Phenotypic expression of the 3120+1G>A mutation in non-Caucasian children with cystic fibrosis in South Africa

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Abstract

Introduction: Cystic fibrosis (CF) is the most common genetic disorder in Caucasians. Presentation of CF in non-Caucasians is less well studied.

Objective: This audit was undertaken to determine the phenotypic expression of the 3120+1G>A mutation in black and mixed race children in South Africa.

Method: A multi-centre retrospective chart review of clinical, laboratory and spirometry data of non-Caucasian CF patients in four CF centres in South Africa was collected. Data was collected at diagnosis and after a five-year follow up period. Ethical approval was granted for the study.

Results: A total of 30 participants were enrolled of whom 14 (47%) were homozygous and 16 (53%) heterozygous for the 3120+1G>A mutation. The mean age of diagnosis was 13 months. Twenty-four (80%) patients had malnutrition (mean weight z-score -3.6) or failure to thrive (77%) at presentation. Twenty (67%) presented with non-specific abdominal symptoms, whilst fifteen (50%) had recurrent respiratory tract infections. *Pseudomonas aeruginosa* was first detected at a mean age of 21 months. The mean FEV_1 was 73% predicted (95% CI 54.0 - 91.1) at study entry and 68% predicted (95% CI 49.74 - 87.06) at follow up.

Conclusion: Failure to thrive and protein energy malnutrition are the common presenting features of CF in children with the 3120+1G>A mutation. Meconium ileus is a rare presenting feature of CF in black and mixed race children in South Africa.

Introduction

CF is a common severe autosomal recessive disorder in the Caucasian population with an incidence of 1 in 2000 live births. CF occurs as a result of mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene found on the long arm of chromosome 7, first described by Lap-Chee Tsui and colleagues in Toronto [1-3]. Over 1400 mutations have been identified, with p.F508del being the most common mutation in Caucasians [4]. In the South African Caucasian population, p.F508del accounts for up to 81% of all CF alleles [5]. The p.F508del mutation is less frequent in the South African mixed-race (53%) and black African populations where it's rarely detected.

The 3120+1G>A *CFTR* mutation was first reported in three African-Americans CF patients by Macek et al., and has subsequently been shown to account for 9-14% of African-American CF mutations [6]. Analysis of the *CFTR* gene and its expression in African-American CF patients has shown a significantly different profile from that observed in Caucasian CF patients [6]. Previous studies comparing the phenotypic presentation of black and white patients with CF have shown some differences in clinical presentation and morbidity related to CF. Differences include a higher incidence of malnutrition, severe lung disease and a higher rate of meconium ileus in black patients [7-9], with one case series describing the phenotype associated with the 3120+1G>A mutation as presenting with mild to moderate symptoms [10].

In South Africa, the most common CF disease causing mutation in the black South Africans is the 3120+1G>A, which is detected in 46% of all CF alleles, with a carrier rate of 1 in 90 [11]. With this carrier rate and the presence of other mutations, over 1000 black African babies with CF are estimated to be born each year in South Africa [9]. A study conducted in Cape Town, revealed that the 3120+1G>A was the second most common mutation in the mixed race children, after the p.F508del mutation [12].

Until recently, the spectrum of disease in the mixed race and black African population has remained poorly understood. Recognition of CF in an African context is difficult due to the overwhelming burden and high prevalence of poverty-associated conditions with similar presentations namely protein energy malnutrition (PEM), human immunodeficiency virus infection (HIV) and tuberculosis. Therefore, diagnosis of CF and institution of therapy may be delayed, impacting negatively on the outcome.

 We therefore undertook this study with the primary aim being to assess the phenotypic expression of the 3120+1G>A mutation in black and mixed race children with CF in South Africa. A secondary objective was to assess the age of diagnosis and the overall morbidity of children with the 3120+1G>A mutation.

Patients and Methods

Study population

A retrospective chart review of black and mixed race CF patients at four CF centres was conducted, namely, the Paediatric Cystic Fibrosis Clinics at Steve Biko Academic Hospital (SBAH), Pretoria; the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH),

Johannesburg; the Tygerberg Hospital (TBH), Cape Town and the Red Cross War Memorial Children's Hospital (RCWMCH), Cape Town. Subjects were included in the study if there was laboratory confirmation of CF with presence of at least on copy of the 3120+G>A mutation by chromosomal analysis.

Clinical investigations

Clinical data collected included: age at diagnosis, weight, height, body mass index (BMI) {World Health Organization defined z-scores for weight, height and BMI} [13]. All data was collected at diagnosis and after a five year follow up period.

Laboratory investigations

Data collected included: screening sweat chloride conductivity (Nanoduct^{TM} Neonatal Sweat Analysis System, Wescor, Inc., South Logan, UT, USA) and faecal elastase (ELISA kit (ScheBo® Pancreatic Elastase 1 Stool Test). Results of sputum microbiology were collected. The colonisation status of the airway pathogens identified was also noted. Colonisation was defined as the persistence of a pathogen on two or more sputum samples over a period of six months as defined in the Leeds Criteria for chronic infection [14].

Pulmonary function parameters for all subjects over the age of six years were collected for forced expiratory flow in one second (FEV_1) and forced vital capacity (FVC) [ViasysSpiroPro Jaeger Spirometer Cardinal Health, Hoechberg, Germany]

Ethical clearance

Ethic approval to access the patient records was obtained from the Research Ethics committees of the University of Pretoria, Faculty of Health sciences Witwatersrand University and Research Ethics Committee of the University of Cape Town.

Results

A total of 30 patients of whom 20 (67%) were black Africans were included in the study. There were 53% males. Of the total number of subjects the patient distribution per site was: 47% (RCWMCH), 27% (SBAH), 20% (CMJAH) and 6% (TBH). Fourteen (47%) of the participants were homozygous for the 3120+G>A mutation, whilst sixteen (53%) were heterozygous. The baseline characteristics of the participants are summarised in Table 1.The mean age at diagnosis was13 months (95% CI 6.57; 19.42). At presentation the

subjects were stunted and severely underweight with height and weight z-scores of (-2.61; 95% CI -3.89; -1.33) and (-3.60; 95% CI -4.60; -2.60], respectively (Table 2).

The majority of participants (97%) were pancreatic insufficient at diagnosis. In twenty-three (77%) participants the original diagnosis at presentation was protein energy malnutrition. Fifteen patients (50%) already had respiratory symptoms at diagnosis. Pulmonary function tests were available in thirteen subjects (43%) with a mean FEV_1 of 72.5 % predicted (95% CI 53.9;   91.1) at baseline. *Staphylococcus aureus* (40%) and *Pseudomonas aeruginosa (Pa)* (60%) were the most common pathogens colonising the airway. The mean age of first identification of *Staphylococcus aureus* and *Pa* was 17 months and 21 months of age, respectively. Gastrointestinal complications were identified at presentation in 26.7% with liver cholestasis present in 10% of participants.

Table 1: Baseline data of black and mixed race children with cystic fibrosis with a 3120+1G>A mutation.

Variable	Frequency	Percentage (%)
Gender (M/F)	16	53
Mean age diagnosis (months)	13	
Ethnic group		
Mixed race	10	33
Black African	20	67
Mutation		
Homozygous 3120+1G>A	14	47
Heterozygous 3120+G>A	16	53
Clinical presentation		
Respiratory tract infection	15	50
Failure to thrive	24	80
PEM	23	77
Abdominal symptoms	20	67
Other	9	30
Pancreatic insufficiency	29	97

PEM: Protein energy malnutrition; Abdominal symptoms: included meconium ileus, rectal prolapse and chronic diarrhoea.

On 5 year follow up, six (20%) of the participants had died and there were twenty-three survivors (77%) (Table 3). Of the 6 patients who died, three demised from respiratory failure (two colonised with *Pa* and one *Staph aureus*), one each from overwhelming sepsis, liver

failure and hypovolemic shock from gastroenteritis. In one patient outcome was unknown as the patient was lost to follow up.

Table 2: Baseline clinical parameters of children with a 3120+1G>A cystic fibrosis at diagnosis and follow up.

Variable	Presentation (mean; 95% CI)	Follow up (mean; 95% CI)
Age (months)	13.0 (6.6; 19.4)	69.2 (44.9; 93.5)
WAZ	-3.6 (-4.6; -2.6)	-1.2 (-2.5; 0.6)
HAZ	-2.6 (-3.9; -1.3)	-2.4 (-3.3; -1.6)
BMI	12.94 (11.9; 14.0)	14.7 (13.1; 16.3)
FEV_1 % predicted	72.5 (54.0; 91.1)	68.4 (49.7; 87.1)

HAZ: height for age z-score; WAZ: weight for age z-score; BMI: body mass index; FEV_1 : Forced expiratory flow in one second.

On follow up, there was improvement in all the growth parameters (Table 2). Lung function decline in those with Pa was more severe than in those without Pa; FEV_1 decline of 15% versus 4%, respectively, over the five year follow up period. One patient developed CF-related diabetes, this patient was a compound heterozygote with the 3120+1G>A/ p.F508del mutation.

Table 3: The follow up of black and mixed-race children with cystic fibrosis

	Frequency	Percentage (%)
Respiratory cultures		
Pseudomonas aeruginosa	13	43
Staph Aureus	13	43
Complications		
Respiratory	13	46
Abdominal	8	27
Liver dysfunction	3	10
Poor growth	3	10
Outcome		
Alive	23	77
Dead	6	20
Unknown	1	3

The most common complications at follow up were respiratory in nature (46%) with recurrent chest infections defined as more than 4 exacerbations per year for the study period. Abdominal complications were the second most common (27%), which included distal

intestinal obstruction, meconium ileus equivalent and rectal prolapse. Only 10% of subjects developed liver cholestasis and had poor growth on follow up.

Discussion

There is a wide range of phenotypic presentations in CF with striking differences between black and white patients, mostly being noted in gastrointestinal manifestations and nutritional status [15]. Previous studies have suggested that the 3120+1G>A is a milder mutation with more abdominal symptomatology, especially meconium ileus. This mutation has been more commonly observed among black patients, reflecting the lower prevalence of p.F508del in that group [15]. In the current study, we have shown that the mean age of diagnosis of the study population was over the age on one year. In the majority of patients failure to thrive and protein energy malnutrition were the most common presenting features. Abdominal symptoms were a presenting feature in only a third of the study population. Moreover, the acquisition of Pa colonisation was within the first two years of life. Almost all the participants had pancreatic insufficiency at presentation.

There is a delay in the diagnosis of children with CF in our population. This is in contra-diction to African-American children where the mean age of diagnosis is eight months [8]. This can be attributed to the lack of awareness of CF (which believed to be rare in this population group), poor access to medical care and missed diagnosis as malnutrition secondary to conditions of poverty .The classic triad for a CF diagnosis is recurrent or persistent respiratory symptoms, pancreatic insufficiency and poor weight gain [16]. A previous study of 181 CF patients in South Africa showed that only 4.6% of patients presented with all 3 features thus limiting its value in this context [17]. This may account for the delay in diagnosis in the current cohort. Another diagnosis limiting factor is that in order for CF to be confirmed, identification of the $CFTR$ gene mutations is necessary, and in the current mutational analysis panel used in South African, only 76% and 46% of mutations are detected in the mixed race and black African CF, respectively [17]. Genome wide testing is also unavailable to the majority of patients due to cost restraints. This implies that a significant number of patients are missed due to inability to identify 2 disease-causing $CFTR$ mutations [18].

Pulmonary disease is the main predictor of morbidity and mortality in CF [19]. Pulmonary function parameters, particularly FEV_1 have been shown to be a reliable outcome measure in most studies and is used as a predictor of lung disease progression and mortality [19]. Van den Branden et al, demonstrated that nutrition remained a strong predictor for accelerated decline in FEV_1 [20]. Morrow et al, in a longitudinal 8 year follow up of children in

Cape Town, demonstrated a 20% improvement in the median pulmonary function scores over the follow up period [21]. They ascribed this to the improved multidisciplinary team approach in the management of CF patients. The current study revealed a decline of FEV_1 of between 3%/year in those with *Pa* compared to 0.8%/year in those not colonised with *Pa*. This decline is slightly higher than that reported by Morrow *et al*, where the rate of FEV_1 decline was 0.43%/year [21]. These results indicate that 3120+1G>A mutation may confer more severe lung disease when compared to a cohort of whom the majority largely had the p.F508del mutation

The strength of this study is that all possible children with the 3120+1G>A mutation in South Africa were included and should serve as a study of the phenotypic presentation and longitudinal follow up of non-Caucasian children with the 3120+1G>A mutation. The study has a number of limitations, one being the small number of participants. There were also few participants who provided pulmonary function data.

In summary, failure to thrive and protein energy malnutrition are the commonest presenting features of CF in black and mixed race South African children with the 3120+1G>A mutation Increased awareness of CF in non-Caucasian African children is therefore necessary. Clinicians should consider CF when investigating children who present with unexplained malnutrition in this population group.

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