Skin pigmentary anomalies and mosaicism for an acentric marker chromosome originating from 3q

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Abstract

We report on a 22 year old man with hyperpigmentation distributed along the lines of Blaschko in whom cytogenetic analysis showed mosaicism for an unusual supernumerary marker chromosome. The patient was of normal intelligence and was not dysmorphic. The marker was present in 30% of his lymphocytes and in 6% of his skin fibroblasts from a dark area, while fibroblasts from a light area showed a normal karyotype, 46,XY.We have identified the origin of the marker using fluorescence in situ hybridisation (FISH) with whole chromosome painting probes and YAC specific clones. The marker was found to consist of duplicated chromosome material from the distal part of chromosome 3q and was interpreted as inv dup(3)(qter→q27.1::q27.1→qter). Hence, this marker did not include any known centromeric region and no alpha satellite DNA could be detected at the site of the primary constriction. The patient was therefore tetrasomic for 3q27-q29 in the cells containing the marker chromosome. We postulate that, in our case, pigmentary anomalies may result directly from the gain of specific pigmentation genes localised on chromosome 3q.
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Keywords: pigmentary anomalies; acentric marker chromosome; mosaicism; tetrasomy 3q

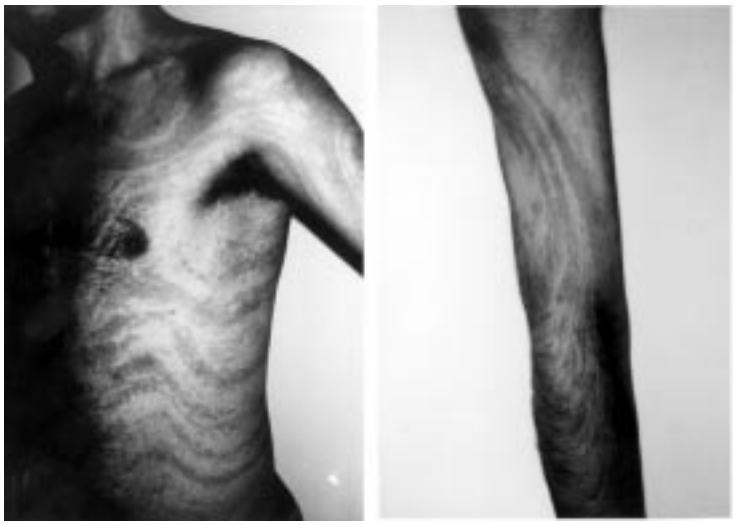

Figure 1 Photograph of the patient (reproduced with permission).

The lines of Blaschko are linear areas of skin differentiated from adjoining skin only by a difference in pigmentation. They are aligned along the axis of limbs and circumferentially around the trunk, and are thought to represent the tracts of migration of melanocytes. The pattern outlined by Blaschko lines shows genetic mosaicism that has arisen in the embryo before the development of neural folds and reflects the dorsoventral outgrowth of two genetically different populations of cells.[1]

Numerous and diverse chromosomal abnormalities have been reported in patients with pigmentary anomalies distributed along the lines of Blaschko, characterised by the presence of mosaicism in peripheral blood lymphocytes or skin fibroblast cultures.[2–5] Many patients have, in addition, various patterns of multiple congenital anomalies and mental retardation. Chromosomal aberrations may result in abnormalities of pigmentation because of the altered expression of genes in the pigmentary pathway, which map to a number of different chromosomes.[6 7]

We report here on a patient who displayed skin pigmentary anomalies distributed along the lines of Blaschko and was mosaic for a small supernumerary marker chromosome. The introduction of molecular cytogenetic techniques has greatly increased the possibility for full characterisation of marker chromosomes. Fluorescence in situ hybridisation (FISH) techniques show that markers can be derived from a variety of chromosomes.[8 9] Using FISH, the results of the present study showed that the marker originated from chromosome 3 and was an unusual acentric marker chromosome lacking detectable alpha satellite DNA. Alpha satellite DNA is believed to be important for centromeric function, because of its presence at all human centromeres.[10]

Materials and methods

CYTOGENETIC ANALYSIS

Chromosome analysis was performed on phytohaemagglutinin stimulated peripheral lymphocyte and skin fibroblast cultures after GTG, RHG, and C banding using standard procedures. Skin biopsy was obtained from both light and dark areas. An EBV transformed lymphoid cell line was established.

FLUORESCENCE IN SITU HYBRIDISATION

Fluorescence in situ hybridisation (FISH) was carried out on mitotic preparations according to Pinkel *et al*[11] with whole chromosome painting probes (CAMBIO) and alpha satellite

probes either for all human centromeres (ONCOR) or specific for chromosome 3 (D3Z1-ONCOR). Additional FISH studies were performed with 30 yeast artificial chromosome (YAC) clones positive for microsatellite markers known to map to chromosome 3.[12] All YACs were supplied by the CEPH library. A subsequent FISH analysis was carried out with three selected YACs spanning the marker breakpoint region (883F3, 781F8, 753C2). All probes were labelled with biotin and detected with avidin FITC and a PI counterstain.

Case report

A 22 year old man with no relevant personal history and of normal intelligence was referred because of pigmentary cutaneous anomalies. The patient was healthy and not dysmorphic. His parents were unrelated and no pigmentary disorder was present in his family. The onset of the pigmentary anomalies was recorded at the age of 10-12 years. This was not preceded by inflammation or vesicles. First both legs were affected, then the whole body, except for the palms, soles, eyes, and mucous membranes. Slight itching was present. Physical examination was otherwise normal. The pigmentary anomalies were characterised by bilateral, asymmetrical, linear, parallel, and hyperpigmented brown macular streaks, following the lines of Blaschko (fig 1). There was no cutaneous atrophy, but hair growth was decreased on affected areas. A brown spiral colouration was visible on his occiput.

Results of chest *x* rays, ophthalmological examination, heart and abdominal echography, and routine biological investigations were normal. Skin biopsy showed a diffuse basal layer hyperpigmentation.

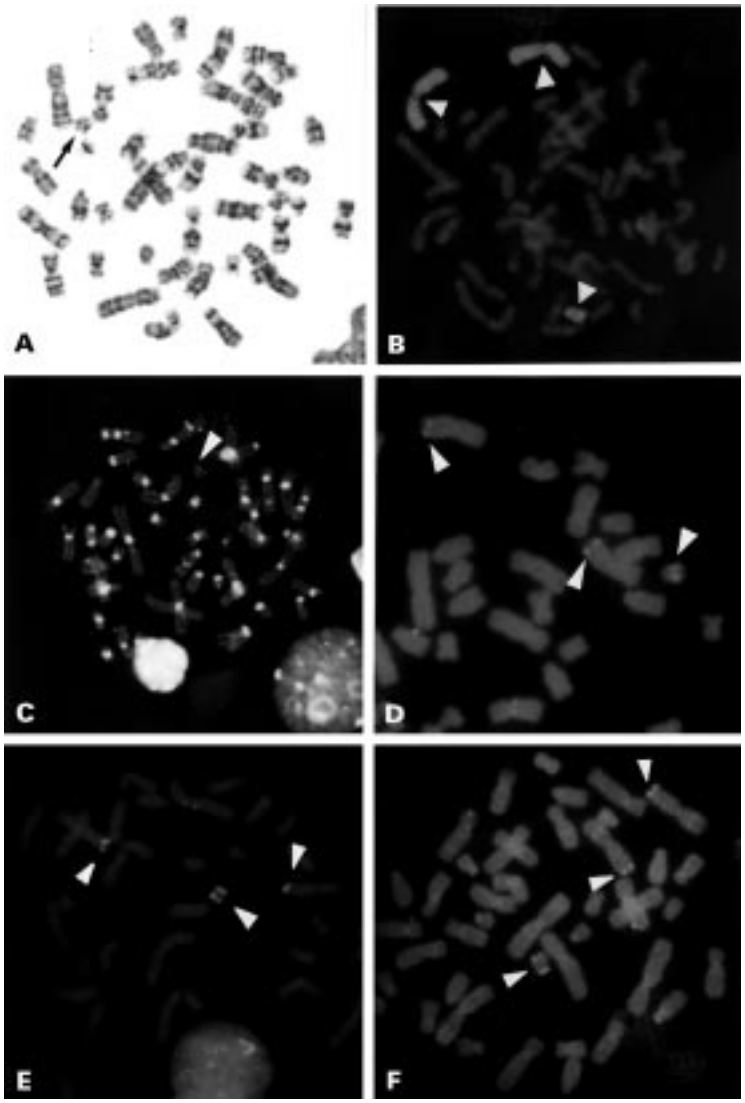

Figure 2 Chromosomes of the patient and FISH analysis of the marker chromosome. (A) G banded chromosomes; arrow indicates an additional marker chromosome. (B) Hybridisation of the chromosome 3 painting probe to the normal 3 and marker chromosomes (arrows). (C) FISH with the all chromosome centromeres probe. All chromosomes showed signal except the marker (arrow). (D) Partial metaphase following FISH with YAC from the 3q27 region. The two 3q27 bands had coalesced (arrow). (E) Partial metaphase following hybridisation with YAC probes from the 3q28 region. Arrows point to double signals on the marker and a single signal on the normal 3. (F) FISH with YAC probes from the 3q29 region.

Results

Cytogenetic analysis showed two populations of cells: a normal male cell line and a line containing 47 chromosomes with an extra supernumerary marker chromosome (fig 2). The marker was present in 30% of the lymphocyte metaphases and in 6% of the fibroblasts from hyperpigmented skin. It was absent in fibroblasts from the normally coloured skin which showed a normal 46,XY karyotype in 200 mitoses analysed. The marker of the G group size was acrocentric without satellites and had no visible C band. The banding pattern was non-specific and could not be attributed to any defined chromosome region. The marker was tested by FISH with many chromosome specific libraries. FISH with a chromosome 3 specific library painted the marker chromosome entirely, as well as the two normal chromosomes 3, indicating that the marker originated from chromosome 3 (fig 2). The chromosome 3 alpha satellite probe did not hybridise with the marker but did hybridise to the normal chromosomes 3. An all human centromeres probe hybridised to all chromosomes except the marker, indicating the absence of alpha satellite sequences on the marker (fig 2).

Further characterisation was achieved using YAC specific probes from chromosome 3. The marker was not labelled with the probes localised to 3p, but showed positive labelling with the probes (883D12, 760F3, 770A8) mapped to the distal 3q region from 3q27 to 3q29. The last negative YAC (806D8) was mapped to 3q26.3. The breakpoint was found to be localised in 3q27.1 using three selected YACs (883F3, 781F8, 753C2). Each positive probe produced double and symmetrical signals on the marker chromosome (fig 2). The hybridisation pattern suggested that the marker chromosome was an inverted duplication of the distal 3q region, interpreted as 3qter→q27.1::q27.1→3qter. The two 3q27.1 bands had coalesced, becoming a large positive band in the middle of the marker with the duplicated 3q29 bands lying in the two most distal regions of the marker. The patient's karyotype was: 46,XY/47,XY,+inv dup(3)(qter→q27.1::q27.1→qter). He was thus

mosaic for pure tetrasomy 3q, which, to our knowledge, has never been described before. The parents were not available for testing.

Discussion

Pigmentary anomalies have their origin all along the path which leads from melanoblast production to the melanocytes and their dysfunctions. The pattern of distribution in cutaneous dyspigmentation may vary, but often follows the lines of Blaschko, as in our patient. The lines of Blaschko consist of transverse bands on the trunk, following a sigmoid curve over the abdomen, a V shaped curve over the back, and showing a marked midline effect. On the limbs they follow a perpendicular pattern and on the scalp they form a spiral. It is now well known that Blaschko's lines can be explained on the basis of cellular mosaicism, chromosomal mosaicism, or mosaic distribution of single gene mutation.[13] The pattern is caused by the random arrangement of melanoblast precursors along the length of the neural crest and by their relative migration potential. This particular shape is the result of the type of embryonic growth of cutaneous tissue. Two clonal genetically different populations of precursor cells begin to proliferate in a transvere direction, starting from the primitive streak each side of the neural tube. The transverse clonal proliferation of cells interferes with the longitudinal growth and increasing flexion of the embryo.These complex movements result in the bizarre pattern of the lines of Blaschko.[1]

Our patient had a late, stable dyspigmentation following Blaschko's lines. Different skin pigmentary dysplasia distributed in a linear pattern could be ruled out as follows. Incontinentia pigmenti (IP), whose vesiculobullous first stage is sometimes absent but never late in appearing, is an X linked dominant gene defect with lethality in hemizygous males. The "acquired pigmented atrophic band-like dermatose following Blaschko's lines" of Moulin *et al*[14] could be ruled out because there was no atrophic lesion in our patient, as could the "familial progressive hyperpigmentation" of Chernosky *et al*,[15] affecting one mother and three of her children born from two different partners, as lesions were present at birth and rapidly spread in the first two years of life. The closest skin disease previously described is the "linear and whorled nevoid hypermelanose" of Kalter *et al*,[16] consisting of 1 to 5 mm coloured, homogeneous macules producing a reticulated form with asymmetrical streaks and whorls. Lesions appear during the first weeks of life, sometimes later, affecting boys and girls. There is no previous inflammation or papular lesions. Lesions spread moderately during the first two years then stabilise and mucous and palmoplantar areas are spared. Congenital anomalies are often present. Histological examination shows a non-specific increased pigmentation of the basal layer. Cytogenetic analysis of cultured fibroblasts from light and dark skin of patients was normal. Patients with skin pigmentation disorders are not described precisely enough clearly to differentiate dyspigmentation following Blaschko's lines from other pigmentary disorders.

The clinical findings of chromosomal mosaicism associated with dermatoses are diverse, most often including dysmorphic features, various patterns of multiple congenital anomalies, mental retardation, asymmetrical and abnormal growth patterns, and skin pigmentary anomalies; patients present with streaks of hyperpigmented skin or linear hypopigmentation described as hypomelanosis of Ito.[17] However, there have not been any consistent chromosomal findings. Similar pigmentary dysplasias may be associated with different chromosomal anomalies involving autosomes or sex chromosomes, and different pigmentary disorders may be associated with identical chromosomal anomalies. In their series, Ohashi *et al*[4] described two patients mosaic for trisomy 18; one had hypopigmented lesions, the other hypo- and hyperpigmented skin anomalies. Thomas *et al*[3] reported seven patients; only one was of normal intelligence and he had been assessed because of first degree hypospadias and linear hypopigmented lesions and lymphocytes and skin fibroblasts showed 45,X/46,XY mosaicism.These reports indicate that multiple genes are involved in pigmentation and map to a number of different chromosomes.

The patient described in this report was mosaic for an extra supernumerary marker chromosome in lymphocytes and in fibroblasts from hyperpigmented skin; his karyotype from light skin was totally normal. We only examined lymphocytes and fibroblasts cytogenetically as it was not feasible to study cultured keratinocytes or melanocytes. Melanocytes are not easy to grow and the patient was not available for further biopsies.Therefore, we are not absolutely sure that the melanocytes of the hyperpigmented or normal areas show the marker chromosome.

The marker was identified by FISH using panels of whole chromosome painting probes as originating from chromosome 3. Markers derived from chromosome 3, identified by an alpha satellite probe specific for chromosome 3, have been reported in four cases.[18–20] They were much smaller than in our case. In one of two cases of Muller-Navia *et al*,[20] further characterisation showed that the marker derived from the juxtacentromeric region of 3p. Skin pigmentary anomalies were not described in any of these four reports. The first association of a skin disorder with a chromosomal abnormality in chromosome 3 was reported by Jokiaho *et al*.[21] They described a 3q27-qter deletion in a girl with meningocele, facial dysmorphism, and skin lesions following the lines of Blaschko. Happle[22] suggested that the eruption observed in this girl appeared to be a new skin disease related to the extremely uncommon deletion 3q27-qter. The similar 3q chromosomal segment was involved in our marker chromosome.

The marker clearly has a functional centromere that is C band negative and forms a distinct primary constriction. FISH using an alpha satellite probe either for all human

chromosomes or specific for chromosome 3 failed to detect a signal on the marker, which was similar to an acentric marker chromosome. Occasional markers devoid of alphoid sequences detectable by FISH have been reported.[18 19 23–25] It was suggested that these marker chromosomes had truncated alpha satellite regions which may be impossible to visualise with FISH. Voullaire *et al*[26] and Maraschio *et al*[27] have both reported a novel mechanism for the formation of acentric marker chromosomes, derived from chromosome 10[26] and from chromosome 3.[27] Markers were associated with a ring chromosome and formed by the joining of the acentric portions, distal to the centric region, forming the ring. Activation of a latent intercalary centromere was suggested as the mechanism of formation. In our case and in that of Maraschio *et al*[27] the supernumerary chromosome derived from chromosome 3, but with a primary constriction localised in a different region. Further investigations of the marker derived from chromosome 10 have been performed at a molecular level by du Sart *et al*.[28] They provided evidence in support of the formation of the marker centromere through the activation of a latent centromere that exists within the normal human genome. The new centromere is composed of non-satellite DNA, suggesting that the alpha satellite DNA is not mandatory for centromere function. Hence, an ancient centromere sequence might exist at distal 3q and was activated through the chromosome rearrangement in our patient.

In our case the marker chromosome was identified as an inverted duplication, derived from 3q, interpreted as 3qter→q27.1::q27.1→qter, without alpha satellite DNA. These uncommon types of marker have recently been reported. They were found to be inversely duplicated segments from the distal region of 8p[29] and from distal 15q[30] without the centromeric region. The authors concluded that these findings strongly indicate that intercalary ancient centromere sequences are present and could be activated through chromosome rearrangements. These kinds of marker can be generated by a single "U type" exchange event involving chromosome breakage and subsequent reunion between the sister chromatids. A U type exchange can also be generated by a simple abnormal event in DNA replication, as suggested by Wik Sjostedt *et al*.[31] These theories have been postulated concerning the formation of dicentric isochromosomes and include the formation of an acentric fragment which, because of the lack of a functional centromere, is assumed to be lost. It could be speculated that this acentric fragment might somehow be retained, if it acquired or activated sequences that would function as a new centromere.[30] The formation of this type of marker chromosome may have been overlooked in the past, because of difficulties in resolving the rearrangements.

The patient described here was thus mosaic for pure tetrasomy 3q, which to our knowledge has never been described before. However, trisomy for distal 3q has been reported, and produces a distinct and recognisable phenotype.[32] It resembles the phenotype in the Cornelia de Lange syndrome and includes hirsutism, synophrys, seizures, severe mental retardation, and slow growth. Duplications may be of variable length, usually within the region 3q21-3qter. Ireland *et al*[33] proposed that 3q26.3 is the 3q duplication critical region and that the de Lange gene maps in this area. Van Essen *et al*,[34] reviewing published reports, suggested that trisomy of genes located in the distal part of 3q26 and the proximal part of 3q27 are essential for the characteristic phenotype in partial trisomy 3q syndrome. Our patient had mosaic tetrasomy of a 3q segment distal to the critical region of the duplication 3q syndrome, explaining why none of the features associated with this syndrome was observed. However, it was surprising that mosaic tetrasomy 3q27-qter had no major impact on the phenotype, apart from skin pigmentary anomalies distributed along the lines of Blaschko. Karyotype analysis from biopsies of light and dark areas of the skin showed that the light skin contained only normal cells, but there was a positive correlation between the abnormal karyotype from dark skin and the pigmentary abnormalities. The cytogenetic abnormality may be directly responsible for the linear dyspigmentation. Our observation lends support to the suggestion that this 3q27-qter region interferes with regulation of pigmentation and is probably the location of a gene(s) responsible for skin pigmentation. In addition, skin lesions in a girl with a deletion 3q27-qter were reported by Jokiaho *et al*.[21]

Although numerous observations of supernumerary chromosomes with various cytogenetic morphologies have been published, their clinical significance in individual cases has been a major problem. In a large multicentre study 1/2500 de novo small marker chromosomes were detected among 377 353 amniocenteses during a 10 year period and abnormal phenotypic outcome was seen in 13% of cases.[35] There is a correlation between mental retardation, the size, its euchromatic content, and the mutant origin of a marker.[36] In our case, the patient was intellectually normal. The absence of severe phenotypic consequences is most likely because of the mosaicism and the marker could be restricted to moderately affected tissues/organs. Therefore, our case confirms the relationship between chromosomal mosaicism and anomalous skin pigmentation, particularly when it follows the lines of Blascko, and stresses the importance in these cases of careful evaluation, which should include a lymphocyte karyotype and karyotypes from skin biopsies from dark and light skin.

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