

**CYSTIC FIBROSIS IN BLACK AFRICAN CHILDREN IN SOUTH AFRICA: A CASE  
CONTROL STUDY**

BY

**SANDRA KWARTENG OWUSU**

Student Number KWRSAN001

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UNIVERSITY OF CAPE TOWN  
MPHIL(PAEDIATRIC PULMONOLOGY)  
FACULTY OF HEALTH SCIENCES

UNIVERSITY OF CAPETOWN

Supervisor : Dr Marco Zampoli

Division of Paediatric Pulmonology  
Red Cross War Memorial Children's Hospital

University of Cape Town

Co-Supervisor –(1) Associate Professor Aneesa Vanker

Division of Paediatric Pulmonology  
Red Cross War Memorial Children's Hospital

University of Cape Town

Co-Supervisor –(2) Associate Professor Diane Gray

Division of Paediatric Pulmonology  
Red Cross War Memorial Children's Hospital

University of Cape Town

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## TABLE OF CONTENTS

<b>CYSTIC FIBROSIS IN BLACK AFRICAN CHILDREN IN SOUTH AFRICA: A CASE CONTROL STUDY</b> .....	<b>1</b>
<b>TABLE OF CONTENTS</b> .....	<b>2</b>
<b>DECLARATION</b> .....	<b>3</b>
<b>ABSTRACT</b> .....	<b>4</b>
<i>BACKGROUND</i> .....	4
<i>METHODS</i> .....	4
<i>RESULTS</i> .....	4
<i>CONCLUSION</i> .....	4
<b>ACKNOWLEDGEMENTS</b> .....	<b>5</b>
<b>LIST OF TABLES</b> .....	<b>6</b>
<b>LIST OF FIGURES</b> .....	<b>7</b>
<b>ABBREVIATIONS</b> .....	<b>8</b>
<b>CHAPTER 1: ACCEPTED FOR PUBLICATION FORMAT</b> .....	<b>9</b>
<b>CYSTIC FIBROSIS IN BLACK AFRICAN CHILDREN IN SOUTH AFRICA: A CASE CONTROL STUDY</b> .....	<b>9</b>
<i>INTRODUCTION</i> .....	10
<i>METHODS</i> .....	11
<i>Study design and setting</i> .....	11
<i>Study population</i> .....	11
<i>Clinical information at diagnosis</i> .....	12
<i>Outcomes</i> .....	12
<i>Statistical analysis</i> .....	12
<i>RESULTS</i> .....	13
<i>Demographic and clinical information at diagnosis</i> .....	13
<i>Outcomes</i> .....	16
<i>DISCUSSION</i> .....	18
<b>REFERENCES</b> .....	<b>21</b>
<b>APPENDIX 1: DATA GATHERING FORM</b> .....	<b>24</b>
<b>APPENDIX 2: DEPARTMENTAL REVIEWER COMMENTS</b> .....	<b>29</b>
<b>APPENDIX 3: ETHIC APPROVAL, UCT</b> .....	<b>31</b>
<b>APPENDIX 4: ETHICS APPROVAL, WITS UNIVERSITY</b> .....	<b>32</b>
<b>APPENDIX 5: AUTHOR'S GUIDELINES</b> .....	<b>34</b>
<b>APPENDIX 6: REVIEWER COMMENTS</b> .....	<b>44</b>

## DECLARATION

I, Dr. Sandra Kwarteng Owusu (student number - KWRSAN001), hereby declare that the work on which this thesis is based, is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 25<sup>th</sup> October, 2019

## ABSTRACT

### *Background*

Cystic fibrosis (CF) is described more commonly in Caucasian populations in whom p.Phe508del is the most common mutation. There is a paucity of data of CF in black African children. The aim of this study was to describe and compare the presentation and outcomes of black African children with CF to those with p.Phe508del genotype.

### *Methods*

A retrospective case-controlled study was conducted from January 2000 – March 2018 of children with CF attending two CF centres in South Africa. Presentation, genotype, nutrition and pulmonary function outcomes of black African children were compared to matched controls with the p.Phe508del mutation.

### *Results*

Thirty-four black African children (cases) with median age of diagnosis (5.5 months, IQR 2.0-15.0) were matched to 34 controls. Among cases, 3120+1G->A *CFTR* mutation was most commonly identified; homozygous n=22 (64.7%) and heterozygous=7(20.5%). Compared to controls, cases at diagnosis were more malnourished and fewer presented with neonatal bowel obstruction [cases n=2 (5.9%) vs. controls n=10 (29.4%); p = 0.03]. Nutrition and pulmonary function (FEV1 in children  $\geq$  6 years) outcomes and changes over time from ages 3-16 years were similar in both groups; median FEV1 z-score at age 6,10 and 14 years was -0.9 ( $\pm$ 1.5), -1.8 ( $\pm$ 2.0) and -1.8 ( $\pm$ 1.9) respectively for all patients. Deaths were recorded in three cases (8.8%) and one control (2.9%) (p = 0.6).

### *Conclusion*

Black African children with CF were more malnourished at diagnosis, and fewer presented with neonatal bowel obstruction. Cases and controls had comparable nutritional, pulmonary function and early mortality outcomes.

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## LIST OF TABLES

Table 1: Demographic and clinical information of cases and controls at time of CF diagnosis and during follow-up period. ....	13
Table 2: Details of early mortality cases.....	18

## LIST OF FIGURES

Figure 1: Changes in mean BMI Z score over the study period between cases and controls. Anova current effect $F(7,7) = 0.14$ ; $p = 0.99$ . .....	16
Figure 2: Change in mean HAZ throughout the study period in cases and controls. Anova current effect $F(7, 21) = 0.79$ ; $p = 0.6$ . .....	17
Figure 3: Change in mean WAZ from diagnosis to age 10. Anova current effect $F(5, 30) = 0.65$ ; $p = 0.7$ .....	17



## ABBREVIATIONS

CF:	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
RWMCH	Red Cross War Memorial Children's Hospital
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
WHO	World Health Organisation
SA	South Africa
WAZ	Weight for age Z-score
HAZ	Height-for-age Z score
WFHZ	Weight for height Z score
SAM	Severe acute malnutrition
PI	Pancreatic Insufficient
FEV1	Forced Expiratory volume in the first second
BMI	Body mass index
PERT	Pancreatic Enzyme Replacement Therapy
HIV	Human immunodeficiency virus
ADHD	Attention deficit hyperactivity disorder
SA	Staphylococcus aureus
PA	Pseudomonas aeruginosa
GLI	Global Lung Initiative
SES	Socio economic status
NRF	National Research Foundation.

# CHAPTER 1: ACCEPTED FOR PUBLICATION FORMAT

## CYSTIC FIBROSIS IN BLACK AFRICAN CHILDREN IN SOUTH AFRICA: A CASE CONTROL STUDY

Sandra Kwarteng Owusu <sup>a,d</sup> , Brenda M. Morrow <sup>c</sup> , Debbie White <sup>b</sup> , Susan Klugman <sup>b</sup> , Aneesa Vanker <sup>a</sup> , Diane Gray <sup>a</sup> , Marco Zampoli<sup>a</sup>,

- a. Division of Paediatric Pulmonology, Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital, University of Cape Town South Africa
- b. Department of Paediatric Pulmonology, Charlotte Maxeke Johannesburg Academic Hospital, Faculty of Health Sciences, University of the Witwatersrand South Africa
- c. Department of Paediatrics and Child Health, University of Cape Town South Africa
- d. Department of Child Health and Paediatrics, Komfo Anokye Teaching Hospital Kumasi Ghana.

**Corresponding Author:** Marco Zampoli, Division of Paediatric Pulmonology, Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital, University of Cape Town South Africa. Telephone +27 83 320 8088

**Email** [m.zampoli@uct.ac.za](mailto:m.zampoli@uct.ac.za)

*Background:* Cystic fibrosis (CF) is described more commonly in Caucasian populations in whom p.Phe508del is the most common mutation. There is a paucity of data of CF in black African children. The aim of this study was to describe and compare the presentation and outcomes of black African children with CF to those with p.Phe508del genotype.

*Methods:* A retrospective case-controlled study was conducted from January 2000 – March 2018 of children with CF attending two CF centres in South Africa. Presentation, genotype, nutrition and pulmonary function outcomes of black African children were compared to matched controls with the p.Phe508del mutation.

*Results:* Thirty-four black African children (cases) with median age of diagnosis (5.5 months, IQR 2.0-15.0) were matched to 34 controls. Among cases, 3120+1G->A *CFTR* mutation was most commonly identified; homozygous n=22 (64.7%) and heterozygous=7(20.5%). Compared to controls, cases at diagnosis were more malnourished and fewer presented with neonatal bowel obstruction [cases n=2 (5.9%) vs. controls n=10 (29.4%); p = 0.03]. Nutrition and pulmonary function (FEV1 in children  $\geq$  6 years) outcomes and changes over time from ages 3-16 years were similar in both groups; median FEV1 z-score at age 6,10 and 14 years was -0.9 ( $\pm$ 1.5), -1.8 ( $\pm$ 2.0) and -1.8 ( $\pm$ 1.9) respectively for all patients. Deaths were recorded in three cases (8.8%) and one control (2.9%) (p = 0.6).

*Conclusion:* Black African children with CF were more malnourished at diagnosis, and fewer presented with neonatal bowel obstruction. Cases and controls had comparable nutritional, pulmonary function and early mortality outcomes.

### *Key Words*

Cystic Fibrosis, Black African children, South Africa,

### *Introduction*

Cystic fibrosis (CF), is a severe life-limiting, autosomal recessive disorder identified in 1938(1). CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene located on chromosome 7q31.2. **Currently six classes of *CFTR* mutations have been described. The first three mutations are associated with severe CF disease, this is due to complete absence of *CFTR* protein on the cell wall of epithelial cells or complete lack of function although present. The most common mutation worldwide is the pPhe508del mutation a class two mutation in which *CFTR* protein that is produced is abnormally folded and gets destroyed by the endoplasmic reticulum. This is a mis sense amino acid deletion.**(2) The *CFTR* gene plays a primary role in chloride and bicarbonate transport with secondary effect on sodium transport(3,4). Severity of CF depends partly on the class of *CFTR* mutation, however genotype-phenotype correlates suggest additional genetic factors such as complex alleles, modifier genes and epigenetic factors significantly influence the phenotypic expression of CF(5). Cystic fibrosis is more prevalent among populations of European descent where the most commonly occurring mutation is p.Phe508del. Among non-Caucasian populations, a wide range of mutations **has** been reported with p.Phe508del mutation occurring less commonly. Cystic fibrosis is less prevalent among populations of African ancestry and has been sporadically reported in Kenya, South Africa(6,7)and more recently in Sudan and Brazil(8,9). The reported incidence of CF is 1 in 15,100 among African Americans (10). The true incidence of CF in sub-Saharan African is unknown (11).

**Due to markedly improved knowledge on cystic fibrosis disease entities over the past six decades the median age of survival has increased from a few months to more than 40 years in advanced countries. In the past death from CF was as a result of meconium ileus and malnutrition secondary to pancreatic malabsorption.**(12–14).

**In the 21<sup>st</sup> century death occurs mainly from respiratory failure in individuals who have not undergone lung transplant. The major achievements in life expectancy has been due to early institution of airway clearance techniques, aggressive management of airway infections and addressing nutritional deficits**(3,15,16).

The 3120+1G->A mutation was first reported by Macek and colleagues (17) among a small group of African Americans with CF. Subsequently it was also reported among South Africans by Carles et al(18). The 3120+1G->A mutation, **is a severe class I nonsense mutation(19) and** accounts for up to 14% of CF mutation in African Americans and native Africans(17). There are few and conflicting reports of the spectrum of CF phenotypes in African children. In a previous case series,

people with the 3120+1G->A mutation were noted to have mild sinopulmonary symptoms and many presented with abdominal symptomatology, especially meconium ileus (20). By contrast, in a comparative study between black and white American patients with CF, meconium ileus was reported less commonly in the black individuals (21). Masekela et al, in a South African study, reported failure to thrive and protein energy malnutrition as common presentations of CF in individuals with 3120+1G->A mutation. All were pancreatic insufficient and acquired *Pseudomonas aeruginosa* airway infection by their second year of life (22). Pulmonary function outcomes were noted to be poorer in black patients with CF compared to Caucasian patients in the study by Hamosh *et al* (21).

Cystic fibrosis has been extensively studied and characterised among Caucasians outside South Africa (23)(24) and also among Caucasians in South Africa(11)(25). Although awareness among clinicians of the existence of CF in black African children is improving, there remains a paucity of data on CF disease in black Africans(26). Improved knowledge of CF in African populations may lead to earlier diagnosis and improved outcomes. The primary aim of this study was therefore to compare the genotype, clinical presentation, pulmonary function and nutritional outcomes in black African children diagnosed with CF in SA to matched cases with p.Phe508del genotype. We hypothesized that genotype **would be different but presentation and clinical outcomes would be similar.**

## Methods

### Study design and setting

A retrospective case-control study (1:1 ratio) was conducted to compare CF disease in black African children and children with the p. Phe508del *CFTR* mutation. The study was conducted in two paediatric CF centres in SA: Red Cross War Memorial Children's Hospital (RWMCH), Cape Town, and Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg. The study was approved by Human Research Ethics committees of the University of Cape Town (HREC 586/2017) and University of the Witwatersrand (Protocol M171149). The requirement for obtaining informed consent was waived as this was a retrospective study.

### Study population.

All black African children diagnosed with CF between 1 January 2000 and 31 March 2018, were included. The diagnosis of CF during the study period was confirmed on international CF Foundation consensus criteria: phenotypical manifestations of CF, or a family history of CF *and* either i) two known disease-causing *CFTR* mutations identified *or* ii) two positive sweats tests (sweat chloride > 60 mmol/L)(27). In circumstances where sweat chloride measurements were not available, a diagnosis of CF was considered likely if at least two sweat conductivity tests were > 80mmol/L in the presence of phenotypic manifestation compatible with CF. *CFTR* mutation panel testing most widely available in South Africa during the study period was Elucigene CF29v2 (Elucigene Diagnostics, Manchester, UK). Further testing in some cases was performed independently where financial resources were available.

Matched controls homozygous with p.Phe508del *CFTR* mutation were identified from medical records at the respective CF centres using the same CF diagnosis criteria. Children heterozygous with p.Phe508del *CFTR* mutation and another mutation (excluding 3120+1G>A) were selected as controls if no suitable p.Phe508del homozygous control was identified. Index cases were further matched with controls according to the following criteria: diagnosis age (within 12 calendar months); gender; and year of diagnosis (within 12 calendar months).

#### Clinical information at diagnosis

Data was extracted from the medical records and existing local CF clinic databases from the two study sites. Demographic and country of origin details, genotype and sweat test information was recorded for cases and matched controls. The modes of presentation at the time of diagnosis, including presence of neonatal bowel obstruction, family history of CF, predominantly respiratory disease, predominantly gastrointestinal disease or both were recorded. The World Health Organisation's (WHO) Anthro or Anthro Plus calculator (version 3.22 January 2011) was used to calculate weight-for-age (WAZ); height-for-age (HAZ) and weight for height (WFHZ) z-scores and body mass index (BMI) and BMI z-scores for children under and over 6 years of age respectively. All anthropometric measurements were standardly taken at the time of diagnosis and at subsequent follow-up visits. Participants with faecal elastase level <200ug/ml or those on pancreatic enzyme replacement therapy (PERT) due to steatorrhea symptoms were classified as pancreatic insufficient (PI).

#### Outcomes

Best calendar-year annual measurements of weight and height were documented for each study participant at ages 1, 3, 6, 8, 10, 12, 14 and 16 years, where available and applicable. Corresponding best annual pre-bronchodilator forced expiratory flow in one second (FEV1) for participants six years and older was also documented. Ethnic-specific Global Lung Initiative (GLI) equations for were applied to calculate FEV1 Z-scores for age (28).

The age at first documented *Pseudomonas aeruginosa* infection was recorded as well as age of chronic *P.aeruginosa* infection as per the modified Leeds's classification (29). Similar information on *Staphylococcus aureus* airway infection was also recorded. Mortality outcomes and causes of death were documented where information was available.

#### Statistical analysis

Data were tested for normality using the Shapiro-Wilks W test, and variables are presented throughout as median (interquartile range, IQR) or n (%) as appropriate for non-normally distributed data, and as mean  $\pm$  standard deviation (SD) for normally distributed data. Comparisons between cases and controls were conducted using Mann-Whitney U or t-tests for independent variables for continuous variables, and chi square tests (with Yates correction where applicable) for categorical variables. Repeated measures analysis was conducted using ANOVA (one- way and between- and within-groups designs) for measures of lung function and nutritional status at different time periods, after testing for normality of residual distribution. A p-value of less than

0.05 was regarded as statistically significant. Statistica version 12 (StatSoft, USA) was used for analysis.

## Results

### Demographic and clinical information at diagnosis

Thirty-four (n=21; 61.8% male) black African children (cases), matched to 34 controls, were recruited from the two CF centres: 15 cases and controls from RCWMCH and 19 cases and controls from CMJAH. Median age of diagnosis (5.5 months, IQR 2.0-15.0), and pancreatic insufficiency status (n=59, 86.8%) was similar in cases and controls (Table 1).

The most common *CFTR* mutation identified in cases was 3120+1G>A, identified in either homozygous or heterozygous state in 23 (67.6%) and 6 (17.6%) cases respectively with an allele frequency of 55/68 (81%); in one case the two mutations identified were Gly458Val/p.Ser466X, and four cases (11.8%) had no mutations identified (Table 1). Among the six cases who were heterozygous for the 3120+1G->A mutation, there were three cases in whom the second mutation could not be identified, two cases with c.1585.3T>G and one with p.Arg792X as the second mutation. Five cases were children of parents originating from other African countries.

Compared to controls, cases at diagnosis were significantly more malnourished, with lower BMI-Z, WAZ, HAZ, and WFHZ scores (Table 1). Mode of presentation was similar except that cases were significantly less likely to present with neonatal bowel obstruction (Table 1).

Table 1: Demographic and clinical information of cases and controls at time of CF diagnosis and during follow-up period.

Variable	Cases (n=34) n (%)	Controls (n=34) n (%)	P-value
Age at diagnosis months (median, IQR)	5.0 (2.0 – 15.0)	6.0 (3.0 – 15.0)	1
Male gender	21 (61.7)	17(50.0)	0.7
<b>Country of origin</b>			
SA	29 (85.2)	34(100)	0.2
Ghana	1(2.9)	0	
Malawi	2(5.9)	0	
Zimbabwe	1(2.9)	0	
Mozambique	1(2.9)	0	

<b>Variable</b>	<b>Cases (n=34) n (%)</b>	<b>Controls (n=34) n (%)</b>	<b>P-value</b>
Sweat chloride mmol/L	111.8 ± 19.0 (n=11)	108.8 ± 11.6 (n=15)	0.6
Sweat conductivity mmol/L	103.2 ± 30.5 (n=12)	101.9 ± 38.0 (n=8)	0.7
<b>CFTR mutations</b>			
p.Phe508del homozygous	0	25(73.5)	
p.Phe508del heterozygous	0	9(26.5)	
3120+1G->A homozygous	23 (67.6)	0	
3120+1G->A heterozygous	6 (17.6)	0	
Other mutations (number of alleles)	Unknown (11) c.1585-3T>G (2) * p.Arg792X Gly458Val* p.Ser466X	p.Arg1162X (2) Gly1173GlnfsX21* 3272-26A>G p.Trp846X p.Arg553X p.Gly542X p.Leu881IlefsX22 (2)	
<b>Pancreatic insufficient</b>	n=30 (88.2%)	n=29 (85.3%)	1
<b>Nutritional status at diagnosis</b>			
BMI-Z	-3.1 ± 2.2	-1.2 ± 3.2	0.009
WAZ	-4.3 ± 2.0	-2.6 ± 1.9	0.0008
HAZ	-3.5 ± 2.5	-2.1 ± 2.2	0.02
WFHZ	-3.0 ± 2.1	-1.6 ± 2.2	0.02
<b>FEV1 Z score</b>			
Age 6	-0.9 ± 1.9	-0.9 ± 1.3	0.4
Age 10	-2.8 ± 2.05	-1.1 ± 1.3	0.08
Age 14	-1.4 ± 1.3	-2.3 ± 2.6	0.2
<b>Presenting symptoms</b>			

<b>Variable</b>	<b>Cases (n=34) n (%)</b>	<b>Controls (n=34) n (%)</b>	<b>P-value</b>
Predominantly respiratory	7(20.5)	7(21.9)	0.8
Predominantly GIT*	9(26.47)	4(12.5)	0.6
Combined GIT* and respiratory	16(47.1)	9(28.2)	0.1
Neonatal bowel obstruction	2(5.9)	10(31.3)	0.03
Family history/neonatal screening	0	2(6.3)	0.4
<b>Airway infections</b>			
Ever PA infection	21(61.7)	24(70.6)	0.6
Ever SA infection	26(76.5)	31(91.2)	0.2
Age at first PA (median, IQR)	11 (5.5 – 48.5)	22.0 (12.0 – 32.0)	0.7
Chronic PA infection	6 (17.6)	5 (14.7)	1
Other significant infections	16 (47.1)	20(58.8)	0.5
<b>Comorbid conditions</b>	6(17.6)	5(14.7)	0.4
Learning difficulties	2(5.9)	1(2.9)	
ADHD	0	3 (8.8)	
Congenital heart disease	1(2.9)	0	
HIV exposed	2(5.9)	1(2.9)	
Gunshot injury to abdomen	1(2.9)	0	
<b>Deaths</b>	3(21.4)	1(7.1)	0.6

**Legend:** **IQR**- Interquartile range, **WAZ**- Weight for age Z-score, **HAZ** Height for age Z-score, **WFAZ**-Weight for age Z-score, **BMIZ**-Body mass index, **GIT**-Gastro intestinal, **ADHD**- Attention deficit Hyper activity disorder, **HIV**-Human immunodeficiency virus: SA- Staphylococcus aureus ; PA- Pseudomonas aeruginosa; Categorical data are presented as n (%) and continuous data as mean  $\pm$  SD unless otherwise indicated; \* *CFTR* variants of unknown clinical significance or not reported in *CFTR2* database.



## Outcomes

Although nutritional status was significantly poorer at diagnosis in cases compared to controls (Table 1), there were no significant differences between groups in the change over time (Figures 1-3) and by three years of age there was no longer a significant difference in nutritional status between groups, for any outcome measures (BMI Z score, HAZ or WAZ).

There were no differences between groups in the change of FEV1 over time ( $p = 0.5$ ); and there were also no differences in FEV1 Z scores between groups at specific ages (Table 1). Median age (15.0 months, IQR 7.0-32.0) of first documented *P.aeruginosa* infection and prevalence ( $n=11$ , 16%) of chronic *P.aeruginosa* infection was similar in cases and controls (Table 1).

There were three early (8.8%) deaths among cases (two with shock and respiratory failure, and one of unknown cause) and one (2.9%) early death in the control group, due to severe malnutrition and sepsis at the time of diagnosis (Table 2).

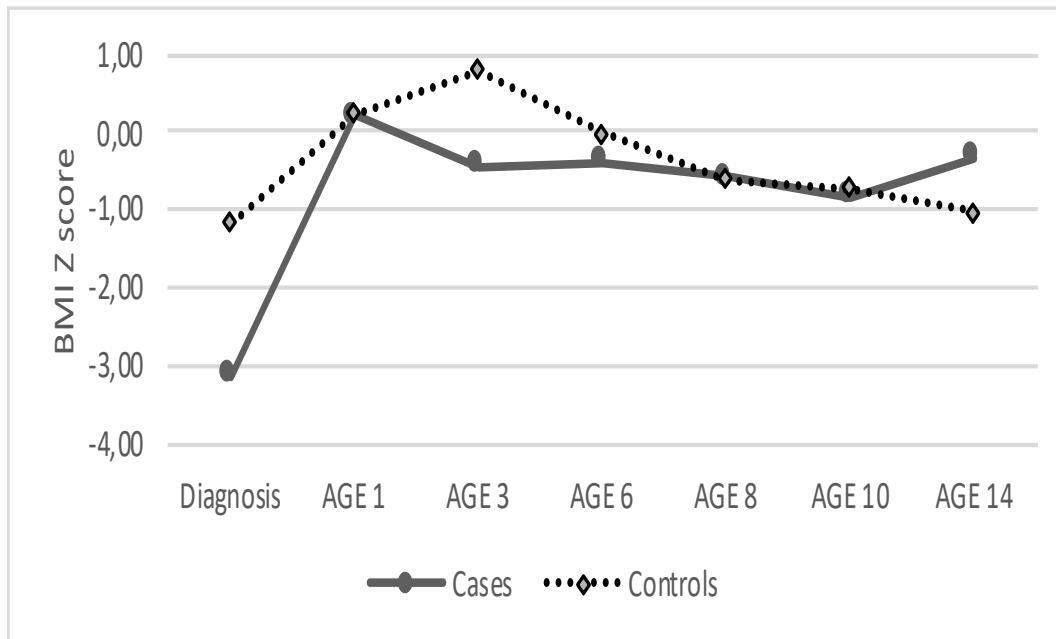


Figure 1: Changes in mean BMI Z score over the study period between cases and controls. Anova current effect  $F(7,7) = 0.14$ ;  $p = 0.99$ .

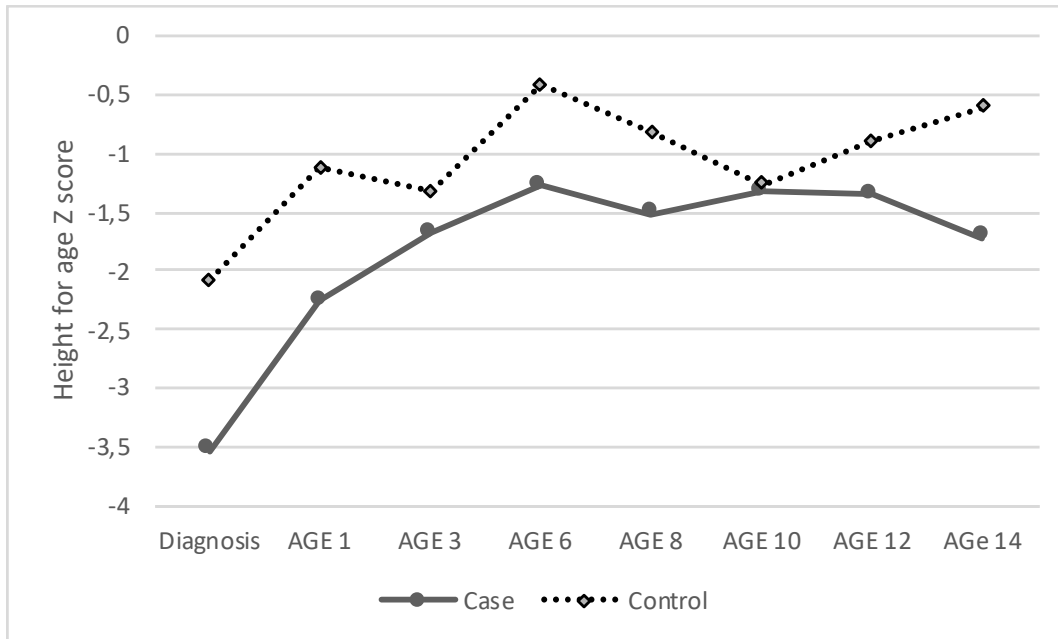


Figure 2: Change in mean HAZ throughout the study period in cases and controls. Anova current effect  $F(7, 21) = 0.79$ ;  $p = 0.6$ .

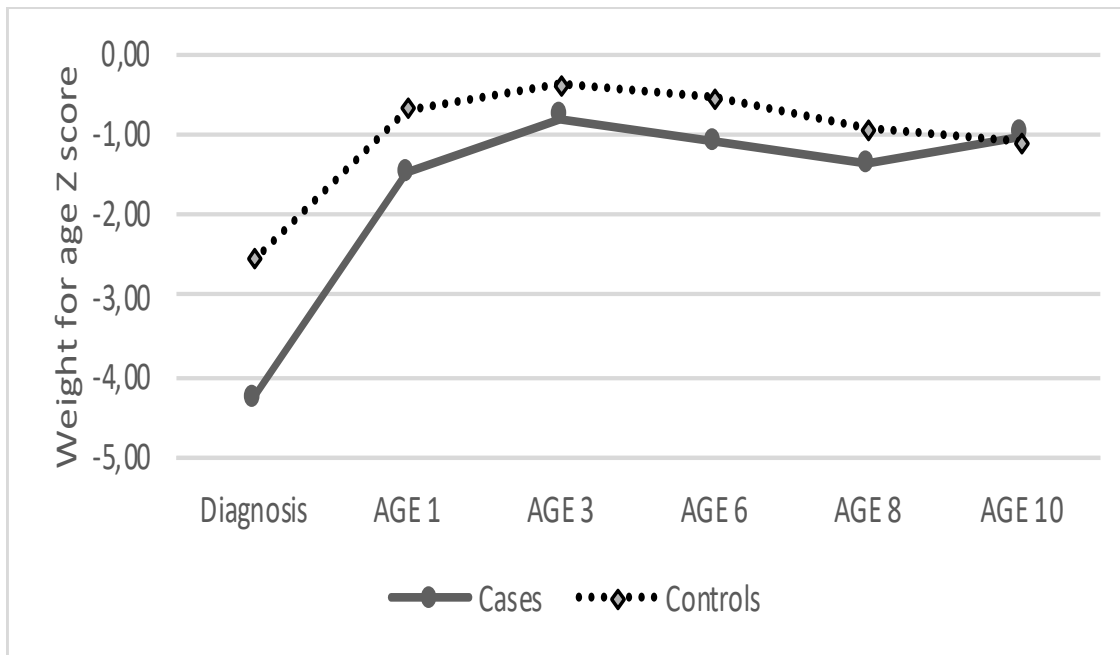


Figure 3: Change in mean WAZ from diagnosis to age 10. Anova current effect  $F(5, 30) = 0.65$ ;  $p = 0.7$ .

Table 2: Details of early mortality cases

	<b>Patient 1</b>	<b>Patient 2</b>	<b>Patient 3</b>	<b>Patient 4</b>
Case/Control	Case	Case	Case	Control
Sex	Male	Female	Male	Female
Affected Sibling	Unknown	Yes	Yes	Yes
Year of presentation	2005	2007	2009	2008
Age at presentation (months)	2	2	2	4
Age at the time of death (months)	5	2	3	5
Mode of presentation	*SAM/Recurrent Pneumonia	SAM	SAM/ Recurrent Pneumonia	Family History
Sweat chloride	120	Not done	Not done	122
Genotype	Negative		3120+1G->A homozygous	p.Phe508del homozygous
Pancreatic Status	*PI	PI	PI	PI
Airway Infections	Staphylococcus aureus Serratia	Not documented	Not documented	Staphylococcus aureus
Cause of Death	Sepsis	Sepsis	Severe pneumonia	Sepsis

SAM -Severe acute malnutrition, PI- Pancreatic insufficient

### *Discussion*

This is the largest series reported to date on CF presentation and disease course among black African children. Our findings highlight the features of CF disease among black African children for which there was previously a paucity of data. Despite some differences observed in presentation, clinical course and outcomes over time were similar to a matched cohort with p.Phe508del genotype in this non-screened population. This is important as it demonstrates comparable outcomes are achievable with appropriate care, regardless of genotype or race, even in SA which according to a World Bank report is the most unequal society in the world (30). **These findings confirm our hypothesis that the genotypes are different among cases and controls, found**

and mode of presentation maybe different. Despite these differences long term management outcomes were similar for both groups.

At diagnosis, cases were found to be more malnourished and fewer presented with neonatal bowel obstruction than controls with the p.Phe508del *CFTR* mutation. Our finding agrees with earlier reports by Masekela et al (2013) in SA (22), Prapphal,(31) and Hamosh et al, in the United States (21), that among black ethnic populations, severe malnutrition and failure to thrive is more likely to be present at diagnosis with lower incidence of meconium ileus. Although socioeconomic status (SES) was not documented, the higher prevalence of malnutrition among cases at diagnosis in our study may relate to lower SES and poverty due to social and wealth inequality gaps aligned with racial demographics that persist in SA society today (30). Lower SES and social deprivation are important determinants of poorer CF-related outcomes throughout the world(32,33). The occurrence of **three early infant deaths among cases** due to respiratory tract infection and severe malnutrition with complications may reflect a failure to recognise CF in the differential diagnosis **of children with severe malnutrition in the SA setting in the non-Caucasian population(cases)**. Although malnutrition was more severe amongst cases at diagnosis, long term nutritional outcomes in our study were comparable; suggesting that severe malnutrition at diagnosis may not have long-term impact. However, severe malnutrition in infants is an important risk factor for sepsis and may lead to early infant deaths as observed in our cohort. In the absence of newborn screening, early diagnosis, targeted management and appropriately timed interventions are key to optimising clinical outcomes of CF regardless of genotype or SES.

CF is a disease with extensive genetic heterogeneity (24). The 3120 +1G>A, a severe class I nonsense mutation(19), was the commonest *CFTR* mutation occurring among black Africans with CF (cases) in this series, both in homozygous and compound heterozygote states. This confirms previous findings that it is the most common pathogenic *CFTR* mutation in black African people with CF in the sub-Saharan Africa, with reported allele frequency ranging between 11-46% (34) of tested alleles, which is lower than found in this series (81%). We speculate **that this** higher allele frequency represents a more accurate picture than previous reports which were derived from smaller samples (7,11,22) .The 3120+1G->A mutation is believed to be a founder mutation that may have existed long ago among African populations and may have spread to many different continents through migration (35–37).**This *CFTR* mutation was initially reported by Macek (35), among African Americans and later by Carles and colleagues in SA (18)**. Our current study also identified unknown, novel or other *CFTR* mutations occurring with 3120+1G->A as compound heterozygotes: c.1585.3T>G and p.Arg792X. This suggests the existence of several undescribed *CFTR* mutations among black African populations yet to be documented. Our series also suggests that genotype confirmation of CF diagnosis is possible in the majority of black Africans in sub-Saharan Africa where, except for SA, access to CF diagnosis tests including sweat testing is not available. The parents of five children with CF were immigrants from neighbouring African countries where CF had not been previously described. This suggests that CF must occur in these countries, but affected children are either dying early or misdiagnosed with other conditions.

Our study has several important implications for future CF-related care and research in Africa. Firstly, understanding the disease spectrum and unique *CFTR* mutation profile in black Africans will guide the development of appropriate CF molecular diagnosis approaches including newborn screening strategies. Secondly, recognition that 3120+1G->A is the most prevalent *CFTR* mutation in black Africans has important treatment implications in the era of *CFTR* modulator therapies where to date no effective compound for class I nonsense *CFTR* mutations has been registered for clinical use (38,39). This has implications for the progression of disease and long-term survival outcomes for patients with this mutation. Further research in the regard is warranted.

The strength of this study lies in the number of cases recruited from the two largest paediatric CF centres in SA. The study is limited by the relatively young population, with no follow up to adulthood, and the small number of patients for whom spirometry could be documented in the long term. Another limitation is that all cases and controls were those already diagnosed with typical CF and managed at CF centres, so this series may have excluded milder disease phenotypes or children who died before CF was diagnosed and is therefore not truly reflective of the whole CF population. Environmental exposures, chronic therapies and compliance are potential confounding factors that could have affected outcomes but we could not evaluate these in this retrospective study as this information was not accurately recorded in the medical records. Next generation sequencing is not widely available in routine care in SA but may have identified additional *CFTR* variants in those without genetic confirmation of CF. Further studies are warranted to document the genotype and phenotype trajectories over time in all ethnic groups with CF in SA.

In conclusion, black African children with CF were more malnourished at the time of diagnosis, and fewer presented with neonatal bowel obstruction than patients with the p. Phe508del *CFTR* mutation. However, nutritional and pulmonary function outcomes were comparable over time. A high index of suspicion for CF amongst health workers is needed for black African infants presenting with unexplained malnutrition and other symptoms of CF disease. Continual education, improved CF awareness and improved access to CF diagnosis testing are needed in Africa.

### **Acknowledgements**

This research was supported by the African Paediatric Fellowship Programme; the University of Cape Town and the National Research Foundation (NRF) of South Africa.

**Conflicts of Interests:** None declared

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### APPENDIX 1: DATA GATHERING FORM

	ID	Date of Data Capturing __/__/__
	CF Clinic Site	<input type="checkbox"/> 0 RCWMCH <input type="checkbox"/> 1 CMH
	DOB	Hospital Number
Q1	Date of diagnosis	__/__/__
Q2	Age in months at diagnosis	
Q3	Sex	<input type="checkbox"/> 0 Male <input type="checkbox"/> 1 Female
Q4	Country of Origin (Country of origin of parents)	<input type="checkbox"/> 0 SA <input type="checkbox"/> 1 Zimbabwe <input type="checkbox"/> 2 Malawi <input type="checkbox"/> 3 Mozambique <input type="checkbox"/> 4 Botswana 5 Other: _____
Q5	Sweat (chloride) Conductivity concentration	<input type="checkbox"/> -----mmol/l <input type="checkbox"/> -----mmol/l
Q6	Genotype	<input type="checkbox"/> 0 Delta F508 homozygous <input type="checkbox"/> 1 3120+1GA homozygous <input type="checkbox"/> 2 3120+1GA /Other: _____ <input type="checkbox"/> 3 3120+1GA/unknown <input type="checkbox"/> 4 Unknown/unknown <input type="checkbox"/> 5 Other; _____
Q7	Feecal elastase levels ----- ug.ml	<input type="checkbox"/> 0 PS <input type="checkbox"/> 1 PI

Q8	Mode of Presentation	<input type="checkbox"/> 0 Neonatal bowel Obstruction <input type="checkbox"/> 1 Predominantly GIT symptoms: malnutrition <input type="checkbox"/> 2 Predominantly Respiratory symptoms <input type="checkbox"/> 3 Combined GIT and Respiratory symptoms <input type="checkbox"/> 4 Family history/newborn screen <input type="checkbox"/> 5 Other
----	----------------------	---

**Longitudinal Outcome Data (From diagnosis to 31<sup>st</sup> March 2017)**

Q9a	Visit 0 Date :	Weight (Kg)	Height(cm)	FEV1 (L) (> 6yrs)
Q9 b	Visit 1 Date:			
Q9c	Visit 2 Date:			
Q 9d	Visit 3 Date:			
Q 9e	Visit 4 Date:			
Q 9f	Visit 5 Date:			
Q 9g	Visit 6 Date:			
Q 9h	Visit 7 Date:			
Q 9i	Visit 8 Date:			
Q 9 j	Visit 9 Date:			

Q 9 k	Visit 10 Date:			
Q 9l	Visit 11 Date			
Q 9m	Visit 12 Date			
Q 9n	Visit 13 Date			
Q 9 o	Visit 14 Date			
Q 9p	Visit 15 Date			
Q9q	Visit 16 Date:			
Q9 r	Visit 17 Date			
Q9s	Visit 18 Date			
Q9t	Visit 19 Date			
Q9 u	Visit 20 Date:			

Q10a	Alive on 31 March 2017	<input type="checkbox"/> 1 Yes <input type="checkbox"/> 0 No
Q10b	If no then  Date last seen: _____	<input type="checkbox"/> 0 Died <input type="checkbox"/> 1 Transfer to another facility <input type="checkbox"/> 2 Lost to follow up.
Q10c	If patient died, state the cause of death.	
Q11a	Has patient ever had pseudomonas	<input type="checkbox"/> 1 Yes <input type="checkbox"/> 0 No
Q11b	Date of first pseudomonas infection	
Q11c	Age at first pseudomonas infection	
Q11d	Chronic pseudomonas colonization	<input type="checkbox"/> 1 Yes <input type="checkbox"/> 2 No
Q11e	Date at pseudomonas colonisation	
Q12f	Age at first pseudomonas colonization	
Q12a	Has patient ever had any staphylococcal infection	<input type="checkbox"/> 1 Yes <input type="checkbox"/> 2 No
Q12b	Date at first staphylococcal infection	
Q12c	Age at first staphylococcal infections colonization	

Q13a	Other significant infections	1 Yes 0 No
Q13b	List significant	
Q14a	Other comorbidity	1 Yes 0 No
Q14b	List Co morbidities	

## APPENDIX 2: DEPARTMENTAL REVIEWER COMMENTS

Review of Proposal:

UCT Department of Paediatrics and Child Health  
Clinical science review: QUANTITATIVE

Study Title:

**Cystic Fibrosis in ethnic African Populations in South Africa- A Retrospective case control study.**

Name of reviewer (not to be shown to researcher): **ANON**

Date submitted:

Date returned:

Please mark the appropriate column:

	Yes	Unclear	No	NA
1. <b>Abstract/Summary - is this complete?</b>	<input checked="" type="checkbox"/>			
2. <b>Background and literature review:</b> Is the setting and the current practice described? Are gaps in the literature identified? Is the problem and purpose of the study clearly stated? Is it appropriately detailed depending on the research method chosen, and does it discuss the major concepts being studied? <b>Comment:</b>	<input checked="" type="checkbox"/>			
3. <b>Objectives</b> – Are objectives clearly stated and linked to the purpose? <b>Comment:</b>	<input checked="" type="checkbox"/>			
4. <b>Methodology:</b>				
<b>a. Design</b> – Will the design answer the research question? <b>Comment: But it depends on numbers of children in each arm. Low numbers may make it difficult to judge differences. Would it not be better to to have two control patients if possible?</b>	<input checked="" type="checkbox"/>			
<b>b. Sample</b> – Are the subjects and/or documents to be reviewed well described? Are there clear inclusion and exclusion criteria? Is the sample representative and is there a rationale for sample size (eg power analysis)? <b>Comment: See above. Sample size is not a sample – it is all available patients</b>	<input checked="" type="checkbox"/>			
<b>c. Data</b> – Is there clear description of which data will be collected? By whom? Is storage and security of data clear? Is each objective linked to a specific item or set of items of data? If field/community workers are collecting data, what training will they receive? <b>Comment: The nutrition/growth parameters are inadequate. There is an opportunity to measure BMI/WFH (much better measures of the nutrition of child with CF) that should be taken. WHO has charts for young children)</b>	<input checked="" type="checkbox"/>			
<b>d. Analysis</b> – Is the statistical analysis described – (including electronic programme e.g. Excel, Stata, other; probability levels, confidence boundaries) <b>Comment:</b>	<input checked="" type="checkbox"/>			
5. Are the limitations stated, and appropriate to the study design, population and sample size? <b>Comment:</b>	<input checked="" type="checkbox"/>			

6. Is the <b>scope of the study feasible</b> within the available time and resources? <b>Comment:</b>	<input checked="" type="checkbox"/>			
7. <b>Ethical Considerations</b> – Are the ethical considerations well described? Are research subjects assured of confidentiality, anonymity and respect? Are possible adverse effects or risks associated with the audit outcomes described? <b>Comment:</b>	<input checked="" type="checkbox"/>			
8. <b>References</b> – Are all references included with the same referencing format throughout? <b>Comment:</b>	<input checked="" type="checkbox"/>			
9. <b>Appendices</b> – Consent and assent form (if appropriate)? Budget? <b>Please note that Institutional permission must be obtained before the study can be conducted</b> <b>Comment:</b>	<input checked="" type="checkbox"/>			
10. Timeline? <b>Comment:</b>				<input checked="" type="checkbox"/>
11. <b>Postgraduate Degree</b> – Is the study for postgraduate degree purposes? If so, is it appropriate for the degree level? <b>Comment:</b>				<input checked="" type="checkbox"/>

**General:**

It would be useful to have some idea of how many patients are likely to be recruited. The danger is that there will be so few that nothing will be learnt.

Recommendation: Approved   Conditional  Not approved

## APPENDIX 3: ETHIC APPROVAL, UCT



UNIVERSITY OF CAPE TOWN  
Faculty of Health Sciences  
Human Research Ethics Committee



Room E52-24 Old Main Building  
Groote Schuur Hospital  
Observatory 7925  
Telephone [021] 406 6492  
Email: [sumayah.ariel@uct.ac.za](mailto:sumayah.ariel@uct.ac.za)  
Website: [www.health.uct.ac.za/fhs/research/humanethics/forms](http://www.health.uct.ac.za/fhs/research/humanethics/forms)

04 August 2017

**HREC REF: 586/2017**

**Dr M Zampoll**  
Division of Paediatric Pulmonology  
Paediatrics & Child Health  
Red Cross Hospital  
Rondebosch

Dear Dr Zampoll

**PROJECT TITLE: CYSTIC FIBROSIS IN ETHNIC AFRICAN POPULATIONS IN SOUTH AFRICA- A RETROSPECTIVE CASE CONTROL STUDY (M.Phil.-candidate-Dr S Owusu)**

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

**Approval is granted for one year until the 30<sup>th</sup> August 2018.**

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: [www.health.uct.ac.za/fhs/research/humanethics/forms](http://www.health.uct.ac.za/fhs/research/humanethics/forms))

***We acknowledge that the student, Dr S Owusu will also be involved in this study.***

**Please quote the HREC REF in all your correspondence.**

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

signature removed

**PROFESSOR M BLOCKMAN**  
**CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE**

Federal Wide Assurance Number: FWA00001637.

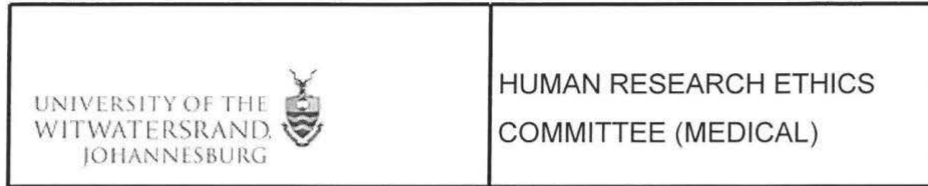
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

HREC 586/2017



## APPENDIX 4: ETHICS APPROVAL, WITS UNIVERSITY



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

**TO:** Dr D White et al  
School of Clinical Medicine  
Department of Paediatrics and Child Health  
Charlotte Maxeke Johannesburg Academic Hospital

E-mail: [Debbie.White@wits.ac.za](mailto:Debbie.White@wits.ac.za)

**CC:** Supervisor: Not applicable <>  
and <[HREC-Medical.ResearchOffice@wits.ac.za](mailto:HREC-Medical.ResearchOffice@wits.ac.za)>

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

E-mail: [Iain.Burns@wits.ac.za](mailto:Iain.Burns@wits.ac.za)

**DATE:** 13/02/2018

**REF:** R14/49

**PROTOCOL NO:** **M171149** (*This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study*)

**PROJECT TITLE:** *Cystic fibrosis in children with African ethnicity:  
a retrospective case control study*

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

signature removed

MSWorks2000/Iain0007/Clearscan.wps



**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**  
Office of the Deputy Vice-Chancellor Research & Post Graduate Affairs

**MEMORANDUM**

(This memo is not a clearance certificate)

**TO:** **Dr Debbie White et al**  
Department of Pediatrics  
E-mail: [Debbie.white@wits.ac.za](mailto:Debbie.white@wits.ac.za)

**FROM:** **Zanele Ndlovu**  
Administrative Officer: Human Research Ethics Committee (Medical)  
Tel: 011 717 2700  
E-mail: [zanele.ndlovu@wits.ac.za](mailto:zanele.ndlovu@wits.ac.za)

**DATE:** 21 December 2017

**REF:** **R14/49**

**PROTOCOL NO: M171149** (This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)

The protocol below was considered at a meeting of the Human Research Ethics Committee (Medical) on **Friday 24 November 2017**. The Committee requires the following amendments/corrections/information from you before your application can be approved.

**Project Title:** Cystic Fibrosis in children with African Ethnicity: A Retrospective Case Control Study

**Conditions: Provisionally approved subject to:**  
(This memo is not a clearance certificate – not yet cleared)

- Permission/s:
  - Providing written permission to do the study from the CEO of CMJAH.

**NB:**

1. This memo is not a clearance certificate, no research should commence prior to obtaining a clearance certificate.
2. **Please submit two hard copies of the following to the Research Office:**
  - **Covering letter:** list all the conditions above and write your response below the each condition and attach relevant documentation listed above, highlight/track any changes made.
  - **Signed declaration** confirming that data has not been collected for the study.
  - **Amendments** must be sent within **3 months** after submission. Application pending amendments on which no action has been taken by the Principal Investigator will be removed from the agenda. This will deem the application null and void; a new resubmission will be required.
  - The default in the research office is hard copies. **Email submission will not be considered.**
3. **Delivery Address for amendments:**
  - Research Office, Faculty of Health Sciences, Phillip Tobias Building, 3<sup>rd</sup> Floor, Office 301, 29 Princess of Wales Terrace, Parktown, 2193

## APPENDIX 5: AUTHOR'S GUIDELINES

AUTHOR INFORMATION PACK 19 Nov 2017 [www.elsevier.com/locate/jcf](http://www.elsevier.com/locate/jcf) 1

### JOURNAL OF CYSTIC FIBROSIS

The Official Journal of the [European Cystic Fibrosis Society](http://www.ecfs.eu)

**AUTHOR INFORMATION PACK**

#### TABLE OF CONTENTS

- Description
- Audience
- Impact Factor
- Abstracting and Indexing
- Editorial Board
- Guide for Authors

p.1

p.1

p.1

p.1

p.2

p.3

ISSN: 1569-1993

#### DESCRIPTION

The Journal of Cystic Fibrosis is the official journal of the [European Cystic Fibrosis Society](http://www.ecfs.eu). The journal is devoted to promoting the research and treatment of cystic fibrosis. To this end the journal publishes original scientific articles, editorials, case reports, short communications and other information relevant to cystic fibrosis. The journal also publishes news and articles concerning the activities and policies of the ECFS as well as those of other societies related to the ECFS.

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Pediatricians, pulmonologists, gastroenterologists, internists, microbiologists, pharmacologists, immunologists, psychologists, basic scientists, physiotherapists, dieticians and nurses dealing with the investigation and treatment of cystic fibrosis.

#### IMPACT FACTOR

2016: 4.727 © Thomson Reuters Journal Citation Reports 2017

#### ABSTRACTING AND INDEXING

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EMBASE

Science Citation Index

ScienceDirect

Scopus

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Reference to a dataset:

[dataset] [5] Oguro M, Imahiro S, Saito S, Nakashizuka T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1; 2015. <https://doi.org/10.17632/xwj98nb39r.1>.

Note shortened form for last page number. e.g., 51–9, and that for more than 6 authors the first 6 should be listed followed by 'et al.' For further details you are referred to 'Uniform Requirements for Manuscripts submitted to Biomedical Journals' (*J Am Med Assoc* 1997;277:927–34) (see also [Samples of Formatted References](#)).

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Online Journal, "Cystic Fibrosis Research News" will be launched in early 2015. This journal will compile lay summaries of full length articles and short communication style papers at the time of acceptance of a paper in JCF. The initiative aims to foster enhanced knowledge by patients, their families and other members of the lay community about research advances published in JCF.

The Journal will be published in English and be made available on the ECFS website ([www.ecfs.eu](http://www.ecfs.eu)) and will be open access. All authors will receive an email at the time of acceptance to highlight this requirement before an accepted paper can be published in JCF. The inaugural Editor in Chief is Dr

Harry Heijerman, previous Editor in Chief of JCF. He has assembled a skilled Editorial Board including members of the CF health care and scientific communities and members of the lay community.

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## APPENDIX 6: REVIEWER COMMENTS

### REVIEWER COMMENTS

Reviewer #1: Cystic fibrosis is a life-limiting, multisystemic, autosomal recessive disorder. Its incidence varies and is higher in Caucasian communities of Northern European ancestry. More than 2000 variants of the Cystic Fibrosis Transmembrane Regulator (CFTR) gene have been reported so far, and the variant F508del remains the most common. Cystic fibrosis (CF) in black African population is less frequently encountered and has been poorly described. In this paper, the authors compared a cohort of CF black African children with matched controls carrying the variant F508del to determine differences in clinical presentation and outcomes in black African children diagnosed with CF.

Below are some suggestions for the authors to consider.

Major comments:

1. The objectives of the study were not clearly stated in a dedicated section (both in the abstract and core text). Although the aim of the study is shortly mentioned in the introduction, the authors could consider including a specific section describing the primary and secondary objectives in more detail. What aspects of the clinical presentation are they exactly looking for? Which outcomes will be compared between the two groups (e.g. lung function decline, BMI, exacerbation rates, mortality)? Did the authors postulate hypotheses about the differences they were expecting between the cases and the controls?

**Response: Thank you for these suggestions. We have revised the last paragraph of Introduction to reflect the aims and hypothesis of this study as suggested: “The primary aim of this study was therefore to describe and compare the genotype, clinical presentation, pulmonary function and nutritional outcomes in black African children diagnosed with CF in SA to matched cases of children in South Africa with. p.Phe508del genotype. We hypothesized that genotype is different but presentation and clinical outcomes would be similar”.**

2. The authors mentioned that children heterozygous with p.Phe508del mutation and another mutation were selected as controls if no suitable p.Phe508del homozygous control was identified. In these cases, was the second mutation a mutation with residual function or not? If so, can the authors comment on the potential impact on the results by choosing these controls?

**Response: We did not include the details of the “Other” mutations in p.Phe508del heterozygotes as we believed the similar proportion of pancreatic sufficient cases in each group (88.2% cases, 85.3% controls) demonstrates sufficient evidence of equal prevalence of mutations with residual function in each group. For completeness we now have listed all the other mutations in control group too, table 1.**

3. Comorbid conditions listed in Table 1 include health issues that are not necessarily associated with CF (ADHD, HIV, etc.). It might have been interesting to include other comorbidities related to CF such as rhinosinusitis, diabetes or distal intestinal obstruction syndrome (DIOS) to determine if their prevalence seems to be different between the two groups.

**Response: We included these non-CF related comorbidities to highlight these socioeconomic and other factors in our setting that could potentially have influenced CF-related outcomes. There were no cases of diabetes or DIOS in this cohort. Rhinosinusitis is a very common symptom in all children in SA and therefore not commonly recorded in the CF medical records.**

4. FEV1 is typically expressed as a percent predicted by comparing the person's value in litres to a healthy reference population taking into consideration age, height, race, and sex. A less common method is to report Z-scores (number of standard deviations from the mean value). Can the authors comment about why they have not presented the FEV1 data as % predicted and chose to use Z-scores instead?

**Response: We recognise that FEV1 has been conventionally reported as percent predicted. Global Lung Initiative (GLI) ethnic-specific reference equations and expression of LF as z-scores, however, are preferred for two reasons: 1) there was no normative spirometry data for black African children prior to GLI. Historical reference equations for “African-Americans” are unsuitable for African children and result in under estimation of lung function; 2) Expression of lung function in standard deviations is considered by GLI more appropriate as it removes any age-related bias and is a better representation of normative LF distribution. We refer the reviewer to recent commentary on this matter which supports our view that z-scores are preferable to percent predicted:**

<https://breathe.ersjournals.com/content/9/6/462>.

**Not sure whether to present % predicted values- my sense is will be same?**

5. Significant differences in nutritional status based on height, weight and BMI Z-scores were reported between the two groups. Although statistically significant, do the authors consider these differences clinically significant? It would be useful to include some discussion in the manuscript about the clinical importance of this difference, if there is one.

**Response: We do consider these differences as clinically significant as malnutrition in infancy predisposes to severe infection and sepsis. Although numbers are too small for meaningful analysis, it is important to note that all CF-related deaths reported in this cohort were sepsis-related and occurred at or soon after the time of CF diagnosis. An important contributing factor was likely to have been severe malnutrition. We have added a comment about this in the second paragraph in Discussion: “However, severe malnutrition in infants is an important risk factor for sepsis and may lead to early infant deaths as observed in our cohort. In the absence of newborn screening, early diagnosis, targeted management and appropriately timed interventions are key to optimising clinical outcomes of CF, regardless of genotype or SES.”**

6. The authors mentioned some limitations of their study and commented about the potential impact of low socioeconomic status on their results. Did they identify other possible confounding factors (e.g. environmental exposures, chronic lung therapies, compliance, etc.)? Could they comment about selection bias, confusion bias and missing data?

**Response: We thank the reviewer for highlighting these additional limitations. Although environmental exposures, chronic therapies and compliance are potential confounding factors, we could not evaluate these in this retrospective study because this information was not accurately recorded in the medical records. We believe the cases accurately represent all black African children diagnosed with CF at the two centres over the study period as both CF clinics keep comprehensive historical medical records. However, this data will have excluded any children who died before CF was diagnosed or considered as a cause of illness or malnutrition. A higher case: control ratio (e.g. 1:2) would have been preferable and eliminated any selection bias in controls but we were limited by the small number of matched controls in our setting. We have highlighted these additional limitations in the Discussion.**



7. Some ideas and discussion points are uneasy to grasp and fully understand as the phrasing is sometimes difficult to follow or words seem to be missing. For example: "Firstly, understanding the disease spectrum and unique CFTR mutation profile in black Africans will guide the development (of?) future diagnosis strategies including newborn screening approaches."

**Response: We have rephrased this statement as follows: "Firstly, understanding the disease spectrum and unique CFTR mutation profile in black Africans will guide the development of appropriate CF molecular diagnosis approaches including newborn screening strategies."**

Minor comments:

1. The authors may consider to uniformize the format better. For example, they should probably use the same nomenclature for the p.Phe508del mutation; it varies in the text and both p.Phe508del and phe508del are used intermittently. Titles, punctuation and spaces between words/sentences should also be uniformized.

**Response: Thank you for pointing out these inconsistencies. We have addressed and corrected this in the manuscript. Where possible, we have used the protein sequence nomenclature or legacy name (where protein sequence not reported)- which is more familiar to most readers.**

2. It would have been interesting to include a table summarizing the FEV1 Z-scores instead of only describing a portion of them in the text.

**Brenda- could we maybe add this? Not sure if permissible to add another table?**

3. Figure 1-3: When printed in black and white, the distinction between the lines is impossible to perceive in the figures.

**Can we replace the colours with other data point markers along the lines?**

4. The section title "conclusion" should be presented as the others, in bold and numbered.

**Response: We have reformatted all headings and sub-headings without numbering now.**

Reviewer #2: This is an interesting and important report proving that African cases with CF may have the same quality of life and life expectancy when properly treated and diagnosed early. The authors also disputed previous reports on the "worse" course of CF in Africans, based on small and/or clinically heterogeneous cohorts.

Generally the paper is well written, but several additional issues need to be addressed:

1) the paper did not specify genetic testing and which variants were previously tested?

**Response: genetic testing available in South Africa for routine diagnostic use over the study period varied from single variant PCR (p.Phe508del) as first step, to the Elucigene CF29v2 kit which includes 29 common variants including c.2988+1G>A. In a few cases clinicians were able to do next generation sequencing through private funding or pro-bono offers by private laboratories. We have now included a sentence in Methods describing the genetic testing. Where possible, we have used the protein sequence nomenclature or legacy name (where protein sequence not reported)- which is more familiar to most readers.**

2) proper variant nomenclature should be used and indicated according to CFTR1 database

**Response: Thank you for highlighting this oversight. We have revised all variant nomenclature according to current CFTR database nomenclature where this was provided.**

3) additional CFTR variants should be discussed in terms of their clinical annotation in CFTR2.org, is there any differences, naturally where applicable?

**Response: we have now listed all the other variants in table 1 and highlighted with a \* those not reported (1) or variants with unknown significance (2).**

4) the authors did not discuss whether consanguinity was present in examined 3120+1 G>A homozygotes.

**Response: there was no consanguinity in this cohort. Consanguinity is extremely rare in the local population.**

5) sweat chloride concentrations / it is not clear how these were established (conductance versus pilocarpine iontophoresis)?

**Response: The two CF centres used different sweat collection and analysis techniques. Both techniques however stimulate sweat glands with pilocarpine iontophoresis. The centre in Cape Town performs Gibson and Cooke collection method with sweat chloride analysis while the centre in Johannesburg collect and analyse sweat using the Macroduct (Sweatcheck) Conductance analyser. This centre does not have facilities to measure sweat chloride in the collected sweat sample and therefore conductance (> 80 mmol/L) was considered diagnostic for CF as per recommended guidelines.**

6) how *P. aeruginosa* bronchial colonisation was established?

**Response: respiratory samples are routinely collected at clinic visits and submitted for CF pathogen cultures. We adopted and cited (ref no 22) the Modified Leeds criteria to define colonisation.**

7) how do the authors explain family history of previous CF-related deaths in given families (e.g. due to lower socioeconomic status, neglect)?

**Response: This particular family did not return for genetic counselling after the death of the first sibling and were untraceable until the second child presented. Poor SES and limited insight into genetic diseases as well as untraceable contact numbers were likely factors.**