic vulva	No	Yes					
Age at menarche	<11 years	11-17 years	>17 years				
Irregular menses	No	Yes	N/A				
Other female genital abnormalities							
Tanner score	1	2	3	4	5		
Upper Limb Malformations							
Ulna abnormality							
*Absent	No	Left	Right	Radiographic	confirmation:	No	Yes
*Hypoplastic	No	Left	Right	Radiographic	confirmation:	No	Yes
*Other	No	Left	Right	Radiographic	confirmation:	No	Yes
Radius abnormality				Radiographic	confirmation:	No	Yes

*Absent	No	Left	Right	Radiographic	confirmation:	No	Yes
*Hypoplastic	No	Left	Right	Radiographic	confirmation:	No	Yes
*Other	No	Left	Right	Radiographic	confirmation:	No	Yes
Specify							
Clinodactyly	No	Left	Right				
Polydactyly	No	Left Pre	Right Pre				
		Post	Post				
Absent first metacarpal	No	Left	Right	Radiographic	confirmation:	No	Yes
Thumb abnormality				Radiographic	confirmation:	No	Yes
*Absent	No	Left	Right				
*Hypoplastic	No	Left	Right				
*Other	No	Left	Right	Specify			
Hyperextensible thumb	No	Left	Right				
Stiff thumb	No	Left	Right				
Triphalangeal thumb	No	Left	Right	Radiographic	confirmation:	No	Yes
Proximally inserted thumb	No	Left	Right				
Transverse palmar crease	No	Left	Right				
Middle finger length (mm-centile for age)	>97th	50th-97th	10th-50th	3rd-10th	<3rd		
Bone age -Left wrist	Normal	Delayed	Accelerated	Unknown			
Radial pulse palpable	Left	Right					
Other upper limb abnormalities							

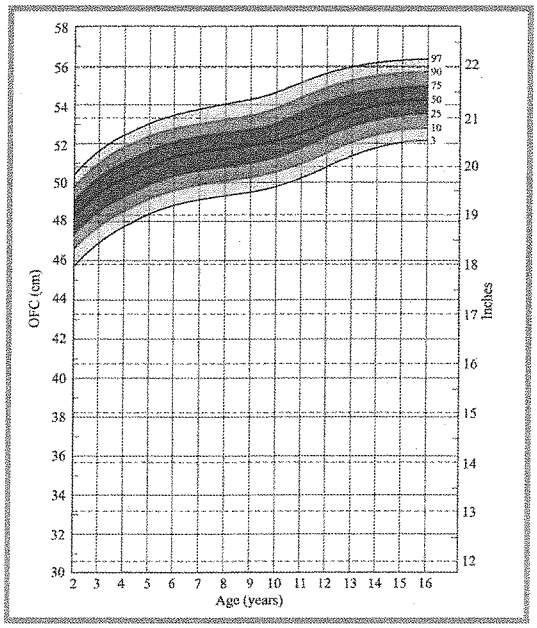
Lower limb abnormalities							
Developmental dysplasia of the hip	No	Left	Right				
Toe syndactyly	No	Left	Right	Specify			
Pes planus	No	Left	Right				
Polydactyly	No	Left Pre	Right Pre				
		Post	Post				
Other lower limb abnormalities							
Vertebral abnormalities							
Scoliosis	No	Yes					
Kyphosis	No	Yes					
Vertebral abnormalities	No	Yes		Specify			
Sprengel deformity	No	Left	Right				
Klippel-Feil deformity	No	Yes					
Pigmentary Changes							
Café au lait spots (>2 patches, each >5mm)	No	Yes					
Hyperpigmentation	No	Yes		Specify			
Hypopigmentation	No	Yes		Specify			
Other pigmentary changes							

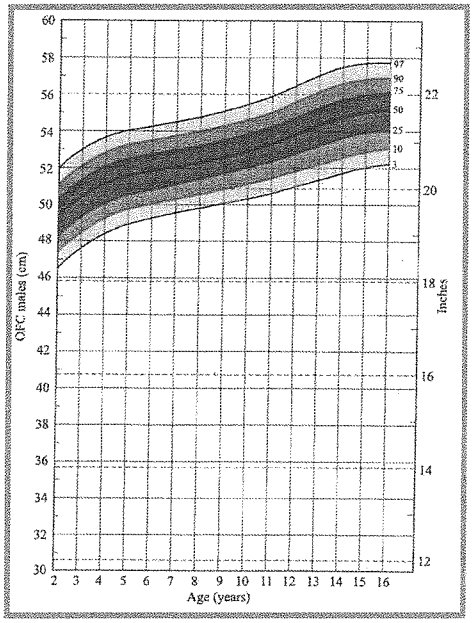
APPENDIX D – Growth & physical parameter charts



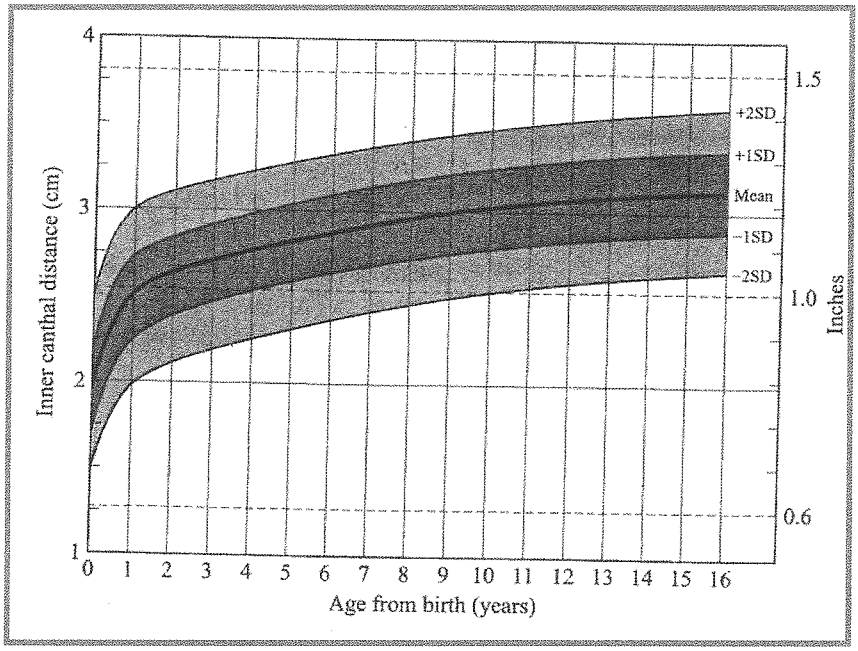


Weight for age and height for age charts for males and females aged 2 to 20 years.
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000) <http:www.cdc.gov/growthcharts>

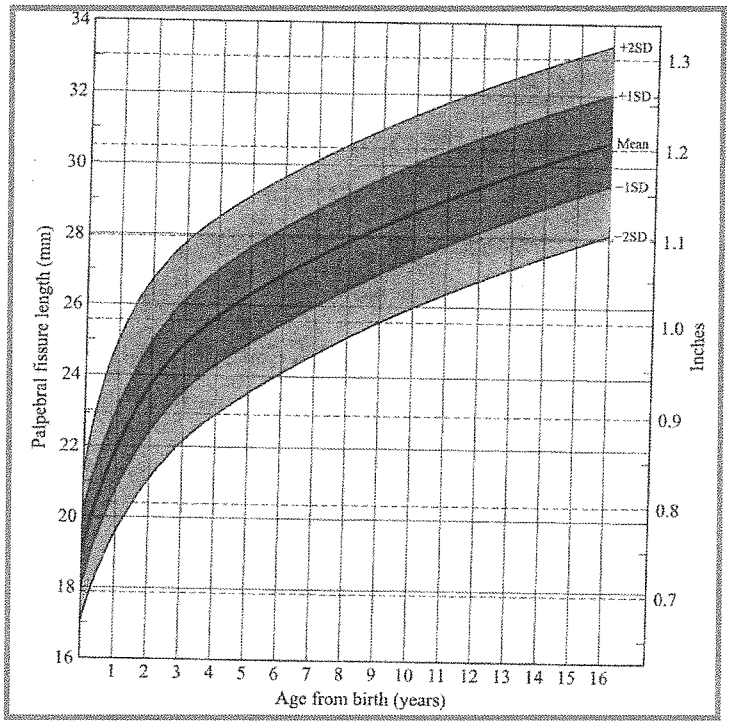




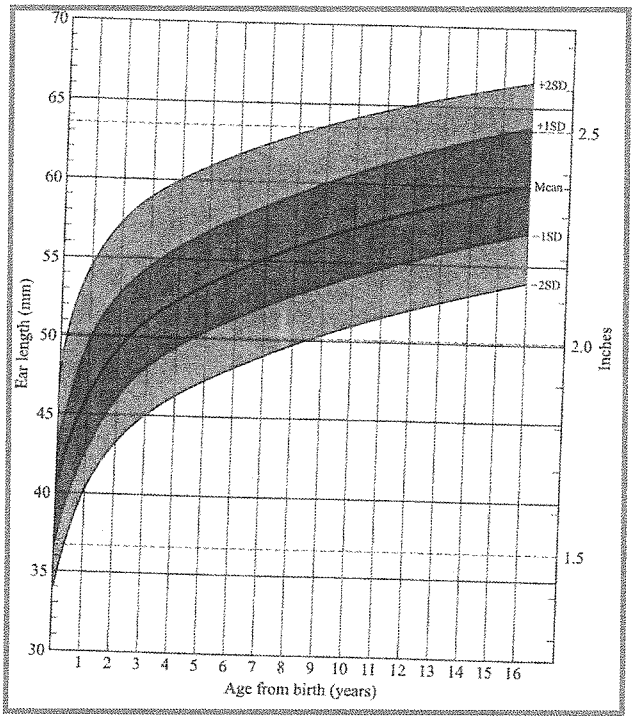
Head Circumference for age charts for males and females from 2 years of age
SOURCE: Hall JG, Allanson JE, Gripp KW *et al.* (2007). Handbook of Physical Measurements (2nd ed.)



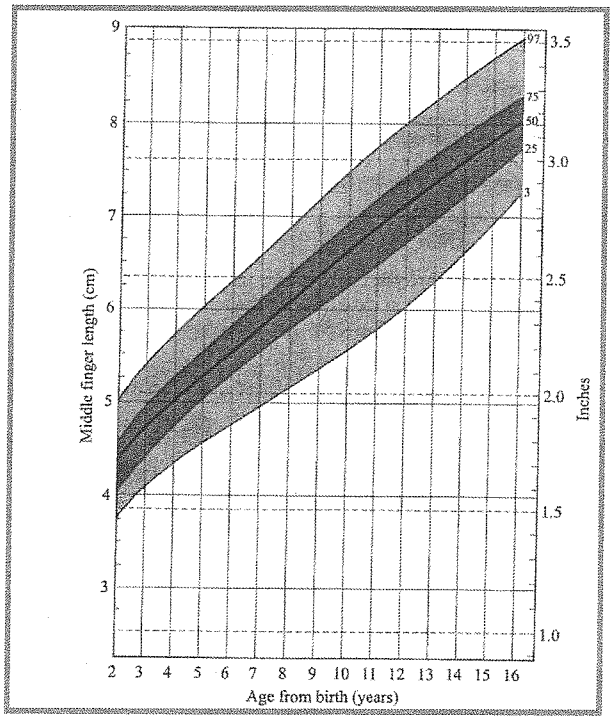
Inner canthal distance chart for age in males and females from birth
SOURCE: Hall JG, Allanson JE, Gripp KW *et al.* (2007). Handbook of Physical Measurements (2nd ed.)



Palpebral fissure length for age chart in males and females from birth
SOURCE: Hall JG, Allanson JE, Gripp KW *et al.* (2007). Handbook of Physical Measurements (2nd ed.)



Ear length for age in males and females from birth
SOURCE: Hall JG, Allanson JE, Gripp KW *et al.* (2007). Handbook of Physical Measurements (2nd ed.)



Middle finger length for age chart in males and females from birth
SOURCE: Hall JG, Allanson JE, Gripp KW *et al.* (2007). Handbook of Physical Measurements (2nd ed.)

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