The 22q11.2 Deletion Syndrome in Congenital Heart Defects

Prevalence of Microdeletion Syndrome in Cameroon

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ABSTRACT

Background: The 22q11.2 deletion syndrome is amongst the most common microdeletion syndrome in humans. Its prevalence remains unknown in sub-Saharan Africa, and its clinical features are underreported for people of African descent.

Objective: We have investigated the prevalence of the 22q11.2 deletion syndrome in patients with congenital heart defects in Cameroon.

Methods: A total of 70 of 100 cases of congenital cardiac malformation with echocardiographic evidence were examined prospectively and tested for the 22q11.2 deletion, using multiplex ligation-dependent probe amplification and fluorescence in situ hybridization.

Results: Two of 70 patients (2.8%) were found to have 22q11.2 deletion. Both cases had conotruncal heart defects and exhibited extracardiac features of the 22q11.2 deletion syndrome that were either classical (e.g., puffy upper eyelids, bulbous tip of the nose) or less identifiable (telecanthus, hooding of eyelids and prominent nasal bridge).

Conclusions: The report shows that the prevalence of the 22q11.2 deletion syndrome in patients with heart malformations in Cameroon (2.8%) is similar to that of various world populations. The clinical phenotypes will contribute to the Global Atlas for dysmorphology. "Omics" technologies offer much promise in genetic/genomic screening of severe global health problems.

Congenital heart defects (CHDs) are the most common group of birth defects in humans, accounting for about one-third of all major congenital anomalies [1]. They occur in about 1% of all live births irrespective of ethnic backgrounds, socioeconomic conditions, and geographic barriers [2-4]. Etiologies of CHD are multiple, typically categorized in genetic and nongenetic factors. Nongenetic factors include teratogenic exposures during pregnancy [5] and perhaps epigenetic alterations, as supported by recent studies [6]. Identifiable genetic etiologies are reported to be as high as ~40% in syndromic CHD, including single gene disorders, chromosomal anomalies, and copy number variations [1,7].

The 22q11.2 deletion syndrome is the most frequent microdeletion syndrome, and is one of the most common genetic causes of CHD, responsible for 1.5% to 5% of all CHD at birth [8-10]. Its clinical presentation is extremely variable. Affected individuals present with a wide range of features, including CHD (~76%), most commonly conotruncal defects (tetralogy of Fallot in 16% to 20%, ventricular septal defect in 14% to 25.5%, interrupted aortic arch type B in 13% to 52%, and truncus arteriosus in 6% to 35%), palatal abnormalities (~69%), immune deficiency (~77%), learning difficulties (70% to 90%), hypocalcemia (~50%), renal anomalies (~31%), characteristic craniofacial dysmorphism, and other features [8,11,12]. Autism spectrum disorder is reported in ~20% of children and psychiatric disorders, in particular Schizophrenia is present in ~25% of adults [13].

The 22q11.2 deletion syndrome is caused by a recurrent 1.5- to 3-Mb microdeletion of chromosome 22, within the q11.2 region (DiGeorge chromosomal region). The deletion is thought to be caused by nonallelic homologous recombination, due to the presence of low copy repeats that flank the region and predispose to ectopic recombination during meiosis [14]. The majority of affected individuals (85%) have a typical 3-Mb deletion, encompassing approximately 30 contiguous genes. Approximately 15% of affected individuals have small atypical deletions (~1.5 Mb). Both typical and atypical deletions include the *TBX1* gene. *TBX1* haploinsufficiency is thought to be responsible for a number of features of the condition, in particular, conotruncal heart defects [15,16]. Complex chromosomal rearrangements involving 22q11.2

The authors report no relationships that could be construed as a conflict of interest.

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GLOBAL HEART

VOL. 12, NO. 2, 2017
ISSN 2211-8160/$36.00.
http://dx.doi.org/10.1016/j.gheart.2017.01.003

TABLE 1. Clinical features that should lead to the consideration of cytogenetic analysis for detection of 22q11.2

Presence of 1 of the following	Presence of 2 or more of the following core features	Presence of 1 core feature plus 1 of these associated features
Conotruncal cardiac anomaly such as tetralogy of Fallot, interrupted aortic arch, truncus arteriosus, or major aortopulmonary collateral arteries	Characteristic facial abnormalities: broad bulbous nose, square-shaped tip of nose, short philtrum, telecanthus, low-set ears, and so on	Long slender fingers and hands
Parent of an affected child	Nonconotruncal congenital cardiac defect	Short stature
	Learning difficulties/developmental delay	Hypotonia
	Cleft palate, velopharyngeal insufficiency, or swallowing difficulty	Renal abnormalities or Potter sequence
	Hypocalcaemia	Psychiatric (especially bipolar) disorders
	Immunodeficiency or thymic hypoplasia	Family history of congenital heart defects

Adapted from Tobias et al. [18].

are found in <1% of patients with typical features, and point mutations in *TBX1* have been proposed as a rare cause of the condition [16,17].

The condition is sporadic in about 93% of cases. In the remaining 7% of individuals, the deletion is inherited in an autosomal dominant manner from a parent [15]. The diagnosis of the 22q11.2 deletion syndrome is made on the combination of suggestive clinical features and detection of a deletion within the DiGeorge chromosomal region [17,18].

The disease prevalence and clinical presentation varies between ethnic groups worldwide [9,15], but remain largely unknown in sub-Saharan Africa. We report here on the prevalence and clinical features of the 22q11.2 deletion in patients with CHD in Cameroon.

MATERIALS AND METHODS

Ethical approval

The study was approved by the institutional Human Ethics Committee of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1. Written informed consent was obtained from the parents of patients for publication of the cases and accompanying images and photos.

Patients

From December 2010 to March 2012, patients were referred from the various regions of Cameroon with structural congenital heart defects. Cardiac echography was performed by a pediatric cardiologist, and all patients were prospectively reviewed and examined by a medical geneticist at the Medical Genetics Unit of the Yaoundé Gynaeco-Obstetric and Pediatric Hospital. All patients presented their echocardiography report and underwent clinical genetics evaluation as per guidelines of Tobias et al. [18] (Table 1), and relevant morphological features were recorded.

Cytogenetic and molecular analysis

Genomic deoxyribonucleic acid samples were extracted from peripheral blood, following instructions from the commercial kit (Puregene blood kit, Qiagen, Germantown, Maryland), at the molecular diagnostic laboratory of Yaoundé Gynaeco-Obstetric and Pediatric Hospital. Heparinized lymphocytes from peripheral venous blood (0.5 to 1.0 ml each) of patients were used for the cytogenetic studies in accordance with standard procedures. G-banding karyotype at the resolution of 400 to 550 bands was performed in 70 individuals. For 70 patients, multiplex ligation-dependent probe amplification was used for the detection of the 22q11.2 deletion, using MRC—Holland P250-A1 and -B1 kits (Amsterdam, the Netherlands). Fragment Profile software and Excel software (Microsoft, Redmond, Washington) using specific macros by MRC-Holland were used for the visualization and result analysis. Positive results for the deletion (Fig. 1A) were then confirmed/replicated by fluorescence in situ hybridization, using DiGeorge (Tuple) Region Probe Dual Colour (Qbiogene, Carlsbad, California), performed according to the procedure established by the manufacturer; this probe is specific to the *HIRA (TUPLE1, DGCR1)* region at 22q11. The hybridization signals were documented with a Zeiss-Axiophot fluorescent microscope (Zeiss, Oberkochen, Germany) and analyzed with CytoVision 3.52 software (Leica Biosystems Inc., Buffalo Grove, Illinois) (Fig. 1B). Cytogenetic and molecular analysis were performed at the Cytogenetic Laboratory of the Geneva University Hospitals.

RESULTS

Prevalence of 22q11.2 deletion syndrome among Cameroonian children with CHD

A total of 100 consecutive patients with structural congenital heart defects were enrolled in the study. The mean age of the study population was 3.7 years (range 1 month to 25 years). There were 56 male and 44 female

patients. Twenty-eight patients had conotruncal heart defect, and 72 had nonconotruncal heart defects (Table 2).

Among 100 cases, 70 patients underwent cytogenetic and molecular analysis: 20 cases of conotruncal heart defects and 50 cases of nonconotruncal heart defect. No genetic abnormality was observed in the 65 parents of patients analyzed. Of 70 patients, 2 patients (2.8%) had the typical 3-Mb hemizygous 22q11.2 deletion (Table 2, Fig. 1). For both patients, GTG-banded metaphases from peripheral blood lymphocyte cultures with a resolution of 400 to 550 bands showed normal 46, XY karyotypes.

Craniofacial features in 2 African children with 22q11.2 deletion syndrome

Case 1 was a 5-year-old boy showing prominent forehead, sparse eyebrows, puffy upper eyelids, low set ears with over folded upper helix, depressed and wide nasal bridge, bulbous tip of the nose, anteverted nares, long philtrum, and retrognathia (Figs. 2A and 2B). Case 2 was a 2.5-year-old boy showing a broad face, puffy upper eyelids, epicanthic folds, depressed and wide nasal bridge, broad bulbous tip of the nose, and retrognathia (Figs. 2C and 2D). Both patients presented with learning difficulties, with palatal insufficiency, and without cleft, characterized by a nasal speech. In both cases, no renal anomalies or hypocalcemia were found.

DISCUSSION

To the best of our knowledge, a single other report exists in sub-Saharan Africa on the prevalence of the 22q11.2 deletion syndrome in individuals with CHD, largely of mixed ancestry from Cape Town, South Africa, and has revealed a prevalence of 4.8% [19]. Several studies aiming at elucidating the prevalence and clinical characteristics of affected individuals with 22q11.2 deletion have been conducted in other population groups, in particular from well-resourced countries [9,11]. The paucity of information about affected individuals of African ancestry may be explained by the limited access to diagnostic facilities and health care in low- and middle-income countries, and perhaps the lack of formal medical genetic services in many sub-Saharan African countries. The present report has been made possible through the recent establishment of a medical genetic service in Cameroon [20].

The 22q11.2 deletion syndrome shows wide variability in penetrance and expressivity, hence cases differ from each other even in identical twins [21,22]. Characteristic clinical features include CHD, in particular conotruncal heart defects and specific craniofacial dysmorphism [17,18]. McDonald-McGinn et al. [23] have demonstrated that characteristic craniofacial features in this condition are not equally specific in all population groups. In fact, facial features are present in the majority of affected Caucasians, especially individuals of northern European descent, but offer no hint to the diagnosis in the majority of affected individuals of African-American descent [23]. Unlike other continents, very few African studies exist on the clinical phenotype of 22q11.2 deletion syndrome in individuals of African descent [24,25]. The 2 cases reported here presented some classical craniofacial features of the 22q11.2 deletion syndrome, such as puffy upper eyelids, ears anomalies, bulbous tip of the nose, and retrognathia (Fig. 2), whereas some other features were less identifiable, such as telecanthus, hooding of eyelids, and prominent

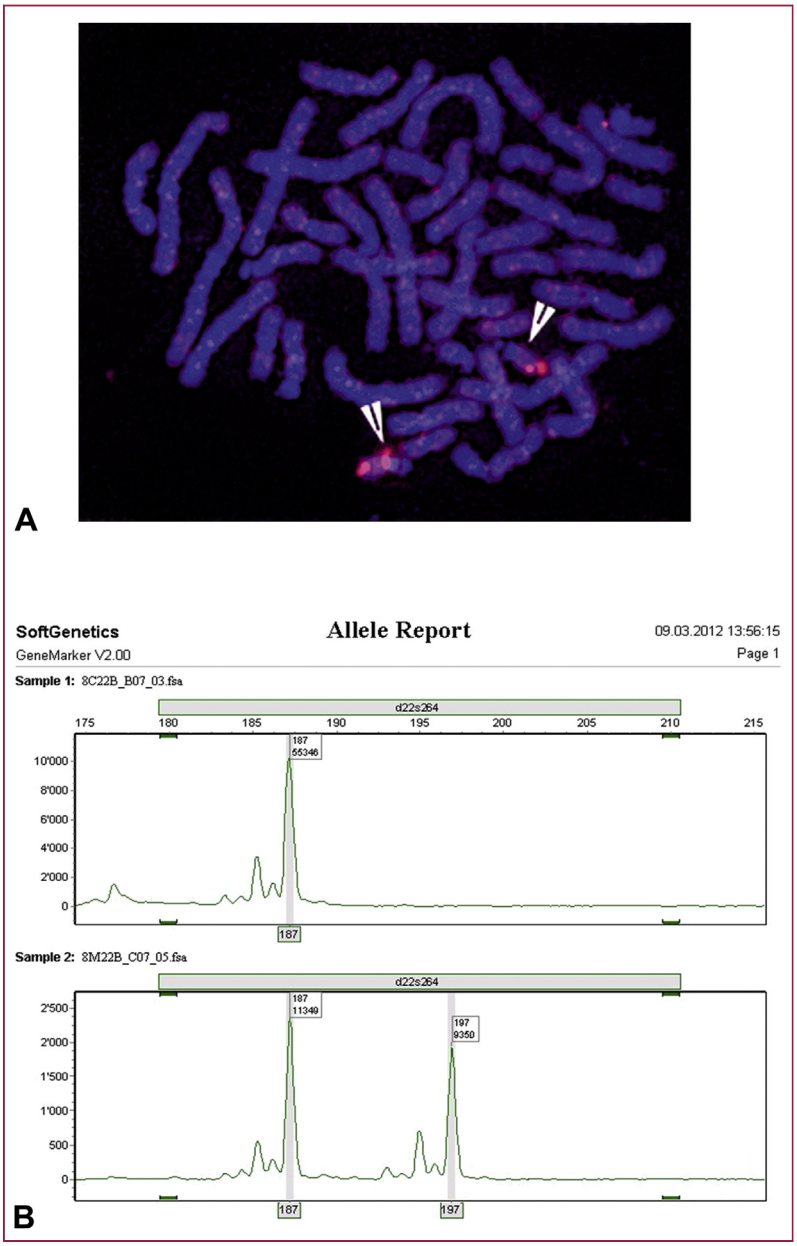

FIGURE 1. Molecular cytogenetic analysis. For 70 patients, multiplex ligation-dependent probe amplification (MLPA) was used for the detection of the 22q11.2 deletion (A), and confirmed in both cases by fluorescence in situ hybridization (FISH) (B).

TABLE 2. Profile of congenital heart defects

Type of Defect	Frequency		
	Children	Adolescents/Adults	Total, %
Ventricular septal defect	23 (23)	3 (3)	26
Tetralogy of Fallot	18 (18)	1 (1)	19
Patent ductus arteriosus	14 (14)	1 (1)	15
Atrial septal defect	6 (6)	1 (1)	7
Atrioventricular septal defect	5 (5)	0	5
Truncus arteriosus	3 (3)	0	3
Other	24 (24)	1 (1)	25
Total, %	93	7	100

Values are n (%) unless otherwise indicated.

nasal bridge. The nasal bridge, in particular, seemed to be remarkably depressed in the 2 cases, in opposition to the prominent nasal bridge usually found in affected individuals form other ethnic backgrounds, especially in Caucasians and Asians [23]. Similar difficulty of using classical facial dysmorphism criteria, for clinical suspicion of 22q11.2 deletion syndrome, was also reported in children of mixed ancestry in South Africa [19]. Therefore, the illustration photographs in the present report will contribute to fill a gap for Global Atlas for dysmorphology, as it is more and more internationally recognized that ethnic-specific clinical features should be taken into account for identification of human malformation syndromes in diverse populations [26,27]. Indeed, a recent report from our group suggests that for sub-Saharan Africans, clinical suspicion of Williams-Beuren syndrome should be mostly on the basis of behavioral phenotype and structural heart defects, and less on the classical craniofacial dysmorphism [28].

The 22q11.2 deletion syndrome is known to be the most common chromosomal microdeletion syndrome associated with CHD in humans, with a prevalence of 1 in 4,000 live births in various parts of the world [9,29]. Its occurs in about 18% of individuals with conotruncal heart defects, and up to 20% of individuals with tetralogy of Fallot, including nonsyndromic cases (6%) [8,30]. In this hospital-based study, we found that 2.8% of patients with CHD were positive for 22q11.2 deletion, all of them presenting with some characteristic extracardiac manifestations of the condition, besides conotruncal cardiac defects (Table 3). Halder et al. [31] reported a frequency of 6.16%

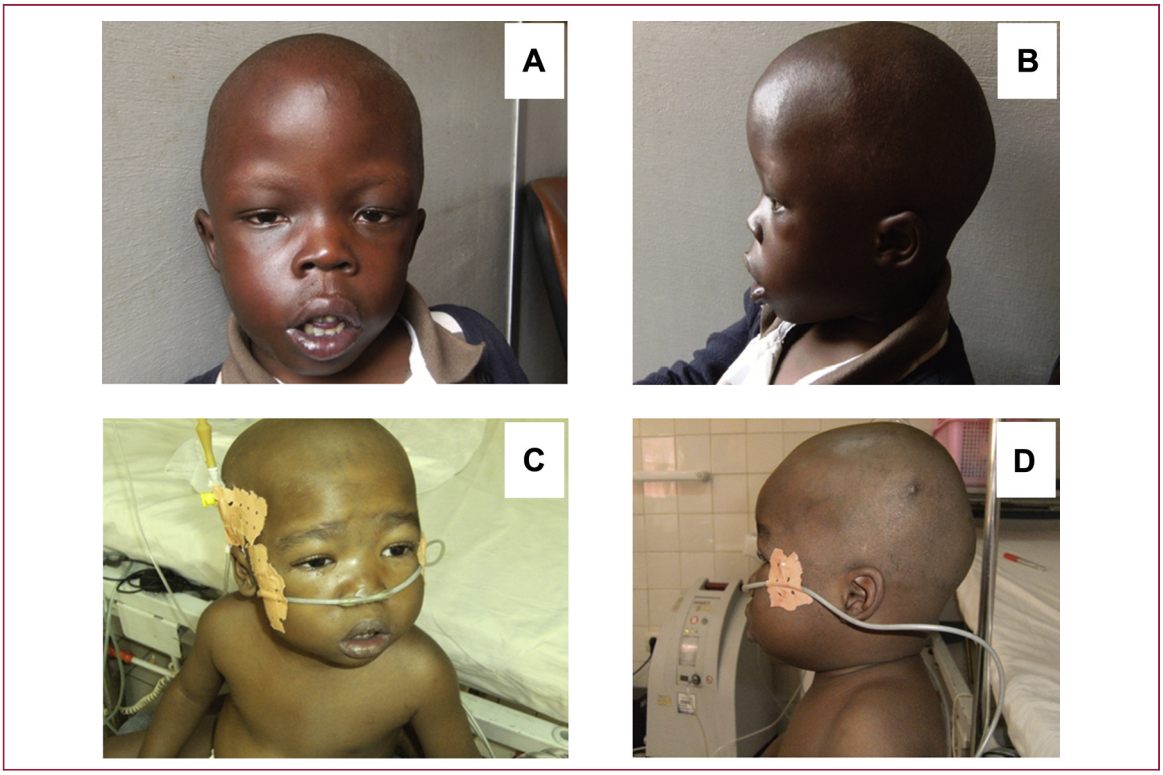

FIGURE 2. Craniofacial features in 2 African children with 22q11.2 deletion syndrome. Case 1: Front (A) and profile (B) views of the 5-year-old boy showing: prominent forehead, sparse eyebrows, puffy upper eyelids, low-set ears with overfolded upper helix, depressed and wide nasal bridge, bulbous tip of the nose, anteverted nares, long philtrum, and retrognathia. Case 2: Front (C) and profile (D) views of the 2.5-year-old boy showing: broad face, puffy upper eyelids, epicanthic folds, depressed and wide nasal bridge, broad bulbous tip of the nose, and retrognathia.

TABLE 3. Clinical features of patients with 22q11.2 deletion

Case	Age, yrs	Sex	Cardiac Abnormalities	Extracardiac Abnormalities
1	5	Male	Tetralogy of Fallot	Nonspecific craniofacial dysmorphism Short stature Learning difficulties
2	2.5	Male	Truncus arteriosus	Nonspecific craniofacial dysmorphism

of 22q11.2 deletion in 146 patients with CHD in a cardiac referral center in North India. Likewise, Rosa et al. [32] reported a frequency of 2% in 147 patients with CHD investigated for 22q11.2 deletion in a pediatric cardiac intensive care unit in Brazil. Although our study was not carried out in a cardiac center, the frequency obtained lies within the range reported in other populations. Despite this similarity, we believe that the low rate of pre-natal detection of CHD as well as the limited access of these patients to appropriate diagnosis and care, which is a reality in sub-Saharan Africa, may have contributed to a possible underestimation. In fact, it is reasonable, in the context of Cameroon, to anticipate that some patients with severe CHD may have died before having the opportunity to be assessed by a cardiologist or to access a tertiary hospital. Moreover, the underestimation could also be impeded by limited health professional training and knowledge regarding genetic conditions, as we previously reported in Cameroon [33], causing a poor referral pattern.

Possible limitations of the present study are the non-exploration of the precise size of the deletion, which could have helped in establishing a genotype-to-phenotype correlation. Both patients were reported to have 46, XY karyotypes, without detecting structural anomalies, ruling out in both cases an unbalanced familial chromosomal translocation. However, additional investigations of their parents for 22q11 deletion could have been performed to provide a more accurate recurrence risk assessment during genetic counseling, because 7% of patients with the 22q11 deletion syndrome have inherited the deletion from 1 parent [15]. Last, but not specific to the study, prevalence on the basis of clinical cohort cannot be necessary generalizable, and the proportion of 22q11.2 deletion syndrome reported here could be different in a population-based study.

Despite these limitations, the study supports the recommendation that health practitioners should raise an index of suspicion of the 22q11.2 deletion syndrome in the presence of conotruncal heart malformations with/without facial dysmorphism, with a prompt referral to the medical genetics unit. The study also supports the need to improve the capacity for global genomic medicine practice, and in sub-Saharan Africa in particular, as most rare genetic diseases frequency are likely to be similar everywhere.

CONCLUSIONS

The study has suggested that frequency of 22q11.2 deletion syndrome among patients with congenital heart defect in Cameroon is close to that of various populations in the world. The clinical phenotypes reported here will contribute to the Global Atlas for dysmorphology. Awareness should be raised by health practitioners for suspicion and screening of the 22q11.2 deletion syndrome in patients with conotruncal malformations with/without craniofacial dysmorphism. The development of a medical genetic service in Cameroon offered the opportunity, with international collaboration, to have access to molecular cytogenetic technology that was essential in identifying the deletion. Therefore, "omics" technologies offer much promise in genetic/genomic screening of severe global health problems.

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