Study of Chromosomal Microarray in Children with Multiple Congenital Anomalies

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ABSTRACT

Introduction: Congenital anomalies are the leading cause of infant mortality and can cause long term disability. Therefore aim of present study is to find out copy number variants (CNV) by chromosomal microarray analysis in children with multiple congenital anomalies.

Material and methods: Present study was conducted in the department of Paediatric Medicine, SPMCHI, SMS Medical College Jaipur between June 2015 to May 2016. A broad spectrum of clinical phenotyping measures, including a comprehensive and standardized clinical genetic evaluation was applied. Physical examination, craniofacial anthropometry, standardized somatic morphological characterization and were performed and statistical analysis was done.

Result: Mean age of onset of symptoms was 8.68±2.133 months with male: female ratio was 1.22:1. Out of 20 children, sixty two CNVs over 400 Kb were observed in all patients, ranging between 0 and 7 CNVs per patient. 40% belonged to group 1 (clear clinical relevance related to the phenotype), 60% to group 2(benign or polymorphic).

Conclusion: Our recommendation based on current evidence is to offer CMA as the first-tier genetic test, in place of G-banded karyotyping, for patients with unexplained MCA.

Keywords: Congenital Anomalies, Chromosomal Microarray, Children, Karyotyping

INTRODUCTION

Congenital anomalies are the leading cause of infant mortality and disability affecting two to three of every 100 live newborns.[1] Congenital anomalies can contribute to long-term disability which may have significant impacts on individuals, families, health-care systems and societies.

The most common, severe congenital anomalies are heart defects, neural tube defects and Down syndrome. Approximately 40-60% of congenital malformations have no known origin. About 20% of birth defects are likely to result from genetic and environmental factors combined. 7.5% are caused by single gene mutations, 6% are caused by chromosome abnormalities, and 5% are caused by maternal illness and/or substance use.

Infants with multiple congenital anomalies (MCA) are typically infants with: two or more major malformations (e.g., a neural tube defect, cardiac defect, missing limb), or three or more minor malformations (e.g., syndactly, a club foot, abnormally formed pinna).[2]

Several factors must be considered in assessing MCA etiology like maternal health history, prenatal history, family history, and careful and detailed physical examination of the infant.[3]

In recent years, the etiological study of developmental disorders has been enriched with the clinical use of microarray-based techniques. In developed countries, molecular karyotyping or chromosomal microarray (CMA) is considered the first-line technique for the analysis of patients with multiple congenital anomalies, non syndromic developmental delay/intellectual disability, and autism spectrum disorders.[6] In contrast, in developing nations, detection of chromosomal anomalies is still performed mainly by conventional cytogenetic techniques. GTG (G-bands by trypsin using Giemsa) banding karyotyping in lymphocytes has been mainly used to identify chromosomal abnormalities with a resolution equal or greater than 5-10 mega bases (5-10 Mb).[4]

Fluorescent *in situ* hybridization (FISH) is available for a limited number of diseases caused by chromosomal micro deletions/micro duplications and has a resolution of 2-5 Mb in metaphase and between 50-150 Kb in interphasenuclei.[4] Other molecular techniques have been developed to look for small micro deletions/micro duplications, such as multiplex ligation-dependent probe amplification (MLPA).[5]

Comparative Genomic Hybridization (CGH) was developed to measure the alterations in dosage of DNA sequences throughout the entire genome in a single experiment.[6] CGH has been applied for the study of human diseases, given that gene dosage variations occur in many conditions from cancer to developmental abnormalities. Therefore, detection and mapping of copy number abnormalities provide an approach for associating aberrations with the phenotype and for localizing candidate genes. Microarray formats for array-based Comparative Genomic Hybridization technique (array-CGH) have been developed over the last 10 years.[7] There are many advantages in array-CGH technique application compared to conventional cytogenetic and other molecular cytogenetic approaches.[8]

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Therefore aim of present study is to find out copy number variants (CNV) by chromosomal microarray analysis in children with multiple congenital anomalies which are not explained by any syndromic disorder.

MATERIAL AND METHODS

Present study was a hospital based observational study conducted in the department of Paediatric Medicine, SPMCHI, SMS Medical College, Jaipur between June 2015 to May 2016. Sample size was calculated at 95% confidence level assuming copy number variants can be found out in 73% of the children with multiple congenital anomalies as per the study of **Guillermo Lay-Sona et al**, J Pediatric (Rio J). 2015 at relative allowable error of 15% minimum 20 children of age ≤18 years were required as sample size. Final sample size included 20 children.

Patients less than 18 years attending indoor and outdoor are included while patients with defined genetic syndromes with multiple congenital anomalies were excluded from the study. A broad spectrum of clinical phenotyping measures, including a comprehensive and standardized clinical genetic evaluation was applied. Physical examination, craniofacial anthropometry, standardized somatic morphological characterization and were performed. Comparison was made between the growth of different parts of the body, for example, to see whether head circumference, height and weight are at the same percentile or at different percentiles.

2 ml EDTA sample was collected from each patient and sent to genetic lab SMS medical college jaipur where sample was stored at 4 degree Celsius. Chromosomal microarray enable us to perform high-resolution genome-wide DNA copy number analysis. It also provides genotyping information, enabling detection of loss of heterozygosity (LOH), which can be used to detect UPDs. In general, copy-number variants are assigned the following interpretations; (1) Abnormal (e.g., well-established syndromes, de novo variants, and large changes). (2) VOUS. (3) Likely benign. Diagnostic yield was defined as the number of patients with abnormal variants divided by the total number of patients tested and was derived directly from each original study.

Quantitative data was expressed as mean and standard deviation while qualitative data was expressed as percentage and proportions. Chi-square tests and Z- score were performed to analyze differences in proportions of categorical variables between two or more groups. P value < 0.05 was considered as the cut-off value for significance. All analyses were performed using IBM SPSS statistics, version 2.0, for Windows.

RESULT

Table 1 shows the demographic profile of study subjects. Among those 20 patients who completed the study, the mean age of onset of symptoms were 8.68±2.133 months. Maximum subjects (35%) belonged to age group of 1-12 months followed by 1-5 year age group (30%). In present study, males (55%) outnumbered female (45%).

Table 2 shows the phenotypic profile of study subjects. Most

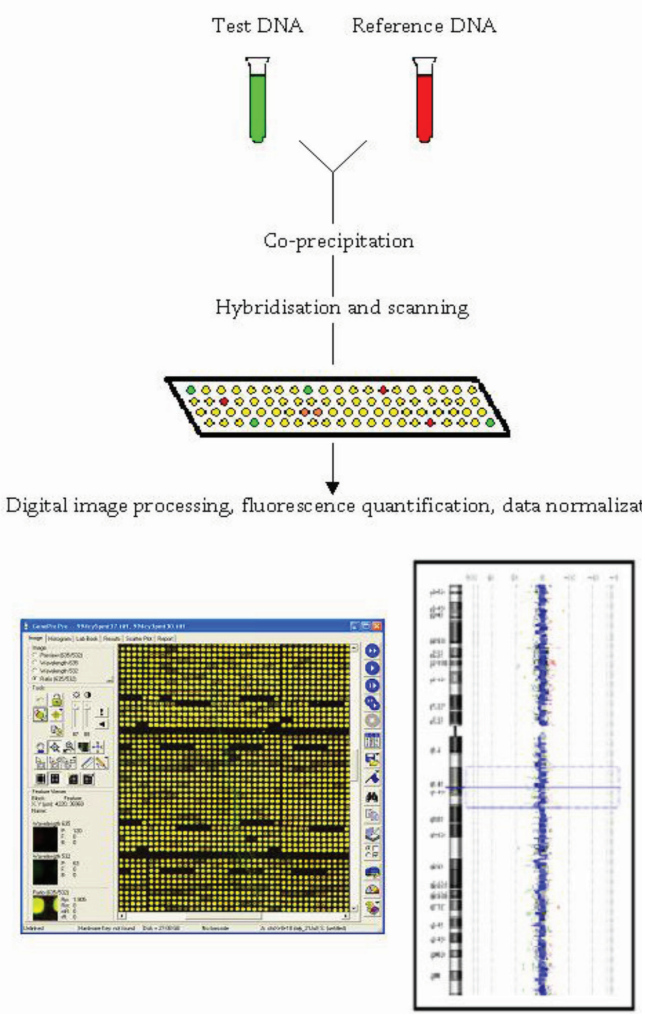
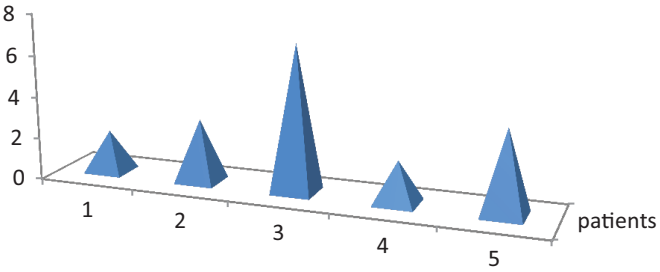


Figure-1: Schematic representation of an array-CGH experiment.



Graph-1: Number of chromosomes involved in present study

common associated phenotypic feature found was facial dysmorphism (50%) and congenital heart disease (50%) followed by Microcephaly (45%).

Figure 1 shows that all subjects have involvement of at least 1 chromosome and 3 chromosomes involvement was most common in present study.

This pie chart shows that gains were found at 82% of total common number variants and in 40% of cases, pathological common number variants were found.

DISCUSSION

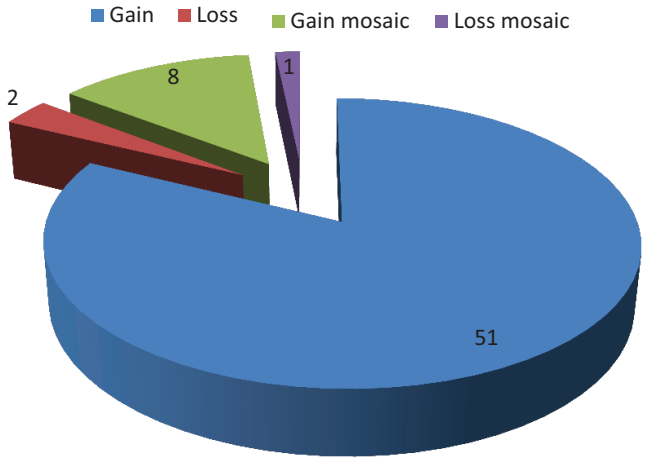
Chromosomal anomalies are a major cause of perinatal morbidity and mortality. Since the late 1960's full

S.No.	Variable	Cases		
		No.	%	
1	Age group			
	0-1 month	4	20%	Mean age= 8.68±2.133 (5-16 months)
	1-12 months	7	35%	
	1-5 years	6	30%	
	>5 years	3	15%	
2	Gender			
	Male	11	55%	
	Female	9	45%	

Table 1: Demographic Profile of the children in present study

Phenotype	Cases	
	Number	Percentage
Microcephaly	9	45%
Facial dysmorphism	10	50%
Congenital cataract	3	15%
Cleft palate/Pierre robin sequence	6	30%
Congenital heart disease	10	50%
Kidney malformation	3	15%
Limb anomaly	6	30%
Skeletal anomaly	2	10%
Short stature	3	15%
Skin defect	2	10%

Table-2: Distribution of children according to phenotypic profile



Graph2: Characterization of common number variants found

conventional G-band karyotyping has been the mainstay of excluding and diagnosing structural karyotypic abnormality. Despite significant improvements in cytogenetic resolution over the last 25 years, conventional full karyotyping can only detect anomalies down to a resolution of 5-10 Mb.[9]

Fluorescence in Situ Hybridization (FISH) is a useful adjunct to full conventional karyotyping when a high degree of suspicion of a specific chromosome anomaly is present (e.g. Di George syndrome and a cardiac anomaly) or when clarification of a difference seen on karyotype is sought. The next development was array Comparative Genomic Hybridization (aCGH) / Chromosomal Microarray (CMA). CMA combined the CGH technique with the use of microarray.[10] It was used to identify submicroscopic deletions or duplications in children with multiple congenital anomalies, undiagnosed learning difficulties and mental retardation.[11-12]

CMA has many advantages when compared with the chromosome testing strategy described above. CMA utilizes uncultured cells reducing the "turnaround time of the results" and is amenable to automation and high throughput analysis that provide the potential for positive health economic effects. Microarrays will become quicker to analyze as chromosomal variants detected will be compared to local (within the laboratory), national and international databases. Most importantly it is of "high resolution" allowing detection of submicroscopic deletions or duplications though the specific choice of CMA platform determines the resolution of the test.[13-14]

The most significant current potential disadvantage of CMA is the identification of novel, previously unreported, variants of unknown significance (VOUS) that can cause difficulties in clinical management because of the uncertainty of the relationship between the VOUS and likely clinical effect. Clinical geneticists, pediatric neurologists, and developmental pediatricians are increasingly ordering CMA to obtain a genetic diagnosis for their patients with unexplained DD/ID, ASD, and MCA. A specific genetic diagnosis facilitates comprehensive medical care and accurate recurrence risk counseling for the family.

Similar to our results, a recent meta-analysis of CMA on 13,926 subjects with ID and/or MCA, most of whom had normal conventional cytogenetic studies, reported an overall diagnostic rate of 10% for pathogenic genomic imbalances.[15] Another retrospective analysis of 36,325 patients with DD estimated that a pathogenic abnormality could be detected in ~19% of unselected DD/ID patients via genome-wide array-based assays with a 30–70 kb median probe spacing.[16]

In our study, males (55%) outnumbered females (45%) and mean age of the children were 8.68±2.133 months. Study by **Guillermo et al**[17] observed results similar to present study with median age of 4.2 years.

In our study, out of 20 children, 62 CNVs over 400 Kb were observed in all patients, ranging between 0 and 7 CNVs per patient. Of the total, 2 were losses and 51 were gains. The size of chromosomal imbalances ranged from 420.9 Kb to 25.2 Mb. These 62 CNVs were classified into two groups based on their clinical interpretation. According to

the classification adopted 40% belonged to group 1 (clear clinical relevance related to the phenotype), 60% to group 2 (benign or polymorphic).

A study by ***Guillermo et al*** [17] Fifty-five CNVs over 400 Kb were observed in 29 of 40 patients (72.5%), ranging between 0 and 4 CNVs per patient. Of the total, 11 were losses and 44 were gains. 21.8% belonged to group 1 (clear clinical relevance related to the phenotype), 1.8% to group 2 (unclear relevance), and 76.4% to group 3 (benign or polymorphic). Losses were predominant in group 1, while gains were predominant in group 3. In study done by **Shaw et al**[18] twelve copy number abnormalities were identified in 12 patients (24% of the total): seven deletions (six apparently de novo and one inherited from a phenotypically normal parent) and five duplications (one de novo and four inherited from phenotypically normal parents). Altered segments ranged in size from those involving a single clone to regions as large as 14 Mb.

Krepischi-Santos AC[19] conducted a study in which chromosome imbalances not previously detected in normal controls were found in 30 patients (31%) and at least 16 of them (17%) seem to be causally related to the abnormal phenotypes. Eight of the causative imbalances had not been described previously. **Mnten et al**[20] conducted a study in which submicroscopic chromosomal imbalances were detected in 28 of the 140 patients (20%) and included 18 deletions, seven duplications, and three unbalanced translocations. Seventeen of 24 imbalances were confirmed de novo and 19 were assumed to be causal. Excluding subtelomeric imbalances, their study identified 11 clinically relevant interstitial submicroscopic imbalances (8%).

In study done by **Rosenberg et al**[21] imbalances never observed in control chromosomes were detected in 20 patients (25%): seven were de novo, nine were inherited, and four could not have their origin determined. **Baris et al**[22] studied 373 patients at Children's Hospital Boston who had normal chromosomal analysis and were tested with this targeted array-based comparative genomic hybridization over a 1-year period from November 1, 2004 to October 31, 2005. These patients were tested because of a suspicion of chromosomal abnormalities based on their clinical presentation. Thirty-six patients (9.7%) had abnormal array-based comparative genomic hybridization results. Twenty patients (5.4%) had potentially pathogenesis genomic imbalances and 16 patients (4.3%) had copy number variations that are not believed to be pathogenesis.

Shaffer et al[23] presented the results of array CGH in 8,789 clinical cases submitted for a variety of developmental problems. Of these cases, 6.9% showed clinically relevant abnormalities, 1.2% showed benign copy-number variants (polymorphisms), 2.5% showed recurrent alterations of unclear clinical significance-many of which are likely to be polymorphisms-and 1.4% showed novel alterations of unclear relevance.

CONCLUSION

The present study detected over 40% pathogenic alterations in this cohort, which is in the upper range that has been reported in the literature. Our recommendation based on current evidence is to offer CMA as the first-tier genetic test, in place of G-banded karyotyping, for patients with unexplained MCA. CMA testing is not inexpensive, but the cost is less than the cost of a G-banded karyotyping plus a customized FISH test such as sub-telomeric FISH and the yield is greater.

Limitation of the study:

Parent's microarray was not done so differentiation between pathological and benign copy number variants was effected and sample size was very small.

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