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Unusual precocious diagnosis of Duchenne muscular dystrophy by SNP-array in a 6-month old baby

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ABSTRACT

Microdeletions and microduplications are involved in many cases of malformations, developmental delay (DD), autism (A) and growth disturbances (GD). Molecular karyotyping (MC) performed by microarray technology is a valuable method frequently used to elucidate the etiology of these clinical expressions, essentially contributing to the diagnosis of rare genetic diseases produced by DNA copy number variations (CNVs). MC has offered a higher diagnostic yield for genetic testing of patiens with unexplained DD, A and GD than a G-banded karyotype, primarily because of its higher sensitivity in detecting submicroscopic deletions and duplications. In addition, the molecular karyotype approach using the SNP-array method also allows highlighting the regions of loss of heterozygosity and uniparental disomy, which are the basis of some genetic syndromes.

In this paper we will present a rare case of a 6 months old boy, referred for MC due to global developmental delay, axial hypotonia, with limbs hypertonia, heart malformation (atrial septal defect). SNP-Array analysis identified a 606,3 Kb microdeletion in the Xp21.1 region, that contains 39 exons of dystrophin gene. This finding was confirmed also with aditional MLPA testing.

In conclusion, it is an unusual, precocious diagnosis of Duchenne muscular dystrophy, during first year of life, referred for atypical presentation, main clinical feature being global developmental delay.

Keywords: developmental delay, Duchenne muscular dystrophy, SNP-srray

INTRODUCTION

Duchenne muscular dystrophy (DMD) is a severe, progressive neuromuscular disease X-linked, caused by mutations in the dystrophin gene. In addition to skeletal muscle pathology, DMD is characterized by cognitive and behavioral disorders in males, such as: cognitive impairment (IQ<70 in 30%), reading deficits phonological dyslexia-like (40%), attention-deficit/hyperactivity disorder (ADHD) (32%), anxiety disorder (27%), autism spectrum disorders (ASD) (15%), epilepsy (6.3%), and obsessive-compulsive disorder (OCD) (4.8%) (1).

Here we present the case of a six months old boy who was referred to the geneticist for global developmental delay, axial hypotonia, moderate hearing loss and heart malformation (atrial septal defect). SNP-Array analysis detected a deletion of 606.3 Kb / 39 exons in the Xp21.1 DMD gene region.

CASE PRESENTATION

It is the second child of a young, healthy parents, from a high risk pregnancy, with repeated bleeding episodes. He was born at 38 weeks by cesarean section, with suspicion of placenta praevia. Parameters at birth were weight 2850 g and length 50 cm, Apgar score 7. He had neonatal hypoxia, which necesitates oxygen and ventilation. Afterthat, in the first week

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of life he had feeding difficulties, and necessitates 5 days of gavage. From medical history, we mention at 3 weeks interstitial pneumonia and at 2 months old bronchiolitis. Hearing evaluation- initially bilateral REFER results, but with normal findings at the further otorhinolaryngoly hearing tests. Ecocardiograpy revealed atrial septal defect. Transfontanellar echography had normal result.

The child had at 6 months old for the first time genetic evaluation, main concern being global developmental delay. At clinical examination noted dysmorphic facies, plagiocephaly, microretrognatia, long philtrum, low set ears. Right hand single palmar creases, axial hypotonia with limb hypertonia. He was not able to hold his head, although the kinetotherapy was initiated at 3 months old. Genetic consultation raised suspicion of a possible genetic syndrome, due to clinical findings and heart malformation.

At the clinical reexamination at the age of 1 year, the patient presented with global developmental delay, hypotrophia, weight of 8 kg (< P5), length 71 cm (P5), skull perimeter 46 cm (P25), good tone, rolls out dorsal in ventral decubitus and vice versa, says a few monosyllabic words.

To establish the diagnosis, the molecular karyotype was recommended and analyzed by SNP-array testing. For this, the DNA sample was isolated from peripheral blood (by means of KIAamp DNA Mini Kit, Qiagen) and analyzed with the HumanCytoSNP-12 v2.1 Analysis BeadChip Kit (Illumina). The SNP-array scan was performed with the NextSeq550 equipment (Illumina) and BlueFuse Multi 4.5 Software (32178) was used for final analysis of the SNP-Array data. A 606.3 Kb hemizygous microdeletion was detected in the Xp21.1 region, containing 39 exons of the dystrophin gene (DMD, OMIM 300377) (fig. 1).

Molecular karyotype formula, according to ISCN 2016 was: arr[GRCh37]Xp21.1(32244312_32850589) x0.

The deletion detected in the DMD gene affected 39 exons, including exons 5-43, which was confirmed by MLPA (multiplex ligation-dependent probe amplification). Thus, the detected deletion change is predictable to lead to a change in the reading frame (frameshift) and the synthesis of a truncated, aberrant, non-functional protein (DMD NM_000109.4).

DISCUSSION

In this paper we have described a rare case of a 6-months old boy, referred for genetic testing in the first year of life due to global developmental delay, axial hypotonia and heart malformation (atrial septal defect), MC identifying a large 39 exon deletion of dystrophin gene by SNP-array.

The DMD gene, which is involved in the pathogenesis of Duchenne (OMIM 310200) and Becker (OMIM 300376) muscle dystrophies and cardiomyopathy, dilated, 3B (OMIM 02045), with X-linked recessive transmission, is localized in Xp21 region, being the longest human gene, with 2.4 Megabases of DNA representing 1% of the chromosome X DNA and consists of 79 exons which encodes a 3685 amino acids protein called dystrophin (2).

Numerous types of mutations in the DMD gene have been reported. Thus, out of 7,149 mutations in the DMD gene analyzed in the TREAT-NMD DMD Global database, 80% (5,682) were large rearrangements, of which 86% (4.8940) were deletions and 14% (784) duplications (of 1-n exons). The remaining 21% patients carry small mutations, out of which, half are nonsense mutations (3).

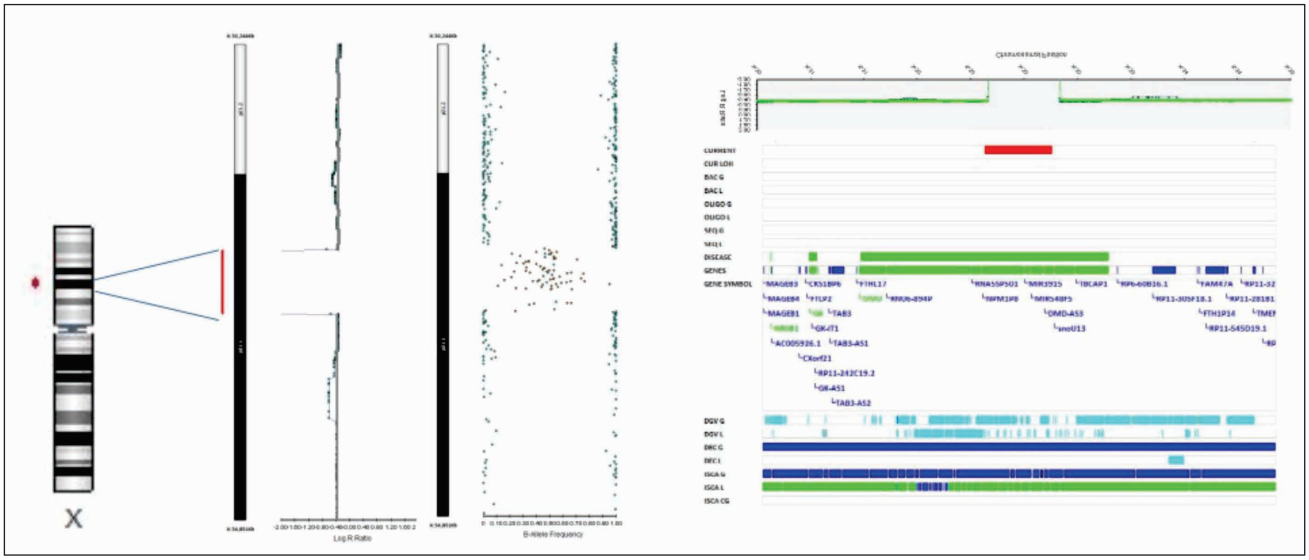

FIGURE 1. Image of the 606.3Kb microdeletion detected in the Xp21.1 region (32244312_32850589), containing at least 39 exons of the Dystrophin gene (DMD, OMIM 300377)

The DMD mRNA of the largest isoform of dystrophin is 14 kb which is only 0.6% of the total weight of the gene. The DMD gene expression is complex, several promoters being active and leading to the expression in a tissue-specificity manner. The full length dystrophin isoform is expressed in all striated skeletal, smooth and cardiac muscles and consists of 4 domains: N-terminal domain (codified by exons 2–8), Central rod domain (exons 9–61), Cysteine-rich domain (exons 64–70) and C-terminal domain (exons 71–79). Other shorter dystrophin isoforms are expressed in a tissue-specificity manner, as follows:

- the Dp260 isoform (from a promoter localised in intron 29) is mainly expressed in retina,
- Dp140 expressed in the CNS (Central Nervous System) and kidney from a promoter in intron 44,
- Dp116 is predominantly expressed in Schwann cells from a promoter localised in intron 55,
- Dp71 isoform, expressed from an intron 62 promoter, is ubiquitously expressed, although it is known to have an important role within the CNS (4,5,6,7).

In case of our patient, the microdeletion detected by SNP-Array in the DMD gene affected 39 exons, including exons 5-43. Microdeletion was confirmed afterthat with a different method as MLPA. The breaking points of the microdeletion were located in introns 4 and 43 of the DMD gene, both introns being large, the intron 4 has 21,395 bp (32,862,899-32,841,505), intron 43 being larger, of 70,465 bp (32,305,645-32,235,181). This out-of-frame microdeletion is predictable to lead to the transcription of a small, aberant mRNA composed of exons 1-4, then exon 44 attached by splicing, with frameshift and the appearance of a stop codon, corresponding to a short, defective, truncated Duchenne protein, containing only a small fragment of the N-terminal domain (codified by exons 1-4). Thus, the defective DMD gene in our patient will not be able to express the longest isoform protein, Dp427, predominantly expressed in brain, skeletal muscle and cerebellar Purkinje cells. DNA sequencing and functional studies would be needed to pinpoint the expression of the defective DMD gene in our patient. In absence of these tests, we can assume that only small DMD protein isoforms are synthesized: Dp140, Dp116 and Dp71.

The role of dystrophin protein in the development and functioning of the CNS seems to be complex and incompletely understood. It was suggested that the absence of dystrophin might be related to increased CNS excitability, comorbidity between epilepsy and dystrophinopathies was frequently reported, ranging from 3.1% to 12.3% (8). Other several studies showed a great variation in cognitive deficits up to severe learning disability and with a disproportionate effect on verbal working memory skills, which are not progressive as muscular symptoms are (9).

Anyway, it seems to be clear that the absence of this protein leads to developmental and functional deficits of CNS at macro-level, such as behavioral alterations and/or cognitive impairment, as well as the micro-level such as alterations in synaptic plasticity, GABA-ergic functioning (4,9).

In families without history of DMD, a patient is usually diagnosed at the age of about 4-5 years (10). Late diagnosis of DMD can have detrimental consequences for both the patient and his family. In this case, the patient loses the opportunity to benefit as early as possible from current therapies developed for patients with certain types of mutations in the DMD gene (such as Ataluren therapy for nonsense mutations), in order to improve the prognosis, quality and duration of life, as well as enrollment in clinical trials. Prenatal counseling is very important for the family in such cases (5,7).

Due to X-linked recessive inheritance model, the mother testing was recommended in order to establish maternal or “de novo” origin of this microdeletion, and then to ascertain familial recurrence risk and the most appropriate options of prenatal diagnosis in further pregnancies.

Van Dommelen et al. (10) reported significant delay in motor activity and communication milestones in their study on a sample of 76 young males with DMD versus 12,414 young males from a control group. In these patients, significant delays in walking, sitting, crawling, standing, walking, speaking their first word and constructing sentences, and lower gross motor and language scores were reported. Thus, the delay in development was noticeable already from the age of 2-3 months of the DMD patients investigated in their study.

In our case, due to an adequate genetic consultation and the proper application of SNP-Array technology, the child was diagnosed much earlier, in the first year of life, before the onset of major muscular muscular manifestations, this being an advantage in establishing an early adequate therapeutic and life management of the patient and for reproductive counseling.

CONCLUSIONS

We suggest, in agreement with previous publications, that identifying early developmental patterns that could be associated with DMD as early as possible is very important for these patients, for whom guidance to genetic counseling may be crucial for

their lives. We also want to emphasize the remarkable usefulness of molecular karyotype investigation through SNP-Array technology, which through its wealth of information can be a particularly valuable aid in the early diagnosis of these patients, crucial for adequate early medical care for patients with DMD and their families.

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