Original Article

Investigation of Copy Number Variation in Children with Conotruncal Heart Defects

Carla Marques Rondon Campos[1], Evelin Aline Zanardo[3], Roberta Lelis Dutra[3], Leslie Domenici Kulikowski[2,3], Chong Ae Kim[2]

Universidade Federal de Mato Grosso[1], Cuiabá, MT; Universidade de São Paulo[2]; Departamento de Patologia – Laboratório de Citogenômica – LIM 03 – Universidade de São Paulo[3], São Paulo, SP – Brazil

Abstract

Background: Congenital heart defects (CHD) are the most prevalent group of structural abnormalities at birth and one of the main causes of infant morbidity and mortality. Studies have shown a contribution of the copy number variation in the genesis of cardiac malformations.

Objectives: Investigate gene copy number variation (CNV) in children with conotruncal heart defect.

Methods: Multiplex ligation-dependent probe amplification (MLPA) was performed in 39 patients with conotruncal heart defect. Clinical and laboratory assessments were conducted in all patients. The parents of the probands who presented abnormal findings were also investigated.

Results: Gene copy number variation was detected in 7/39 patients: 22q11.2 deletion, 22q11.2 duplication, 15q11.2 duplication, 20p12.2 duplication, 19p deletion, 15q and 8p23.2 duplication with 10p12.31 duplication. The clinical characteristics were consistent with those reported in the literature associated with the encountered microdeletion/microduplication. None of these changes was inherited from the parents.

Conclusions: Our results demonstrate that the technique of MLPA is useful in the investigation of microdeletions and microduplications in conotruncal congenital heart defects. Early diagnosis of the copy number variation in patients with congenital heart defect assists in the prevention of morbidity and decreased mortality in these patients. (Arq Bras Cardiol. 2015; 104(1):24-31)

Keywords: Heart Defects Congenital; Genetic Variation; DNA, Truncus Arteriosus; Heart Septal Defects, Ventricular.

Introduction

Congenital heart defects (CHD) are the most common group of structural abnormalities at birth, with an estimated prevalence of 1-5% of life births, constituting one of the main causes of infant morbidity and mortality[1,2].

Genetic factors are important in the complex etiology of CHD[3]. Mendelian and chromosomal syndromes occur in 20% of the cases of CHD. The underlying genetic mechanisms accounting for the remaining 80% are poorly understood[4,5].

Conotruncal malformations represent a heterogeneous group of cardiac malformations involving the ventricular outflow tract and the arterial pole of the heart. These malformations compromise the development of the outflow tract of the heart and are responsible for roughly 10-25% of all CHDs diagnosed at birth[1,6].

Following sequencing of the human genome, a new type of genomic alteration was discovered – the copy number variation – and its association with cardiac malformations was established[7,8].

Copy number variations (CNVs) are defined as fragments of deoxyribonucleic acid (DNA), greater than or equal to a kilobase (kb), present in variable number in a genome[9,10].

CNVs are an important part of the genetic diversity in relation to evolution and disease susceptibility; hence, its detection and association with characteristics and phenotypes are an important step to better understand the etiology of the disorder[11,12]. CNVs that span multiple genes may affect other major organs beyond the heart. Since CHDs may be the first defect to be detected in the patient, the CNV in CHD patients can lead to early diagnosis and treatment of extracardiac symptoms[13].

Molecular analysis with the multiplex ligation-dependent probe amplification (MLPA) technique has been used to determine copy number variation. This technique detects several microdeletion/microduplication syndromes associated with CHD and could be used as a diagnostic test to detect relevant variations in the number of copies and identify syndromic patients[13].

This study investigated the presence of copy number variation in children with conotruncal heart defect and their parents, and associated the genetic and clinical findings with those described in the literature.

Campos et al.
CNV and Conotruncal Heart Defect

Original Article

Methods

For the prospective and descriptive study, 39 patients with conotruncal and aortic coarctation congenital heart diseases were included. A convenience sampling was used.

The patients were referred from the outpatient pediatric cardiology and neonatal intensive care unit (ICU) of the *Hospital Universitário Julius Müller* and other hospitals located in Cuiabá, Mato Grosso. Data were collected between March and November 2012.

The inclusion criterion was the presence of lesions in the echocardiographic examination: *truncus arteriosus*, tetralogy of Fallot, interrupted aortic arch, pulmonary atresia and ventricular septal defect, transposition of the great arteries and coarctation of the aorta. Patients with abnormal G-band karyotype were excluded.

The study was approved by the Ethics Committee in Research of the School of Medicine, University of São Paulo and by the Ethics Committee of the University Hospital Júlio Müller, Federal University of Mato Grosso. A signed informed consent form was obtained from patients or parents, depending on the age of the patient.

A clinical record was filled for each individual. The assessment of dysmorphic features was subsequently analyzed in photographs or in person by a geneticist.

In patients with copy number variation, a clinical and laboratory evaluation of the parents was included.

Peripheral venous blood of the probands and their families was collected for classical cytogenetics and MLPA.

Molecular study by multiplex ligation-dependent probe amplification (MLPA)

Blood samples were sent to the cytogenomics laboratory of the School of Medicine of the University of São Paulo, where the MLPA technique was performed.

MLPA reactions were conducted according to the manufacturer's protocol using the kits SALSA P036, P070, P064 and P250 (MRC-Holland®, Amsterdam, the Netherlands) with some modifications for higher yield of the reagents.

The P036 and P070 kits detect subtelomeric changes. The P064 kit is recommended for major microdeletion syndromes, detecting changes in 22q11.2 and other critical regions for some syndromes.

The P250 kit is specific for DiGeorge syndrome, detecting microdeletions/duplications in 22q11.2. It contains probes for other regions involved in the occurrence of heart disease.

A total of 250 ng of genomic DNA (5 ul) from each patient was added to a microtube and taken to the thermocycler (Veriti® ThermalCycler – Life Technologies) for denaturation at 98°C for 15 minutes. Then, a mixture of probes (specific for each kit) and buffer solution were added to the methylated DNA for the hybridization process of the MLPA probes to DNA at 60°C for three hours. Buffered solutions, along with the enzyme ligase were added to the hybridization solution to allow binding of the probes to each specific target region to 54°C for 15 minutes. In the last step, the reagents for PCR were added to the binding solution for the amplification of only the fragments connected by the ligase. Thus, the amplified products were placed in microplates along with a molecular weight marker (LIZ – GS600) and Hi-Di Formamide (Life Technologies) and carried to the automated sequencer ABI 3500 (Life Technologies) for the reactions of fragment analysis.

In all MLPA reactions, at least three normal controls were used. The data were generated by the automated sequencer ABI 3500 (Life Technologies), at the Network of Multiuser Equipment of the immunology laboratory of the *Instituto do Coração* – Incor – HC / USP.

The analysis of the results of the MLPA reaction was performed with the software Gene Marker® (SoftGenetics LLC, State College, PA –www.softgenetics.com). The results were considered abnormal when the relative peak size was lower than 0.75 (deletion) or greater than 1.25 (duplication) when compared with normal samples.

Results

The study included 39 patients with congenital heart disease. Of these, 23 (59%) were males and 16 (41%) were females. Their ages ranged from two days to 19 years, with an average of five years and seven months. Only two mothers reported a previous diagnosis by gestational ultrasound of the fetus with congenital heart disease.

Among the diseases observed, most patients had tetralogy of Fallot, which was present in approximately 56% of the cohort. Other heart diseases included transposition of the great arteries (23%), coarctation of the aorta (10.3%), double outlet right ventricle (7.7%), pulmonary atresia and ventricular septal defect (2.6%) – Table 1.

Patients without changes in the MLPA

CNVs were not detected in 32/39 patients (82.05%). Familial recurrence of congenital heart disease was observed in 2/32 patients.

Extracardiac abnormalities were observed In 15/32 patients (46.8%) with normal MLPA, and the clinical signs and symptoms observed were: facial dysmorphias, cleft palate, strabismus, stroke, seizure, facial paralysis, hearing loss, vitiligo, epilepsy, autism, subglottic stenosis, asthma, frequent infections of the upper and lower airways, omphalocele, color blindness, feeding difficulties, growth failure, gastroesophageal reflux, cryptorchidism, speech disorder, learning disabilities and hypocalcemia. Frequent airway infections occurred in

Table 1 – Distribution of the types of heart defects in the cohort

Type of heart defect	N (%)
Tetralogy of Fallot	22 (56.4%)
Transposition of the great arteries	9 (23%)
Coarctation of the aorta	4 (10.3%)
Double outlet right ventricle	3 (7.7%)
Pulmonary atresia and ventricular septal defect	1 (2.6%)
Total	39 (100%)

Original Article

six cases; other extracardiac changes occurred sporadically in the patients.

Of the 32 patients, five died, and all of these patients who died had been admitted to a neonatal intensive care unit awaiting transfer to a center specialized in heart surgery.

Patients with changes in the MLPA

With the MLPA technique using four kits, it was possible to detect microdeletions/microduplications in 7/39 patients (17.9%), including two cases of deletion (19p deletion, 22q deletion) and five cases of duplication (two cases of 15q duplication, 20p duplication, 22q duplication, 8p duplication and 10p duplication).

Clinical presentation

The ages of the seven patients who presented CNVs ranged from 19 days to 10 years. Three presented with tetralogy of Fallot, two had double outlet right ventricle, one had transposition of the great arteries and one had coarctation of the aorta. All seven patients had extracardiac manifestations.

Of the seven patients, three were admitted to the neonatal intensive care unit and died (Table 2).

Investigation of the parents

The investigation of the parents was conducted in six families of patients with abnormal MLPA. Of these, we obtained material from both parents (father and mother) in four families and, in two cases, only material from the mother. None of them presented changes similar to their children's CNVs.

Description of seven cases with abnormalities

15q11.2 duplication

Female patient, child of non-consanguineous parents, father with 30 years of age and mother of indigenous origin, 31 years of age, with four children, the third child had a complex congenital heart disease and died. The child was born by cesarean delivery at 38 weeks gestation, weighing 2,165 g (below the 3rd percentile for age), measuring 44 cm (3rd percentile for age), with head circumference of 32 cm (between the 3rd and 10th percentiles for age) and Apgar 9 and 9. The echocardiography identified severe coarctation of the aorta, patent ductus arteriosus, atrial septal defect and several ventricular septal defects.

At the age of three months, her weight was 3,030 g (between the 3rd and 15th percentiles for age), her length was 49 cm (between the 3rd and 15th percentiles for age) and her head circumference was 36 cm (much below the 3rd percentile for age). The facial changes observed were small, almond-shaped eyes, thin upper lip, micrognathia, microcephaly, presence of neonatal teeth and narrow ear canal.

Other abnormalities also present were duodenal atresia, left appendix, constipation, feeding difficulties, failure to thrive, hypertonia, neuropsychomotor development delay, change in body temperature regulation and frequent airway infections. Cranial computed tomography showed atrophy and cortical dysplasia. The patient died at the age of four months, shortly after being discharged home from the ICU.

20p12.2 duplication

Neonate with 19 days of age, male, first child of healthy, non-consanguineous mother and father with 20 and 24 years, respectively. He was born by cesarean delivery at 39 weeks gestation, weighing 3,800 g (75th percentile for age), length of 52 cm (50th percentile for age), head circumference of 34 cm (25th percentile for age) and Apgar 10. Echocardiography diagnosed double outlet right ventricle, large atrial septal defect, rudimentary ventricular chamber, aortic arch hypoplasia, patent ductus arteriosus and aortic stenosis.

Physical examination detected facial findings, such as bitemporal flattening, elongated face, ocular hypertelorism, wide nasal dorsum, flat nasal tip, anteverted nostrils and micrognathia, also presenting short neck, and elongated fingers and feet.

He remained in the ICU and died awaiting transfer to undergo heart surgery.

19p deletion

Male patient, second son of healthy, non-consanguineous parents, mother and father with 32 and 33 years of age, respectively.

He was born by cesarean section at 39 weeks gestation, weighing 3,450 g (50th percentile for age), measuring 49 cm (between the 25th and 50th percentiles for age), with a head perimeter of 33.5 cm (50th percentile for age), and with diagnosis of tetralogy of Fallot.

At the age of six years, he weighed 26,500 g (between percentiles 85th and 97th for age) and measured 123 cm (85th percentile for age).

On physical examination he presented dysmorphic facial features such as wide nasal dorsum, flattened nasal bridge and almond-shaped eyes. Asthma was reported as an extracardiac finding. The neuropsychomotor development was normal.

15q duplication

Male patient, the only child of non-consanguineous and healthy mother and father with 36 and 26 years, respectively, and without reported heart disease in the family. He was born by cesarean section with 30 weeks gestation, weighing 1,015 g (10th percentile for age) and measuring 38 cm (10th percentile for age).

At the age of two years and 11 months, he presented the following anthropometric measurements: weight of 14,000 g (between the 50th and 85th percentiles for age), length of 82 cm (below the 3rd percentile for age) and head circumference of 46 cm (below the 3rd percentile for age).

On echocardiography, he presented tetralogy of Fallot. On physical examination, dysmorphic facial features were detected: ocular hypertelorism, broad nasal dorsum, flattened nasal bridge, anteverted nostrils, long and flat nasolabial philtrum and microcephaly.

The clinical abnormalities evidenced were growth deficit and delayed neuropsychological development.

Campos et al.
CNV and Conotruncal Heart Defect

Original Article

Table 2 – Characteristics of patients with CNV

N	Age	Gender	CHD	Clinical features	CNV	Outcome	Current age/death
MC001	3 m	F	Co. Aorta	Facial dimorphism Duodenal Atresia Sick neonatal, Atrophy, cerebral dysplasia Frequent ILA, DNPD	15q 11.2 dup	Death	5 m
MC003	6 y	M	TOF	Facial dysmorphism Asthma	19p del	Amb	8 y
MC006	19 d	M	DORV	Facial dysmorphism	20p12.2 dup	Death	23 d
MC011	2 y	M	TOF	Facial dysmorphism Growth deficit DNPD	15q dup	Amb	4 y
MC015	6 y	F	TOF	Broad forehead Fusiform fingers	8p23.2 dup and 10p12.31 dup	Amb	8 y
MC030	10 y	M	TGA	Facial dysmorphism GERD Frequent ILA Learning difficulty	Dup2 2q11	Amb	12 y
MC039	5 m	F	DORV	Facial dysmorphism Umbilical hernia Feeding difficulty Growth deficit GERD Velopharyngeal insufficiency Thymic aplasia DNPD	22q11 del	Death	6 m

y: years; amb: ambulatory; CoA: coarctation of the aorta; d: day; CHD: congenital heart defect; del: deletion; dup: duplication; DORV: double-outlet right ventricle; F: female; ILA:infection of the lower airways; m: month; M: male; DNPD: delayed neuropsychomotor development; GERD: gastroesophageal reflux; TGA: transposition of the great arteries; TOF: tetralogy of Fallot.

22q11.2 deletion

Female infant, the first child of healthy, non-consanguineous father and mother with 29 and 24 years of age, respectively, without family history of heart disease. During pregnancy, the mother developed gestational diabetes requiring insulin, and the only abnormality observed on gestational ultrasonography was polyhydramnios.

She was born by cesarean section at 37 weeks gestation, weighing 2,500 g (between the 10th and 25th percentiles for age), measuring 44 cm (10th percentile for age), with a head circumference of 33.5 cm (50th percentile for age) and Apgar 8 and 8. At the age of five days, a heart murmur was detected, echocardiography showed double outlet right ventricle, atrial septal defect, ventricular septal defect and mild pulmonary stenosis. She was admitted to the ICU and underwent the first cardiac surgery.

At the age of five months, her weight was 4,200 g (between the 15th and 50th percentiles for age), her length was 55 cm (below the 3rd percentile for age) and her head circumference was 36.5 cm (below the 3rd percentile for age).

The physical examination detected dysmorphic facial features: ocular hypertelorism, large eyes, broad nasal dorsum, flat nasal tip with anteverted nostrils, long nasolabial philtrum, thin upper lip with inverted "V", micrognathia, flattened earlobe, fingers tapered and elongated and umbilical hernia.

Clinical changes highlighted were difficulty of feeding, growth deficit, neuropsychomotor development retardation (she could only hold her head).

Imaging tests showed gastroesophageal reflux and thymic aplasia. An evaluation with an ENT specialist showed velopharyngeal insufficiency. Laboratory exams identified serum calcium within the normal range.

The patient died after heart surgery at the age of six months.

She had a deletion of 3.0 Mb, therefore a typical *de novo* deletion for DiGeorge syndrome, since the parental MLPA was normal.

22q11.2 duplication

Male patient, an only child of a single mother, healthy, 35 years old, without report of heart disease in the family. He was born by cesarean section at 40 weeks and six days gestation, weighing 4,425 g (above the 90th percentile for age) measuring 50 cm (between the 10th and 25th percentiles for age), with head circumference 37.5 cm (above the 97th percentile for age) and Apgar 8 and 10.

The echocardiography showed transposition of the great arteries, atrial septal defect and patent ductus arteriosus. Heart surgery was performed at the tenth day of life.

At the age of 10 years he weighed 40 kg (between the 85th and 90th percentiles for age), measured 142 cm (between

Original Article

the 50th and 85th percentiles for age) and had a head circumference of 52.5 cm.

On physical examination, subtle phenotypic changes were observed, such as broad nasal base, long nasolabial philtrum and thin upper lip, with a diagnosis of gastroesophageal reflux and frequent respiratory infections. The neuropsychomotor development was normal, but he had learning disability.

8p23.2 and 10p12.31 duplication

Female patient, six-years old, an only child of separated parents, no history of consanguinity or heart disease in the family, healthy mother with 23 years of age.

She was born by normal delivery at 40 weeks gestation, weighing 3,150 g (25th percentile for age) with a diagnosis of tetralogy of Fallot, mild tricuspid regurgitation and moderate pulmonary insufficiency. She underwent heart surgery at the age of four.

She currently weighs 20.7 kg (between the 15th and 50th percentiles for age), measures 117 cm (between the 15th and 50th percentiles for age) and has a head circumference of 51 cm. On physical examination were evidenced wide forehead and fusiform fingers. The neuropsychomotor development was normal and did not show other clinical changes.

Discussion

Recent studies have shown that CNVs occur in significant proportion in patients with CHD. In syndromes involving CHD as part of the clinical spectrum, cardiac malformation is often the first symptom to appear[13].

In the present study we detected CNVs in 7/39 cases: 22q11.2 deletion, 22q11.2 duplication, 15q11.2 duplication, 20p12.2 duplication, 19p deletion, 15q duplication and 8p23.2 duplication with concomitant 10p12.31 duplication.

Currently, several studies have established the relevance of CNVs in the etiology of CHD. Association has been demonstrated of both syndromic CHD and isolated CHD and chromosomal imbalances[13-15].

The karyotype was normal in all subjects of this research, which was also observed by Thiempont et al[16], who detected 30% of rare CNVs in patients with CHD and other birth defects with normal karyotypes.

CNVs are also known to be involved in the genesis of neurodevelopmental and neurocognition disorders such as intellectual disability, schizophrenia and autism spectrum[17]. In this study we observed the presence of delayed neuropsychomotor development in only two of the seven patients who presented CNV. Richards et al[18], studying CNV on 40 individuals with CHD, observed that the risk of causal CNV increased to 45% in those individuals with neurological abnormalities or developmental delay. Cooper et a[19], confirming this relationship when they analyzed CNV in 575 children with CHD and intellectual disabilities showed significant increase in the number of CNVs in children with CHD.

Our study identified five cases of duplication and deletion of only two cases of deletion, similar to the study by Erdogan et al[20], who while researching CNV in 150 individuals with isolated CHD, detected 18 rare CNVs, most of which were duplications in contrast to those found in the syndromic CHD, which are predominantly deletions. Additionally, 44% were family members, also occurring in parents without evidence of CHD, perhaps indicating that these CNVs increase the susceptibility to CHD, but require other factors to manifest the phenotype. This demonstrates that rare CNV can be an important genetic contributor to isolated CHD.

Initially, the patients in this study appeared to have only CHD, however a more careful clinical examination and phenotypic evaluation showed extracardiac changes possibly part of a syndrome. Several studies have demonstrated the presence of CNVs in non-syndromic CHD[21].

Recently, Warburton et al[15] studying 223 children with conotruncal heart defect and hypoplastic left heart syndrome, found 22q11.2 deletion in nine children and 33 de novo CNVs. In our study we detected a 22q11.2 deletion in only one patient (2.5%).

The 22q11.2 deletion syndrome (22q11DS), also known as DiGeorge or velocardiofacial syndrome, is considered the most common of the syndromes of human microdeletion and contains multiple genes. The estimated prevalence is 1: 4,000 live births[8].

A submicroscopic chromosomal deletion is detected by FISH, MLPA or chromosomal microarray analysis. The vast majority of cases (90 percent) showing deletion of approximate size of three million bases (Mb) leads to deletion of approximately 45 genes, as presented by the patient of the study[8].

The only patient in this study with 22q11 deletion was initially carrier of isolated conotruncal heart defect. After detailed physical examination, several phenotypic changes that are part of 22q11.2 deletion syndrome were observed. In most studies that have sampled a population of individuals with isolated conotruncal heart defect, it was later observed in a more detailed clinical examination the presence of subtle phenotypic changes not evident at the first examination[22].

The clinical presentation of the 22q11 deletion syndrome can be extremely variable. Early detection of deletion is important for the treatment of anomalies and for investigation of associated malformations and prevention of neuropsychological and immune deficiency problems. In most cases, the 22q11 deletion occurs de novo in the family, but inheritance of parental microdeletion can occur in 6-28%. Affected parents may experience mild phenotype. For this reason, testing for 22q11 deletion should be offered to all parents of affected children for the purpose of genetic counseling[23,24]. In the patient in this study, the parents were also surveyed, and the MLPA in both resulted normal.

The patient in question presented as heart defect a double outlet right ventricle and died after the second surgery. Congenital heart defect is one of the most frequent manifestations of the 22q11.2 deletion syndrome. Furthermore, it is the leading cause of death in most patients with this syndrome.

The identification of genetic factors for CHD is important to provide genetic counseling for parents who plan to have

Campos et al.
CNV and Conotruncal Heart Defect

Original Article

other children. The risk of recurrence for many CHDs is 2-6%, and the recurrence risk of CHD increases significantly when parents are carriers of deletion/duplication[18], information that becomes relevant to conduct the genetic counseling of these parents or the patient, upon reaching adulthood and deciding to start a family.

In recent years, dozens of clinically relevant chromosome microdeletions and microduplications have been described in humans, often associated with mental retardation, autism and/or physical malformations. Once these small genomic arrangements are generally below the detection limit of optical microscopy, it is essential to use molecular diagnostic procedures to provide an explanation for the symptoms and signs observed and provide clinical and genetic prognostic for patients and their families. In developed countries, molecular tests, particularly comparative genomic hybridization (CGH) array, have become the gold standard for such laboratory diagnosis. However, these tests are very costly and rely on the availability of expensive equipment which must be updated frequently. As a result of these high costs, patients in developing countries have no access to testing and are often not diagnosed, with great loss to their families[25].

The MLPA is an established technique for detecting known CNVs. The cost of the MLPA is substantially lower than the array CGH and, with respect to fluorescence in situ hybridization (FISH), is fast, easy and economical, with a simple kit capable of performing simultaneous search of multiple anomalies. Therefore, it can be used for detecting variation in the number of copies clinically relevant in patients with apparently non-syndromic CHD, causing early identification of patients with genomic disorders[13,26,27].

If the diagnosis of CHD is established early, i.e., intrauterine, better preparation for birth with earlier treatment would improve the prognosis in terms of morbidity and mortality, yet we observe that most mothers of patients in this study, even having performed prenatal and pregnancy ultrasound, were unaware of the diagnosis of fetal cardiac defects. In a state with a shortage of beds in neonatal intensive care units for the treatment of these neonates and which does not offer heart surgeries of greater complexity, these patients are probably dying without diagnosis and treatment. This can be evidenced in our sample, in which all patients who were admitted to the intensive care unit died awaiting transfer to a center specializing in heart surgery, demonstrating the importance of early diagnosis in the impact of high mortality in these individuals.

There is still controversy in the literature about to which group of individuals should be promoted the routine investigation of variation in copy –number in patients with isolated heart defect or in those with other extracardiac changes. What can be seen of consensus that a thorough physical examination looking for dysmorphic signs, especially in children with conotruncal heart defect, can assist in the decision to carry out molecular tests. The benefits provided would be adequate genetic counseling, evaluation and management of problems more effectively, resulting in improved quality of life of the individual and the family.

Conclusions

The MLPA technique is useful in the investigation of microdeletions and microduplications in conotruncal congenital heart defects.

Early diagnosis of CNVs in patients with CHD assists in the prevention of morbidity and reduction of mortality in these patients. A thorough clinical evaluation in every patient with CHD is essential to detect other associated congenital anomalies.

This work highlights the need for the physician, when facing a child with heart defect, to be alert to the possibility of genetic abnormalities, with knowledge of new techniques that enable the diagnosis.

A limitation of this study is the sampling method used, the convenience, as the small sample size, which allows us to consider the results found only for the population in question.

Author contributions

Conception and design of the research and Critical revision of the manuscript for intellectual content: Campos CMR, Kulikowski LD, Kim CA; Acquisition of data and Writing of the manuscript: Campos CMR; Analysis and interpretation of the data: Campos CMR, Zanardo EA, Dutra RL.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Study Association

This article is part of the thesis of Doctoral submitted by Carla Marques Campos, from Universidade de São Paulo e Universidade Federal de Mato Grosso.

Original Article

References

1. Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol. 2002;39(12):1890-900.

2. Wang E, Sun S, Qiao B, Duan W, Huang G, An Y, et al. Identification of functional mutations in GATA4 in patients with congenital heart disease. PLoS One. 2013;8(4):e62138.

3. Pierpont ME, Basson CT, Benson Jr DW, Devriendt K, Digilio MC, Goldenberg P, et al; American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young. Genetic basis for congenital heart defects – current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. Circulation. 2007;115(27):3015-38.

4. Bittel DC, Butler MG, Kibiryeva N, Marshall JA, Chen J, Lofland GA et al. Gene expression in cardiac tissues from infants with idiopathic conotruncal defects. BMC Med Genomics. 2011; 4:1-10.

5. Soemedi R, Wilson IJ, Bentham J, Darlay R, Töpf A, Zelenika D, et al. Contribution of global rare copy-number variants to the risk of sporadic congenital heart disease. Am J Hum Gen. 2012;91(3):489-501.

6. Vaidyanathan B, Kumar S, Sudhakar A, Kumar RK. Conotruncal anomalies in the fetus: referral patterns and pregnancy outcomes in a dedicated fetal cardiology unit in South India. Ann Pediatr Cardiol. 2013;6(1):15-20.

7. Gelb BD, Seidman CE. The good SHP2 association: a porthole into the genetics of congenital heart disease. Circ Cardiovasc Genet. 2012;5(3):271-3.

8. Ware SM, Jefferies JL. New genetic insights into congenital heart disease. J Clin Exp Cardiolog. 2012 Jun; 15; S8. pii:003.

9. Castellani CA, Melka MG, Wishart AE, Locke ME, Awamleh Z, O Reilly L, et al. Biological relevance of CNV calling methods using familial relatedness including monozygotic twins. BMC Bioinformatics. 2014;15:114.

10. Li X, Chen S, Xie W, Vogel I, Choy KW, Chen F, et al. PSCC: sensitive and reliable population-scale copy number variation detection method based on low coverage sequencing. PLoS One. 2014;9(1):e85096.

11. Xu Y, Peng B, Fu Y, Amos CI. Genome-wide algorithm for detecting CNV associations with disease. BMC Bioinformatics. 2011;12:331.

12. Valsesia A, Macé A, Jacquemont S, Beckmann J, Kutalik Z. The growing importance of CNVs: new insights for detection and clinical interpretation. Front Genet. 2013;4:92.

13. Sorensen KM, El-Segaler M, Fernlund E, Errami A, Bouvagnet P, Nehme N, et al. Screening of congenital heart disease patients using multiplex ligation-dependent probe amplification: early diagnosis of syndromic patients. Am J Med Genet. 2012;158A(4):720-5.

14. Breckpot J, Thienpont B, Arens Y, Tranchevent LC, Vermeesch JR, Moreau Y, et al. Challenges of interpreting copy number variation in syndromic and non-syndromic congenital heart defects. Citogenet Genome Res. 2011;135(3-4):251-9.

15. Warburton D, Ronemus M, Kline J, Jobanputra V, Williams I, Anyane-Yeboa K, et al. The contribution of de novo and rare inherited copy number changes to congenital heart disease in an unselected sample of children with conotruncal defects or hipoplastic left heart disease. Human Genet. 2014;133(1):11-27.

16. Thienpont B, Mertens I, de Ravel T, Eyskens B, Boshoff D, Maas N, et al. Submicroscopic chromosomal imbalances detected by array-CGH are a frequent cases of congenital heart defects in selected patients. Eur Heart J. 2007;28(22):2778-84.

17. Silversides CK, Lionel AC, Constain G, Merico D, Migita O, Liu B, et al. Rare copy number variations in adults with tetralogy of Fallot implicate novel risk gene pathways. PLoS Genet. 2012;8(8):e1002843.

18. Richards AA, Santos IJ, Nichols HA, Crider BP, Elder FF, Hauser NS, et al. Cryptic chromosomal abnormalities identified in children with congenital heart disease. Pediatr Res. 2008;64(4):358-63.

19. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, et al. A copy number variation morbidity map of developmental delay. Nat Genet. 2011;43(9):838-46.

20. Erdogan F, Larsen LA, Zhang L, Tumer Z, Tommerup N, Chen W, et al. High frequency of submicroscopic genomic aberrations detected by tiling path array comparative genome hybridization in patients with isolated congenital heart disease. J Med Genet. 2008;45(11):704-9.

21. Greenway SC, Pereira AC, Lin JC, DePalma SR, Israel SJ, Mesquita SM, et al. De novo copy number variants identify new genes and loci in isolated, sporadic tetralogy of Fallot. Nat Genet. 2009;41(8):931-5.

22. Goldmuntz E, Clark B, Mitchel LA, Jawad AF, Cuneo BF, Reed L, et al. Frequency of 22q11 deletion in patients with conotruncal defects. J Am Coll Cardiol.1998;32(2):492-8.

23. Cuturilo CA, Drakullic D, Stevanovic M, Jovanovic I, Djukic M, Miletic-Grkovic S, et al. A rare of interrupted aortic arch type C and microdeletion 22q11.2. Eur J Pediatr. 2008;167(10):1195-8.

24. McDonald-McGinn DM, Emanuel BS, Zackai EH. 22q11.2 deletion syndrome. Gene Reviews. Last update: 16 December 2005. [Cited in 2014 Jan 28]. Available from: http://peds.stamford.edu/rotations/genetics_article_22q11_2_deletion_sys.

25. Stofanko M, Gonçalves-Dornelas H, Cunha PS, Pena PS, Vianna-Morgante AM, Pena SD. Simple, rapid and inexpensive quantitative fluorescent PCR method for detection of microdeletion and microduplication syndromes. PLoS One. 2013;8(4):e61328.

26. Fernández L, Lapunzina P, Arjona D, López Pajares I, Garcia-Guereta L, Elorza D, et al. Comparative study of three diagnostic approaches (FISH, STRs and MLPA) in 30 patients with 22q11.2 deletion syndrome. Clin Genet. 2005;68(4):373-8.

27. Dutra RL, Honjo RS, Kulikowski LD, Fonseca FM, Pieri PC, Jehee FS, et al. Copy number variation in Williams-Beuren syndrome: suitable diagnostic strategy for developing countries. BMC Res Notes. 2012;5:13.