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Original article

A girl with an atypical form of ataxia telangiectasia and an additional *de novo* 3.14 Mb microduplication in region 19q12

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ABSTRACT

A 9-year-old girl born to healthy parents showed manifestations suggestive of ataxia telangiectasia (AT), such as short stature, sudden short bouts of horizontal and rotary nystagmus, a weak and dysarthric voice, rolling gait, unstable posture, and atactic movements. She did not show several cardinal features typical of AT such as frequent, severe infections of the respiratory tract. In contrast, she showed symptoms not generally related to AT, including microcephaly, profound motor and mental retardation, small hands and feet, severely and progressively reduced muscle tone with slackly protruding abdomen and undue drooling, excess fat on her upper arms, and severe oligoarthritis. A cranial MRI showed no cerebellar hypoplasia and other abnormalities. In peripheral blood samples she carried a *de novo* duplication of 3.14 Mb in chromosomal region 19q12 containing six annotated genes, *UQCRFS1*, *VSTM2B*, *POP4*, *PLEKHF1*, *CCNE1*, and *ZNF536*, and a *de novo* mosaic inversion 14q11q32 (96% of metaphases). In a saliva-derived DNA sample only the duplication in 19q12 was detected, suggesting that the rearrangements in blood lymphocytes were acquired. These findings reinforced the suspicion that she had AT. AT was confirmed by strongly elevated serum AFP levels, cellular radiosensitivity and two inherited mutations in the *ATM* gene (c.510_511delGT; paternal origin and c.2922-50_2940del69; maternal origin). This case suggest that a defective ATM-dependent DNA damage response may entail additional stochastic genomic rearrangements. Screening for genomic rearrangements appears indicated in patients suspected of defective DNA damage responses.

1. Introduction

Patients with genomic instability disorders generally display recognizable patterns of manifestations that allow a clinical diagnosis. In some cases, however, cardinal signs of a particular syndrome may be entirely absent (e.g. radial aplasia in some patients with Fanconi anemia) [1]. In such cases, and in the presence of atypical symptoms, clinical geneticists are often inclined to request a genome-wide screening test (such as karyotyping, array-CGH) in addition to direct tests for differential diagnoses. Genome-wide segmental aneuploidy screening tests often provide unexpected and novel findings which prompt a complicated process of interpretation [2].

Here we present a 9-year-old girl with profound developmental retardation and severely and progressively reduced muscle tone, who showed several cardinal signs of ataxia telangiectasia (MIM: 208900) but also features apparently at variance with that diagnosis. This case prompted dual screening procedures, on the one hand for chromosomal rearrangements and segmental aneuploidies in peripheral blood and saliva, and on the other hand determination of the serum alpha-fetoprotein level, cellular radiosensitivity testing by flow cytometry, and mutation analysis of the *ATM* gene. SNP array-based segmental aneuploidy screening revealed a *de novo* 3.14 Mb microduplication in chromosomal region 19q12, while the latter studies confirmed the diagnosis of AT. We discuss the complex clinical phenotype of this patient in

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light of the biochemical and genomic findings and the diagnostic challenges resulting from mutations at two independent loci.

2. Methods

Metaphases were prepared from short term (72 h) phytohemagglutinin (PHA)- and DSP30-stimulated blood cultures and analyzed with classic GTG-banding at the 300–500 band level [3]. FISH with the break-apart probe for the *TCRA/D* locus at 14q11 (LSI TCR alpha/delta dual color break apart probe, Abbott) as well as the break apart probes for the *TCL1* [4] and *BCL11B* loci was performed according to Przybylski et al., 2005 and Ventura et al., 2006 [5,6]. To determine whether the genome of the patient contained submicroscopic segmental aneuploidies we analyzed DNA samples of the patient and both her parents using Infinium HumanHap370 Genotyping BeadChip SNP arrays (Illumina, San Diego, CA, USA) as described [7].

Serum alpha-fetoprotein (AFP) level was determined according to standard procedures [8,9]. To test for cellular radiosensitivity, peripheral blood lymphocytes were left untreated or exposed to ionizing radiation of 1.5 Gy in G0/G1 phase. Thereafter, lymphocytes were PHA-stimulated and grown for 72 h in the presence of 0.1 mM bromodeoxyuridine (BrdU). Cultures were then terminated, the cells were lyzed, nuclei stained with Hoechst 33258 and ethidium bromide (EB) and assayed by two-parametric flow cytometry for cell cycle analysis [10–12].

Capillary sequencing of the *ATM* gene and *ATM* cDNA was performed using the primers and PCR conditions provided by Sandoval and co workers (1999) [13]. Mutations were evaluated using Alamut ™ (Interactive Biosoftware, Rouen, France). Multiplex ligation-dependent probe amplification for the *ATM* gene was performed using the MLPA assay kits P041-ATM-1 and P042-ATM-2 according to the instructions of the supplier (MRC-Holland, Amsterdam, The Netherlands).

3. Case report

The patient was born to a healthy 31-year-old mother being unrelated to the 30-year-old father. Her 13-year-older maternal half-sister had scoliosis but was otherwise healthy. Except for her maternal grandmother being diagnosed with breast cancer, her family history for genetic diseases was unremarkable. During the first weeks of pregnancy, her mother had smoked about 20 cigarettes per day, but less later on. Following an uncomplicated pregnancy, she was born after 36 weeks of gestation. Her birth weight was 2050 g (10th percentile), her length 43 cm (10th percentile), and her occipitofrontal circumference (OFC) 31 cm (10th–25th percentile). Developmental delay including speech delay was noted in early infancy. She started walking, albeit very unstably, between 18 and 24 months of age. From 18 months of age her parents noted her frequent "tipping over". From 2 years of age on, she suffered from rheumatoid arthritis of her knee joints and was treated with oral non-steroidal anti-inflammatory drugs. At age 6 years her oligoarthritis had become more severe and decreased immunoglobulin levels were noted. Specifically, her IgA was <20 mg/dl (normal range: 70 to 400), IgE < 1 U/l (normal range: <90 U/l) and her IgG was 75 mg/dl (normal range: 590 to 1430). In addition, she showed a lowered number of total leucocytes (5.43×10^3; normal range: $6.8–10 \times 10^3$), T-lymphocytes (1.23×10^3; normal range: $1.8–3.0 \times 10^3$), T-helper cells (0.28×10^3; normal range: $1.0–1.8 \times 10^3$), NK-cells (0.1×10^3; normal range: $0.2–0.6 \times 10^3$), B-lymphocytes (0.26×10^3; normal range: $0.7–1.3 \times 10^3$), and a strongly lowered T4/T8 ratio (0.26; normal range: 1.0–1.6). These data are consistent with a cellular form of immunodeficiency. Following negative blood tests for rheumatoid factor (RF), antinuclear antibody (ANA), and HLA-B27, she was diagnosed with acute juvenile idiopathic arthritis and common variable immune deficiency (CVID). Her treatment included infusions of immunoglobulins at regular intervals, high dose oral non-steroidal anti-inflammatory medication, and punctures of the knees with injections of the corticosteroid analog triamcinolon hexacetonide into the joints.

On medical genetic evaluation at the age of 7 years and 2 months she presented with ataxia, severe muscular hypotonia, and motor and mental retardation. Hypotonia also included the muscles of her abdominal wall with the bowels protruding. She had microcephaly (OFC 47.8 cm; 1 cm below 3rd percentile), short stature (height 113.5 cm; 1.5 cm below 3rd percentile), but normal weight (20 kg; 10th percentile). Her parents showed no microcephaly and were within the normal range for height and weight. She was hyperopic and wore glasses. She showed a high forehead, hypotelorism, deep-set orbital cavities, sagging skin below her eyes, a high palate, and retrognathia (Fig. 1). She rarely closed her mouth and salivated continuously and profusely. On her upper arms, she had excess subcutaneous fat . Her hands were small, but otherwise unremarkable. Her feet were short (foot length about 17 cm corresponding to an age of 3–4 years) and narrow, and her toes frequently rolled in to compensate for instability while standing upright.

She showed various signs of ataxia, including a broad-based, wobbly gait, a shaky stand and oculomotor apraxia with sudden fits of very rapid nystagmus and uncoordinated, partially rotary eye movements. Occasionally she revealed dystonic movements of her hands. In addition, she left an overall impression of marked muscular weakness and hypotonia. She spoke in sentences of one or two words at most and in a dysarthric voice that was weak to the point of being inaudible. When excited or absorbed into play she could suddenly breathe stronger and change to a normal voice for short time. Her parents reported no clinical crises and no developmental regression. In a cranial MRI no cerebellar hypoplasia or other gross abnormalities were noted. Several EEG examinations were unremarkable.

At age 9 years and 3 months her hypotonia had further deteriorated, such that she had lost her ability to walk and to stand without support. Also her lack of agility and coordination had further deteriorated. At this point she showed traces of telangiectasia around her upper eyelids. Flow cytometric analysis of a peripheral blood sample showed no signs of leukemia. Overall she impressed as a microcephalic child with short stature and severe ataxia, oculomotor apraxia, progressive muscular weakness, and developmental retardation, not fitting into classical AT (Fig. 1).

4. Results

The complex clinical presentation of our patient prompted us to perform karyotyping and a SNP array screen for segmental aneuploidies. In PHA-stimulated lymphocytes, obtained at an age of 7 years and two months, our patient showed besides four metaphases with a regular female karyotype a clonal paracentric inversion of chromosome 14 with breakpoints in 14q11 and 14q32. FISH to metaphases carrying the inv(14) using a break apart probe ruled out that the inversion affected the IGH locus in 14q32. Thus, the karyotype was described as: 46,XX,inv(14)(q11.2q32.1)[96].ish inv(14)(q11.2q32.1)(3'IGH+,IGHV+)[5]/46,XX[4].

At 9 years and 3 months of age PHA-stimulated and DSP30-stimulated lymphocyte cultures were again karyotyped and showed in all metaphases from the PHA-stimulated cultures the inv(14), and additionally in 25 out of 27 metaphases an entirely heterochromatic (C-band positive) marker chromosome. Thus, the karyotype from the PHA-stimulated cultures was now described as: 47,XX,inv(14)(q11.2q32.1),+mar[25]/46,XX,inv(14)(q11.2q32.1)[2]. Remarkably metaphases from the DSP30-stimulated cultures did not show the inv(14) or any other clonal changes Fig. 2.