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De novo 13q12.3-q14.11 deletion involving BRCA2 gene in a patient with developmental delay, elevated IgM levels, transient ataxia, and cerebellar hypoplasia, mimicking an A-T like phenotype

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A Novel clinical phenotype associated with a 13q12.3q14.11 deletion

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ABSTRACT

We report a child with a de novo ~12 Mb deletion on chromosome 13q12.3q14.11, characterized by array-CGH, and spanning ~50 genes. Clinical features included developmental delay, dysmorphisms and cerebellar ataxia. Beside ataxia, the infant had features reminiscent of Ataxia-Telangiectasia syndrome (A-T), including oculocutaneus telangiectasiae, recurrent upper airway infections and cellular radiosensitivity. The latter may be associated to the deletion of the *BRCA2* gene within the lost 13q region. The deletion of the 13q12.3q14.11 is the second deletion syndrome associated with chromosomal instability, besides the 12p11.3 deletion involving the Rb locus. In addition the knowledge of genes within a deleted interval can help to explain the observed phenotypes and facilitates optimal medical management by planning future cancer screening and treatment for the patient affected.

Commento [A B1]: Non mi sembra sia vero, ci sono altre sindromi da instabilità cromosomica

Commento [A2]: vago

INTRODUCTION

Deletion of the long arm of chromosome 13 is a rare condition characterized by a wide range of clinical features, including developmental delay, mental retardation, growth retardation with microcephaly, hypotonia, trigonocephaly, facial dysmorphism, limb defects such as hypoplastic or absent thumbs and anogenital anomalies [Allderdice et al.,1969; Grace et al., 1971]. The clinical phenotype varies according to the location and size of the deletion. The involvement of the critical band 13q32 allows to define three major phenotypic groups [Brown S et al., 1995]. Distal deletions are closely associated with the more severe phenotypes, whereas proximal deletions cause less anomalies but include retinoblastoma [Kivela et al., 2003]. Haploinsufficiency of the *ZIC2* gene, which maps on the critical 13q32 region, has been associated with holoprosencephaly (HPE) - [Brown LYet al., 2001].

We report on a novel clinical phenotype characterized by immunodeficiency with hyper IgM, mild and transient cerebellar ataxia, increased frequency of chromosomal breaks, telangiectasia and freckles associated with microcephaly, developmental delay, dysmorphisms, scheletric anomalies and spontaneous fractures, in the absence of molecular alterations of ataxia-telangiectasia mutated (*ATM*) and meiotic recombination 11 (Mre11) genes. Array CGH identified a 13q12.3-q14.11 deletion including the *BRCA2* gene.

CLINICAL REPORT

The proband is a 17-year-old Caucasian male referred to our Immunodeficiency Center at the age of 3.3 years for recurrent upper airway infections, mild ataxia, ocular telangiectasia and developmental delay. He was the second child of three healthy children of non-consanguineous parents. The family history was unremarkable. He was born at term after an uncomplicated pregnancy by caesarean section. His birth weight was 2.5 Kg. Fetal movements were reported as normal. A developmental delay was observed since the first months of life. The child was able to sit up and walk at 14 and 18 months, respectively. At the age of 27 months, he could pronounce 10 words. At 20 months of age, his neurological exam showed mild dysmetria and gait ataxia, muscle hypotonia, trunk titubation and developmental delay. Brain CT at 24 months of age revealed moderate enlargement of cisterna magna ventricular system due to cerebellar vermis hypoplasia. During the follow up, brain magnetic resonance imaging (MRI), performed at 10 years of age, confirmed the moderate hypoplasia of the caudal part of the cerebellar vermis with dilatation of adjacent CSF spaces**.** Electroencephalography was normal.

Commento [A B3]: vale la pena specificarlo?

At 3.3 years of age, the physical examination revealed a height of 94 cm (25-50th centile), weight of 12.5 Kg (10th centile) and head circumference of 48.1 cm (<5th centile). Dysmorphic features included -long face with sparse hair, hypotelorism, dental abnormalities, high-arched palate with hypertrophic gums, oculocutaneous telangiectasias and facial freckles (Figure 1). Mild ligament

hyperlaxity of upper limbs, clinodactyly of the 5th finger on both hands, asymmetry of the lower limbs and dorsal scoliosis were also noted**.**

Since the age of 3 months, the patient had recurrent and frequent episodes of wheezing. The ear-nose-throat evaluation revealed bilateral transmission hearing loss. Ophthalmologic examination was normal and negative for retinoblastoma signs.

Routine metabolic assays, such as serum amino acids, urine organic acids, acyl carnitine and serum alfa-fetoprotein levels were within a normal range. Fragile X CGG repeat test was normal.

During the long neurological follow up a progressive improvement of gross and fine motor performances was noted. Tendon reflexes were normal. After 12 years of age, cerebellar ataxia disappeared, but aa mild dysmetria and adiadochokinesia persisted. together with the developmental delay, characterized by impairment of cognitive and speech skills, motor stereotypies and obsessive behavior. A mild to severe mental retardation was confirmed by Wechsler Intelligence Scale for Children (WISH-R) (IQ 40). At the age of 17 yr., a painful swelling of the left foot, persisting after treatment with steroids and anti-inflammatory drugs, was observed. An X-ray of the foot revealed the presence of sequential atraumatic fractures of the II, III and IV metatarsus (Fig. 1B).

Commento [A4]: È necessaria?

MATERIALS AND METHODS

Cytogenetic and molecular genetics

Karyotype was performed by standard GTG banding at 550 bands resolution (ISCN 2009). Array-CGH was performed with a 44 K whole-genome oligonucleotide microarray (Agilent) following the manufacturer protocol. Array data were visualized using the Genome Workbench standard edition ver. 5.0 (Agilent) and compared with the human genome reference sequence hg19 (Feb. 2009).

Real-time quantitive PCR was performed to confirm array-CGH data. We designed a set of primers and probe specific for the exon 8 of gene ARNTL2 (ref seq NM_020183.3), provided by the Roche Diagnostics Universal probe Library software (http://www.universalprobelibrary.com). Amplification was performed in a total volume of 20 µl containing 2X TaqMan Universal PCR Master mix (P/N 4324018, Applied Biosystems), 1X RNaseP kit (20X, VIC dye, P/N 4316844), 0.2 µM of forward and reverse (5'- cgatgccctatgtgacaatg and 5'- ccccctctgcttctaagtaattc) and 0.1 µM UPL probe #46. For each sample a triplicate mix was prepared and aliquoted in three different tubes. The thermal cycling conditions were: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles 15 sec 95°C and 1 min 60°C. The PCR was performed on a 7500 fast apparatus (Applied Biosystems). The number of gene copies was determined using the comparative delta Ct method [1].

RESULTS

Immunological evaluation

The immunological evaluation revealed increased IgM serum levels (between 240-528 mg/dl, normal range 60-234 mg/dl), with a progressive decrease in the first seven years of life to stabilize at ~300 mg/dl, always above the normal upper limit (Figure 2). IgG and IgA were always normal. The patient -had normal counts of total white blood cells and lymphocytes. The immunophenotype, evaluated by flow cytometry, revealed normal -CD3+, CD4+, CD8+, CD56+, CD3+HLA-DR+ cells even though the number of CD3+CD4-CD8- (DN) increased over the time (Table I). Autoantibodies to anti-thyroglobulin (TGB-Ab), anti-thyroid peroxidise (TPO-Ab), anti-nuclear (ANA), anti-double stranded DNA (dsDNA), and transglutaminase were all negative. The proliferative response to mitogens (PHA and CD3 cross-linking (CD3 XL)), was evaluated by thymidine uptake from cultured cells pulsed with 0.5 µCi [^{3}H]thymidine (Amersham International) 8 h before harvesting. As illustrated in figure 2B, the response to CD3 XL was abnormal in the first 9 years of life, corresponding to 1.5 – 11% of the control. The response to PHA was initially low, corresponding to 23-53 % of the control, but variable over the time and became normal by 5 years of age. The responses persisted normal thereafter. To better define the pathogenesis of hyper IgM, the in vitro evaluation of Class Switch Recombination (CSR) was studied. CD40 triggering induced a normal B cell proliferation (data not shown) and *in-vitro* isotype switch, even though at a lower extent than control (ADD ANNE DURANDY).

Commento [A B5]: Un tempo questo era accettabile, per mancanza di spazio nella rivista. Adesso ci sono pagine e pagine di supplementary, quindi o mettete l'esperimento o togliete il testo.

Chromosomal analysis, genetic studies, array-based comparative genomic hybridization

ATM and Mre11 protein levels were normal and no point mutations in the *ATM* gene were found. Increased frequency of chromosomal breaks after exposure to gamma radiations was noted**.** Preliminary routine karyotype on peripheral blood lymphocytes were followed by whole genome array CGH (44K, average probe spacing 43 Kb) following the manufacturer's recommendations (Agilent Technologies, Santa Clara, CA, U.S.A.). Array CGH revealed an interstitial deletion at 13q12.3-q14.11 with breakpoints at 31,955,213 bp and 43,918,723 bp (Figure 3) and a 43-167 kb deletion at 12p11.3p11.22 between 27,182,294 and 27,826,732 bp-. About 50 genes are located in the 13q deleted region (National Center for Biotechnology Information, hg19, Fig. 3D), and 12 genes are located in the 12p11.3 region. The 12p deletion was confirmed by real time PCR using a XXX assay and showed to be inherited by the asymptomatic father

Commento [A6]: Il test CSA è negativo!

Commento [A7]: Non è stata verificata la delezione nei genitori, quindi possiamo supporre sia de novo data la sua grandezza

Discussion

We report -a 17-year-old boy who exhibited a complex clinical phenotype, characterized by a transient form of immunodeficiency with hyper IgM, and cellular radiosensitivity. Clinically, a transient cerebellar ataxia, associated to a mild cerebellar atrophy, was èresent with ocular teleangiectasiae and freackles, psychomotor and speech delay, dysmorphic features and skeletal abnormalities. Spontaneous fractures were also documented during the follow up. ArrayCGH revealed a de novo deletion at 13q12.3-q14.11, involving *BRCA2* but not the Rb locus. The patient also had a smaller 12p11.3 deletion not considered of clinical relevance since it was inherited from the asymptomatic father.

Commento [A B8]: Non è una caratteristica clinica, la separerei

Few patients carrying deletions in the region 13q12.3-q14.11 are reported in the literature with variable phenotypes. In two patients reported by Ballarati (Ref 2007) the deletion overlapped the one herein described. These patients share with our case the presence of cerebellar hypoplasia, skeletal abnormalities and mild mental retardation. An additional patient with a 13q13.3-13qter deletion also had cerebellar hypoplasia, thus suggesting the presence in the area of a gene implicated in cerebellar development (Quélin (2009)). Drummond-Borg et al. [2002] reported on a case of 13q12.1-q14.1 deletion in a child with dysmorphic features, growth retardation and developmental delay. However, in this case, ataxia and cerebellar anomalies were not reported and no data on immune response and radiosensitivity are available.

Commento [E9]: Bisognerebbe inserirlo nello schema

In our patient, hyper IgM levels were the immunological hallmark of the early phase of the syndrome, associated with reduced proliferative response to mitogens. The geneticsof hyper IgM comprises five subtypes characterized by impaired class-switch recombination(REF). It should be noted that in our case the initial evaluation of class switch recombination (CSR) following X stimulation (ask Ann) revealed a normal IgG production thus ruling out the overall integrity of the CSR B-cell machinery. The pathogenesis of a few of them is still unknown and, indeed, it may involve the DNA repair mechanisms. It has been reported that fibroblasts from patients affected with a still molecularly undefined HIGM show an increased radiosensitivity, even though less marked than that of patients suffering of other DNA repair defects, such as A-T or Artemis deficiency (Peron S JEM 2007). Moreover, B cell lymphoma and leukemia have been reported in these patients, strongly suggesting a DNA repair defect (Durandy et al, personal communication), So far, in none of the reported cases of hyper IgM syndrome the chromosomal aberration herein described has been reported.

Commento [A B10]: Quanti, "è vago a few"

As for the radiosensitivity reported in the patient here described, an increased cytotoxic effect of X-irradiation has already been documented in patients carrying alterations of chromosome 13, as deletions, trisomy, inversion, or translocation, (Nove J, 1981;), being referred to the Rb locus and never in the region of the deletion here described. Thus far, several genes have been associated to altered cellular radiosensitivity, as *ATM, p53, BRCA1, BRCA2, DNA-PK* [McKinnon, 2007]. Our

patient, initially clinically classified as AT, had some features reminiscent of diseases due to DNA repair defects, and, on the other hand-A-T patients may show increased levels of IgM (cercare REF). The Nijmegen Breakage Syndrome (NBS), DNA LIG4-deficiency and Fanconi anemia (FA) also share the radiosensitivity and cancer predisposition[McKinnon, 2007]. -

Commento [A B11]: ? non vedo il nesso

BRCA1 and BRCA2 proteins are also involved in the repair of DSB by homologous recombination (HR) and their alteration confers high risk for breast and ovarian cancer[Barwell et al., 2007]. Of note, BRCA2 is also intimately related to Fanconi Anemia (FA), in that BRCA2 biallelic mutations have recently been identified in group D1 FA (FANCD1) patients. BRCA2 interacts with FA proteins and belongs to a complex functional network, which includes ATM, Rad3 related (ATR) proteins and BRCA1(Wang, Nature 2007) all implicated in DSB**.**

Two further genes, HMGB1 and RFC3, localized in the 13q region, involved in apoptosis and tissue homeostasis, could also be connected with the increased radiosensitivity. Unfortunately, no human model of disease related to alteration of HMGB1, is available. As for RFC3, the only information available is that two subjects with a chromosome 7;13 translocation involving this gene, showed language and cognitive disorders.

Commento [A B12]: Perché?

Commento [A B13]: Quindi il paziente deve avere queste caratteristiche cliniche.

In conclusion, we describe for the first time a novel clinical phenotype due to a de novo 13q deletion, characterized by an atypical form of hyper IgM, a transient cerebellar ataxia, mimicking some features of a milder A-T clinical phenotype, which

is part of a more complex disease, consisting of mental retardation, dysmorphic features, skeletal abnormalities and spontaneous bone fractures. As supported by several reports including a fetus showing a de novo mosaic deletion of 13q13.3, showing cerebellar hypoplasia and microcephaly associated with multiple abnormalities, we localize the critical chromosomal segment responsible for cerebellar development at 13q13.3.