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ORIGINAL ARTICLE

Mutational Screening and Prenatal Diagnosis in Cornelia de Lange syndrome

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Abstract Phenotypic variability and the lack of a diagnostic marker have complicated the rapid diagnosis and genetic counseling for Cornelia de Lange syndrome (CdLS). The clinical features of CdLS are striking and easily recognizable by characteristic facial dysmorphism, upper-extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, and gastrointestinal abnormalities with severe mental retardation. The molecular diagnosis is essential for predicting prognosis and genetic counseling in the affected family, especially while planning the next pregnancy. We report here from India six cases of CdLS and how precise mutational screening in two cases helped in prenatal diagnosis and proved significant in prevention of recurrence in the affected family.

Keywords Cornelia de Lange syndrome · Prenatal diagnosis · Mental retardation

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Introduction

Cornelia de Lange syndrome (CdLS), is a clinically heterogeneous developmental disorder characterized by various congenital anomalies [1, 2]. The distinctive facial features include synophrys, long eyelashes, micrognathia, depressed nasal bridge with an up-tilted nasal tip and anteverted nares, thin upper-lip with down-turned corners of the mouth, and posteriorly rotated low-set ears. Other frequent features include hearing loss, ophthalmologic findings (ptosis and myopia), palatal abnormalities, genitourinary abnormalities (cryptorchidism and hypospadias), cardiac septal defects, and congenital diaphragmatic hernias. Growth retardation is an almost universal finding in CdLS and typically has a prenatal onset. Mental retardation in CdLS is often severe, with a mean IQ of 53 (range 30–86) [3]. Many patients also demonstrate autistic and self-injurious behavior [4]. The prevalence of CdLS is estimated to be as high as 1/10,000 [5]. In Indian scenario, the parents of affected children often face the difficulties of accurate diagnosis and prenatal diagnostic facilities due to lack of adequate molecular laboratory infrastructure and genetic counseling expertise.

Mutational Analysis

Mutations in the NIPBL [OMIM #608667] gene were identified in clinically diagnosed CdLS patients. Through the combined use of genome wide linkage-exclusion

analysis and the mapping of a chromosomal rearrangement on chromosome 5p13, NIPBL was identified as a CdLS disease gene which has 46 coding exons and spans 188 kb [6, 7]. Nonsense, missense, frameshift, and splicing mutations have been identified in the NIPBL gene. Recently, intragenic deletions of one or more exons of NIPBL have been reported in approximately 2 % of patients with a clinical diagnosis of CdLS. The NIPBL mutations were found in clinically diagnosed 56 of 120 (47 %) patients, and reported in more severely affected CdLS patients with growth, development and limb anomalies. Patients with a missense mutation are seen more mildly affected than those with a truncating mutation [6].

Mutations in the SMC1A [OMIM #300590] gene have also been identified in 5 % of CdLS patients (about 9 % of those negative for NIPBL mutations). SMC1A has 25 coding exons. Only missense mutations and in-frame deletions have been identified in the SMC1A gene. It is reported that patients with mutations in NIPBL tend to be more severely affected than those with mutations in SMC1A. No patients with mutations in SMC1A have so far been reported with limb reduction defects.

Genetic Counseling

CdLS is inherited in an autosomal dominant manner or in an X-linked manner. NIPBL mutations are inherited in an autosomal dominant pattern, while SMC1A mutations are X-linked and have been found in both males and females. The vast majority of affected individuals have a de novo mutation; fewer than 1 % of individuals diagnosed with NIPBL-related CdLS have an affected parent. The risk to sibs of a proband depends on the genetic status of the parents, and hence mutational screening in parents is necessary. Each child of an individual with NIPBL-related CdLS has a 50 % chance of inheriting the mutation. When the parents are clinically unaffected, the risk to the sibs of a proband has been estimated to be 1.5 % because the possibility of germline mosaicism exists. The risk to sibs of a proband with NIPBL-related CdLS depends on the carrier status of the proband's mother.

Prenatal Diagnosis

Prenatal diagnosis is feasible for pregnancies at increased risk for CdLS if the mutational diagnosis in index case is precisely done. Analysis of DNA extracted from fetal cells obtained by chorionic villus sampling (CVS) at 10–12 weeks gestation or by amniocentesis (16–18 weeks gestation). High-resolution ultrasound examination to follow growth and to evaluate the limbs, heart, diaphragm, palate, and other organs is offered to families in which a disease-causing mutation has not been identified. Reported prenatal ultrasound findings of the affected CdLS are as below.

- Increased nuchal translucency in the first trimester [8, 9].
- Growth failure, which typically presents in the second trimester.
- The typical in utero facial profile of a fetus with CdLS, consisting of micrognathia, a prominent upper-lip, and a depressed nasal-bridge with somewhat anteverted nares [10–12].
- Maternal serum PAPP-A level may be low in the first and second trimester, if the fetus has CdLS [13–15].

Materials and Methods

There were total six clinically diagnosed cases of CdLS studied. The medical genetic evaluation included birth history, family history, dysmorphology examination, psychological testing, cytogenetic analysis, and molecular testing. The age range was 6 months to 6 years and the mean age was 3.5 years, when the parents brought the affected children to us. The informed consent of parents was taken for ethical approval.

Psychological Evaluation

The psychological evaluation was conducted by a clinical psychologist using standard battery of tests, which included: Infant Bayley Scale for Development, Vineland Social Maturity Scale, Wechsler Intelligence Scale for Indian children or Kamat's Binet Test of Intelligence to find out the Development, Social and Intelligence Quotient (D.Q., S.Q., or. I.Q.). The cases were classified as mild, moderate, or severe MR, according to the guidelines given by American Psychiatric Association [16].

Cytogenetics

Phytohemaglutinin stimulated lymphocyte cultures for G-banding were set up using 2 ml sodium heparinized intravenous blood; harvested and the slides for metaphase study were prepared, according to the standard method [17]; the slides were stained using Giemsa [18]. The separate lymphocyte cultures were also set up for High Resolution Banding using Ethidium bromide method [19], which shows more chromosomal bands (about 400–600), in comparision with the routine G-Banding Method (about 250–300). It helps in studying structural variations better than G-banding, especially in the absence of FISH facilities. Metaphases were studied under oil imersion lens (100×) in Zeiss microscope and were captured using

KaryoImager Version V 1.0. For each sample, 40–50 metaphases were screened for chromosomal anomalies, which were designated according to the standard nomenclature [20].

Molecular Study

The 47 exons in the NIPBL gene were screened for mutations by direct sequencing. Primer pairs were designed to amplify exons, exon/intron boundaries, and short flanking intronic sequences. Mutational analysis of the amplimers was performed using conformation-sensitive gel electrophoresis (CSGE) with standard protocols. PCR products corresponding to all altered migration patterns (shifts) on CSGE were purified using QIAquick PCR purification kit (Qiagen) and were sequenced bidirectionally on an ABI 377 sequencer.

Based on dysmorphic characteristic features and mutational analysis, the patients were diagnosed to have CdLS. Some young mothers opted for prenatal genetic counseling, further mutational analysis and future pregnancy monitoring. Details of 2 of 6 cases is given below.

Case 1: This was a 4-year-old male, born of FTND, Birth wt 2.25 kg, with h/o maternal cousin uncle with mental retardation, and had no epilepsy; He had both mental and motor delayed milestones. On psychological assessment, the child had mild retardation with intellectual quotient (IQ) was found to be 55.

Dysmorphic features consisted of microcephaly (HC 42 cm) with temporal bulge, short stature, low set ears, bow-shaped upper-lip, long eyelashes, lowest ears, hirsuitism, lacramal duct blocked (Fig. 1a), hypoplastic middle-phalanx of little and middle finger, Simian crease (Fig. 1b). X-ray skull examination revealed premature closure of coronal suture indicative of craniostenosis. Urinary screening tests for mental retardation were negative. The chromosomal analysis revealed 46,X invY (Fig. 1c, d). Mutational analysis showed a missense mutation in exon 28 of NIPBL gene: V1800D.The mutation was de novo as both parents were found to be negative for NIPBL gene mutation.

Case 2: 6-months-male, born of FTND, Birth wt 3.2 kg, On day 3 had neonatal jaundice and was kept in incubator for 5–6 days; History of consanguineous marriage of 3rd degree. At 2.5 months-right focal convulsions with multiple episodes; DQ < 35; severe microcephaly, short stature, laryngomalacia(inpiratory stridor), long philtrum, long eyelashes, hypertelorism, scolosis, increased tone, flexion deformity of knee/units, hirsuitism (Fig. 2a). MRI brain revealed hypomyelination, corpus callosal hypoplasia, ventriculomegaly with mild atrophy, CT Scan brain showed early fusion of the lambdoid sutures, rest of the cranial sutures are open, there was mild fullness of lateral ventricles, EEG showed mild slowing and ECG was normal, X-ray PBH with hip joints (lateral view) was normal, Vision test suggested BE nystagmus, BE mypoic tessellated fundus with extensive peripapillary atrophy. No metabolic abnormality was detected in the urinary screening tests for mental retardation. The chromosomal analysis revealed 46,XY, 9qh+ (Fig. 2b). Molecular study suggested no mutation in NIPBL gene in the patient, but test for SMCIA gene mutation was not conducted and the rare possibility exists.

Out of six cases, mutational screening for NIPBL gene was feasible in three cases only due to financial constraint. One of three cases was positive which showed de novo mutation on gene sequence analysis. The other two cases were negative for the NIPBL mutation indicating the possibility of other rare genes involved. The whole genome approach of microarray technology is perhaps the better cost-effective approach in such cases when common mutations are not detected.

Prenatal diagnosis: In the case 2, the couple had come back to us when the mother was 5 weeks pregnant for the second child and asked for prenatal diagnostic test. As the disease-causing mutation was not identified in this case, prenatal diagnosis using amniotic fluid for mutational analysis was not required in the current situation but pregnancy monitoring with fetal anomaly or malformation scan was conducted. Fetal MRI done at 22 weeks also confirmed the absence of typical dysmorphic features of CdLS. The genetic counseling helped to give the couple the recurrence risk of CdLS, which lessened their anxiety. The Obstetric Gynecologist and the Radiologist monitored the entire pregnancy with frequent prenatal fetal scans for dysmorphology and the couple had a normal healthy child.

Discussion

Cornelia de Lange is one of the common genetic syndromes with classical dysmorphic features and mental retardation next to Down syndrome and Fragile-X syndrome. It is therefore essential to have the genetic and mutational testing available to the parents for confirmed diagnosis and prevention of recurrence risk. It was interesting to note that NIPBL gene mutation was detected in 30 %(1 of 3 cases) in the present study, which is in agreement with the published report [6].

Even though in India molecular diagnosis is not advanced enough to offer the mutational detection testing for various rare genetic syndromes, we can prevent the birth of a child with congenital malformations or birth defects by appropriate fetal anomaly scan monitoring and prenatal diagnosis. Hence, a dire need of routine triple marker screening and ultrasonography (for fetal anomaly

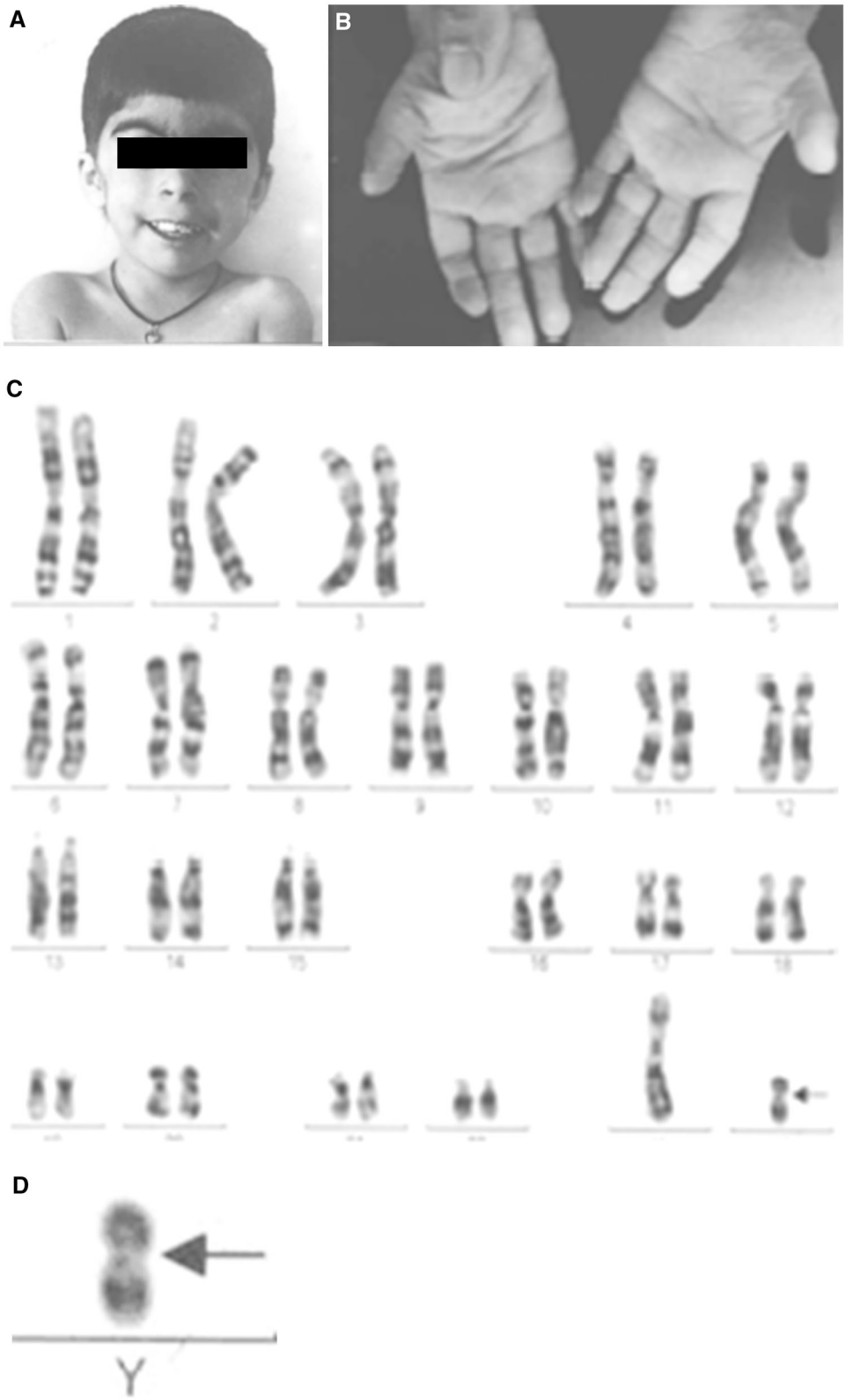

Fig. 1 **a** Case 1 of Cornelia de Lange syndrome, **b** Simian crease observed in case 1 patient, **c** Karyotype with 46,X,inv Y, **d** Inverted chromosome Y

detection) in all the pregnant women is strongly recommended. The genetic counseling to the affected family members/parents reduced their anxiety when the recurrence risk was informed to them. The various ways of monitoring the pregnancy despite the inadequate health infrastructure of prenatal DNA testing was explained to

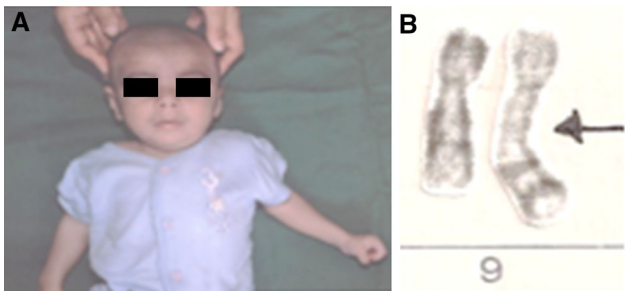

Fig. 2 **a** Case 2 of Cornelia de Lange syndrome, **b** Increase in the heterochromatin region in long arm of chromosome 9

them. At the same time neurodevelopmental therapy to the infants and special training to the young children was provided under the same roof caring for mentally retarded children. In conclusion, it is strongly suggested that wherever possible the mutational screening in the index case or in CVS/amniotic fluid for prenatal diagnosis should achieved by prior case discussion with medical geneticist so as to offer the best possible help and guidance to the affected parents.

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