

# Importance of the fibroblast chromosomal analysis in suspected cases of mosaicism: experience of a Clinical Genetics service

*Importância da análise cromossômica dos fibroblastos em casos suspeitos de mosaicismo: experiência de um serviço de Genética Clínica*

*Importancia del análisis cromosómico de los fibroblastos en casos sospechosos de mosaicismo: experiencia de un servicio de Genética Clínica*

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## ABSTRACT

**Objective:** To verify clinical characteristics and cytogenetic findings of patients suspects of having mosaicism and submitted to chromosomal analysis of lymphocytes and fibroblasts through GTG-Banding karyotype.

**Methods:** This is a retrospective analysis of the patients evaluated in the Genetic Clinic of Complexo Hospitalar Santa Casa de Porto Alegre of the Universidade Federal de Ciências da Saúde de Porto Alegre, from 1975 to 2009 (clinical data and results of cytogenetic exams were evaluated).

**Results:** Fifteen patients were enrolled, being six males (40%) with ten days to 14 years-old. Alterations in the chromosomal analysis of blood were observed in four patients (26.7%) and included one case of balanced translocation [t(2;9) pat] and three cases of mosaicism [mos 45,X/46,X,+mar; mos 46,XY,r(12)/45,XY,-12/47,XY,r(12),+r(12) and mos 46,XY/47,XY,+9]. The patients were then submitted to skin karyotype to confirm or to identify a chromosomal mosaicism. The main reasons for such suspicion in patients with blood karyotype without mosaicism were the presence of hemihypertrophy (n=5) and skin spots following the Blaschko lines (n=4). Mosaicism was confirmed in two cases and identified in another two (two cases of mos 46,XX/47,XX,+22). The mos 46,XY/47,XY,+9 was not verified in the fibroblast study.

**Conclusions:** Our results highlight the tissue variability that is characteristic of chromosomal mosaicism, as well as confirm the importance of the evaluation of a second tissue for the diagnosis. Clinical findings, as limb asymmetry and pigmentary anomalies following the Blaschko lines, are strongly indicative of mosaicism.

**Key-words:** mosaicism; chromosome aberrations; cytogenetic analysis; pigmentation disorders; hypertrophy.

## RESUMO

**Objetivos:** Verificar características clínicas e achados citogenéticos de pacientes com suspeita de mosaicismo submetidos à avaliação cromossômica por meio do cariótipo por bandas GTG de linfócitos e fibroblastos.

**Métodos:** Realizou-se uma análise retrospectiva dos pacientes avaliados no Serviço de Genética Clínica do Complexo Hospitalar Santa Casa de Porto Alegre da Universidade Federal de Ciências da Saúde de Porto Alegre, entre 1975 e 2009, por meio da coleta de dados clínicos e resultados da avaliação citogenética.

**Resultados:** A amostra foi composta de 15 pacientes, seis (40%) do sexo masculino, e idades variando de dez dias a 14 anos. Na análise cromossômica do sangue, alterações foram observadas em quatro pacientes (26,7%), incluindo-se um caso de transloca-

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ção balanceada [t(2;9)pat] e três de mosaicismo [um caso, respectivamente, de mos 45,X/46,X,+mar; mos 46,XY,r(12)/45,XY,-12/47,XY,r(12),+r(12) e mos 46,XY/47,XY,+9]. Com o objetivo de confirmar ou mesmo identificar um mosaicismo cromossômico, os pacientes foram submetidos posteriormente ao cariótipo de pele. Os principais motivos pelos quais os pacientes com cariótipo do sangue sem mosaicismo apresentaram tal suspeita foram a presença de hemi-hipertrofia (n=5) e de manchas hipocrômicas seguindo as linhas de Blaschko (n=4). Mosaicismo foi confirmado em dois casos e identificado em outros dois (dois casos de mos 46,XX/47,XX,+22). O mos 46,XY/47,XY,+9 não foi verificado no estudo dos fibroblastos.

**Conclusões:** Os resultados ilustram a variabilidade tecidual característica dos casos de mosaicismo cromossômico, bem como confirmam a importância da avaliação de um segundo tecido para a determinação diagnóstica. Achados clínicos, como assimetria de membros e anomalias pigmentares seguindo as linhas de Blaschko, são fortemente indicativos da presença de mosaicismo.

**Palavras-chave:** mosaicismo; aberrações cromossômicas; análise citogenética; transtornos da pigmentação; hipertrofia.

## RESUMEN

**Objetivos:** Verificar características clínicas y hallazgos citogenéticos de pacientes con sospecha de mosaicismo sometidos a la evaluación cromosómica mediante cariotipo por bandas GTG de linfocitos y fibroblastos.

**Métodos:** se realizó un análisis retrospectivo de los pacientes evaluados en el Servicio de Genética Clínica de UFCSPA/CHSCPA, en el periodo de enero de 1975 a junio de 2008, mediante la recolección de datos clínicos y resultados de la evaluación citogenética.

**Resultados:** La muestra estuvo compuesta por 15 pacientes, 6 de ellos (40%) del sexo masculino y edades variando entre 10 días y 14 años. En el análisis cromosómico de la sangre, se observaron alteraciones en 4 (26,7%) y se incluyó 1 caso de translocación balanceada [t(2;9)pat] y 3 de mosaicismo [1 caso, respectivamente, de mos 45,X/46,X,+mar; mos 45,XY,-12/46,XY,r(12)/47,XY,r(12),+r(12) y mos 47,XY,+9/46,XY]. Con el objetivo de confirmar, o incluso identificar un mosaicismo cromosómico, los pacientes fueron sometidos posteriormente al cariotipo de piel. Los principales motivos por los que los pacientes con cariotipo de la sangre sin mosaicismo presentaron tal sospecha fueron la presencia de hemihipertrofia (n=5) y de manchas hipocró-

micas siguiendo las líneas de Blaschko (n=4). Se confirmó el mosaicismo en 2 casos y se identificó en otros 2 (2 casos de mos 47,XX,+22/46,XX). El mos 47,XY,+9/46,XY no fue verificado en el estudio de los fibroblastos.

**Conclusiones:** Los resultados demuestran la variabilidad tejidual característica de los casos de mosaicismo cromosómico, así como confirman la importancia de la evaluación de un según tejido para la determinación diagnóstica. Hallazgos clínicos, como asimetría de miembros y anomalías pigmentares siguiendo las líneas de Blaschko son fuertemente indicativos de la presencia de mosaicismo.

**Palabras-clave:** mosaicismo; aberraciones cromosómicas; análisis citogenético; trastornos de la pigmentación; hipertrofia.

## Introduction

Chromosomal mosaicism is a reasonably common phenomenon, but it is rarely identified. It is believed that the incidence of cases that lead to significant phenotypical effects is greater than 1 in 10,000<sup>(1)</sup>. The condition is defined as the presence of two or more cell lineages with different chromosomal constitutions in a single person<sup>(1-3)</sup>. Description of mosaic status was made possible by advances in genetics which, with relation to chromosome analysis techniques, occurred from the 1950s onwards. An understanding of mosaicism's etiology is of great importance because it permits correct interpretation of laboratory results, diagnosis and clinical management, with the possibility of predicting phenotype in some cases. A thorough knowledge of the condition's mechanism provides the basis for stating that each and every genetic disease can exist in a mosaic form<sup>(1)</sup>.

Clinical investigations are usually limited to karyotyping of blood cells. However, the diagnostic difficulties that occur in many cases of mosaicism confirm the importance of analyzing a second tissue type. The skin is generally used in these situations. However, there are very few laboratories in Brazil with the infrastructure and technical capacity necessary to conduct this type of analysis. Furthermore, there is a paucity of studies of the subject in our country and those that do exist are basically descriptions of case histories<sup>(4-6)</sup>.

The objective of our study was therefore to describe the clinical characteristics of patients with suspected mosaicism and their cytogenetic findings after chromosome karyotyping by GTG banding of lymphocytes and fibroblasts, performed at a clinical genetics service.

## Methods

This study investigated all patients with suspected mosaicism seen at the clinical genetics service of UFCSPA/CHSCPA from its creation in January of 1975 until January of 2009. Patients were only enrolled if both peripheral blood and skin fibroblast karyotyping had been done. Patients were excluded if the clinical descriptions in their medical records were incomplete or if their skin had not been karyotyped.

A retrospective analysis was conducted of these patients' clinical and cytogenetic characteristics after systematically harvesting data from their medical records and filling in a standardized protocol. These data basically consisted of patient age at presentation; sex; cause of referral to the genetics department; referring department; reason for karyotyping skin and results of karyotype analyses.

Routine blood analysis is conducted at the UFCSPA cytogenetics laboratory using GTG-banding karyotype. This technique basically consists of preparing cell cultures with a lymphocyte-stimulating mitogenic (phytohemagglutinin), applying colchicine and a hypotonic shock, fixing the cells with Carnoy's solution and preparing the slides for staining G bands with trypsin and Giemsa (GTG).

When fibroblast karyotypes are requested they are prepared from skin biopsies. After the biopsy, the skin specimen is initially placed in a flask containing culture transport medium. It is then transferred to a small Petri dish, in which it is chopped up using a scalpel, to achieve mechanical dissociation of the cells. The material is then seeded into flasks containing culture medium and placed in an oven. After cultures have grown, they are treated in the same way as the blood samples, starting with administration colchicine.

Both karyotyping methods are analyzed using a Zeiss Axioskop microscope. Results were reclassified in line with the standards published in 2009, the International System for Human Cytogenetic Nomenclature (*ISCN*), which is a standardized nomenclature for the description of chromosomal abnormalities<sup>(7)</sup>. This study was approved by the Research Ethics Committee at UFCSPA.

## Results

Fifteen of the patients seen at the clinical genetics service had both blood and skin karyotyping conducted as part of their cytogenetic workup. Six of them were male (40%) and 9 were female (60%). Age at first consultation varied from 10 days to 14 years (median of 1.7 years, see Table 1). The

majority were referred by the pediatrics department (n=11 - 73.3%) and 4 (26.7%) were referred by other specialties. Primary motives for referral were: appearance suggestive of a syndrome (33.3%); hemihyperplasia (20%), delayed neuropsychomotor development (20%); seizures (13.3%) and pigmentation anomalies (6.7%).

Chromosome analysis of the blood detected abnormalities in 4 patients (26.7%), including one case of balanced translocation of chromosome 2 and 9 of paternal origin [t(2;9)pat] and 3 cases of mosaicism [one mos 45,X/46,X,+mar (mosaicism for one lineage with monosomy of the X chromosome and another with a missing sex chromosome and presence of a marker chromosome); one mos 45,XY,-12/46,XY,r(12)/47,XY,r(12),+r(12) (mosaicism for three different lineages with chromosome 12 ring) and one mos 47,XY,+9/46,XY (trisomy of chromosome 9 in mosaic)]. The number of metaphases analyzed varied from 20 to 226 (median of 33) (see Table 1 and Figure 1).

With the objective of confirming chromosomal mosaicism, or even of identifying it for the first time, the patients also had their skin karyotyped. The reasons for suspicion of mosaicism in patients without mosaic blood karyotypes were hemihyperplasia (n=5); hypochromic lesions following Blaschko's lines (n=4); multiple malformation syndromes combined with delayed neuropsychomotor development (n=3); limb hyperplasia (n= 2); hyperchromic warty lesions following Blaschko's lines (n= 1) and café-au-lait spots respecting the midline (n=1) (see Table 1 and Figure 1). Fibroblast chromosome analysis identified mosaicism in 2 of these patients (16.7%) [2 cases of mos 47,XX,+22/46,XX (trisomy of chromosome 22 in mosaic)]. Two of the patients with mosaicism in the blood karyotype (n=3) also had mosaicism in the analysis of skin [mos 45,X/46,X,+mar and mos 45,XY,-12/46,XY,r(12)/47,XY,r(12),+r(12)], while the mos 47,XY,+9/46,XY case was not confirmed. The number of metaphases analyzed for the fibroblast chromosome analysis varied from 10 to 124 (median of 48, see Table 1), and the most common biopsy site was the forearm.

## Discussion

Abnormal cell lineages do not tend to pass through the sieve of natural selection. The short period during which embryo cleavage progresses through the stages of morula and blastocyst tends to be the period of greatest selection. Many

**Table 1** - Clinical and cytogenetic characteristics of the patients in the sample.

N	Sex	Age	Blood KTP	Reason for skin KTP	skin KTP
1	F	5y	46,XX[33]	Hemihyperplasia + hypochromic lesions following Blaschko's lines	mos 47,XX,+22[40]/46,XX[8]
2	F	4m	46,XX[42]	Hemihyperplasia + hypochromic lesions following Blaschko's lines	mos 47,XX,+22[3]/46,XX[7]
3	F	1y 8m	46,XX[31]	Café-au-lait spots respecting the midline	46,XX[52]
4	F	4a	46,XX[75]	Hypertrophy of right leg + hyperchromic and warty lesions following Blaschko's lines	46,XX[43]
5	F	14y 9m	46,XX,t(2;9)pat[40]	Hemihyperplasia	46,XX,t(2;9)pat[57]
6	M	2m 28d	46,XY[226]	Multiple malformations + DNPD	46,XY[63]
7	M	7m	46,XY[27]	Hemihyperplasia	46,XY[124]
8	F	11m 24d	mos 45,X[41]/46,X,+mar[28]	Mosaicism in blood + hypochromic lesions following Blaschko's lines	mos 45,X[23 ]/46,X,+mar[26]
9	M	7y	mos 45,XY,-12[8]/ 46,XY,r(12)[73]/ 47,XY,r(12),+r(12)[2]	Mosaicism in blood + café-au-lait spots	mos 45,XY,- 12[11]/46,XY,r(12)[2]/ 47,XY,r(12),+r(12)[3]
10	M	1y 13d	46,XY[32]	Hemihyperplasia	46,XY[40]
11	F	6y 1m	46,XX[20]	Multiple malformations + DNPD	46,XX[28]
12	F	3y 10m	46,XX[27]	Hypertrophy of left leg + hypochromic lesions following Blaschko's lines	46,XX[51]
13	M	6y 5m	mos 47,XY,+9[4]/46,XY[50]	Mosaicism in blood	46,XY[59]
14	F	10d	46,XX[34]	Multiple malformations + DNPD	46,XX[35]
15	M	5y	46,XY[27]	Hypochromic lesions following Blaschko's lines	46,XY[60]

\*M: male; F: female; d: days; m: months; y: years; KTP: karyotype result. The number in brackets after the karyotype results indicates the number of metaphases detected with this chromosome constitution; /: separates different chromosome lineages; DNPD: delayed neuropsychomotor development.

genetically abnormal embryos are halted at this point, because many abnormalities are lethal<sup>(8,9)</sup>. Extensive mosaicism can also prevent progression to the blastocyst stage<sup>(10)</sup>. Cell lineages with abnormalities that do survive beyond this point may be able to continue up to the end of the first and start of the second trimesters, which is when the pressure of selection is brought to bear once more and is also when loss of a fetus is considered to be a clinical miscarriage. Some abnormalities can only, or almost only, exist in the mosaic state, since the non- -mosaic forms are lethal during the intrauterine period. Examples of this include mosaicisms involving a normal lineage and one with trisomy of chromosome 9 or trisomy of chromosome 22, as was identified in three patients in our sample.

A small degree of confined mosaicism may not provoke particularly severe phenotypical effects, but this depends on

the type of tissue involved and how abnormal it is. For example, a localized area of a given cell type in the brain could theoretically cause a neurological dysfunction; an abnormality involving a gonad or part of one could lead to the formation of unbalanced gametes and conception of a child with polymalformations. It is plausible to hypothesize that undetected islets of mosaicism involving small numbers of cells may be common. Perhaps we are all mosaics<sup>(11)</sup>. This tissue variability can also be illustrated by our patients with trisomy of chromosome 9 or trisomy of chromosome 22, whose mosaicism was only identified in one of the tissues typed.

Therefore, the scope of mosaicism is very wide and the distribution of different cell lineages can be highly variable. This depends on the point at which the mitosis error takes place: the earlier on in embryogenesis that the mutation occurs, the greater the

probability of a substantial fraction of the total being abnormal and the greater the degree of difference from the normal phenotype<sup>(1)</sup>. In contrast, a later mutation may give rise to mosaicism confined to just a few tissues, or even to a single organ<sup>(3)</sup>. Other factors that can also have an influence are the type of mutation; the specific type(s) of cells involved, and the destination of the particular cell lineage that resulted (migration and selection)<sup>(2,11,12)</sup>.

Chromosomal mosaicism, in turn, can present clinically as a recognized phenotype, such as in tetrasomy 12p/Pallister-Killian syndrome and in Down Syndrome mosaicism, or as a dysmorphic and intellectually affected person with chromosomal analysis findings of normal lymphocytes, as was suspected in patients 6, 11 and 14 of our sample. The mosaic state is very often associated with milder forms of clinical characteristics. For example, some people who have Turner and Klinefelter syndromes in mosaic will be fertile, in contrast with those who only have the abnormal lineage. Despite all of these features, clinical diagnosis of chromosomal mosaicism is difficult and many cases are only identified after cytogenetic analyses<sup>(1)</sup>.

The principle clinical signs that arouse a suspicion of somatic mosaicism are abnormal growth and skin pigmentation. The abnormal cell lineage may distort the normal growth pattern, leading to hypoplastic and asymmetrical areas<sup>(1,2)</sup>. This effect is highly variable and can involve part of a limb, a whole limb or one side of the body (hemihyperplasia)<sup>(2)</sup>, as was observed in 5 of our patients (see Figure 1). Hemihyperplasia is truly the most commonly reported finding in the literature. Hemihypoplasia, in turn, can also manifest, although in practice differentiating this from hemihyperplasia of the contralateral side can be difficult<sup>(1)</sup>.

Patients with chromosomal mosaicism may also exhibit pigmentation abnormalities (hyperpigmentation or hypopigmentation)<sup>(2)</sup>. The typical phenotype has hypopigmentation in unilateral or bilateral whorls and stripes, following Blaschko's lines<sup>(13)</sup>. These lines were first described in 1901, by the German anatomist Alfred Blaschko and correspond to the paths along which skin cells migrate during normal development of the body. However, other types of pigmentation patterns have also been observed, such as chequerboard, zosteriform, dermatomal or plaque-like<sup>(13,14)</sup>. This phenotype is usually given the name of hypomelanosis of Ito and five of the patients in our sample had this pattern (patients 1, 2, 8, 12 and 15).

In the majority of cases of hypomelanosis of Ito, the abnormal pigmentation of the skin is interpreted as hypopigmented lesions. This is essentially evident when there are large areas of unaffected skin. However it is often difficult to decide whether hypopigmented or hyperpigmented lesions are present,



**Figure 1** - Images of patients showing (A) hypopigmentation in stripes on the left forearm (patient 12) and (B) on the lateral surface of the abdomen (patient 6), and (C) hyperplasia of the right leg (patient 5).

especially when lesions are larger and mixed with normal skin<sup>(13)</sup>. A Wood's lamp can aid with detection, especially with Caucasians (the marks are easier to see in dark-skinned people)<sup>(1)</sup>. In some cases, onset of pigment anomalies is described at birth, but they may not be detected until 2-5 years in other cases (in general they become more visible after exposure to the sun)<sup>(1,2,5,13)</sup>.

Although the term "syndrome" is often used with relation to hypomelanosis of Ito - suggesting a single condition - the current concept is that it is actually the cutaneous (phenotypical) expression of chromosomal or gene mosaicism<sup>(15)</sup>. In other words it is a sign or a symptom and not a single syndrome<sup>(16)</sup>. The hypothesis is that the two different types of skin represent two different genotypes, but this has been difficult to prove, because the majority of studies report a mixture of cell types in biopsies from both types of skin<sup>(16,17)</sup>. One hypothesis that has been proposed to explain this is that disorders that follow Blaschko's lines are due to mutations in epidermal cells (keratinocytes and melanocytes) rather than mutations to fibroblasts in the dermis<sup>(17)</sup>.

In contrast, in linear and whorled nevoid hypermelanosis, another type of neurocutaneous disorder, the skin lesions are hyperpigmented, unlike hypomelanosis of Ito. Nevertheless, cytogenetic analysis often reveals chromosomal mosaicism in both hypomelanosis of Ito and linear and whorled nevoid hypermelanosis<sup>(14)</sup>. It is interesting that patient 4 in our study had marks following Blaschko's lines, but with a specific appearance: they were warty. On the basis of the sum of clinical findings, a diagnosis was later made of epidermal nevus syndrome, also known as Schimmelpenning-Feuerstein-Mims syndrome, which is a condition that is believed to be caused by a lethal dominant autosomal gene mutation that survives because of somatic mosaicism<sup>(18)</sup>.

Chromosomal mosaicism cases are usually identified by karyotyping and confirmation of diagnosis demands careful examination of cells. One accepted cytogenetic definition states that mosaicism is present if two or more cells are identified with the same structural abnormality or the same supernumerary chromosome. On the other hand, if there is a chromosome missing, the same abnormality must be detected in at least three cells. Karyotypes are generally conducted by analyzing lymphocytes from peripheral blood, assessing 15 metaphases per patient. These represent about one billionth of 1% of the total number of cells in an adult, but this tiny fraction is considered a valid representation of the remaining 99.999999999%. In cases where results are negative, but the suspicion of mosaicism remains, the analysis should be increased to 100 metaphases.

There are tables available in the literature that relate the number of metaphases analyzed to the degree of mosaicism that can be excluded to a given confidence interval<sup>(19)</sup>.

However, analysis of samples of a second tissue type can very often be necessary. The skin is usually used because of its accessibility<sup>(2)</sup>. Chromosome abnormalities in the fibroblasts of people with phenotypical abnormalities but normal lymphocytes have been described, and this was the case of our patients with trisomy 22 in mosaic<sup>(1,4,20)</sup>. It would appear logical to take the biopsy from the abnormal region, which is generally an area of abnormal pigmentation or growth and which is what was done with our patients; but the abnormal cells are often found in fibroblasts cultured from both normal and abnormal skin<sup>(1,17)</sup>. Furthermore, skin pigmentation anomalies can be caused by isolated defects of melanocytes or keratinocytes, rather than fibroblasts. It is believed that Blaschko's lines represent the migration paths of melanocytes. If so, melanocyte cultures should be the diagnostic method of choice for assessing suspected cases of chromosomal mosaicism with pigmentation anomalies following Blaschko's lines<sup>(1)</sup>.

The principal cause of chromosomal mosaicism is a non-disjunction in mitosis, which can take place at any stage of a person's embryogenesis and development (the post-zygotic phase)<sup>(3)</sup>. The abnormal cell gives rise to a cell lineage with abnormal chromosomes, which can affect the organism's development. While the normal lineages follow their usual course, monosomal and trisomal lineages can both be generated in the same body. When this involves autosomal chromosomes, the growth of monosomal lineages is disadvantaged and they are supplanted by normal and trisomal lineages. On the other hand, the non-disjunction error that leads to mosaicism may occur in a cell that is initially abnormal - trisomal - and after division give rise to a cell lineage with normal chromosomes. This phenomenon is known as chromosomal recovery. Nevertheless, there could still be an abnormality in this "normal" lineage if both the chromosomes that remain came from the same parent, in a condition called uniparental disomy. This abnormality cannot be detected via karyotyping and molecular tests are needed to diagnose it<sup>(11)</sup>.

Nonetheless, a large variety of cytogenetic abnormalities have been described in mosaicism, including chromosomal deletions, insertions and translocations. This is one of the reasons why hypomelanosis of Ito is not considered a single entity. Abnormalities of the X chromosome are some of the most common<sup>(21)</sup>, but any chromosome can be affected<sup>(1,13-15)</sup>. Mosaicism of trisomies of chromosomes 9 and 22, which was observed in 3 patients in our sample, is considered rare<sup>(20,22,23)</sup>.

Descriptions of mosaicism of chromosome 12 ring are also rare and the patient in our sample was described by Zen *et al* in 2005<sup>(24)</sup>. Mosaicism for mos 45,X/46,X,+mar is a more common chromosomal abnormality, usually seen in association with hypopigmentation or hypopigmentation in stripes distributed along Blaschko's lines, cognitive deficits and other phenotypical findings that are not typical of Turner syndrome, as was observed in patient 8 in our sample<sup>(25)</sup>. The balanced translocation identified in patient 5 was also detected in the father. In this case we cannot rule out the possibility that the patient's hemihyperplasia is the result of low-level chromosomal mosaicism for a specific abnormality, not detected by our analysis, or even that in the case of the child the translocation has undergone some minor rearrangement that could not be detected by the karyotyping techniques employed. Typically, chromosomal abnormalities are only detected in 30-60% of patients diagnosed clinically with hypomelanosis of Ito or with linear and whorled nevoid hypermelanosis<sup>(14)</sup>. In our sample, chromosomal abnormalities were identified in 3 of the 5 patients with these phenotypes (60%).

Chromosomal mosaicism can still escape detection even when different tissue types are analyzed, possibly because it is a very low degree of mosaicism or because it is confined to a tissue-type or site that has not been tested. Other causes include: a) the biopsy specimen only contains normal cells; b) there was negative selection of abnormal cells during cell culturing, and c) the mutation that is present cannot be detected cytogenetically, because it affects a single gene or is a submicroscopic abnormality that can only be identified with molecular cytogenetic tests, such as fluorescent in situ hybridization (FISH) or array comparative genomic hybridization (array CGH)<sup>(2,13,14,16)</sup>.

Therefore, our results illustrate the tissue variability characteristic of cases of chromosomal mosaicism and confirm the importance of testing a second tissue type for diagnostic confirmation. Furthermore, it is important that physicians be aware of the spectrum of clinical findings that chromosomal mosaicism can cause<sup>(1)</sup>. As Hall (1988)<sup>(12)</sup> and Ferreira *et al* (1996)<sup>(4)</sup> have pointed out, findings such as abnormal pigmentation following Blaschko's lines and body asymmetry are clues suggestive of mosaicism.

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