A Malay boy with the Cornelia de Lange syndrome: clinical and molecular findings

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ABSTRACT

The Cornelia de Lange syndrome is a multiple congenital anomaly syndrome characterised by dysmorphic facial features, hirsutism, severe growth and developmental delays, and malformed upper limbs. The prevalence is estimated to be one per 10,000. Recently, several independent groups proved that Cornelia de Lange syndrome is caused by mutations in the NIPBL gene, the human homologue of the Drosophila Nipped-B gene. Here, we present the first clinical case report of a Malay child, a 9-year-old boy with the Cornelia de Lange syndrome. We also report the molecular investigation of the NIPBL gene in this patient.

Keywords: congenital anomalies, Cornelia de Lange syndrome, NIPBL gene, mutation, polymerase chain reaction

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INTRODUCTION

Cornelia de Lange syndrome (CdLS [MIM 122470]), also called Typus Amstelodamensis or Brachmann-de Lange syndrome, is a multiple congenital anomaly syndrome characterised by a distinctive facial appearance, prenatal and postnatal growth deficiency, psychomotor delay, behavioural problems, and malformations of the upper extremities[1]. Cardiac defects and gastrointestinal anomalies are common, and many additional physical features occur, including hearing loss, myopia, palatal abnormalities, genitourinary abnormalities, and congenital diaphragmatic hernias. CdLS has a variable phenotype, with a main division into a classical and mild type, and is known to evolve with age[2].

In 2004, two groups independently detected NIPBL gene mutations to be responsible for CdLS[3,4]. NIPBL is the human homologue of the Drosophila Nipped-B gene, which belongs to the family of chromosomal adherins involved in chromatid cohesion processes and enhancer-promoter communication[6]. The exact function of the human NIPBL gene product, called delangin, is unknown. To date, NIPBL mutations have been identified in 20-56% of CdLS cases[3-5,7-8]. Genotype-phenotype correlations in the study of Gillis et al[7] showed significant differences between patients with and without mutations in terms of the degree of growth retardation and developmental delay. In a recent study on 39 sporadic cases of CdLS from the Netherlands, truncating NIPBL mutations were prevalently detected in CdLS patients of the classical type[5].

CASE REPORT

A nine-year-old boy from Kelantan, Malaysia, was referred to the biweekly medical genetics clinic at the Universiti Sains Malaysia Hospital for undiagnosed malformation syndrome. He was diagnosed to have the classical CdLS, based on the developmental delay, impaired growth in length, and classical facial symptoms, of which the most important ones are the microcephaly, synophrys, long curly eyelashes, long philtrum, thin vermillion of the upper lip, and downturned corners of the mouth (Figs. 1-3). Detailed clinical features are summarised in Table I.

His past medical history included a right orchidopexy and left hernioplasty performed at six years of age for a unilaterally undescended testis and inguinal hernia (Fig. 3). The patient was lost to follow-up until the age of nine years, when he was admitted because of recurrent vomiting after meals. Abdominal computed tomography (CT) showed a huge splenic cyst with multiple spots of splenic infarctions, which was confirmed at subsequent laparotomy and led to splenectomy. He receives (life-long) penicillin prophylaxis. A ventricular septum defect was diagnosed but surgical repair was not needed.

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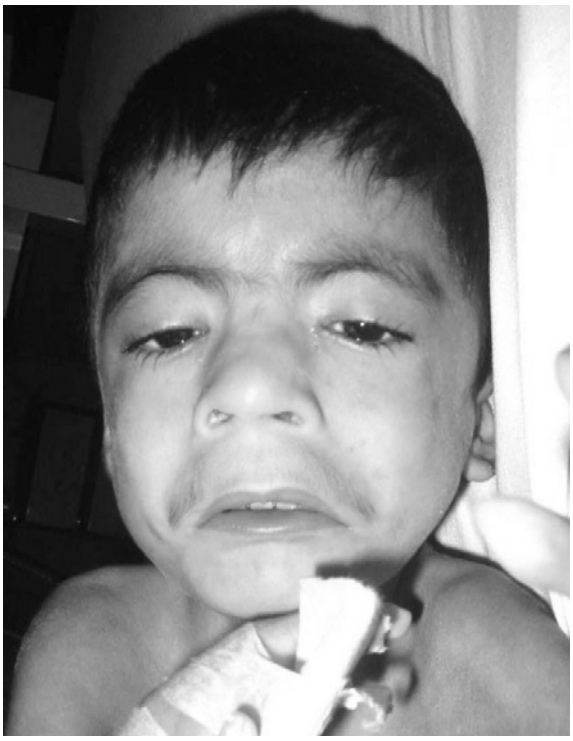

Fig. 1 Photograph shows the characteristic facial features of the Cornelia de Lange syndrome, including arched eyebrows with synophrys, long eyelashes, ptosis, depressed nasal bridge, long philtrum with thin upper vermillion border and micrognathia. Hirsutism is also seen. [Published with written consent from the patient's legal guardian.]

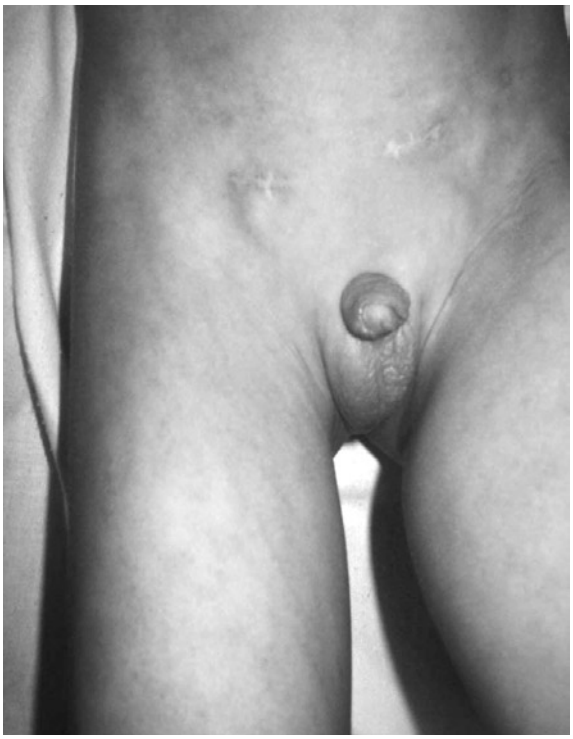

Fig. 3 Photograph shows the small genitalia observed in this patient. Right orchidopexy and left hernioplasty had been performed.

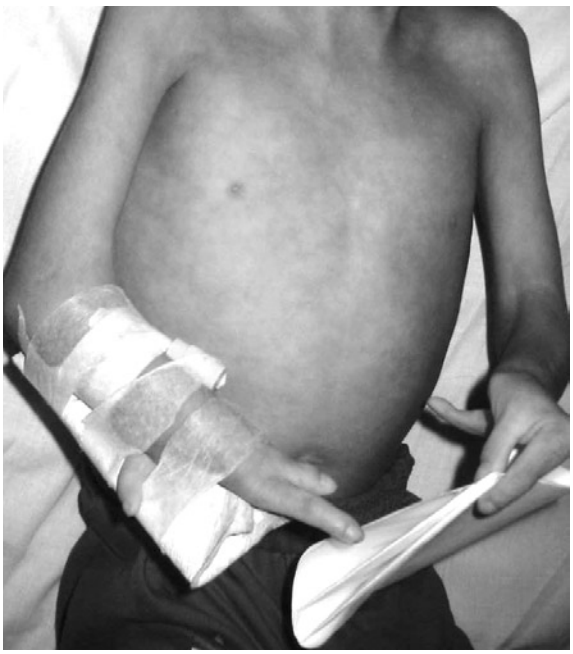

Fig. 2 Photograph shows the variability of upper limb abnormalities, distal reduction defect with missing and fused digits in the same child.

Molecular investigation was performed with the genomic DNA isolated from peripheral blood lymphocytes of the patient according to the manufacturer's instructions (Gentra Systems, Minneapolis, MN, USA). The entire NIPBL coding region (exons 2-47) was screened for mutations. Primer sequences and polymerase chain reaction (PCR) conditions were the same as that mentioned by Bhuiyan et al[5]. Mutational analysis of the amplimers was performed by denaturing high-performance liquid chromatography (DHPLC) (transgenomic wave).

PCR products with altered DHPLC peak were purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and were sequenced bidirectionally on an ABI 377 sequencer. NIPBL sequence from the NCBI (NM_015384) was used as reference sequence. We identified a novel insertion mutation (Fig. 4) in exon 6 of the NIPBL: an insertion of nucleotide "T" at cDNA position 513. This created a premature stop codon and thus, the NIPBL protein was truncated prematurely at peptide position 171. Parental samples were unavailable for investigations.

DISCUSSION

The clinical phenotype of our patient is concordant with the more expressed phenotype (classical facial; expressed prenatal and postnatal growth retardation; expressed microcephaly;

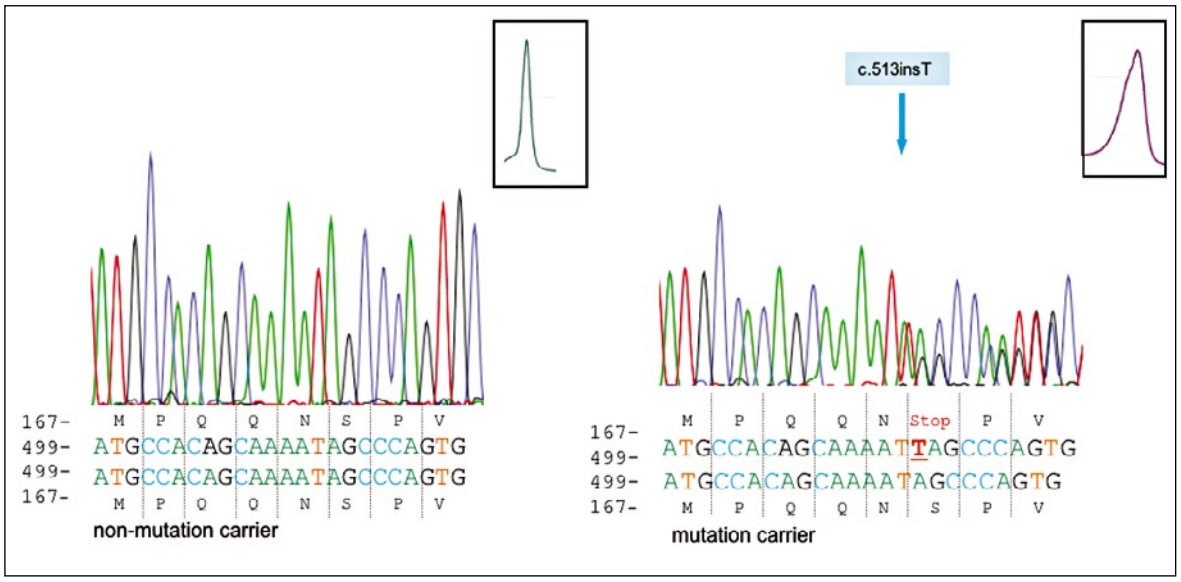

Fig. 4 Screening of the NIPBL gene shows an insertion of nucleotide "T" at cDNA position 513 (NCBI ref: NM_015384) which causes premature termination of the NIPBL mRNA leading to a non-functional NIPBL protein. Left panel shows the nucleotide sequence from a control (wild type). Upper right corners of both sequences (wild type and from the patient) show DHPLC wave patterns for PCR amplified NIPBL exon 6.

Table I. Summary of clinical features of our patient.

Gestation	38 weeks	Small hands	+
Weight at birth	2.2 kg	Limb reduction	+
Prenatal growth retardation[9]	+		(absence of two fingers)
Postnatal growth retardation[9]	+	Single palmar crease	+
Microcephaly	+	Hallux valgus	-
Cognitive functioning	severely impaired	Cutis marmorata	+
Hirsutism	+	Short neck	+
Low posterior hairline	+	Hypoplastic nipples	+
Synophrys	+	Small umbilicus	+
Long, curly eyelashes	+	Cardiac defect	+
Depressed nasal bridge	+		(ventricular septal defect)
Long philtrum	+	Small genitalia	+
Downturned angles of mouth	+	Hypospadias	+
Thin upper vermillion border	+	Cryptorchidism	+
Widely-spaced teeth	+	Inguinal hernia	+
Highly-arched palate	+	GI reflux	+
Micrognathia	+	Splenic cyst	+
Low-set ears	+	Neurosensory deafness	+
Small ears	-		

severely impaired cognitive functioning) predominantly found in patients with truncating mutations. Though parental samples could not be investigated in this study, it seems likely that the mutation has arisen de novo in the patient. As with most mutations arising in the patient, both parents had normal growth and development, with no major clinical features fitting CdLS, and family history was also negative for CdLS.

Germline mosaicism, e.g. the presence of a

mutation in germ cells only (ovary, testis) and not in the body cells of the parents, has not been described in CdLS until now. Parents, siblings and other relatives can now be reassured about the very low recurrence risk for future pregnancies. If parents want, prenatal studies for the same NIPBL mutation can be performed in future pregnancies, to exclude the extremely small risk for germ cell mosaicism. In conclusion, we report here on the first detection of a NIPBL mutation in a CdLS patient from Malaysia with classical CdLS.

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