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CLINICAL CASE REPORT

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Mosaic loss of 15q11q13 in a patient with hypomelanosis of Ito: is there a role for the P gene?

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Abstract We report a patient with mental retardation, behavioral disturbances, and pigmentary anomalies, consistent with the phenotype of hypomelanosis of Ito (HMI), and in whom cytogenetic analysis revealed mosaicism for an unbalanced translocation. His karyotype is 45, XY,–7, –15,+der(7)(7;15)t(q34;q13)/46, XY. He is therefore monosomic for 7q34 to qter and 15pter to q13 in the cells containing the translocation. The human homolog (P) of the p gene (the product of the mouse *pink-eyed dilution* locus) maps to 15q11q13. Loss of this locus is believed to be associated with abnormalities of pigmentation, such as the hypopigmentation seen in patients with deletions of 15q11q13, and the Prader-Willi and Angelman syndromes. Mutations within the P gene have also been associated with tyrosinase-positive (type II) oculocutaneous albinism. Using fluorescence in situ hybridization, we confirmed that our patient is deleted for one copy of a P gene probe in the cells with the unbalanced translocation, and for loci within the region critical for the Prader-Willi/Angelman syndromes. Although hypomelanosis of Ito is a heterogeneous disorder, we postulate that, in our case and potentially in others, this phenotype may result directly from the loss of specific pigmentation genes.

Introduction

Hypomelanosis of Ito (HMI) is a heterogeneous, clinically variable disorder characterized by specific patterns of hypopigmentation on the limbs and trunk; these patterns frequently follow the lines of Blaschko. The cutaneous findings have been associated with other systemic anomalies, particularly of the musculoskeletal and central nervous systems (Glover et al. 1989; Ruiz-Maldonado et al. 1992). Most cases of HMI are sporadic, but a small number of familial cases suggest that this may be an autosomal dominant disorder with variable expression (McKusick 1992). Many different mosaic chromosomal abnormalities have been reported associated with this condition; this has lead some authors to hypothesize that HMI is a manifestation of chromosome mosaicism (Donnai et al. 1988). One form of HMI has been mapped to Xp11 based on multiple reports of patients with X-autosome translocations with breakpoints clustered in Xp11 (Koiffman et al. 1993).

Multiple genes are involved in pigmentation in both man and mouse. More than 50 loci are known in mouse (Silvers 1979; Halaban and Moellmann 1993), and two major pigmentation genes (tyrosinase and P) have recently been cloned in man (Kwon et al. 1987; Rinchik et al. 1993). These genes map to a number of different chromosomes, and many chromosomal aberrations may result in abnormalities of pigmentation because of the altered expression of these genes in the pigmentation pathway.

The P gene is the human homolog of the mouse *pink-eyed dilution locus,* the deletion or mutation of which causes hypopigmentation resulting from a reduction of pigment in the melanocytes (Brilliant 1992; Russell 1949). The P gene has been mapped to 15q11q13 and is hypothesized to be associated with the pigmentary dilution seen in the Prader-Willi (PWS) and Angelman syndromes (AS) (Nicholls 1993 a). Mutations of the P gene are responsible for autosomal recessive (type II) human tyrosinase-positive oculocutaneous and ocular albinism (Rinchik et al. 1993; Lee et al. 1994). In the mouse, the *pink-eyed dilution* mutation results in the reduction of pigment in both coat and eyes. Some p mutant alleles have additional phenotypic features (neurologic deficits, male sterility, reduced female fertility, and cleft palate) (Brilliant 1992). The P polypeptide has been hypothesized to be a component of the melanosomal membrane and may act as a transporter of tyrosine, the amino acid precursor of melanin (Rinchik

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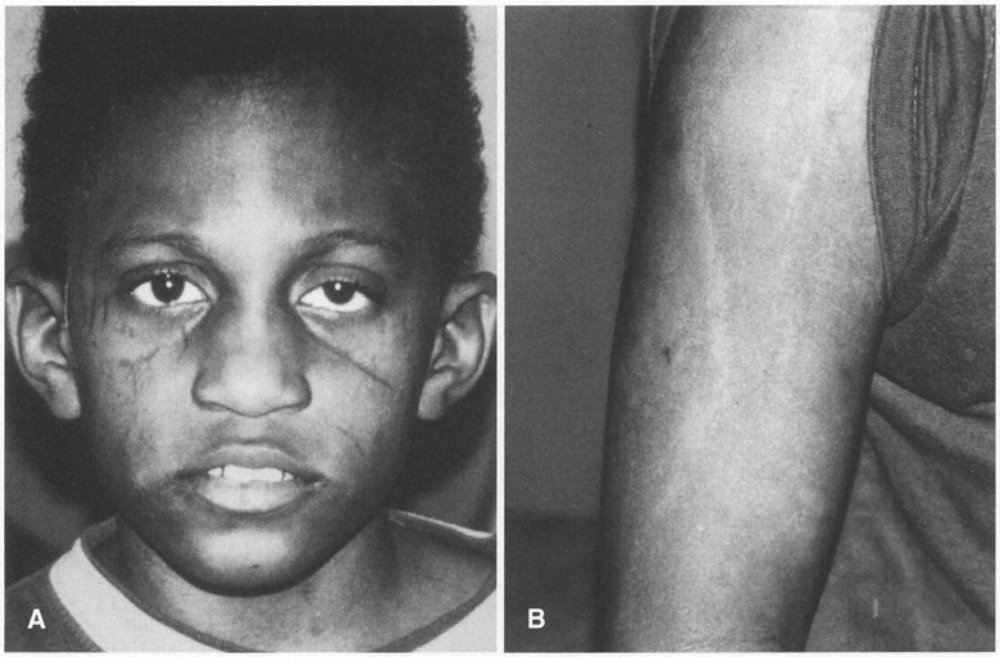

Fig. 1 A Photograph of the patient's face and **B** pigmentary lesions on his arm at 7 years of age (several lesions on his face represent scars from self-inflicted wounds)

et al. 1993). We report our studies of the P gene in a patient with HMI and mosaicism for an unbalanced chromosome translocation that results in a deletion of 15q11q13.

Clinical report

A 7-year-old black male was referred to the genetics and dermatology services for evaluation of mental retardation, facial dysmorphism, maladaptive behavior, and pigmentary abnormalities. He was born at term by a normal spontaneous vaginal delivery after an uncomplicated pregnancy. There was no history of maternal alcohol, tobacco, or other drug use. His birth weight was 2.9 kgs (20th centile). His neonatal course was uneventful. By one year of age, he was noted to be developmentally delayed. At that time, he was diagnosed as having possible neurofibromatosis type 1 (NF1) because of multiple cafe-au-lait macules and pigmentary lesions near the axilla. The family history was negative for NF1, mental retardation, and pigmentary abnormalities, and he has a healthy 6-year-old brother. By two years of age, he was reported to exhibit self-injurious and aggressive behavior that became worse with increasing age.

At 7 years, he was evaluated in a biobehavioral inpatient unit. He was noted to be nonverbal with receptive and expressive language skills estimated to be at the level of a 2–2½ year old. He was able to feed himself and void independently. He had aggressive behavior, including frequent biting of others. He was diagnosed as having oppositional defiant disorder, mental retardation, and attention deficit hyperactivity disorder.

On physical examination at 7 years of age, his height was 44.5 cm (5th centile), occipito-frontal circumference was 50.5 cm (45th centile), and weight was 23.5 kgs (50th centile). He was intermittently cooperative. Notable features included a high forehead with sparse hair in the temporal and occipital regions, ptosis of the left lid, thick helices of both ears, and a flat philtrum (Fig. 1 a). His hands were stubby. There was a single transverse crease on the right palm.

The most striking feature was his skin. He had a swirled and streaky pattern of both hypo- and hyperpigmentation along the lines of Blaschko over his arms (Fig. 1 B), legs, scapular region, and chest; this was more prominent on the right side. Histologic examination of a border between the hypo- and hyperpigmented areas revealed no specific changes. Multiple cafe-au-lait macules were noted on the trunk, thigh, abdomen, and near the left axilla, but no axillary freckling was noted. There were also numerous self-induced scars, scratches, and bruises. A small nevus was noted on the left fourth toe. His nails were normal. The remainder of the examination was unremarkable.

An ophthalmologic evaluation under anesthesia was normal, with normal pigmentation of the retina. Unfortunately, the presence of Lisch nodules could not be fully excluded because of the dilated state of his pupils. A magnetic resonance imaging scan revealed a subarachnoid cyst in the temporal region and no intracranial findings of NF.

Materials and methods

Cytogenetics

Chromosome analysis was performed on phytohemagglutinin-stimulated peripheral lymphocytes by standard techniques. In addition, multiple primary fibroblast cultures were established from a punch skin biopsy and harvested for cytogenetics. Thirty metaphases from blood and 100 metaphases from skin were studied.

Molecular cytogenetics

Fluorescence in situ hybridization (FISH) was carried out using probes from four loci on chromosome 15 according to standard protocols (ONCOR, Gaithersburg, MD). Loci (and their corresponding probes) included D15S11, GABRB3 (Prader-Willi probes A and B from Oncor) and D15S12 (IR10) (provided by J.H.M. Knoll), all of which map to 15q11q13. Probe IR10 lies within the P gene locus (Gardner et al. 1992). The order of these loci on chromosome 15 is: centromere–D15S11–GABRB3–D15S12– –myl– – – –telomere. Myl maps to 15q22 (Oncor). We also used a probe that maps to the subtelomeric region of 7qter (Oncor). For experiments using D15S12, the der(7) was marked using an alpha satellite probe for chromosome 7 (Oncor).

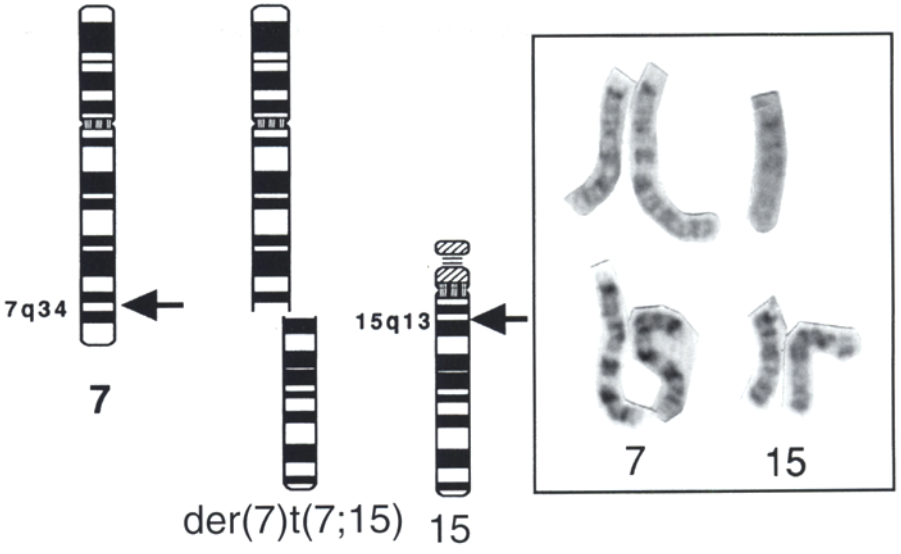

Fig. 2 Idiogram demonstrating the breakpoints leading to the formation of the t(7;15)(q34;p13) in our patient. A partial karyotype of chromosomes 7 and 15 is shown in the *box*

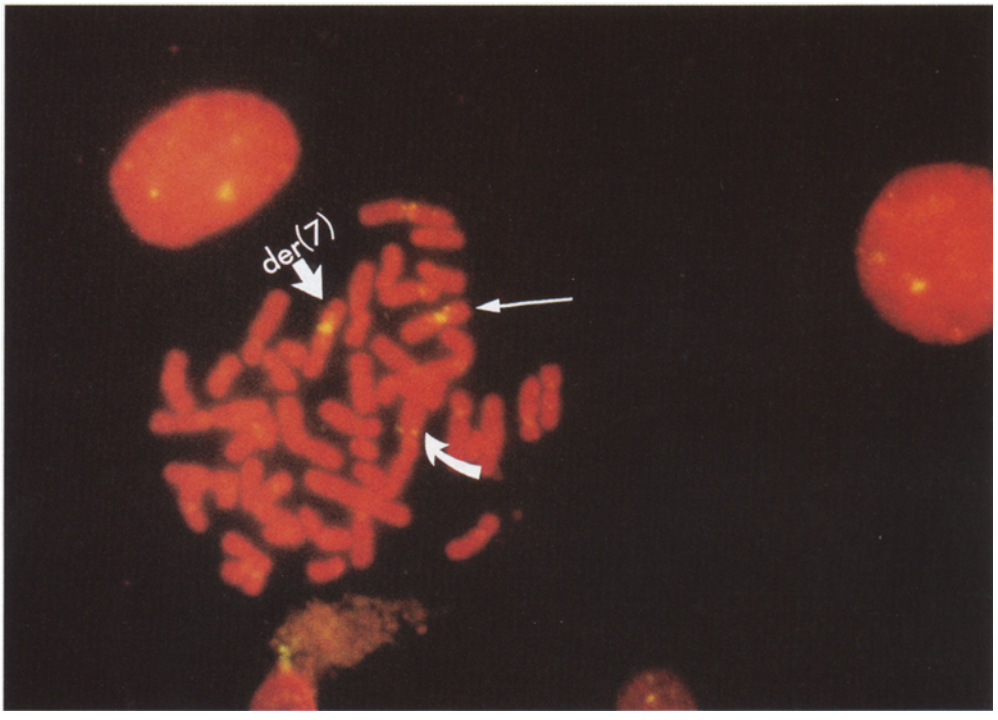

Fig. 3 Metaphase spread from a cell containing the der(7)t(7;15) after FISH with a biotinylated probe for the P gene (probe IR10) and an alpha satellite (centromeric) probe for chromosome 7. The *long straight arrow* marks the normal chromosome 7, which demonstrates a signal corresponding to the centromeric probe. The *curved arrow* marks the normal chromosome 15, which has a signal indicating hybridization of the P gene. The *short arrow* indicates the der(7). Note that the alpha satellite probe has hybridized to the centromere of the der(7), but that there is no hybridization of the P gene, indicating that it is not present on this chromosome

Results

Cytogenetic analysis demonstrated two populations of cells: a normal male cell line and a line containing 45 chromosomes, with an unbalanced rearrangement between chromosomes 7 and 15. The patient's karyotype was 45,XY, –7,–15,+der(7)t(7;15)(q34;q13)/46,XY. Twenty percent (6/30) of peripheral lymphocytes demonstrated the unbalanced translocation between chromosomes 7 and 15. These cells were partially monosomic for 15pter→q13 and 7q34→qter (Fig. 2). Of 100 metaphases from cultured fibroblasts, 80 demonstrated a 46,XY apparently normal male karyotype, and 20 had the unbalanced t(7;15). The level of mosaicism for this unbalanced rearrangement was therefore identical in blood and in skin.

D15S11, GABRB3, and D15S12 (IR10) were all absent from the der(7), and therefore cells with this unbalanced rearrangement were monosomic for these loci (data from D15S12 shown in Fig. 3). Myl was present on the derivative chromosome confirming that this region was not deleted but was translocated to chromosome 7 as predicted from the G-banding pattern. The probe from 7qter was absent, confirming that the patient was partially monosomic for 7qter in cells with the rearrangement.

Discussion

HMI is a heterogeneous group of disorders, all of which are characterized by macular cutaneous hypo- and hyperpigmentation in a distinctive pattern of streaks, whorls, or patches (Glover et al. 1989). This is not preceded by inflammation, vesicles, or verrucous lesions (as in seen in incontinentia pigmenti). There is a great deal of clinical variability. Ruiz-Maldonado et al. (1992) have proposed the following diagnostic criteria. The sine qua non is the congenital or early acquired variation in cutaneous pigmentation, viz., linear streaks or patches involving more than two body segments. The pattern of distribution may vary, but often follows the lines of Blaschko. In addition, this diagnosis requires at least one of the major criteria including an anomaly of the nervous or musculoskeletal system. Minor criteria include either a chromosome anomaly or two or more congenital malformations, other than those of the nervous or musculoskeletal system. A definitive diagnosis is made if the characteristic skin findings and at least one major criterion or two minor criteria are fulfilled. Our patient fulfills these criteria by exhibiting the characteristic skin findings and by showing involvement of the central nervous system and chromosomal mosaicism.

It is interesting to note that our patient was initially diagnosed as having NF1. He does have more than six cafe-au-lait macules, and pigmentation near the axilla. However, there are no neurofibromas or axillary freckling. Lisch nodules have not fully been excluded.

The relationship between chromosomal mosaicism and anomalous pigmentation has been well established in the literature (Turleau et al. 1986; Thomas et al. 1989; Ritter et al. 1990; Sybert et al. 1990; Lenzini et al. 1991). Numerous chromosomal abnormalities have been reported in patients with pigmentary anomalies; however, there has not been a consistent chromosomal finding. Reports associating chromosomal mosaicism and pigmentary anomalies include diploid/triploid mosaicism, autosomal trisomies, reciprocal and Robertsonian translocations, numerous reports of rearrangements involving Xp11, and others including 4p+, –7, del(13)(q11), –14, del(15)(q11), 18p-, r(7), r(10), r(13), r(14), r(15), r(22), r(X) and r(Y), i(12p), trisomy 12p, trisomies 13, 14, 18, and 22, inv(9), and 45,X. Although Ritter et al. (1990) have postulated that HMI is a nonspecific marker for chromosomal mosaicism, we believe that mosaicism for at least some of these regions may result in this phenotype because of the involvement of genes that are critical to normal pigmentary development.

Our patient is monosomic for one of these previously implicated regions (15q11 to q13), which is known to contain a human pigmentation gene. There are two prior reports of patients with HMI and rearrangements involving 15q11. Turleau et al. (1986) reported a female with HMI and mosaicism for a microdeletion of 15q11. That patient presented with a complex sacrococcygeal dysembryoma, seizures, severe cerebral lesions, chorioretinal atrophy, hemiatrophy, mental retardation, and skeletal anomalies. There were no clinical signs of PWS or AS. Bernstein et al. (1979) investigated a female with a complex unbalanced X;15 translocation who presented with mental retardation, short stature, cleft palate, and confluent depigmented areas on the trunk and limbs; these were reported as incontinentia pigmenti. This rearrangement was interpreted as an inversion of Xq25 to Xp11 or Xq11, with loss of Xq25 to qter, and centromeric or proximal long arm breakage of chromosome 15 with translocation of 15q onto the centromeric breakpoint of the X. It was not specifically determined whether there was a deletion of 15q11. Additionally, interpretation of this rearrangement was complicated by incomplete inactivation of the translocated chromosome. Therefore, this patient's cytogenetic rearrangement was compatible with the depigmented areas being caused by a rearrangement or deletion of a presumed locus for pigmentary dyscrasia at Xp11, or a deletion or interruption of a gene at 15q11.

X chromosome abnormalities (mosaicism and/or structural rearrangements) have been found in 60% of the cases of HMI (Koiffman et al. 1993). A locus for one form of pigmentary dyscrasia (described as IP1 in the literature) has been provisionally mapped to Xp11, based on multiple reports of patients with X-autosome translocations with similar breakpoints at Xp11 (Koiffman et al. 1993).

Deletion of 15q11→15q13 is associated with both PWS and AS (Knoll et al. 1989). These two syndromes have distinct clinical presentations. Patients with PWS have hypotonia, developmental delay, and poor feeding in infancy. In early childhood, they develop hyperphagia, obesity, short stature, hypogonadism, small hands and feet, mental retardation, and behavioral disorders. In addition, about 50% of PWS patients with a 15q11q13 deletion have hypopigmentation of their skin, hair, and eyes compared with other family members (Butler 1989). AS patients present with ataxia, seizures, severe mental retardation, hyperactivity, and absence of speech. Hypopigmentation of the skin, eyes, and hair is also a common feature of AS deletion patients (King et al. 1993).

Our patient does not demonstrate major features that are completely consistent with either of these well-known syndromes. He has severe mental retardation, hyperactivity, and absence of speech, which are consistent with a diagnosis of AS, although he does not exhibit the characteristic laughter or abnormal movements. His hypopigmentation pattern is streaky, but does not involve his eyes or hair. There is no history of neonatal hypotonia, nasogastric tube feeding or early childhood obesity as seen in patients with PWS. The PWS or AS phenotype is determined by the parental origin (paternal or maternal) of the deletion, or by uniparental disomy (Nicholls 1993b). Unfortunately, the parents of our patient are unavailable for analysis of the parental origin of the rearrangement. Additionally, the mosaic nature of this rearrangement would complicate such an analysis.

This patient is also monosomic for 7q34 to qter. Monosomy of terminal 7q is a relatively distinct syndrome that includes developmental delay, growth retardation, un-

usual facial features, cleft lip and/or palate, microcephaly, and genital anomalies (Bogart et al. 1990). A critical region for holoprosencephaly has been mapped to 7q36 (Gurrieri et al. 1993). Patients with r(7) have been reported with a variety of dermatologic lesions (Vollenweider et al. 1993), including typical congenital nevi, cafe-au-lait spots, and hemangiomas, and a single patient had achromic spots, although these were not further described (Caramia et al. 1990). Loss of this chromosomal region may account for the cafe-au-lait macules seen in our patient. The finding of achromic spots in a single patient with a r(7) in noteworthy, however; this patient may lack material from 7p and from distal 7q. Moreover, ring chromosomes are unstable and can lead to mosaicism via the loss and duplication of the entire ring chromosome (Kosztolanyi 1987). We cannot completely rule out the possibility that there is a pigmentation gene at distal 7q36, and deletion of one homolog may uncover a recessive mutation on the other chromosome 7. Nevertheless, neither HMI nor hypopigmentation has been reported in patients with simple deletions of distal 7q, so there is no strong evidence for this hypothesis.

In summary, we have identified a patient with HMI who is mosaic for a partial deletion of chromosomes 7 and 15, including the P gene. Deletion of genes specifically involved in pigmentation, such as P, may be one mechanism behind the pathogenesis of the pigmentary dyscrasia seen in HMI patients. We therefore suggest that HMI patients with normal karyotypes should be screened, by FISH, for submicroscopic mosaic deletions of known pigmentation genes such as P or tyrosinase.

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