

CASE REPORT

1p36 Deletions in Two Cases with Thalassemia

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ABSTRACT

Summary: Terminal deletions in the short arm of chromosome one are generally associated with characteristic phenotype with dysmorphic features, including congenital anomalies and mental retardation with various degrees. Different outcomes depend on the sizes and locations of the deleted areas characterized by moderate to severe intellectual disability, delayed growth, hypotonia, seizures, limited speech ability, malformations, hearing and vision impairment and distinct facial features. The symptoms may vary, depending on the exact location of the chromosomal deletion but not a single reported case showed any 'feature of anemia'.

We report here one male and one female individual with partial deletions on chromosome no. one, both at 1p36 region. Cytogenetic analysis of blood lymphocytes was studied with high resolution GTG-banding analysis, using cyto-vision software on their chromosomes. Results revealed 46, XY, del(1)(p36.21) in the male who was also diagnosed as a 'beta thalassemia trait' and the other case was 46, XX, del(1)(p36.3) in the female who was diagnosed as a case of 'HbE-beta thalassemia'.

This report provides additional cases to the growing literature.

Purpose/background: Deletion 1p36 is the most common terminal deletion syndrome with an estimated occurrence of 1:5000 live births. We report two patients with 1p36 deletions among which one shows 'atypical' proximal interstitial deletion at 1p36.21 using HR-GTG banding analysis. Interestingly, both the patients manifest one extra clinical characteristic that is different from those seen in 'classical' monosomy 1p36 syndrome, is 'microcytic anemia'.

Based on the analysis of the clinical and molecular data from our patients and those reported in the literature, we suggest that deletion 1p36.21 chromosomal abnormality may constitute yet another deletion syndrome distinct from the classical distal 1p36 deletion syndrome.

Our aim was to find out further information regarding anemia since there are previously reported cases of anemias associated with this 1p36 region—one is presence of a 'putative tumor suppressor gene' important in the evolution of chronic myelocytic leukemia and the other is one inherited erythroblastopenia, commonly known as 'Diamond-Blackfan anemia' (DBA), caused by mutation in the gene encoding ribosomal protein L11 (RPL11) to answer families' questions in the clinical setting.

Materials and methods: Standard cytogenetic analysis was used with high resolution GTG-banding analysis, using cyto-vision software for karyotyping and high performance liquid chromatography (HPLC) and amplification refractory muta-

tion system (ARMS-PCR) to detect the five common Indian β -thalassemia mutations: [IVS-I-5 (G>C), Cod 15 (G-A), Cod 8/9 (+G), Fr 41/42 (-TTCT) and Cod 26 (G-A)].

Results: Results showed 46, XY, del(1)(p36.21) in the male, additionally diagnosed as a 'beta thalassemia trait' and in another case 46, XX, del(1)(p36.3) in female who was diagnosed as a case of 'HbE-beta thalassemia'.

Conclusion: These two patients with deletion 1p36 represent association with other genetic disorder which is characterized by hematological abnormalities.

Keywords: Cytogenetics, Copy number variation, Microdeletion, Thalassemia, Mental retardation, Microcephaly.

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INTRODUCTION

Thalassemia is one of the most common genetic disorders worldwide.¹⁻⁴ Beta-thalassemias are heterogeneous autosomal recessive hereditary anemias characterized by reduced or absent β globin chain synthesis. Beta-thalassemia phenotypes are variable, ranging from the severe transfusion dependent thalassemia major to the mild form of thalassemia intermedia. Patients with the major form of the disease have severe anemia, microcytic and hypochromic anemia, hepatosplenomegaly, and usually come to medical attention within the first two years of life.⁵ No specific constellation of congenital abnormalities has been associated with thalassemia, and no constitutional chromosomal abnormalities have been identified that predispose to the development of this disease.

We now report two cases of thalassemias with constitutional deletion of 1p36. The most important aims behind the characterization of any 'chromosomal syndrome' should be recognition of the phenotype and to document developmental progress. Until recently, there were few published reports of patients with 'pure' monosomy for terminal chromosome 1p36. We provide two cases with 1p36 deletions association with thalassemia, in order to assist geneticists and other health professionals with the identification of patients and counselling of families with this chromosome abnormality. The constitutional deletion of chromosome 1p36 results in

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a syndrome with multiple congenital anomalies and mental retardation.⁶ Monosomy 1p36 is the most common terminal deletion syndrome in humans, occurring in 1 in 5,000 births.^{7,8} Monosomy in 1p36 is the most common terminal deletion syndrome with an estimated occurrence of 1:5000 live births.⁶ In our present study, patients with 1p36 deletions show 'atypical' proximal interstitial deletions one at 1p36.21 and the other at 1p36.3 using HR-GTG banding analysis, where both the patients manifest 'microcytosis' due to presence of beta thalassemia association, that is different from those seen in 'classical' monosomy 1p36 syndrome.

There are previously reported cases of anemias associated with this 1p36 region—one is presence of a 'putative tumor suppressor gene' important in the evolution of chronic myelocytic leukemia⁹ and the other is one inherited erythroblastopenia, commonly known as 'Diamond-Blackfan anemia' (DBA), caused by mutation in the gene encoding ribosomal protein L11 (RPL11).¹⁰ In our further study, we will try to investigate whether the severity of anemia in both the cases are due to association of 1p36 deletion or not, since both the thalassemia cases are actually mild and intermediate ones, they do not need blood transfusions to survive.

CASE REPORT

In the first case of our study, a 16 years old male was taken to our laboratory for diagnosis of the cause of microcytic anemia. After doing high performance liquid chromatography (HPLC) and deoxyribonucleic acid (DNA) testing, we found that he was a case of 'beta thalassemia trait'. Along with these, he had dysmorphic features, H/O delayed milestones, mental retardation in low degree, and microcephaly (Table 1). So, we were again interested to know his chromosome constitution. In our cytogenetic laboratory, chromosomal studies were performed on the basis of G-banding technique at high resolution. The result showed partial deletion on chromosome no. one at 36.21 position and the karyotype showed 46, XY, del(1)(p36.21) (Fig. 1). Both of his father

and mother were with clinically normal phenotype. The patient is still alive.

In the second case, a 3 years old female child, the first born child, distally related parents, father being 32 years old and mother 26 years old at delivery, was taken to our laboratory for diagnosis of the cause of severe anemia, since she was on occasional blood transfusions. After doing HPLC and DNA testing, we found she was a case of 'HbE-beta thalassemia'. She also had dysmorphic features, severely delayed milestones, mental retardation in high degree, walking problem and vision problem due to bilateral cataract (*see* Table 1). So, we were interested to know her chromosome constitution. In our cytogenetic laboratory, we performed karyotyping by HR-GTG banding analysis. The result showed partial deletion on chromosome no. one at 36.3 position and the karyotype showed 46, XX, del(1)(p36.3) (Fig. 2). But the baby was died after 1½ years at the age of 5 years. Interestingly, she had a sib (younger brother) with 'HbE-beta thalassemia' but without any H/O blood transfusion.

MATERIALS AND METHODS

Cytogenetic analysis was carried out based on phytohemagglutinin-stimulated peripheral blood lymphocyte cultures, of the couples—both the male and the female partner. Lymphocyte culturing and GTG-banding were performed following standard protocols as described by the AGT cytogenetics laboratory manual. Karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN 2005).

HEMATOLOGICAL STUDIES

The participants were evaluated for hemoglobin, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW), hematocrit (Hct). The complete and final

Table 1: Clinical features of two patients with 1p36 deletion

<i>Clinical features</i>	<i>Patient-1</i>	<i>Patient-2</i>
Karyotype	46, XY, del(1)	46, XX, del(1)
Proband	Chromosome-1, p36.21	Chromosome-1, p36.3
Other disease	Beta thalassemia trait	HbE-beta thalassemia
Mutation	IVS 1-5(G-C)/+	IVS 1-5(G-C)/Cod 26
Growth	Developmental delay	Severe developmental delay
IQ	Mentally retarded	Severely mentally retarded
Skull	Microcephaly	Normal
Eyes	Normal	Megalocornea, hypertelorism, downslanting, bilateral cataract
Nose and nasal bridge	Bulbous nasal tip	Bulbous nasal tip
Ear	Normal	Low set
Neck	Short neck	Short neck, no neck holding
Life span	17 years, still alive	Died at the age of 5 years
Mouth and palate	Narrow chin, thick lower lip	High arch, thin lip

screening was done through Hb-variant analysis by HPLC. Hemoglobin variants (HbA, HbF and HbA₂/E) were estimated by HPLC (Variant I, Bio-Rad, USA) using manufacturer's protocol.

MUTATION ANALYSIS

Deoxyribonucleic acid (DNA) was isolated from white blood cells, using a DNA isolation kit for mammalian blood (Qiagen). Patients were screened for five common

β -thalassemia mutations of Eastern India (2, 3, 4) like IVS1-1 (G-T), IVS1-5 (G-C), codon 8/9 (+G), codon 26 (G-A), and Fr 41/42 (-TCTT). The screening was performed by PCR based technique, amplification refractory mutation system (ARMS) and direct DNA sequencing of the β -globin gene was also done in selected cases to further confirmation.

Written consent for evaluation of β -globin mutation was taken from adult participants and in case of children it

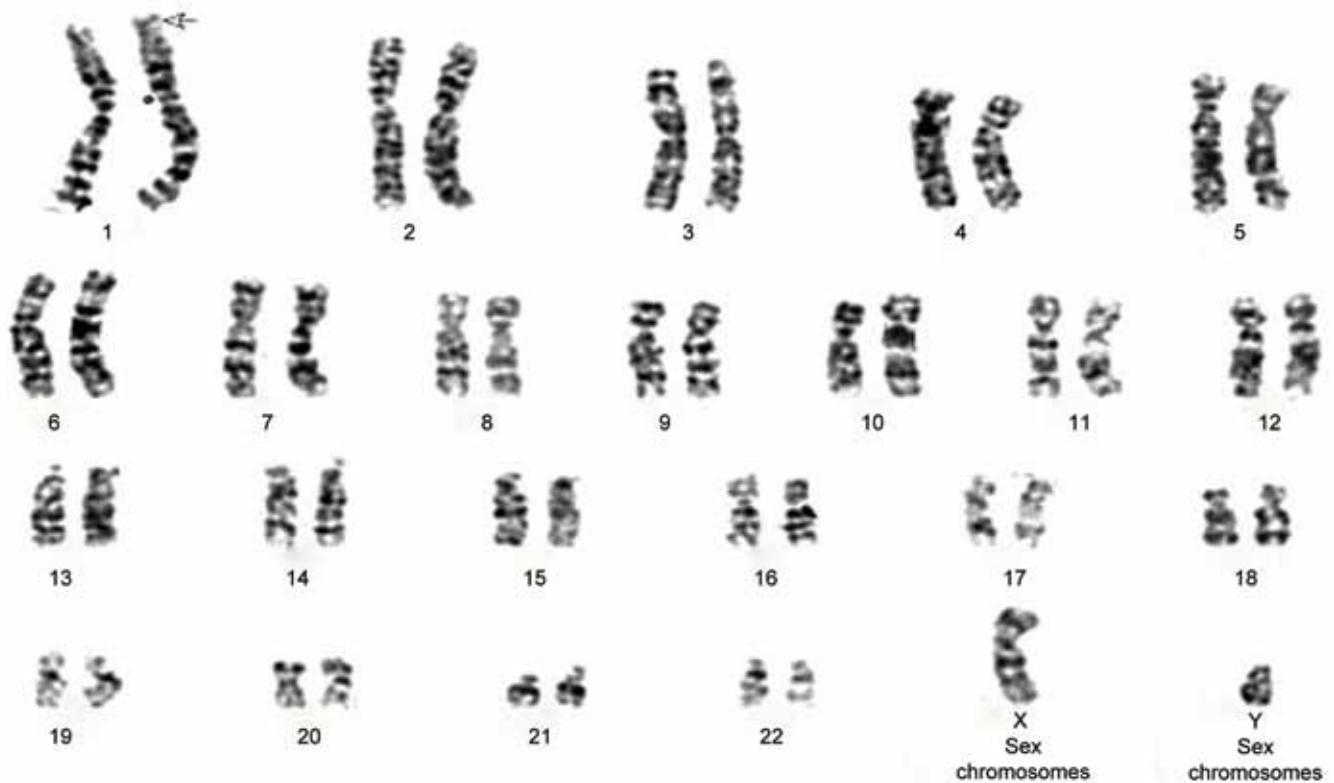


Fig. 1: Karyotype showing deletion in male: 46, XY, del(1)(p36.21)

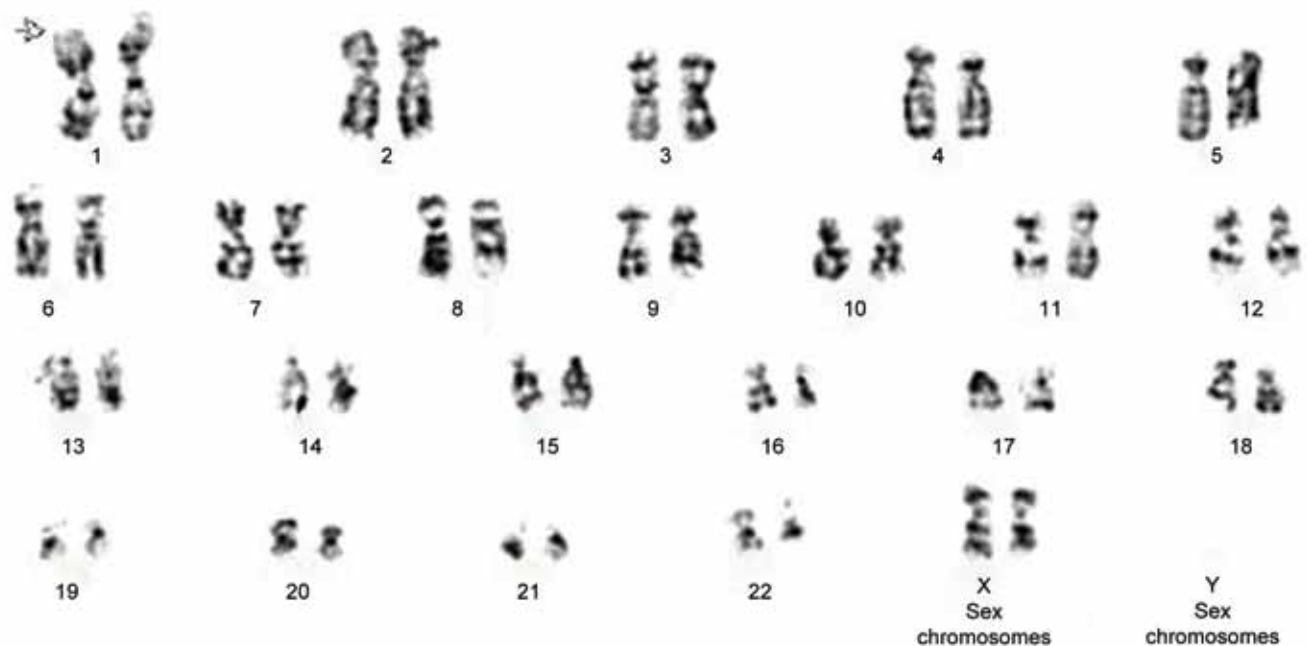


Fig. 2: Karyotype showing deletion in female: 46, XX, del(1)(p36.3)

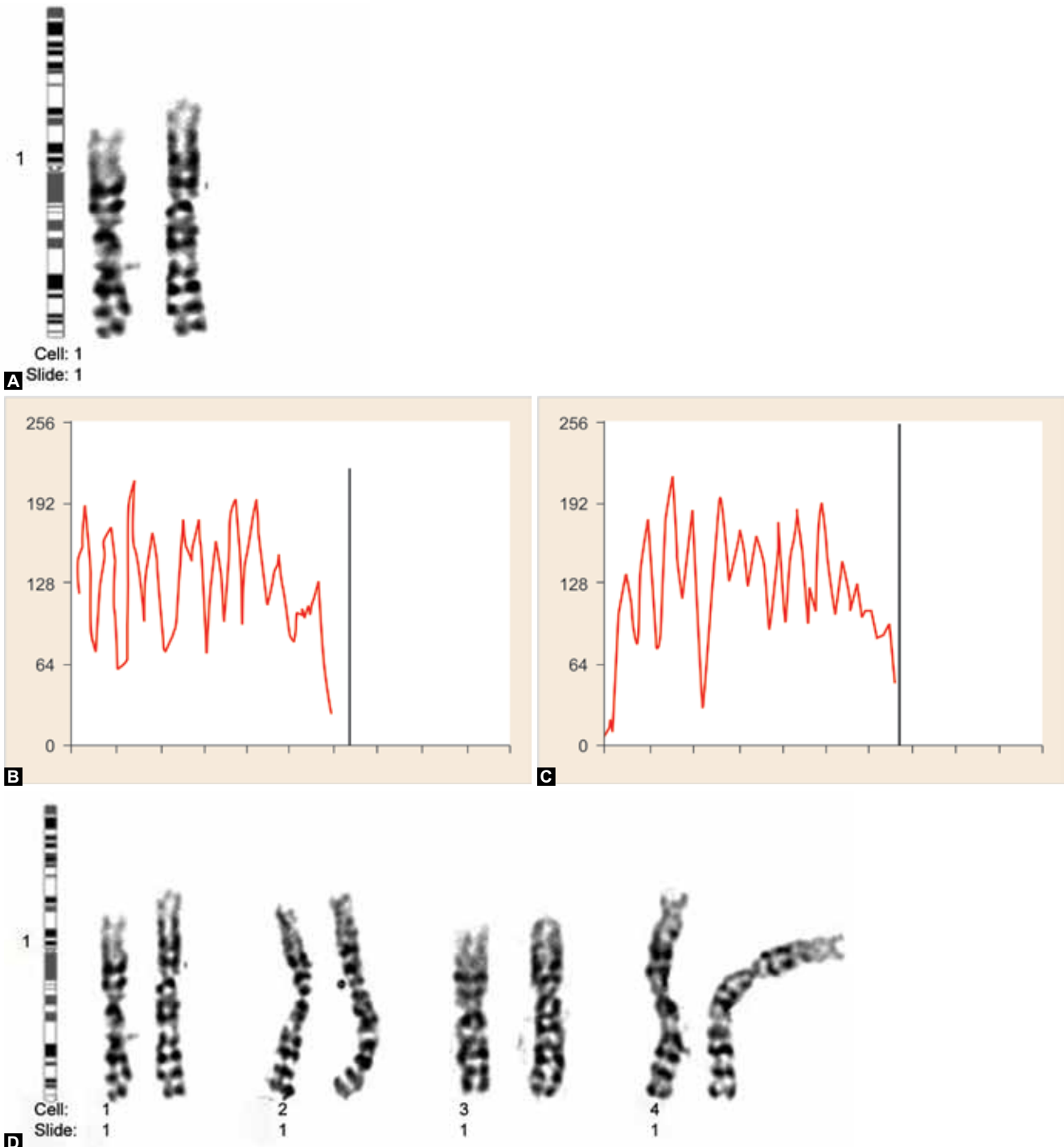
was taken from the parents as per guidelines of institutional ethical committee. Peripheral blood samples were collected from every participant, in vials containing 5 mM EDTA. The handling of all human blood samples was carried out in accordance with the guidelines established by the Local Ethical Committee.

The initial screening of the participants was done by hemoglobin and complete blood count (Hb and CBC) in Automated analyzer (Cell Counter: Medonic 530, EMerck)

and finally the β -thalassemia status was confirmed by HPLC. Only those, who were confirmed of their status by these studies, were taken for evaluation for determining the β -thalassemia mutations.

RESULTS

Chromosomal analysis in both the cases revealed 1p36 deletions—in the ‘beta thalassemia trait’ male the deletion is at 36.21 region of p arm of chromosome one indicating



Figs 3A to D: (A) High resolution banding analysis (done by cyto-vision software) revealed ‘loss of material in chromosome 1 at p36.21’, (B) loss of material in chromosome 1 at p36.21, (C) normal chromosome 1 and (D) multicell analysis of chromosome 1 showing ‘loss of material at p36.21’

the karyotype 46, XY, del(1)(p36.21) (*see* Fig. 1) and in the other case in 'HbE-beta thalassemia' the deletion is at 36.3 region of p arm of chromosome one indicating the karyotype 46, XX, del(1)(p36.3) (*see* Fig. 2).

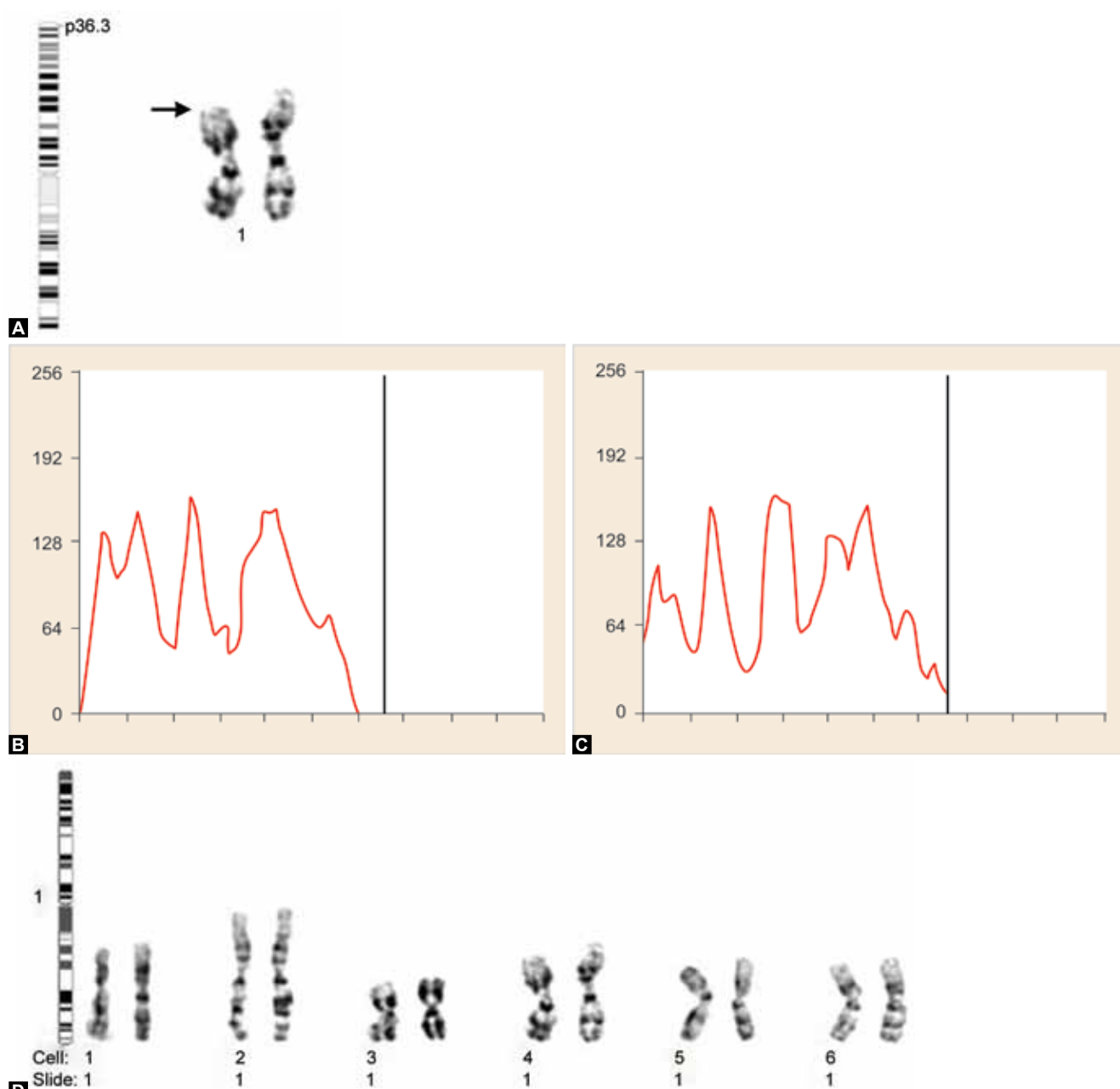
High resolution banding analysis (done by Cyto-vision software) revealed 'loss of material in chromosome one at p36.21' in comparison to their normal ones in the first case (Figs 3A to D), and 'loss of material in chromosome one at p36.3' in comparison to their normal ones in the second case (Figs 4A to D).

Patient 1 showed IVS1-5(G-C)/+ mutation and the patient 2 showed IVS1-5(G-C)/Cod 26 mutation.

To the best of our knowledge, these two are the new 1p36 deletion cases showing 'microcytic anemia' along with the common features of the syndrome.

DISCUSSION

This study describes the cytogenetic analysis of a constitutional deletion involving the distal short arm of chromosome one in two cases with dysmorphic features and developmental and growth delays along with 'microcytic anemia'. By cytogenetic analysis, the deletion appeared to involve 1p36.21 and 1p36.3. The molecular analysis provided



Figs 4A to D: (A) High resolution banding analysis (done by cyto-vision software) revealed 'loss of material in chromosome 1 at p36.3', (B) loss of material in chromosome 1 at p36.3, (C) normal chromosome 1 and (D) multicell analysis of chromosome 1 showing 'loss of material at p36.3'

confirmation that the patients had mutation of thalassemia for which they are showing features of 'microcytic anemia'.

Most deletions in chromosome 1p36 are new mutations, those occur before fertilization, during the formation of gametes (eggs or sperm). There have also been reports of patients with 1p36 deletion syndrome whose parents have a balanced or symmetrical translocation. This means a portion of one chromosome is transferred to another chromosome, so the parent has the '36' portion of chromosome one attached in an alternate location. When this occurs, cell division creates gametes that are missing a piece of 36.

In new mutations, the mechanism causing chromosome breakage is unknown. Deletions of paternal origin (father) are larger than the deletions deriving from the maternal (mother) chromosome. The majority of deletions are maternally derived. There do not seem to be differences in the clinical manifestations (the symptoms or observable conditions which are seen as a result of 1p36) based on whether the deletion is on the paternal or maternal chromosome.

In our reported cases, this deletion has an association with 'microcytic anemia'. There are so many reported cases of anemias associated with this region—one is presence of a 'putative tumor suppressor gene' for chronic myelocytic leukemia and the other is DBA, caused by mutation in the gene encoding ribosomal protein L11 (RPL11). In our further study, we will try to investigate whether the severity of anemia in both the cases are due to association of 1p36 deletion or not, since GATA1 gene is also situated adjacent to this 1p36 region. This GATA1 gene codes for a transcription factor one and its normal function leads to increased proliferation, decreased differentiation, and premature death of immature blood cells. Immature blood cells cannot perform the functions of specialized, mature blood cells. A lack of differentiation causes a shortage of red blood cells (anemia), which may be responsible for production of both of ours thalassemia

cases as severe type, who are actually mild and intermediate ones.

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