Epilepsy Research (2008) **81**, 69—79

journal homepage: www.elsevier.com/locate/epilepsyres

Deletion of the *SCN* gene cluster on 2q24.4 is associated with severe epilepsy: An array-based genotype—phenotype correlation and a comprehensive review of previously published cases

Josef Davidsson[a,*], **Anna Collin**[a], **Mia Engman Olsson**[b], **Johan Lundgren**[c], **Maria Soller**[a]

[a] *Department of Clinical Genetics, Lund University Hospital, SE-221 85 Lund, Sweden*
[b] *Department of Pediatrics, Karlskrona Hospital, Karlskrona, Sweden*
[c] *Department of Pediatrics, Lund University Hospital, Lund, Sweden*

Received 17 December 2007; received in revised form 13 March 2008; accepted 22 April 2008
Available online 9 June 2008

KEYWORDS
Severe epilepsy;
SCN1A;
del(2)(q24);
Array CGH

Summary
Purpose: To characterize a deletion of chromosome 2q at the molecular level in a patient suffering from severe epilepsy resembling severe myoclonic epilepsy of infancy/Dravet's syndrome (SMEI/DS) and to correlate other cases harboring deletions in the same region to morphological and clinical data.
Methods: Array-based comparative genomic hybridization (array CGH) was performed on DNA from the patient. Forty-three previously published cases reporting deletions within region 2q21-q31 were collected and analyzed regarding their cytogenetic and clinical data.
Results: A del(2)(q24.3q31.1) was detected in the patient, spanning a 10.4-megabase (Mb) region between 165.18 and 175.58 Mb, harboring 47 genes. FISH analysis was performed, confirming this deletion. Twenty-two of the 43 previously published cases were seizure-positive. The most common dysmorphic features were ear abnormalities, microcephaly, micrognathia and brachysyndactyly for all patients as well as for solely the seizure-positive and -negative ones. For the 22 seizure-positive cases chromosome subband 2q24.3 constituted the smallest commonly deleted region among the majority of the cases, where subbands 2q22.1 and 2q33.3 represented the most proximal and distal breakpoint, respectively.
Conclusions: Based on the early age of presentation and the severity of the epilepsy reported for the majority of the seizure-positive cases it was concluded that SMEI/DS could be the epileptic encephalopathy associated with deletions within the 2q22.1-q33.3 region, due to haploinsuffiency of *SCN1A* and/or complete or partial deletion of other voltage-gated sodium channel genes caused by the aberration. Furthermore, our study supports that array CGH is a

* Corresponding author. Tel.: +46 46 173398; fax: +46 46 131061.
E-mail address: Josef.Davidsson@med.lu.se (J. Davidsson).

0920-1211/$ — see front matter
doi:10.1016/j.eplepsyres.2008.04.018

competent technique for screening *SCN1A* mutation-negative patients diagnosed with SMEI/DS-like epilepsies and dysmorphic features, generating rapid and high-resolution data of genomic imbalances present in the patients.

Introduction

Since the late 1970s the number of reports of interstitial deletions on the long arm of chromosome 2 (2q) have been steadily growing. Today more than 100 cases have been described, displaying deletions of various sizes and distributions on 2q, and a wide variety of dysmorphic and phenotypic features in the patients (Schinzel, 2001).

Several patients cytogenetically diagnosed with a 2q-deletion affecting bands 2q22-q33 have been described to suffer from epilepsy together with varying dysmorphic traits (Buchanan et al., 1983; Bernar et al., 1985; Takahashi et al., 1985; Ramer et al., 1990; Wamsler et al., 1991; Lurie et al., 1994; Boles et al., 1995; McMilin et al., 1998; Slavotinek et al., 1999; Maas et al., 2000; Pereira et al., 2004; Langer et al., 2006; Madia et al., 2006; Pescucci et al., 2007). However, most of these studies have focused on mapping the genetic aberrations and describing the phenotypic traits of the patients, not with a directed attention towards the epilepsy so commonly seen in association with microdeletions in 2q22-q33.

The first report of seizures associated with a deletion within 2q22-q33 was described in 1983 (Buchanan et al., 1983). Later, by means of microsatellite mapping of families affected by generalized epilepsy with febrile seizures plus (GEFS+) and benign neonatal-infantile seizures (BNIS), an epilepsy-associated locus was identified within 2q24-q33 (Moulard et al., 1999; Malacarne et al., 2001). In addition it was demonstrated that point mutations in *SCN1A* and *SCN2A*, that is part of the voltage-gated sodium channel gene cluster on 2q24 encoding α-subunits, was strongly associated with GEFS+, severe myoclonic epilepsy of infancy/Dravet's syndrome (SMEI/DS), BNIS, borderline SMEI/DS, intractable childhood epilepsy with generalized tonic–clonic seizures, and infantile spasms (Escayg et al., 2000; Claes et al., 2001; Heron et al., 2002; Fujiwara et al., 2003; Ohmori et al., 2003; Wallace et al., 2003). The majority of *SCN1A* point mutations are found in pore-encoding regions consequently giving rise to more severe sodium channel dysfunctions and probably resulting in the fact that the majority of the pediatric point mutation-positive patients are diagnosed with SMEI/DS and not the milder forms of infant epilepsy (Mulley et al., 2005).

The cluster of sodium channel genes within chromosome subband 2q24.1 harbors five genes and the epilepsy seen in the patients positive for a deletion of this region most likely reflect a functional outcome of hemizygosity for one or several of these genes. However, within the plethora of interstitial 2q-deletions, a clinically recognizable genetic syndrome involving epilepsy and associated phenotypes has not been clearly delineated. In addition, a clinically satisfactory description of the type of epilepsy has in many cases not accompanied the studies of seizure-positive patients harboring deletions including band 2q24. In this study we have characterized a del(2)(q24.3q31.1) at the molecular cytogenetic level with tiling resolution (32k) genome-wide array-based comparative genomic hybridization (array CGH) in a patient suffering from SMEI/DS. We have also studied the majority of patients harboring deletions within bands 2q21-q33, described in the literature to date, collecting their clinical data and reported cytogenetic breakpoints, to identify possible dysmorphic traits and seizure characteristics associated with epilepsy of this genetic origin.

Methods

Subjects

Blood samples from the patient and his parents were taken after informed consent.

Cytogenetics

G-banding analysis of karyotypes derived from peripheral blood lymphocytes was performed in standard clinical settings on the patient and both his parents.

Array CGH

DNA was extracted from the peripheral blood cells obtained at the time of diagnosis. Male genomic DNA (Promega, Madison, WI) was used as reference in the hybridization. The 32,000 slides used, containing 32,433 tiling bacterial artificial chromosome (BAC) clones covering at least 98% of the human genome, were produced at the SWEGENE DNA microarray resource center at Lund University, Sweden. Labeling of DNA, slide preparation, and hybridization were performed as described (Jönsson et al., 2007) with minor modifications. Analysis of the microarray images were performed with the GenePix Pro 4.1 software (Axon Instruments, Foster City, CA). For each spot, the median pixel intensity minus the median local background for both dyes was used to obtain the ratio of test gene copy number to reference gene copy number. Data normalization was performed for each array subgrid using lowess curve fitting with a smoothing factor of 0.33 (Yang et al., 2002). The sex chromosomes were excluded when calculating the correction factor in the normalization of the data set. All normalizations and analyses were performed in the Bioarray Software Environment database (BASE) (Saal et al., 2002). To identify imbalances, the MATLAB toolbox CGH plotter and the CGH Explorer software (Lingjaerde et al., 2005) were applied, using moving mean average over three clones and $\log 2$ limits of ≥ 0.2. Classification as gain or loss was based on identification as such by the CGH plotter and also by visual inspection of the $\log 2$ ratios. Ratios $\pm \geq 0.5$ in three adjacent clones were classified as abnormal, with ratios between 0.5 and 1.0 interpreted as duplications/hemizygous deletions and $\pm \geq 1.0$ classified as amplifications/homozygous deletions.

FISH

To confirm the deletion detected on chromosome 2, a total of 3 BAC probes, targeting the 2q24.3-q31.1 region were selected from the UCSC Genome Browser (http://genome.ucsc.edu) (Table 1). BAC DNA was extracted by alkaline lysis with SDS using standard