CASE REPORT

A *de novo em* ~1.3 Mb microdeletion at 17q11.2 associated with Neurofibromatosis-Noonan syndrome

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Abstract

Introduction: Neurofibromatosis-Noonan syndrome is a clinical entity considered an extended Neurofibromatosis phenotype generally caused by different types of intragenic mutations at the NF1 gene. About 5%-10% of patients with neurofibromatosis diagnosis carry chromosomal microdeletions involving NF1, often presenting with a more severe phenotype than that observed in the patients carrying intragenic mutations; however, anticipating the presence of a deletion based only in the phenotype is not straightforward. **Patient and Methods:** Here we investigated by oligoarray-CGH (aCGH) the presence of a submicroscopic genomic rearrangement in a patient with a clinical picture of Neurofibromatosis, and other characteristics compatible with Noonan syndrome. **Results:** The aCGH analysis revealed a germline *de novo* ~1.3 Mb microdeletion at 17q11.2 encompassing other coding genes besides the NF1 gene. **Discussion:** Up to now, the number of reported patients with Neurofibromatosis-Noonan syndrome carrying NF1 microdeletions is quite small. The continuous identification of patients carrying 17q11.2 deletions can help to establish a reliable genotype-phenotype relationship in this syndrome.

Keywords: neurofibromatoses, neurofibromatosis-noonan syndrome, nf1 microdeletion syndrome.

INTRODUCTION

Neurofibromatosis type I is an autosomal dominant disorder that affects 1 in 3.500 live births and is caused by NF1 haploinsufficiency. This gene is located at 17q11.2 and, when mutated, presents complete penetrance and high degree of variable expressivity. Neurofibromatosis-Noonan syndrome (NFNS) is a well-characterized clinical entity[1] and is considered an extended NF1 phenotype generally caused by different types of intragenic mutations at NF1 (nonsense, missense, out-of-frame deletions, small in-frame deletions and large multi-exon deletions)[2,3].

About 5%-10% of patients with neurofibromatosis diagnosis carry microdeletions involving NF1 and surrounding genes; this microdeletion NF1 syndrome is often characterized by a more severe phenotype than that observed in the majority of NF1 patients carrying intragenic mutations, which may include several dysmorphisms (including Noonan-like features), mental impairment, developmental delay, and an excessive number of early-onset neurofibromas[4,5]. Considering that there is an overlap of the clinical signs among patients with NF1 microdeletion, classic NF1 patients and NFNS patients, anticipating the presence of a deletion based only in the phenotype is not straightforward[5-7].

Array-based comparative genomic hybridization (aCGH) has improved the detection of submicroscopic chromosomal abnormalities in different groups of patients with syndromic mental deficiency and/or congenital abnormalities. Here we described a Brazilian patient with normal conventional GTG karyotype and a clinical picture compatible with NF1 and Noonan syndromes. The aCGH investigation for submicroscopic rearrangements revealed a ~1.3 Mb microdeletion at 17q11.2 encompassing, besides the neurofibromingene (NF1), at least another 15 genes.

PATIENT AND METHODS

This study has been conducted according to the local and international regulations of ethics in research. This case report is part of one study that has been approved by the Institutional Committee for Ethics in Research of A.C. Camargo Cancer Center. Written informed consent was obtained from the parents of the patient for publication of this case report and any accompanying images.

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Patient - Clinical description

A 10-year-old female patient presented at diagnosis some degree of developmental delay with learning problems. At external examination, multiple cafe-au-lait spots, axillary freckles and a plexiform neurofibroma were shown. Eye examination confirmed Lisch nodules. Others clinical features included: down-slanting palpebral fissures, hypertelorism, large and bulbous nasal tip, long philtrum, webbed neck and thoracic asymmetry with superior *pectus carinatum* and inferior *pectus excavatum*. Cardiac findings were normal at physical examination. Although she did not have short stature, the clinical findings were compatible with the Neurofibromatosis-Noonan syndrome (NFNS; Figure 1). A previous conventional cytogenetic analysis (GTG-banding) revealed a normal 46, XX karyotype.

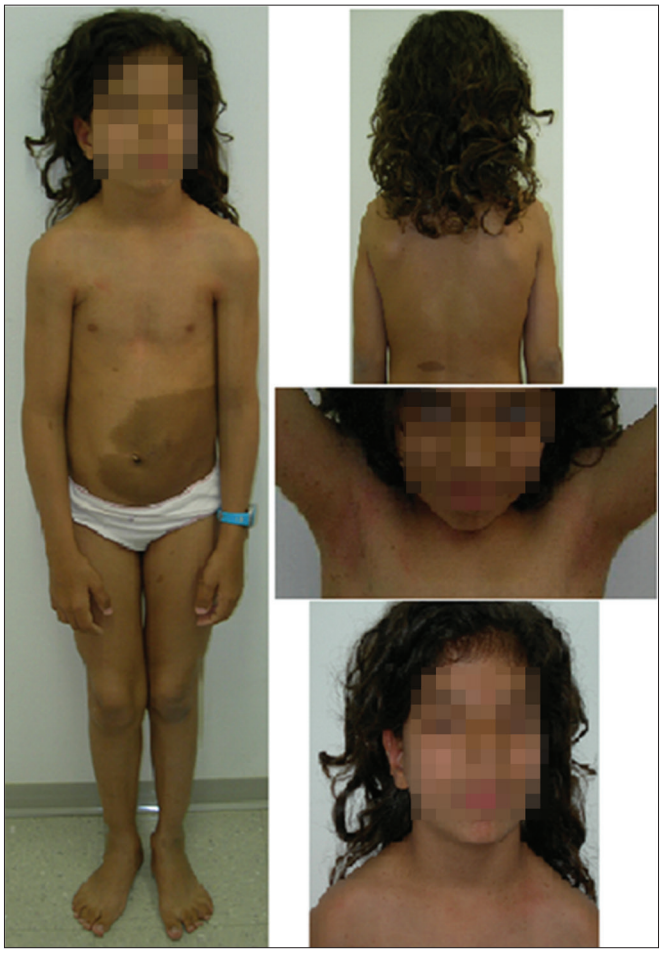

Figure 1. Patient affected by Neurofibromatosis-Noonan syndrome.

aCGH analysis

The investigation of DNA copy number imbalances was performed by aCGH using the whole-genome 180K Agilent SurePrint G3 Human CGH Microarray, which contains ~180.000 oligonucleotides, hybridized according to the manufacturer's protocols, and gives an average spacing between oligonucleotides of ~17 Kb. The analysis was performed on DNA samples from the patient and their parents. Dye-swap aCGH experiments were performed using as reference DNA a commercial sex-matched pool of controls (Promega).

Scanned images of the arrays were processed and analyzed using Feature Extraction software and Genomic Workbench software (both from Agilent Technologies), with the statistical algorithm ADM-2, and a sensitivity threshold of 6.7.

RESULT

The aCGH analysis revealed a ~1.29 Mb microdeletion at 17q11.2, ranging from chr17:29,071,113-30,361,835 nucleotides: [arr 17q11.2 (29,071.113-30.361.835)x1] (according to ISCN 2009, GRCh37/hg 19; Figure 2). The detected 17q11.2 microdeletion involved 13 genes (CRLF3, ATAD5, C17 orf 42, ADAP2, RNF135, NF1, OMG, EVI2B, EVI2A, RAB11FIP4, C17 or f79, UTP6, and SUZ12), two pseudogenes (SUZ12P and DPRXP4), and two miRNA genes (hsa-mir-193a and hsa-mir-365-2). Investigation of DNA samples from the patient's parents using aCGH revealed a normal aCGH profile at the 17q11.2 region, showing that this microdeletion was *de novo*.

DISCUSSION

Due to the overlap of clinical signs among patients with NF1 microdeletion, both classic NF1 and NFNS, it is hard to anticipate the presence of a deletion based only in the phenotype. Although the patient here described did not exhibit short stature, she presented a phenotype compatible with NFNS, with several Noonan-like dysmorphisms, mental impairment, developmental delay, and a plexiform neurofibroma. Accordingly, we performed an aCGH investigation in a patient with a clinical picture compatible with NFNS in order to screen for the presence of a submicroscopic chromosomal deletion. A germline *de novo* ~1.3 Mb microdeletion at 17q11.2 was detected.

The current advance in cytogenetic techniques has allowed the precise detection and characterization of microdeletions in patients with NF1, as witnessed by a recently developed *custom 8x15k* micro*array to refine the mapping of NF1* deletions[5]. Three distinct types were characterized: type-1 (77% of the cases), involving 14 genes in ~1.4 Mb; type-2 (9% of cases), sizing ~1.2 Mb and encompassing 13 genes; and type-3 (4% of the cases), affecting eight genes in ~1 Mb. The 10% remaining microdeletions were considered atypical[7]. Different paralogous regions flanking the NF1 gene contributes to a non-allelic homologous recombination (NAHR) mechanism, which causes the loss of the aforementioned specific size-chromosomal segments[4,7-9].

We used a whole-genome commercial 180K platform with a lower density of probes in the 17q11.2

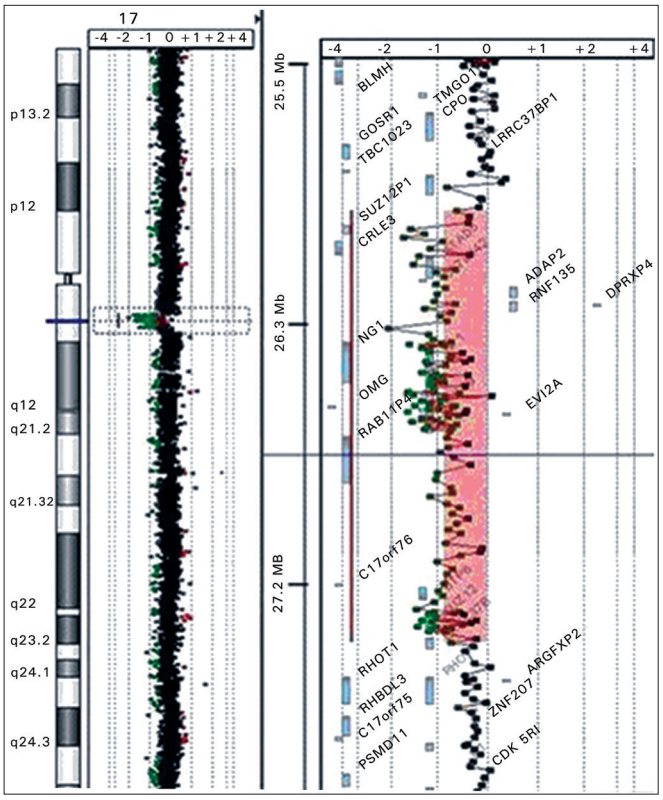

Figure 2. Germline *de novo* 17q11.2 microdeletion encompassing, among others, the NF1 gene, detected in a patient with Neurofibromatosis-Noonan syndrome. Right panel (A) exhibits the chromosome 17 ideogram (image based on the Genomic Workbench software). The blue bar marks the ~1.29 Mb genomic region covered by consecutive oligoprobes with median log2 ratios < -0.8, indicating a heterozygous deletion in the patient. At left, (B)the image shows an enlarged vision of the 17q11.2 deletion in the aCGH profile (red bar), mapped at chr17:29,071,113-30,361,835 (genomic coordinates on hg19) - the encompassed genes are shown as blue boxes.

region when compared to the custom array mentioned above[5]. Nevertheless, it seems that our patient presented a type-2 microdeletion once the number of genes encompassed and the total size of the alteration is quite similar. It has been proposed that type-2 microdeletion originates by an unequal crossing-over between the pseudogene SUZ12Pand the gene SUZ12[7]. However, in our patient, we cannot exclude the possibility that the underlying region at the 3 portion of the deletion was also deleted.

Up to now, the number of patients carrying NF1 microdeletions is quite small. As previously proposed [4,5,7], a large number of patients presenting different chromosome losses might clarify the specificity of the deleted genes to the clinical manifestations and the correct genotype/phenotype relationship. The clinical presentation of our patient compared to that reported for other patients carrying this type of microdeletion revealed a quite similar pattern. In addition, a well characterized cohort of NFNS patients would help to correctly establish the frequency of type-2 mutations to this population.

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