RESEARCH NOTE

Overlap of Patau and Pierre Robin syndromes along with abnormal metabolism: an interesting case study

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Introduction

Patau syndrome in humans is due to trisomy 13, whose frequency is one in every 10,000 newborns (Zergollern *et al.* 1975; Nanjiani *et al.* 2007). Symptoms include cleft lip or palate, clenched hands (with outer fingers on top of the inner fingers), close-set eyes, decreased muscle tone, extra fingers or toes (polydactyl), hole in the iris (Koole *et al.* 1990), mental retardation, severe seizures, skeletal (limb) abnormalities, small head (microcephaly), small lower jaw (micrognathia) and undescended testicles (cryptorchidism). There are often signs of congenital heart disease, such as atrial septal defect (ASD), patent ductus arteriosus, ventricular septal defect (VSD). MRI or CT scans of the head may reveal a holoprosencephaly or other brain related problems (Rosa *et al.* 2011). Often more than 80% of children with trisomy 13 die in the first month (Iliopoulos *et al.* 2006).

Pierre Robin syndrome or sequence or glossoptosis is a well documented facial abnormality in newborns (Jones 1997). It is a congenital condition characterized by a smaller lower jaw than normal, a tongue that falls back in the throat, difficulty in breathing, small opening in the roof of the mouth which often causes choking and also cleft soft palate (Kliegman *et al.* 2007; Breugem *et al.* 2009). An earlier molecular genetic study on a patient with Pierre Robin sequence showed (2, 17) (q23.3; q24.3) translocation in which 17q breakpoint was mapped to 1.13 Mb upstream of *SOX9* gene and 800 kb downstream of *KCNJ2* gene. It was suggested that disorder may be caused by both *SOX9* and *KCNJ2* dysregulation (Jakobsen *et al.* 2007). Benko *et al.* (2009) identified translocations, each sharing a breakpoint at chromosome 17q24. The karyotype of another patient with Pierre Robin syndrome showed 46,XY,t(1;2)(p34;q33) and the 1p breakpoint was found to interrupt the *FAF1* gene within its first intron resulting in low *FAF1* expression in the proband's lymphocytes compared to the control (Ghassibe-Sabbagh *et al.* 2011).

In this paper, phenotypic heterogeneity of an infant showing clinical features of both Patau and Pierre Robin syndromes, along with other metabolic anomalies are discussed. The diverse clinical manifestations (phenotypic heterogeneity), results due to genomic imbalance observed in the subject are discussed in this study.

Case report

A six-month-old female infant with marked retrognathia and inspiratory stridor suggested Pierre Robin syndrome (figure 1). The infant also had multiple congenital anomalies in the form of coloboma in both of the eyes, cleft soft palate, congenital heart disease with bidirectional interatrial shunt and polydactyly in both the hands and feet, clenched fingers, these clinical features indicated Patau syndrome. The infant was born to a nonconsanguineous parents as a preterm through normal vaginal delivery at 31 weeks, a little earlier than the normal 37 weeks gestation time. Low birth weight (< 2500 g) was recorded as 1800 g, length of the baby was 46 cm and head circumference was 30.8 cm. She expired after 17 months which means that the infant lived a little longer than the general survival rate of Patau syndrome cases. Family history ruled out previous occurrence of the complex phenotypes.

Materials and methods

Chromosomal analysis was performed with heparinized peripheral blood drawn from the child after taking written consent from parents. Lymphocyte culture was set up with

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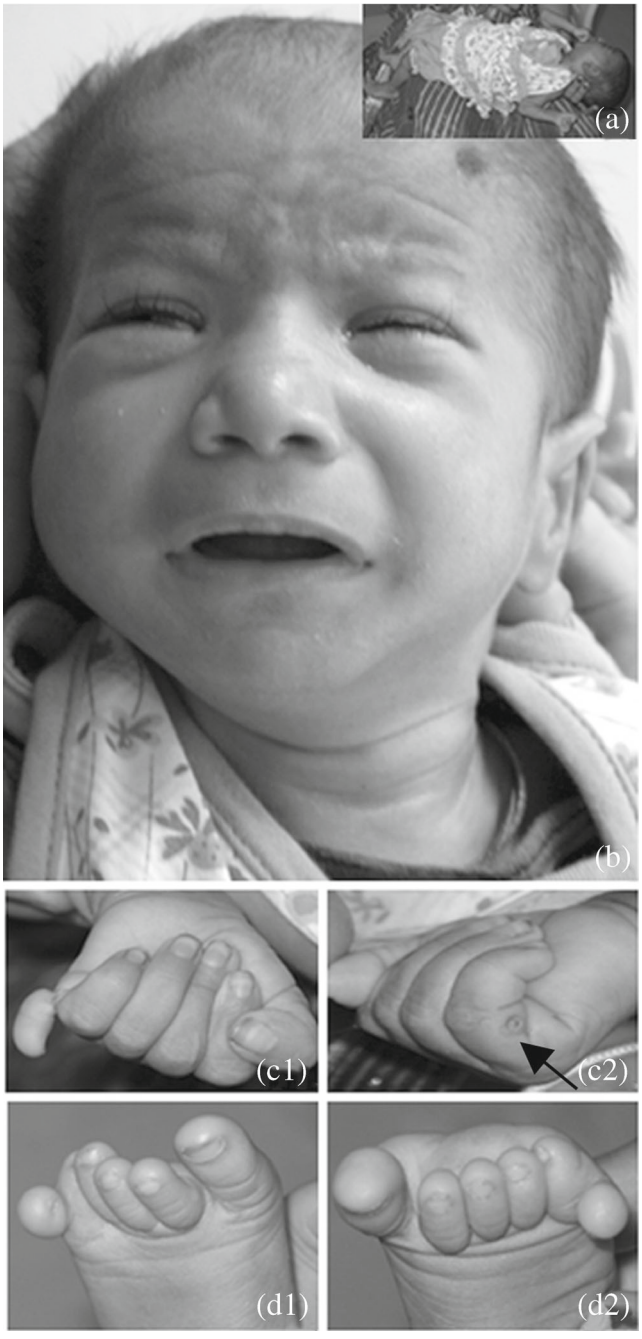

Figure 1. Clinical phenotypes of female child with trisomy 13. (a) Weak female child 06 months old (in inset). (b) Facial appearance showing retrognathia indicating Pierre Robin sequence. (c1) Left hand with one extra digit. (c2) Right hand with one small extra digit (arrow). (d1) Left feet with extra 6th finger. (d2) Right feet with extra 6th finger.

0.3 mL of blood in 5 mL of RPMI-1640 pH 7.2 (Sigma-Aldrich, St. Louis, USA) culture medium supplemented with 10% feotal bovine serum and 0.05 mL of 1 mg/mL phytohaemagglutinin-m (Sigma-Aldrich, St Louis, USA) as a mitogen. Culture was incubated for 72 h at 37°C and arrested at metaphase after 70 h by adding colchicine (0.02 μg/mL). Cells were treated with prewarmed hypotonic solution (4.49 mg/mL KCl and 4.0 mg/mL Na-citrate) for 12 min at 37°C. The cells were collected and centrifuged at 1500 rpm for 5 min and fixed with acetic acid and methanol in 1:3 ratio. Clear cell suspensions were obtained by processing with the fixative 3–4 times. Slides were prepared by laying drops of suspension on alcohol cleaned slides and then exposed to flame for instant drying. G-banding was performed by saline-trypsin-giemsa (STG) method and mounted with DPX (distrene, plsticiser, xylene). Twenty metaphases with 450 G-band resolutions were observed under microscope (Carl Zeiss Microscopy Gmbh, Göttingen, Germany). Karyotyping was done with the help of Ikaros karyotyping system-Metasystems software (MetaSystems GmbH, Altlussheim, German).

Fluorescence *in situ* hybridization (FISH) was carried out using plasmid alphoid probe specific for the chromosomes 13 and 21 which was directly labelled with fluorescein isothiocyanate (FITC) by nick translation (Vysis, Abbott, USA) and hybridized to metaphases. Genomic DNA was isolated from peripheral blood of the patient by salting out method (Miller *et al.* 1988). Microarray was performed on genomic DNA using 2.7 M cytogenetics array (figure 2) (Affymetrix Inc., Santa Clara, USA) following the manufacturer's instructions. Homocysteine was measured by reverse phase high-performance liquid chromatography (HPLC) as described by (Kumar *et al.* 2005) (Shimadzu, Kyoto, Japan). Vitamin B_{12} and folic acid were measured by chemiluminescence method (Immulite 1000 Analyser, SIEMENS Diagnostic Products, Flanders, USA) as per the manufacturer's instructions.

Results and discussion

The infant presented strong clinical ailments like apnoea, tonic spasms, absence of cry, visible reflexes respiratory distress and neonatal sepsis on day two of postnatal life and initially, her survivability was managed with broad spectrum of antibiotics. She showed metabolic abnormalities like dyselectrolemia, hypoglycemia, neonatal-hyperbilirubinemia ($\geq$15 mg/dL bilirubin), hyperhomocysteinemia (HypHcy, 21.2 μmol/L) alongwith low levels of vitamin B_{12} (172 pmol/L) and folic acid (2.6 ng/mL). HypHcy $\geq$15 μmol/L of Hcy, vitamin B_{12} deficiency $\leq$ 220 pmol/L and folic acid deficiency $\leq$ 3.0 ng/mL were taken as a reference, reported by Sukla and Raman (2012) in a healthy population. Impaired folate metabolism was shown to be associated with low birth weight, prematurity and postnatal complications (Black 2008; Sukla *et al.* 2013), also observed in this study. The soft cleft palate could be due to afflicted folate metabolism. Folic acid plays crucial role in early embryonic development and its deficiency often result in clinical ailments like neural tube defects and other orofacial disorders including cleft lip $\pm$ palate (Pitkin 2007; Johnson and Little 2008). She was kept on conservative treatment before being discharged on day 25 from birth but was again readmitted with respiratory complaints. Multiple

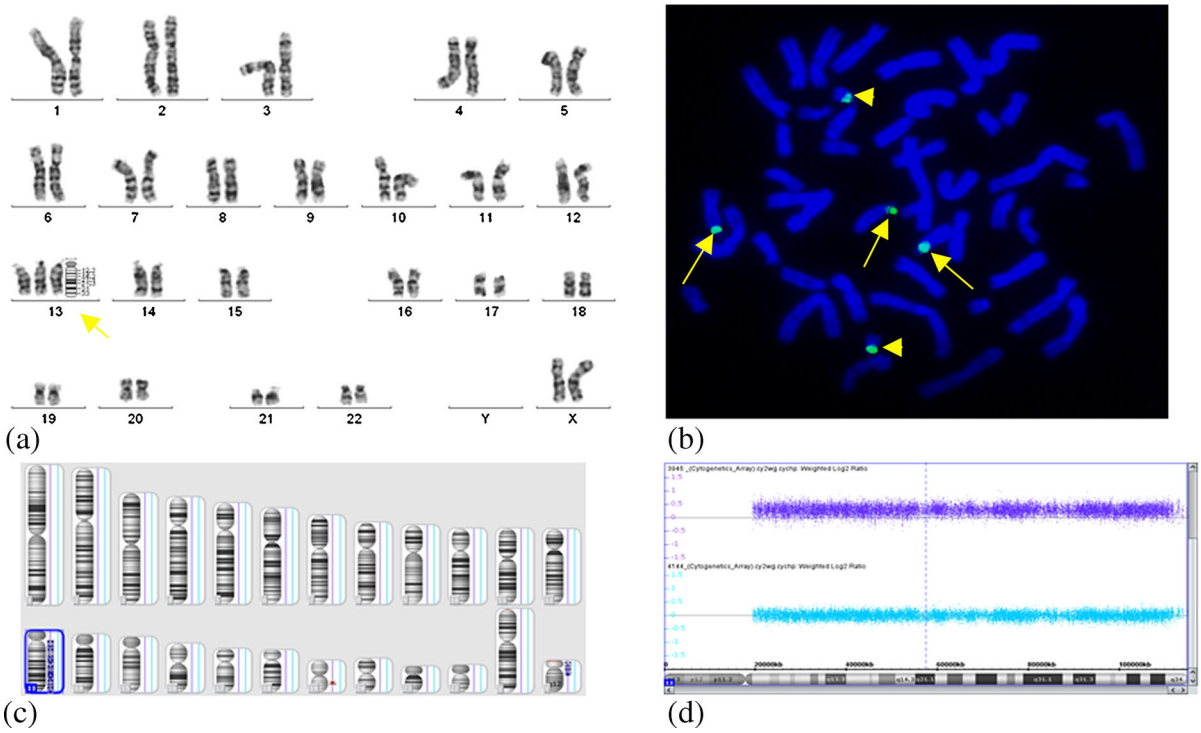

Figure 2. Molecular genetics analysis. (a) Karyotype 47,XX+13 showing extra chromosome 13 (→). (b) FISH showing three signals on pericentromeric region chromosome 13q (→) and two signals on chromosome 21q (►). (c) Oligo based micro-array result of whole genome showing genomic imbalances. (d) Oligo based micro-array result showing extra copy increase (in blue colour) than the control (in sky-blue).

congenital anomalies were noted for which cytogenetic and molecular tests were advised.

Karyotype of the patient revealed three copies of chromosome 13 (47,XX+13) which was further confirmed with the help of FISH using plasmid alphoid probe specific for pericentromeric region of chromosomes 13 and 21. Cytogenetic microarray resulted in three copies of chromosome 13 as expected. In addition, a deletion of 380 kb on 19q chromosome involving *LOC100289650* and a cluster of seven genes pregnancy-specific-beta-1-glycoprotein family (*PSG1, PSG2, PSG5, PSG6, PSG7, PSG10* and *PSG11*) was observed. Thus far, no clinical correlation with the above mentioned malformations have been reported with this pregnancy-specific-beta-1-glycoprotein. On the other hand, there was no gain or loss of the genomic regions like 17q24 or 1p, which was associated with Pierre Robin syndrome in earlier studies (Benko *et al.* 2009; Ghassibe-Sabbagh *et al.* 2011).

This case showed genetic heterogeneity with varied phenotypic characteristics of Patau syndrome, showing clinical complications associated with Pierre Robin syndrome. The subject indeed presented atypical nature with several malfunctions in various metabolic pathways including soft cleft palate, facial anomalies along with amalgamation of impaired folate, bilirubin and glucose metabolism. The possibility of interference occurring in these impaired metabolic pathways resulting in the cumulative phenotype of the disorder remains open. Systematic analysis with convergence of both metabolomics and genomics in detail would give a comprehensive picture to understand the phenotypic heterogeneity in these complex clinical anomalies.

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