



## Clinical research

## Dravet phenotype in a subject with a $\text{der}(4)\text{t}(4;8)(\text{p}16.3;\text{p}23.3)$ without the involvement of the LETM1 gene



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## ARTICLE INFO

## Article history:

Received 27 May 2013

Accepted 8 August 2013

Available online 31 August 2013

## Keywords:

Dravet syndrome

$\text{der}(4)\text{t}(4;8)(\text{p}16.3;\text{p}23.3)$

WHS

## ABSTRACT

We present a patient affected by Dravet syndrome. Thorough analysis of genes that might be involved in the pathogenesis of such phenotype with both conventional and next generation sequencing resulted negative, therefore she was investigated by a-GCH that showed the presence of an unbalanced translocation resulting in a  $\text{der}(4)\text{t}(4;8)(\text{p}16.3;\text{p}23.3)$ . This was an unconventional translocation, different from the recurrent translocation affiliated with WHS and did not involve LETM1.

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### 1. Introduction

Unbalanced translocations involving short-arm of chromosome 4 are a well-known cause of Wolf–Hirschhorn syndrome [1,2] (WHS, OMIM #194190) with the recurrent  $\text{t}(4;8)(\text{p}16;\text{p}23)$  translocation being by far the most frequent one [3]. WHS is a contiguous gene syndrome caused by differently sized partial deletions of the short arm of one chromosome 4. Several literature reports point to a great variability of the WHS phenotype that is dependent on the genomic defect (terminal versus interstitial deletions; pure deletions versus unbalanced translocations; size of the deletion), consisting in the association of severe growth delay, intellectual disability, peculiar facial dysmorphisms, greek warrior helmet profile and seizures. The critical region in the pathogenesis of such disorder is in 4p16.3 between 1.8 Mb and 1.9 Mb [2,4–6], containing WHSC1, WHSC2, and LETM1, located just distal and reported as an excellent candidate gene for seizures. However, there is evidence that another epilepsy-causative gene is located in the 0.4–1.7 Mb 4p distal region whose deletion has been reported in other epilepsy-associated cases [2,4,5]. This distal epilepsy critical region would not only explain the epileptic phenotype in terminal

deletions not involving the LETM1 gene but also gives a better refinement of the contiguous phenotype.

We report a patient clinically described as affected by Dravet syndrome (DRAVET, OMIM: #607208), an early-onset epileptic encephalopathy characterized by generalized clonic, and tonic–clonic seizures that are initially induced by fever and begin during the first year of life. The EEG is often normal at first, but later characteristically shows generalized spike-wave activity and affected individuals show subsequent mental decline and other neurologic manifestations [7].

The patient, who resulted negative after Sanger analysis of SCN1A gene, underwent a Next Generation Sequencing (NGS) analysis for 67 epilepsy-related genes of which 17 genes related to either early infantile epileptic encephalopathies or febrile seizures with negative results. Finally, she was investigated by array-comparative genomic hybridization (a-CGH). This analysis detected a 4p deletion and 8p duplication compatible with an unbalanced 4p;8p translocation whose breakpoints are different from those characterizing the well-known recurrent translocation  $\text{t}(4\text{p};8\text{p})(\text{p}16;\text{p}23)$  [3].

### 2. Clinical report

The female patient is the first child of healthy unrelated parents with no familiarity for epilepsy (her younger brother does not show

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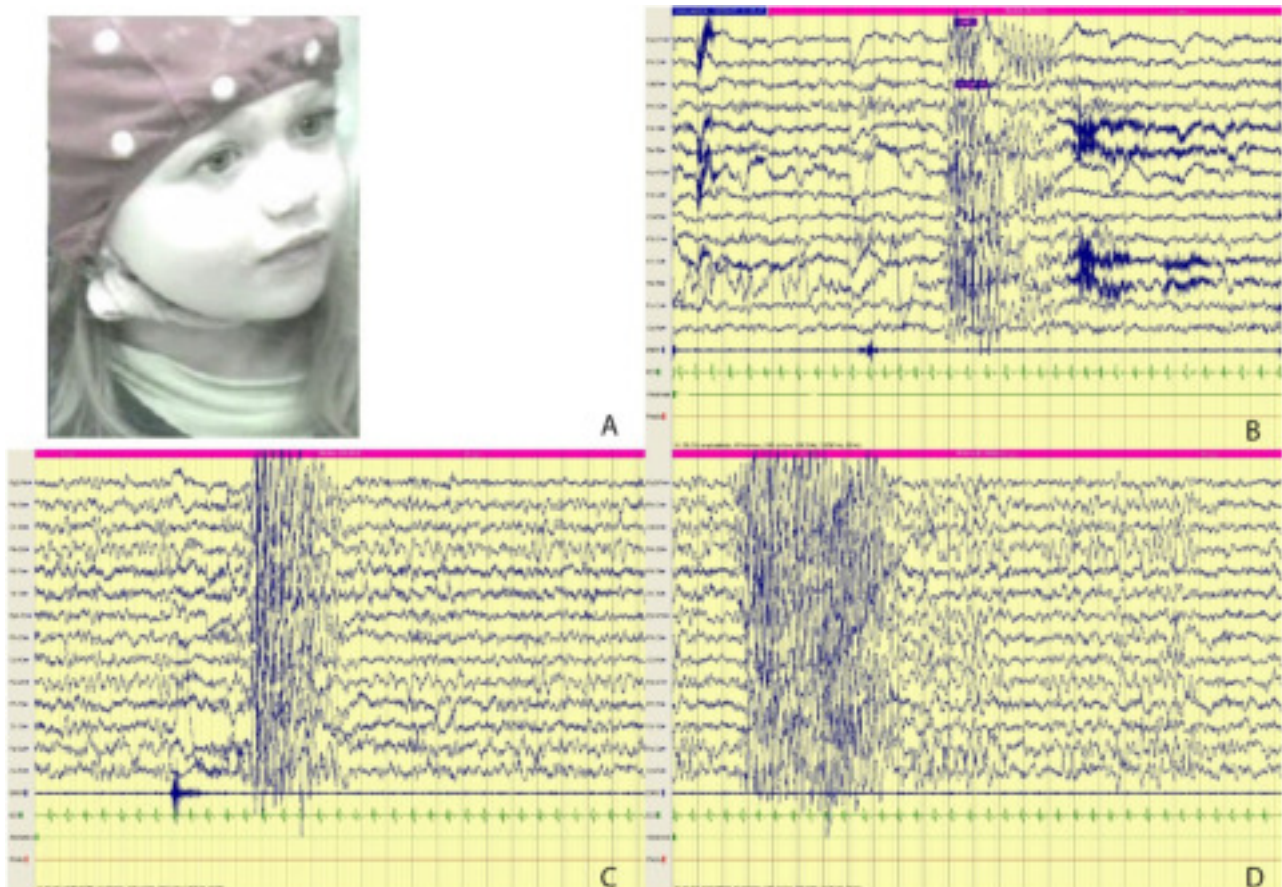
any pathogenic condition) apart from the typical infectious diseases that generally affect children. She was born at 37 weeks of gestation with a vaginal birth and with an average birth weight even though the pregnancy had been characterized by threatened abortion. The APGAR score was 9 (1'), 10 (5'). The patient presented a normal psychomotor development until the age of 5 months, when, during a post vaccination febrile episode, she had a prolonged generalized tonic–clonic seizure. The EEG performed when she was admitted to hospital showed non-specific abnormalities. After only one month, she had an afebrile right-partial seizure, characterized by ocular deviation and subsequent ipsilateral generalized clonus (5') and during the following months she had some generalized tonic–clonic seizures that lasted over 5 min. It was possible to interrupt some of these seizures with the use of BDZ (benzodiazepine), which were given to her each month until she was 12 months old. Two times out of three these episodes occurred when the child moved from a closed cool space to an open one, especially if the outdoor temperature was as high as 35 °C.

Brain MRI resulted to be normal whereas EEG showed poor organization and presence of diffuse abnormalities. In spite of the introduction of oxcarbazepine, her seizures kept worsening, concerning seizure frequency, but also because they acquired a polymorphic semiology. The seizure were long lasting, some presenting hyperpyrexia and they also featured clonuses in the upper and lower limbs. Atypical absences and clonic seizures were frequent, even daily; at that time, we could describe them as diffuse tremors rather than real myoclonic seizures.

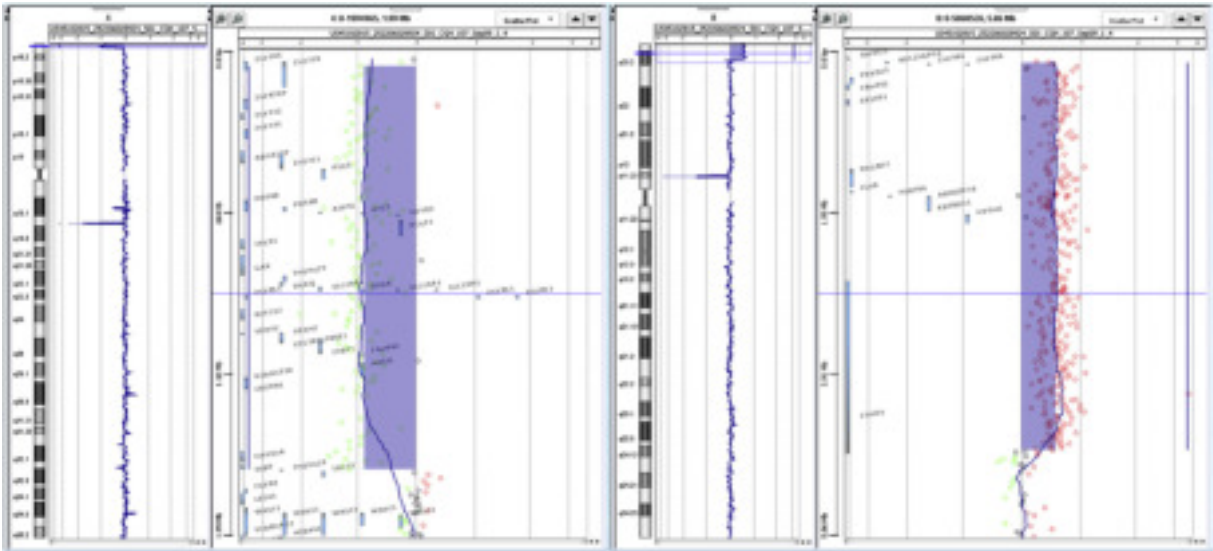
At the age of 14 months, the patient was taken back to our structure to re-assess her case. No facial dysmorphism or

microcephaly (head circumference 46 cm: 50th percentile) was found (Fig. 1a); neurological and psychomotor evaluation (assessed using a standardized Griffiths scale) appeared to be normal (QS 91): autonomous walking was a little uncertain, the expressive language was formed from a number of words (10–20) pronounced with communicative intent. She had basic comprehensive skills to deliver simple commands and good gesture skills (sign with her hand hello). The EEG showed slow activity in the central–right region. A diagnosis of Dravet syndrome was hypothesized based on early onset of symptoms and clinical features. The replacement of oxcarbazepine with clobazam and valproic acid allowed a better control of the seizures over time. The patient is presently 7 years old and with normal growth parameters and head circumference (51.5 cm: 50th percentile). She is currently in monotherapy with valproic acid and has only sporadic febrile tonic–clonic seizures of short-term (<2'). Diffuse hypotonia and mild motor clumsiness are still present. From the cognitive point of view, there is evidence of a slight deterioration. Cognitive test, performed (as assessed by standardized scale WPPSI III) appeared at the lower limit of normal (QIT: 76, QIV: 80, QIP: 82). The EEG showed a discrete organization and spatialization over time, with presence of short bursts of spikes waves sometimes associated with myoclonus and fixity of gaze (Fig. 1b, c, d). There was no evidence of photosensitivity.

Sequencing of all 26 exons of SCN1A including the splice junctions was done by classical Sanger method without detecting any causative nucleotide alterations. Patient's DNA was then analyzed by NGS on a platform including exons and splice junctions of 67 epilepsy-related genes of which 17 known to cause early infantile



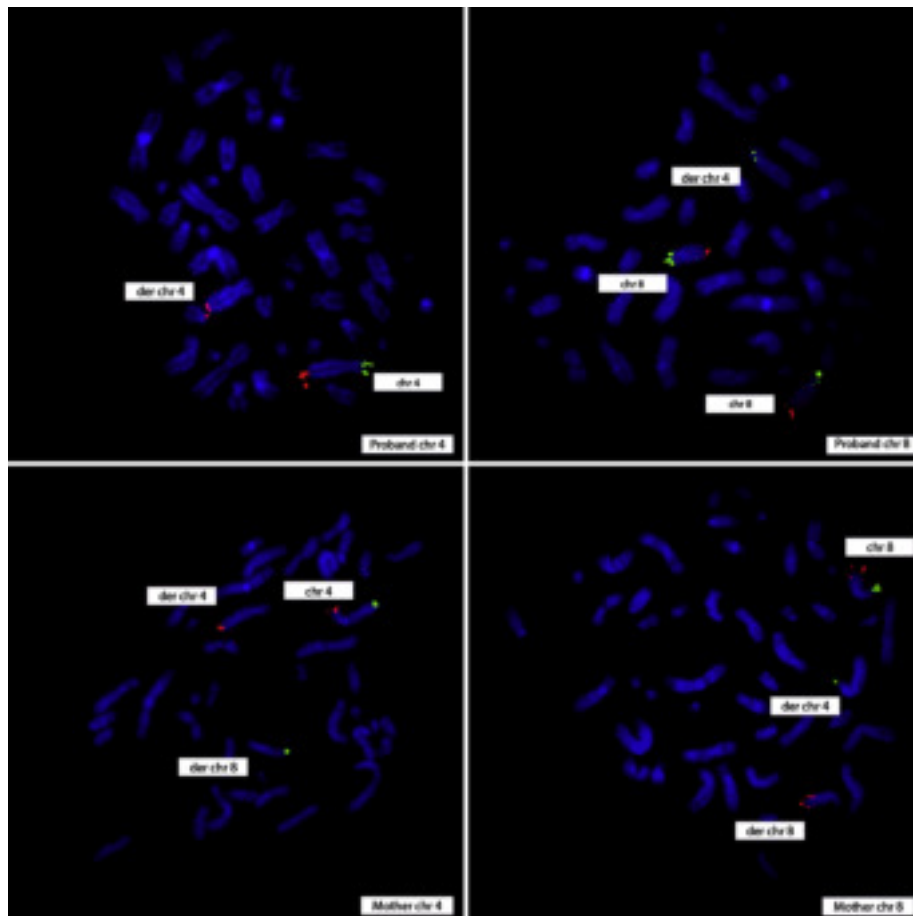
**Fig. 1.** The photo of the proband and EEG findings of three different events with spike wave activity, no myoclonic jerks were recorded. EEG parameters were as follows: speed: 15 mm/s, voltage: 100 mV/cm, muscle: ECG, PNG, right and left deltoids.



**Fig. 2.** a-CGH on proband's DNA performed using the Agilent array 180K (Human Genome CGH Microarray, Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol. Data analysis was performed using Agilent Genomic Workbench Standard Edition 7.0.4.0. The 4p deletion had a log<sub>2</sub> ratio of  $-0.73$  and the 8p duplication a log<sub>2</sub> ratio of  $+0.62$ .

epileptic encephalopathies or febrile seizures (see Table 1 in Supplementary material), including SCN1A, the causative gene for Dravet syndrome [8]. PCDH19, a gene commonly investigated when SCN1A is negative, as well as other 15 genes, known to cause a

similar phenotype, were also negative [9]. This analysis was performed on an Illumina's Genome Analyzer IIX ( $2 \times 150$  bp pair ended) with a median coverage of 447x; findings were then filtered and prioritized [10].



**Fig. 3.** Fish analysis using two Vysis probe sets; left; telomeres of chr 4, right; telomeres of chr 8; left and right upper boxes show images of proband's unbalanced translocation respectively on 4 and 8, left and right bottom boxes show mother's balanced translocation respectively on 4 and 8.



**Fig. 4.** WHS candidate region as defined in the literature (black bar) and the critical region for the epileptic phenotype (green bar). In red deleted regions of patients suffering from epilepsy without the involvement of LETM1 gene while in gray the deleted region of the patient not presenting epilepsy is shown.

The results showed no causative alterations in any of the analyzed genes with all the detected variants described either in dbSNP137 or in our in-house database. To exclude any chromosomal imbalances we continued the analysis by a-CGH detecting a terminal deletion on 4p [del4(p16.3)] of approximately 1.7 Mb, with a breakpoint between 1,715,277 bp and 1,729,442 bp, as well as a terminal duplication on 8p [dup8(p23.3)] of approximately 4.8 Mb, with breakpoint between 4,814,708 bp and 4,833,351 bp (Genome release hg19). This finding pointed to a derivative chromosome der(4)t(4;8)(p16.3;p23.3) (Fig. 2). The imbalance involved genes: ZNF595, ZNF141, ABCA11, PIGG, PDE6B, ATP5I, MFSD7, PCGF3, GAK, TMEM175, DGKQ, SLC26A1, IDUA, FGFRL1, RNF212, SPON2, CTBP1, MAEA, CRIPAK, FAM53A, SLBP, TMEM129, TACC3 (chromosome 4) and ZNF596, FBXO25, C8orf42, ERICH1, DLGAP2, CLN8, ARHGEF10, KBTBD11, MYOM2, CSMD1 (chromosome 8).

Analyses of the parents as well as the proband with FISH (Vysis™; Catalog number: 33-270000, Abbott, Abbott park, IL) using the telomeric probes of chromosomes 8 and 4 not only confirmed the presence of the derivative chromosome but also demonstrated a balanced translocation between the two chromosomes in the healthy mother (Fig. 3).

### 3. Discussion

We described a female patient ascertained at 5 months of age because of epilepsy. From the clinical point of view, the age of seizure onset, their long duration and high frequency in the first year of life, their association with fever and sensitivity to high temperatures, the worsening after the introduction of oxcarbazepine, led us to hypothesize a type of epilepsy within the spectrum of Dravet syndrome. The finding of the chromosome imbalance came much unexpected. Even after the identification of the 4p deletion, a re-evaluation of the patient did not show any phenotypic features of the WHS (severe growth delay, microcephaly, peculiar facial dysmorphisms, Greek warrior helmet profile and epilepsy but with clinical aspects very different from that of Dravet syndrome [11]). In fact, the DNA of our patient has been deeply investigated by different sequencing approaches without finding the causative mutation. However, in our diagnostic protocol all cases that result negative at the NGS epilepsy platform are then investigated by array-CGH with a 180k platform. This way we were able to pick up the genomic imbalance at the bases of the phenotype of our patient.

Translocations t(4;8)(p16;p23) are the most frequent recurrent translocations after t(11;22)(q23;q11). The recurrence of this translocation is due to non-allelic homologous recombination (NAHR) between two pairs of the olfactory receptor (OR)-gene clusters located close to each other, on both 4p16 and 8p23 [3]. In our case, the translocation has breakpoints that are more distal on both chromosomes 4 and 8 where no segmental duplications are present. Thus, the occurrence of this translocation does not depend on NAHR. Subjects carrying the recurrent der(4)

t(4;8)(p16;p23) are invariably affected by the full phenotype of WHS. In these cases, the 4p breakpoint, that may occur either at about 4 or 9 Mb, is proximal to Wolf–Hirschhorn syndrome critical regions WHSCR1 and WHSCR2 [12–14] that lie between 1.8 and 1.9 Mb. LETM1 gene, that is within the WHSCR2 has been considered as the main candidate for epilepsy [15]. However, Maas et al. [2008] [5] reported that a patient with the 1.4 Mb terminal 4p deletion without the LETM1 deletion did present with seizures thus suggesting that another gene in the terminal region may cause the epilepsy (Fig. 4). More recently, other cases [5,16,17] including one in Decipher (#263421) confirmed this hypothesis although not all subjects with comparable 4p deletions distal to LETM1 present with epilepsy [18].

While the interpretation of terminal duplication of 8p23 is difficult as no overlapping terminal duplications in literature exist, duplications of this region has already been reported as a possible benign variant [19]. In fact Brooks et al. [1998] [20] reported on a family segregating a Y;8 translocation, in which several men are trisomic for 8p22–8pter. Their phenotype is remarkably mild with no evidence of epilepsy. Seeing that there are no strong associations with an epileptic phenotype with duplications involving the terminal region of chromosome 8, we believe that the alteration is not involved in the genesis of this epileptic phenotype.

Many considerations have already been made regarding the gene or genes causing epilepsy in patients with 4p deletions smaller than 1.7 Mb [16]. Previously other authors hypothesized that CPLX1, DGKQ, and CTBP1 were possible candidate genes responsible of the epileptic phenotype. The similarities of the epileptic profile to ones associated with SCN1A mutations made us hypothesize that PIGG gene, involved in the biogenesis of GPI anchor proteins, might be responsible. Recent studies on animal models showed that alterations in the biogenesis of GPI anchor proteins can alter expression of Na<sub>v</sub>1.1 encoded by SCN1A [21] gene.

As epilepsy is such a variable disorder that can present itself in different types and different phenotypes, finding a genetic underlying has been a trivial quest. In our case, the extensive genomic investigation was not limited to sequencing but also to highlight copy number variants, which allowed us to discover an unexpected event. The Dravet-like phenotype of our patient, in absence of facial dysmorphisms or other malformations, made clinicians search for a needle in the wrong haystack.

### Conflict of interest

The authors declare no conflict of interest.

### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmg.2013.08.003>.

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