Short communication

Co-occurrence of mosaic supernumerary isochromosome 18p and intermittent 2q13 deletions in a child with multiple congenital anomalies

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A R T I C L E   I N   P R E S S

A B S T R A C T

The present study deals with karyotype–phenotype correlations in a six month old child with multiple congenital abnormalities. Cytogenetic analysis revealed mosaicism of a small metacentric supernumerary marker chromosome with a karyotype mos 47,XY+mar[34]/46,XY[31]. Cytogenetic microarray result showed three copies of chromosome 18p (15,400 kb in size). Moreover, 255 kbp intermittent deletion of chromosome 2q13 involving RGPD5, RGPD6, LIMS3, and LIMS3-LOC440895 was also observed. Correlating microarray data with the mosaic karyotype, the marker chromosome was identified as mosaic isochromosome 18p and was found to be 32,600 kbp in size. Baby resembled clinical characteristics of trisomy chromosome 18p, isochromosome 18p and trisomy chromosome 18. The present study suggested that deletion of evolutionarily conserved developmental genes (RGPD5, RGPD and LIMS3) in the 2q13 region might have contributed to more severity in phenotype as compared to so far such reported cases of 18p trisomy's, as these are involved in nuclear-cytoplasm trafficking, signaling for tissue patterning and differentiation.

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1. Introduction

Isochromosome 18p is a very rare chromosomal disorder with the prevalence of 1 in 140,000–180,000 live births. It is reported to be associated with certain degree of phenotypic variability. It may occur with other submicroscopic genomic imbalances. Isochromosome 18p is due to the presence of two copies of 18p arm on the same chromosome (Brambila Tapia et al., 2010; Dundar et al., 2010; Plaiasu et al., 2011). It is one of the most commonly observed isochromosome with no gender biasness and was for the first time reported by Froland et al. (1963). Isochromosome 18p is a result of nondisjunction in maternal meiosis II (Dundar et al., 2010; Plaiasu et al., 2011) and characterized by non-specific morphological features, low birth weight, microcephaly, low-set ears, strabismus, a small pinched nose, short palpebral fissures, and a small jaw (Kotzot et al., 1996). Developmental delay like motor and mental retardation, varying degrees of contractures and scoliosis, feeding difficulties, rare cardiac and renal malformations are also reported in this disorder (Ramegowda et al., 2006; Bakshi et al., 2006). Except few familial cases, majority of the isochromosome 18p seem to be de novo.

The present case report deals with karyotype–phenotype correlation in a child with multiple congenital abnormalities carrying a mosaic metacentric marker chromosome which was identified as isochromosome 18p with the help of cytogenetic microarray. This child also presented a chromosome 2q13 deletion involving evolutionarily conserved developmental genes RGPD5, RGPD6, LIMS3, and LIMS3-LOC440895.

2. Material and methods

2.1. Case presentation

A six month old male child with multiple congenital abnormalities was referred from Pediatrics Department, Sir Sunderlal Hospital, Banaras Hindu University, Varanasi with complaint of generalized weakness and facial deformity to Centre for Genetic Disorders, Banaras Hindu University, Varanasi. The child was a second baby of healthy non-consanguineous parents. Father and mother’s age was 29 and 22 years, respectively at the time of the case child birth. The mother had one induced abortion due to congenital malformed fetus. The third baby of the parents was healthy without any complication.

Proband was born as full term with intra-uterine growth retardation (IUGR). He had clinical features like craniosynostosis, microcephaly, partial corpus callosum agenesis, hydrocephaly, dysmorphic ears,
ankyloblepharon, deformed nose, unilateral complete cleft of upper lip, micrognathia, abnormal cheeks, popliteal pterygium, adactyly, syndactyly, ectrodactyly and congenital talipes equinovarus (CETV) in feet and hand (Fig. 1A and B) and also suffered hypospadias. As per information given by parents, the child survived for six years.

2.2. Cytogenetic analysis

For cytogenetic study peripheral blood was drawn in heparinized vial from the child after written consent taken from parents. Whole blood culture was performed in RPMI 1640 pH 7.2 (Sigma-Aldrich, Inc., St. Louis, MI, USA) culture medium supplemented with 10% fetal bovine serum (Himedia) stimulated by Phytohaemagglutinin-M (Sigma-Aldrich, Inc., St. Louis, MI, USA) for 72 h at 37 °C. Metaphases were arrested at 70 h with colchicine treatment (Sigma-Aldrich, Inc., St. Louis, MI, USA) (0.02 μg/ml). Cells were harvested by hypotonic treatment and G-banding of chromosome was performed by Saline–Trypsin–Giemsa (STG) method. Sixty five metaphases with 450 G-band resolution were observed under microscope (Carl Zeiss Microscopy Gmbh, Göttingen, Germany) and karyotyping was done with the help of Ikaros karyotyping system—Metasystems software (Carl Zeiss Microscopy Gmbh, Göttingen, Germany) to see chromosomal abnormality.

2.3. Cytogenetic microarray analysis

Genomic DNA was extracted from heparinized peripheral blood of proband by modified salting out method (Miller et al., 1988). Cytogenetic microarray experiment was performed with patient genomic DNA using 2.7 M cytogenetics array (Affymetrix, Inc. Santa Clara, CA, USA) as per manufacturers’ instruction. The array consists of 2,761,979 copy number markers with 1086 bp average marker spacing. Data were analyzed with Chromosome Analysis Suite (ChAS) software (Affymetrix, Inc. Santa Clara, CA, USA).

2.4. Quantitative PCR analysis

In order to validate copy number result obtained from cytogenetic microarray PCR was performed by relative quantification method in the ABI 7500 Real time PCR machine using 2 × SYBR Green Real time PCR master mix (Thermo Scientific, USA). 50 ng DNA was added to 20 μl of 2 × SYBR-green PCR master mix. All of the reactions were performed in triplicate and included non-template control for each gene. The amount of target gene Thymidylate synthase (TYMS) on chromosome 18p was normalized to beta Actin as the reference gene.

3. Results

The child showed phenotypic heterogeneity with congenital malformations resembling clinical characteristics of trisomy chromosome 18, trisomy chromosome 18p and isochromosome 18p (Table 1). However, clinical features like craniosynostosis, partial corpus callosum agenesis, hydrocephaly, ankyloblepharon, abnormal cheeks, left hand popliteal pterygium, adactyly, and ectrodactyly are not yet reported to be associated with chromosome 18 aneuploidy (Table 1).

Karyotype of the child showed a small metacentric supernumerary marker chromosome in 52% (n = 34) of observed metaphases (47, XY+mar) (Fig. 1C). The rest of the 48% (n = 31) metaphases were normal (46,XY) (not shown). Cytogenetic investigation of the father and mother of the child showed 46,XY and 46,XX karyotypes respectively revealing de novo origin of the marker chromosome. Cytogenetic microarray showed one copy gain for 15,400 kbp 18p11.21-18pter chromosomal region (Fig. 1D) and size of marker chromosome was found to be precisely 32,600 kbp. An intermittent deletion of 255 kbp in...
chromosome 2q13 spanning the proximal region chr2:110,664,787–110,538,809 (125,980 bp) and distal region chr2:111,221,158–111,350,533 (129,375 bp) (UCSC Genome Browser, GRCh37/h19, http://genome.ucsc.edu) involving \textit{RGPD5}, \textit{RGPD6}, \textit{LIMS3}, and \textit{LIMS3-LOC440895} genes was observed (Fig. 2A). The presence of other genomic duplications of the pericentromeric region was excluded because of the absence of any gene in that region. Real-time quantitative PCR resulted in ~1.5 fold increase of target gene \textit{TYMS} amount relative to the reference beta Actin gene, which was in concordance with the results of cytogenetic and microarray data analysis.

4. Discussion

Karyotyping of the child revealed 52% mosaicism for metacentric supernumerary marker chromosome \([\text{mos}(47,XY+\text{mar}[34]/46,XY[31])]\). Cytogenetic microarray resulted three copies of the 18p11.21-18pter

| Table 1: Clinical features in the child resembling clinical characteristics of different chromosome 18 aneuploidies. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Features resembling trisomy chromosome 18 syndrome | Features resembling trisomy chromosome 18p syndrome | Features resembling tetrasomy chromosome 18 syndrome | Features only shown by present case child |
| Failure to thrive | Failure to thrive | Failure to thrive | Partial corpus callosum agenesis |
| Microcephaly | Microcephaly | Microcephaly | Hydrocephaly |
| Micrognathia | Micrognathia | Micrognathia | Craniosynostosis |
| Dysmorphic ears | Dysmorphic ears | Deformed nose | Ankyloblepharon |
| Cleft lip and cleft palate | NR | NR | Abnormal cheeks |
| Club feet | Club feet | NR | Ectrodactyly |
| Syndactyly | IUIGR | NR | Left hand popliteal pterygium |
| Hypospadias | NR | NR | |

NR = not reported.
cytogenetic region which was 15,400 kbp (1800 kbp per centromeric region 18p11.1 is not included due to the absence of markers on the array). Thus it was obvious that one copy gain of chromosome 18p was due to the metacentric marker chromosome. This marker chromosome was observed in only 52% of the cells concluding that the metacentric marker chromosome was isochromosome 18p in mosaic form and showed three copy numbers in the cytogenetic microarray experiment result. Fluorescent in situ hybridization (FISH) could not be performed due to insufficient chromosome suspension and unavailability of more blood sample since the child was no longer alive.

Mosaic SMCs are derived from almost all chromosomes to varying degrees (mos 47;+mar/+46) and for chromosome 18, level of mosaicism is 67% (Liewh et al., 2010). Several reports from prenatal diagnosis show varying degrees of mosaicism for isochromosome 18p with unusual clinical features like small head, low-set ears, poorly formed philtrum, epicanthic folds, a pinched nose, high-arched palate, small upper lip, retrorotathia, contracture of fingers, rocker-bottom feet, a short first toe, short broad hallux, hypoplastic penis, mild scoliosis and angulation of clavicles, and mitral valve dysplasia (Abeliovich et al., 1993; Gocke et al., 1986; Verschraegen-Spae et al., 1993; Blennow et al., 1994; Hsu et al., 1996; Pinto et al., 1998; Kim et al., 2009). But, in the present case, clinical features of the child resembled with clinical characteristics of trisomy chromosome 18, trisomy chromosome 18p, and isochromosome 18p (Table 1). Apart from the abovementioned clinical features the child had craniosynostosis, partial corpus callosum agenesis, hydrocephaly, ankyloblepharon, left hand popliteal pterygium, abnormal cheeks, adactyly and ectodactyly.

The child was also having a 255 kbp intermittent deletion of chromosome 2q13 involving RGPD5, RGPD6 and LIMS3 genes which could be correlated with rare clinical features observed in the present study. Deletion of these evolutionarily conserved developmental genes might have added to improper development of the child leading to comparatively severe congenital malformation than other cases with isochromosome 18p. LIMS3-LOC440895 is not reported to have any clinical correlation.

Chromosome 2q13 deletion leads to central nervous disorders for example cortical disruption in Joubert syndrome due to deletion of the NPHP1 gene (Konrad et al., 1996). Deletions in this region have been associated with pervasive developmental disorder (not otherwise specified) and autism spectrum disorder (Shen et al., 2010). Deletion on chromosome 2q13 involving only RGPD5, RGPD6, LIMS3, and LIMS3-LOC440895 genes is not reported. RGPD5/RGPD6 (RANBP2-Like and Grip domain-containing protein) originated from the highly conserved nucleoparin RanBP2 by several genetic rearrangements. RGPD5 (OMIM: 612708) and RGPD6 (OMIM: 612709) are two of the six RGPD genes on chromosome 2q12.3-q13 that resulted from duplication. The C terminus includes GRIP domain from GCC2 and encodes polysaccharide ABC transporter ATP-binding protein. It is associated with the nuclear membrane and is thought to control a variety of cellular functions through its interactions with other proteins (Ciccarelli et al., 2005). LIMS3 (no OMIM Entry) (LIM and senescent cell antigen-like domains 3) is a protein-coding gene which has been conserved through evolution. A LIM domain contains two zinc fingers, each of which binds one zinc ion (Michelsen et al., 1993). It participates in processes of cell–cell and cell–matrix adhesion, formation of multiprotein complexes, and facilitation of cell spreading, migration and survival. In all organisms it is involved in tissue patterning (neural) and differentiation.

Chromosome 2q13 deletion in the present case child overlapped with patients 266503, 577801, 252497, 283054, and 280574 in DECIPHER database (https://decipher.sanger.ac.uk/) but no clinical features were reported for these patients. Patients 289817, 266729, and 280574 shared proximal deleted region of the present case child, of them patient 289817 (absence of seizure) had almost the same deletion (chr2:110,521,667–110,649,116) found at the proximal end in the present case child. Patient 266729 had cognitive impairment, and abnormalities of the kidney and liver with larger deletion (chr2:110,504,318–110,980,108) compared to the proximal deletion of the present case child. No clinical feature was reported for patient 280574. None of the abovementioned clinical features reported in patients of DECIPHER database were presented by the present case child. Patients 272764 and 250370 shared distal deleted region of the present case child (Fig. 2B). Of these cases in DECIPHER, patient 272764 was reported to have clinodactyly, delay speech language, epicantus, short attention span and toenail dysplasia with a larger deletion (chr2:110,783,258–111,368,862) compared to distal deletion of the present case child. For patient 250370 no clinical feature was mentioned.

SMCs lead to phenotypic heterogeneity which varies remarkably with its euchromatic DNA content and level of mosaicism. It becomes very crucial to determine origin and constitution of the marker chromosome for genotype–phenotype correlation and genetic counseling to predict the prognosis. Besides the gross chromosomal anomalies, the submicroscopic deletion and duplications which go unnoticed in conventional cytogenetic methods (due to limited resolutions) are very crucial in explaining the phenotypic variability of a disorder. Cytogenetic microarray seems an important tool for identification of chromosomal origin and accurate delineation of duplicated and deleted regions. It is time saving as well as cost effective when compared with the use of multiple FISH probes and can be effectively maneuvered in clinical diagnosis of several undiagnosed ailments.

To the best of our knowledge there are no reports of mosaic isochromosome 18p with intermittent deletion of duplicated inverted region of chromosome 2q13 that has important developmental genes like RGPD5, RGPD6, LIMS3, and LIMS3-LOC440895.

5. Conclusions

The present study indicates a supernumerary metacentric marker chromosome showing 52% mosaicism in isochromosome 18p leading to phenotypic heterogeneity. Deletion of important developmental genes RGPD5, RGPD6 and LIMS3 in the 2q13 region might have led to rare phenotypic features in the child compared to other published cases of trisomy 18.

Conflict of interest

The authors declare no conflict of interest.

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