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REGULAR ARTICLE

Variation in prevalence of chromosome 22q11 deletion in subtypes of conotruncal defect in 254 children

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ABSTRACT

Aim: To determine the frequency of chromosomal aberrations particularly 22q11 deletion in Indian children ≤2 years with different types of conotruncal malformations and their association with abnormal aortic arch. Additionally, extracardiac features were also studied.

Methods: Conventional cytogenetic and fluorescence *in situ* hybridization analyses were performed in 254 patients with conotruncal defects. Multivariable logistic regression analysis was performed to ascertain extracardiac features helpful in identifying high-risk patients with deletion.

Results: Chromosomal abnormalities were identified in 52 (21%) children, of whom 49 (94%) showed 22q11 deletion and 3 (6%) had abnormalities of chromosome 6, 2 and X. None of the 11/254 children with tetralogy of Fallot with absent pulmonary valve showed deletion. The association of 22q11 deletion with right sidedness of the aortic arch varied with the type of conotruncal defect. The eight extracardiac features in combination showed 93.5% agreement with the presence of deletion.

Conclusion: The extracardiac features along with specific type of conotruncal defect and associated cardiovascular anomaly should alert the clinician for 22q11 deletion testing. However, if deletion analysis is not possible, specific extracardiac features (six dysmorphic facial features, thin long fingers and hypocalcemia) can help to identify an increased risk of 22q11 deletion in patients with conotruncal defect.

INTRODUCTION

The microdeletion of chromosome 22q11 is the most common type of structural aberration found in humans, with an incidence of one in 4000 live births (1). The reduced gene dosage on autosome 22q11 results in a spectrum of congenital defects including DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS) and conotruncal anomaly face syndrome. The 22q11 deletion syndrome is partially characterized by congenital heart defect, aplasia or hypoplasia of parathyroid, cleft palate, hypocalcemia and facial abnormalities. During recent decades with the progress in human genetics, it has been noticed that approximately 90% of DGS/VCFS patients show a common typical deleted region (TDR) of 3 Mb on chromosome 22q11; in 7% of cases, 1.5-Mb deletion within the TDR is noticed (2). In addition, few patients are also found to have smaller atypical deletions (3–5).

The conotruncal heart defects most commonly associated with high frequency of 22q11 deletion include interrupted aortic arch type B, tetralogy of Fallot with pulmonary atresia and truncus arteriosus. Arch anomalies in association with conotruncal defects are found to be more likely among patients with 22q11 deletion as compared to those without deletion. In approximately 90% of cases, 22q11 deletion occurs sporadically, and in about 10%, it is inherited from an affected parent (6). It is possible that genetic and/or epigenetic-modifying factors may play role in such defects (7).

Key notes

- 'Screening of chromosome 22q11 deletion is required when extracardiac features along with specific type of conotruncal defect and associated cardiovascular anomaly are observed. In the case of unavailability of diagnostic facility, particular phenotypic features can be useful in detecting high-risk patients with 22q11 deletion. It also indicates further research to understand the basis for variation in frequency of deletion in subtypes of conotruncal defect.'

As the data on frequency of microdeletion of chromosome 22q11 in specific subtypes of conotruncal defects are limited, we have tried to determine the frequency of chromosomal aberrations specifically 22q11 deletion in 254 Indian children ≤2 years with seven different types of conotruncal malformations and its association with abnormal aortic arch. In addition, extracardiac features were also evaluated.

SUBJECTS AND METHODS

Within the duration of 3 years, 254 consecutive live-born children attending Paediatric Cardiology unit were recruited for the genetic study, based on cardiac diagnosis irrespective of the extracardiac features. As per the author's knowledge, this is the first large published report on 22q11 deletion in children specifically up to 2 years of age with conotruncal defects, because this is the most common age for presentation of conotruncal malformations and subtle phenotypic features as compared to older children and adults. It was conducted at a tertiary care hospital of south India. Informed parental consent was obtained for all patients. The study was prospectively reviewed and approved by the Institutional ethics committee of Amrita Institute of Medical Sciences and Research Centre, Kochi, India.

The seven types of conotruncal defects identified using standard echocardiographic techniques were categorized as follows: tetralogy of Fallot (TOF), tetralogy of Fallot with pulmonary atresia (TOF/PA), tetralogy of Fallot with absent pulmonary valve (TOF/APV), double outlet right ventricle (DORV), conoventricular ventricle septal defect (CVVSD), truncus arteriosus (TA) and interrupted aortic arch (IAA type A, B, C). The patients with numerical aberration of autosomes, noncardiac phenotypic features and those with family history of 22q11 deletion were excluded from the study. The patients were subjected to genetic testing by standard cytogenetic and fluorescence *in situ* hybridization (FISH) techniques using TUPLE1 probe.

The extracardiac features were evaluated by paediatric clinical geneticist in only 226 children. It included systematic physical examination and clinical history. The noncardiac features included dysmorphic facial features, mental retardation, cleft palate, cleft lip and hypocalcaemic status. Specialized imaging for organ defects were not routinely performed and done wherever needed. Nasal endoscopy was not performed routinely to detect submucous cleft palate. In addition, family history was also taken into consideration. Parental screening for deletion status was performed wherever possible. In twelve cases both parents and in four cases only mothers of deleted patients were tested for 22q11 deletion.

Statistical analysis was carried out using standard statistical software SPSS Inc., Chicago, IL, USA (version 11.0). Values were expressed in terms of mean and standard deviation (SD). Chi-square test was carried out to find the statistical significance of the difference in percentage of deletion between patients with right and left aortic arch. Univariate and multivariate logistic regression analyses were performed to ascertain extracardiac features (16 physical features and hypocalcemia) significantly associated with 22q11 deletion. The p value <0.05 was considered significant.

RESULTS

A total of 254 children were included in the study. Their age ranged from 0.1 to 24 months (mean = 6.5; SD = 6.5), and the weight ranged from 1.7 to 12 kilograms (mean = 5.2; SD = 2.2). Twenty-one per cent (53/254) of them were neonates. Four per cent in all were born to consanguious parents. The present study reveals chromosomal abnormalities in 52 (21%) children with conotruncal defect, including 49 (94%) patients with 22q11 deletion and three (6%) had other abnormalities. Monosomy of chromosome 22q11.2 was found in 53% men and 47% women. Thirty-one of deleted and 25% of nondeleted children had low birth weight (<2500 g). The seven types of conotruncal lesions included TOF in 41.7% of patients, TOF/PA in 22%, TOF/APV in 4.3%, DORV and CVVSD in 11.8% each, TA in 5% and IAA in 3.1% of cases. The frequency of 22q11 deletion in conotruncal defects was found to be 19% (49/254). It varied in subtypes of conotruncal defects being maximum (50%) in interrupted aortic arch, whereas no deletion was noted in 11 patients with TOF/APV (Table 1). In the case of IAA, three of four deleted patients had Type

Table 1 Frequency of 22q11deletion and arch sidedness in conotruncal defects

	Deleted patients – percentage (deleted/total)			
Conotruncal defect	Frequency	Right arch	Left arch	p-value
Tetralogy of Fallot	15.1 (16/106)	27.0 (10/37)	8.7 (6/69)	0.021*
TOF with pulmonary atresia	23.2 (13/56)	46.7 (7/15)	14.6 (6/41)	0.027*
TOF with APV	0 (0/11)	–	0 (0/11)	–
Double outlet right ventricle	13.3 (4/30)	0 (0/7)	17.4 (4/23)	0.548 (NS)
Conoventricular VSD	20.0 (6/30)	0 (0/7)	26.1 (6/23)	0.290 (NS)
Truncus Arteriosus	46.2 (6/13)	60.0 (3/5)	37.5 (3/8)	0.592 (NS)
Interrupted aortic Arch	50.0 (4/8)	–	50.0 (4/8)	–
Total	19.3 (49/254)	28.2 (20/71)	15.8 (29/183)	0.026*

TOF, tetralogy of fallot; VSD, ventricle septal defect; APV, absent pulmonary valve; NS, non significant.
*p < 0.05.

B, while one patient had Type A. The association of microdeletion with arch sidedness varied with the type of conotruncal defect (Table 1). The sensitivity, specificity, positive and negative predictive value of right aortic arch for 22q11 deletion were 62.5%, 70%, 27%, 91.3%, and 53.8%, 81.39%, 46.7%, 85.36% in the TOF and TOF/PA, respectively.

Cytogenetic analysis showed normal karyotype in all patients, except three cases. Case 1 was an infant with birth weight 1.3 Kg, his clinical findings included DORV, CVVSD, microphthalmia, micrognathia, microstomia, low set posteriorly placed pinna, sacrococcygeal dimple and had 46,XY,del(6)(q14q21) chromosomal abnormality. Case 2 with 46,XX,del(2)(q34-qter) karyotype, had TOF/APV, microstomia, normal fingers and toes and no ear anomaly. In case 3, the infant had CVVSD with right aortic arch, hypocalcemic seizures, prominent hooked nose, high arched palate, micrognathia, low set small ears, long fingers and toes, the child had 45,XO karyotype. All three infants were born to nonconsanguious parents. In addition, six patients also showed polymorphic variants including 9qh+, 22ps+ and 15ps+. In two cases, maternally derived deletion was observed. In first case, the proband had interrupted aortic arch type A. In second case, the child had TOF and the mother had no heart defect but phenotypic features of 22q11 deletion syndrome.

The multivariable logistic regression analysis showed that eight extracardiac features in combination had 93.5% agreement with the presence of deletion by FISH test (Table 2). Cleft palate, mental retardation and hernia were observed in two children each and seizure in one patient with 22q11 deletion. None of the deleted patient had cleft lip. The four features, i.e. low set ears, dysplastic flared pinna, bulbous nasal tip and thin long fingers were present together in 91.6% (11/12) of cases with deletion. All 44 patients with monosomy of 22q11 showed one or more extracardiac feature of 22q11 deletion syndrome.

DISCUSSION

This is first major study performed in India evaluating the frequency of microdeletion of 22q11 in 254 children particularly ≤2 years of age with conotruncal defects. A high frequency (19%) of deletion of chromosome 22q11 may be attributed to high susceptibility of unequal recombination exchanges between low copy repeat in the 22q11.2 region (8). The study was carried out in a tertiary care hospital and is not reflective of population prevalence. The frequency of chromosome 22q11.2 deletion in conotruncal malformations is found to vary between 7% and 48% (9–11). It may be attributed to the patient selection criteria based on type of cardiac defect, age of diagnosis and the techniques used.

Table 2 Extracardiac features found significant by multivariate analysis

Features	p value	Odd's ratio	95% Confidence interval
Low set ears	0.001	12.7	3.0–53.8
Dysplastic flared pinnae	<0.001	20.9	4.4–99.7
Short palpebral fissures	0.002	11.8	2.5–55.3
Bulbous nasal tip	0.003	10.3	2.2–49.0
Microstomia	<0.001	21.9	4.1–119.1
High arched palate	0.027	4.4	1.2–16.6
Thin long fingers	0.026	4.4	1.2–14.8
Hypocalcemia	<0.001	33.2	6.0–183.9

22q11 deletion in subtypes of conotruncal defects

The frequency of 22q11 deletion in specific lesions like tetralogy of Fallot with absent pulmonary valve and double outlet right ventricle differed with reference to published literature. The reported frequency of interstitial deletion of 22q11 in patients with tetralogy of fallot with absent pulmonary valve is 21%–75% (11,12); however, no deletion was identified in our series of 11 patients. The 22q11 deletion is rarely associated with double outlet right ventricle (13,14), but present data indicates a comparatively high frequency (13%) of deletion. A high association of deletion in Interrupted aortic arch indicates a major role of genetics in the aetiology of such lesions. The knowledge of 22q11 deletion is important based on the type of cardiovascular lesion, because the surgical prognosis is good in the case of deleted patients with tetralogy of Fallot with/without pulmonary atresia and truncus arteriosus, whereas in children with pulmonary atresia with ventricular septal defect and perhaps in interrupted aortic arch, the 22q11 deletion is often associated with surgical challenge (15).

Arch sidedness and 22q11 deletion

Although the frequency of deletion was significantly higher in patients with right arch as compared to normal left arch, there was difference in its prevalence based on the subtypes of conotruncal defect. Previous investigators have shown that right aortic arch increased the risk of deletion in patients with TOF (13) and TOF/PA (9), which was consistent with our study.

Chromosomal aberrations besides 22q11 deletion

A recent study showed that 5% of infants with conotruncal defects had chromosomal abnormalities (13). The presence of other chromosomal aberrations besides 22q11 microdeletion in three patients in this study specifies a need of conventional cytogenetic analysis in conotruncal malformations.

Phenotypic features in conotruncal defects

In spite of wide spectrum of extracardiac features, children with conotruncal defects owing to 22q11 microdeletion were more likely to have dysmorphic facial features (low set ears, dysplastic flared pinna, short palpebral fissures, bulbous nasal tip, microstomia, high arched palate), thin long fingers and hypocalcemia as compared to those without deletion. The cause of this may be developmental anomalies because of deletion. However, the combination of these features varied with cases.

Diagnostic method

The most extensively and routinely used genetic diagnostic method for the detection of 22q11 microdeletions is FISH using TUPLE1 or N25 probe. It is capable of detecting microdeletions usually missed by conventional karyotyping, but fails to detect atypical deletions. However, the advanced techniques like microarray, high-density multiplex ligation-dependent probe amplification, array-based comparative genomic hybridization and FISH using multiple probes are more sensitive and can detect deletion variants both overlapping and nonoverlapping the common typical deleted region within 22q11. Few studies have shown atypical deletion in conotruncal defects (4,5), but they are more common in patients with atypical phenotypes mildly overlapping with the DGS/VCFS features (3,16,17,18). Atypical distal deletions nonoverlapping the typically deleted region of 3 Mb occur at a frequency of 2% in 22q11 deletion syndrome (2). Genetic testing by FISH is still the gold standard (19), as it can detect 96% of deletions in 22q11 deletion syndrome (16) and is not expensive as compared to other techniques like microarray, quantitative real-time PCR and FISH using multiple probes.

CONCLUSION

The study suggests that extracardiac features along with specific type of conotruncal defect and associated cardiovascular anomaly should alert the clinicians for 22q11 deletion testing. However, if deletion analysis is not possible, specific extracardiac features (six dysmorphic facial features, thin long fingers and hypocalcemia) can help to identify an increased risk of 22q11 deletion in patients with conotruncal defect especially in developing and in underdeveloped countries. In addition, screening of chromosomal anomalies at an early age will assist in better management of patients, thus preventing severe complications. The study also indicates a need for further research with large sample size to understand the exact prevalence and basis for variation in frequency of 22q11 deletion in subtypes of conotruncal defects.

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