Analysis of mutations in the PIK3CA and FGFR3 genes in verrucous epidermal nevus*

Análise de mutações nos genes PIK3CA e FGFR3 em caso de nevo epidérmico verrucoso

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DOI: http://dx.doi.org/10.1590/abd1806-4841.20132055

Abstract: Verrucous epidermal nevi are congenital hamartomas composed of keratinocytes and may occur alone or in association with developmental abnormalities. A close relationship between variations in the PIK3CA and FGFR3 genes and the appearance of nevi has been recently reported. Based on that, we performed molecular assays for the identification of E542K, E545G/K and H1047R mutations in the PIK3CA gene and of the R248C mutation in the FGFR3 gene. Interestingly, during the amplification process, we did not observe the PCR product of exon 9 of the PIK3CA gene, a region comprising amino acids 542-545. This strongly suggests the occurrence of a microdeletion of that region and indicates a possible allelic variant, which has not yet being described in the literature.
Keywords: Hamartoma; Mutation; Nevus

Resumo: Os nevos epidérmicos verrucosos são hamartomas congênitos compostos por queratinócitos, que podem ocorrer isolados ou associados a alterações do desenvolvimento. Com a recente observação de uma relação estreita entre variações nos genes PIK3CA e FGFR3 e o aparecimento do nevo, realizamos ensaios moleculares para identificação das mutações E542K, E545G/K e H1047R do gene PIK3CA, e R248C do gene FGFR3. Interessantemente, durante o processo de amplificação não observamos o produto da PCR do exon 9 do gene PIK3CA, região que compreende aos aminoácidos 542-545, sugerindo fortemente a ocorrência de uma microdeleção da região e indicando uma provável variante alélica ainda não descrita na literatura.
Palavras-chave:Hamartoma; Mutação; Nevo

INTRODUCTION

Verrucous epidermal nevi are congenital hamartomas composed of keratinocytes and are classified into epidermolytic and non-epidermolytic. Their prevalence in adults is 0.1 to 0.5%, and there is no predilection for gender or race.[1] They are initially whitish due to maceration by the amniotic fluid. Over time, they acquire the aspect of a velvety streak or of a slightly brownish or pinkish plaque. The lesions are distributed following the Blaschko lines; on the trunk, they tend not to cross the midline.

In this report, we describe a verrucous nevus of the non-epidermolytic type, which should be clinically differentiated from other epidermal nevi, for they

Received on 09.08.2012.
Approved by the Advisory Board and accepted for publication on 04.11.2012.
* Work conducted at Pedro Ernesto University Hospital - University of the State of Rio de Janeiro (Hospital Universitario Pedro Hernesto - Universidade do Estado do Rio de Janeiro / HUPE-UERJ) - Rio de Janeiro (RJ) - Brazil.
Financial Support: None
Conflict of Interest: None

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are usually present at birth, asymptomatic, non-inflammatory and persistent.

Non-epidermolytic verrucous epidermal nevus may originate from different genetic mutations, which have not yet been completely identified. Recent reports in the literature point to a relation between the disease with the fibroblast growth factor receptor 3 gene (*FGFR3*) and the phosphatidylinositol 3-kinase catalytic subunit alpha (PIK3CA) oncogene.[1-4] The mechanism of action of these variants in the development of the disease and phenotypic level have not yet been elucidated; however, such mutations have been described in almost half of cases and have a strong association with oncogenesis.[5]

Histopathology of non-epidermolytic verrucous epidermal nevus shows hyperkeratosis and acanthosis, often associated with papillomatosis and occasionally with focal hypergranulosis and parakeratosis. About 10% of lesions show the pattern of acrokeratosis verruciformis and seborrheic keratosis. They rarely present histological findings characteristic of viral warts, acanthosis nigricans or of the verrucous phase of incontinentia pigmenti.[1]

A variety of developmental abnormalities may occur in association with verrucous epidermal nevus. These abnormalities include localized anomalies such as megalopinna and aplasia cutis of the scalp as well as syndromes such as epidermal nevus syndrome, Proteus Syndrome, McCune-Albright Syndrome, and Klippel-Trenaunay Syndrome.[1]

The various treatment options, which are not always satisfactory, include topical salicylic acid, retinoic acid, calcipotriol, and calcitriol,[1,6] cryotherapy for smaller lesions, and systemic retinoids for bigger and unaesthetic lesions. Patients and caregivers should understand that drug therapy is not curative and that relapses are common when treatment is discontinued.

CASE REPORT

A three-year old black female patient from Rio de Janeiro presented with diffuse hyperchromic macules which were present at birth and became darker and keratotic over time. She was born by vaginal term delivery and presented psychomotor development compatible with her age. Dermatological examination revealed presence of velvety hyperchromic papules on the face, neck, trunk, and limbs (Figures 1 and 2). Distribution of the lesions follows the Blaschko lines in the posterior area of the trunk (Figure 3). No other changes were observed on physical examination.

Histopathological examination of the cervical region showed hyperkeratosis, acanthosis, and papillomatosis, which is compatible with verrucous nevus. Genetic analysis of peripheral blood and of the skin

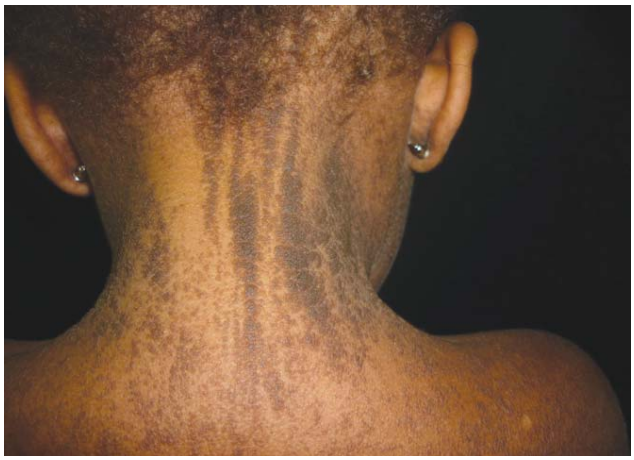

Figure 1: Brownish papules in linear arrangements with a velvety surface in the posterior cervical region

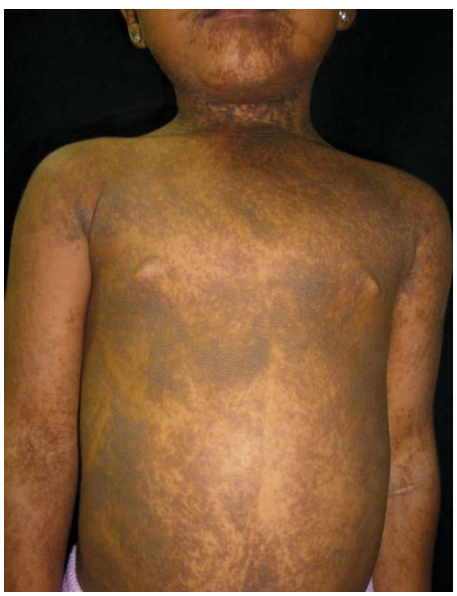

Figure 2: Hyperchromic verrucous lesions on the face, anterior trunk and upper limbs

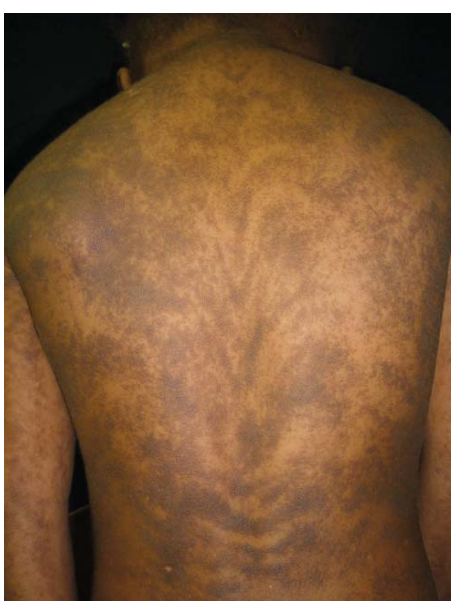

Figure 3: Lesions on the posterior trunk following the Blaschko lines

lesion detected a mutation in the Aa542-545 region of the PIK3CA gene, despite the aspect of deletion. Additional tests will be conducted for clarification. For amplification of the Aa542-545 region, we used downstream 5´CCAGAGGGGAAAAATATGACAA-AG3´ and upstream 5´CCCTTTCATTTTTCTTTTT-TCTTACG´3 primers at the following final concentrations of reagents: 1x Buffer, MgCl2 2.5 mM, dNTPs 0.2mM, primers 0.5µM; polymerase 0.03 U/ µL; Sample ~ 100ng. Thermocycling conditions were 50°C for 2 minutes; 95°C for 10 minutes; 40 cycles of 95°C for 30 seconds, 58°C for 1 minute, 72°C for 1 minute, and 72°C for 10 minutes.

The patient is being regularly monitored and using adapalene gel 0.1% on the face, showing little response.

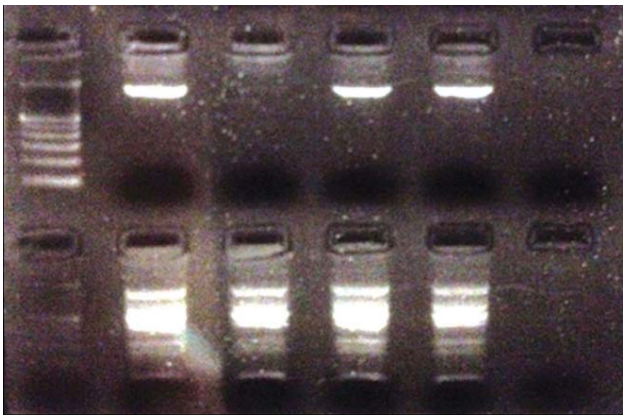

Figure 4: Amplification products of the regions of the PIK3CA gene; Top line, investigation of E542K, E545G or E545K mutations; Bottom line, investigation of H1047R mutation. Columns from left to right: **1)** 100bp DNA Ladder Invitrogen®, **2)** Positive Control, **3)** Patient of the study, **4** and **5)** Other Patients, **6)** Negative Control

DISCUSSION

Classification of verrucous nevus into epidermolytic and non-epidermolytic is done by histopathology. In the case here discussed, the absence of epidermolysis allowed us to classify it as non-epidermolytic verrucous nevus.

Some genetic mutations have been proposed to explain the origin of non-epidermolytic verrucous nevi. The main ones are those in the PIK3CA oncogene and in the FGFR3 gene. During the amplification phase for the discrimination of E542K, E545G/K and H1047R mutations in the PIK3CA gene and the R248C mutation in the FGFR3 gene, we observed absence of the polymerase chain reaction (PCR) product for exon 9 of the PIK3CA gene, a region which corresponds to codons 542-545. The absence of amplification strongly suggests the occurrence of a microdeletion of the region or a gene rearrangement, which has not yet been described (Figure 4). The main function of the exon 9 region of the PIK3CA gene is a helical domain of interaction with other proteins. As the absence of this region has not yet been reported, we can only suggest that the interaction relationship between proteins of this pathway should be in disequilibrium, which could be the primary cause of the disease. Another relevant datum, in case this variant is confirmed, is the possibility that this allele is not correctly identified in heterozygous individuals, since only one allele with the microdeletion still allows amplification and observation of the PCR product and sequencing as a false wild-type. Other mutations investigated for the PIK3CA and FGFR3 genes were not identified.

Despite the need for further molecular studies in order to characterize this mutation, it is likely that this new allele will bring some relevant information about the function of the PIK3CA protein and also contribute to a better understanding and diagnosis of verrucous epidermal nevus. ❑

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How to cite this article: Miranda LQ, Fracaroli TS, Fonseca JCM, Fontenelle E, Curvo RPM, Porto LC, Souto R. Analysis of mutations in the PIK3CA and FGFR3 genes in verrucous epidermal nevus. An Bras Dermatol. 2013;88(6 Suppl 1):S36-8.