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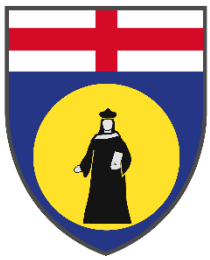

Epileptic features and neuropsychiatric aspects in 2q24.3 deletion syndrome

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1. Abstract

Background: Deletion spanning in the chromosome 2q24.3 region could involve genes encoding for neuronal voltage-gated sodium channels like *SCN1A*, *SCN2A*, and *SCN3A*. These genes play an important role in the aetiology of neurodevelopmental disorders, including epilepsy. We aimed to dissecting genotype-phenotype correlation, trying to infer the contribution of specific genes deletion on the phenotype of patients with 2q24.3 deletions.

Methods: Previously published cases of 2q24.3 deletion syndrome were reviewed and analyzed together with the description of 2 novel patients. Clinical data were collected by face-to-face interview and structured spreadsheet. Instrumental and laboratory tests, including EEG, MRI, and array-CGH were performed for all patients.

Results: 14 patients (6 female) were eligible. The mean age at randomization was 6.93 years (age range, 2.6 - 28 years). Age at seizure onset ranged from 2 to 12 months (mean age, 4.75 months) with no differences between males and females. Different seizure types were recorded including, tonic clonic (57%), myoclonic (35%), multifocal and autonomic (28%) seizures. Autism spectrum disorder and Tourette syndrome were the most frequent associated neuropsychiatric comorbidities, being present in 35% and 7% of the patients, respectively. Array-CGH showed 12 (85%) patients to carry a deletion sized between 112 and 4.800 Kb. Five (35%) patients carried a deletion involving all five sodium channels genes (i.e, *SCN1A, SCN2A, SCN3A, SCN7A,* and *SCN9A*), while 4 (28%) patients showed haploinsufficiency of only the *SCN2A* and *SCN3A* genes. Deletion involving the *SCN1A* gene was found in 11 (91%) patients with seizures, of which 54% were resistant to multiple antiseizure medications.

Conclusions: Haploinsufficiency of the *SCN1A* gene cause the "epileptic phenotype" of patients with 2q24.3 deletion syndrome. Conversely, *SCN2A* and *SCN3A* seem to play a key role in the appearance of associated neurobehavioral disorders, like ASD.

2. Introduction

2q24.3 deletion syndrome is a rare disease that presents a frequency of incidence < 1/1000000 and involves both female and male sexes[1,2].

The 2q24.3 region is located on the long arm of chromosome 2 and inside it is found a 1.4-Mb sodium channel gene cluster (*SCN1A, SCN2A, SCN3A, SCN7A,* and *SCN9A*) whose deletion of a part of the entire grouping is associated with variable epilepsy phenotype, developmental delay, psychiatric disease, or morphological abnormalities. SCN1A, SCN2A, and SCN3A are clustered within 600 kB on human chromosome 2q24.3 and they are highly expressed in neurons and glias throughout the central and peripheral nervous systems[8].

Already in the '80s with the studies of Bernar J. et al.[10] and Chinen et al.[22] it was understood that there was a syndrome associated with the deletion of the 2q24.3 region. The two studies were reported the clinical histories of two pediatric patients presenting dysmorphisms, hypotonia, and in the first case, also, difficulty in feeding and epileptic state. The real change in the study of the syndrome occurred after the introduction, in 2004, of the CGH- array technique, which has led to an increasing number of studies describing the epilepsy phenotype, and/or other clinical features of chromosome 2q24.3 deletion. It has allowed identifying which were the possible genes more important in determining the phenotypic manifestation. In the most recent studies[15,1,21,8], starting from the publication of Madia et al. in 2006[16], neuropsychiatric comorbidities associated with

chromosomal deletion were also studied in more depth, among which autistic spectrum disorders stand out. After having gathered evidence on the genetic cause of the syndrome, and its possible clinical implications, we decided to recruit two new patients presenting the same deletion to perform a cohort study covering the entire casuistry.

2.1 2q24.3 deletion syndrome: clinical features

The deletion of the long arm of chromosome 2 in the specific region 24.3 can present with a variety of phenotypes. The most likely features are seizures, psychiatric diseases, developmental delay, learning difficulty or disability, severe growth failure after birth (possibly with low birth weight), some unusual facial characteristics, and heart involvement.

Around half of the babies are small at birth with low weight, low lenght, reduction of the head circumference (microcephaly) and the growth rate for the head has been shown in some cases to tail off in the first year of life due to early fusion of the bone plates in the skull (craniosynostosis). Cleft palate or lip is also reported quite often and especially the first condition causes difficulties both in feeding and in speech production, requiring surgical repair. Other facial dysmorphisms could be hypertelorism, micrognathia, nasal abnormalities, abnormal external ears or ears set a little lower on the side of the head than is usual, teeth anomalies like late appearance (front teeth in the second year of life, molars at the age of four), failure to fall out to allow adult teeth through, or crooked teeth and mild dental crowding. Visual complications like down slanting and small palpebral fissures, ocular motor apraxia, strabismus, ptosis and coloboma of the iris, retina and optic nerve (a developmental defect usually caused in the womb when the cleft that forms to help the nourishment of the developing eye does not close properly) can be observed too[4,2,5,6,7,8].

Associated with low birth weight feeding and eating problems often concur; indeed, a few babies do not succeed in sucking and/or swallowing. In some case, the condition ease after the neonatal period, but other babies benefit from a period of feeding via a nasogastric tube (**Fig. 1. A; B)** or alternatively with a gastrostomy tube fitted so they can be fed directly through it into their stomach[9,10].

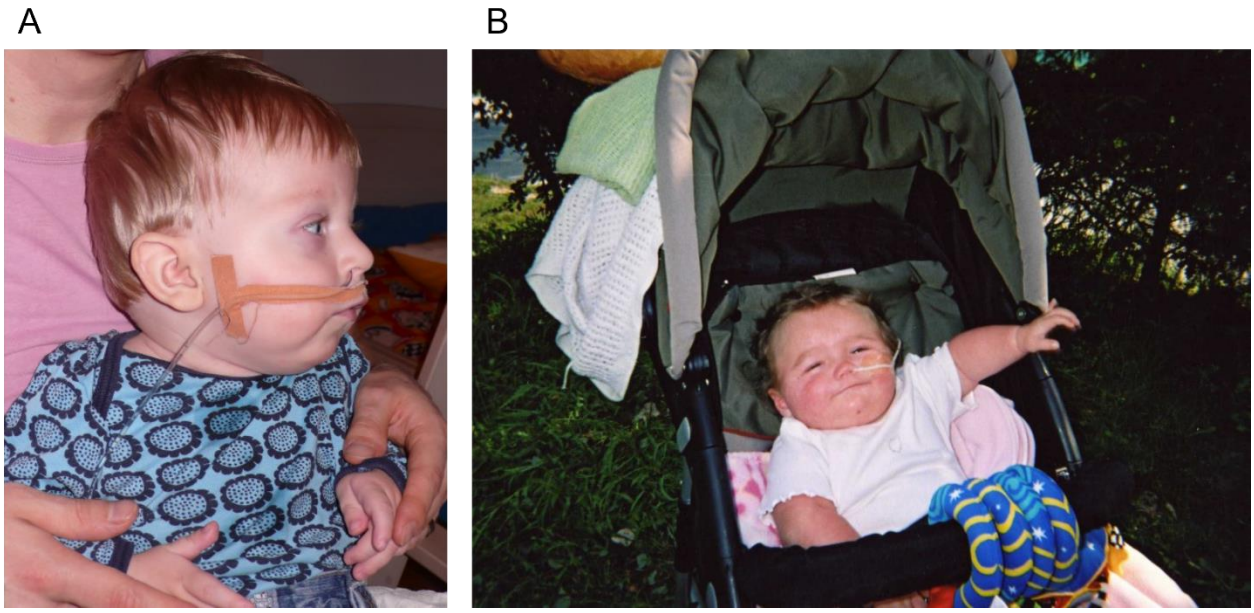

Fig. 1. A; B: child and infant feeding via nasogastric tube (Unique, 2014)

Hypotonia often found in the clinical presentation also affects chewing so while some children move on to solids at the expected age, most are late in weaning and need their food cutting up small or processing for a long time[9,10]. Lastly, gastroesophageal reflux and vomiting are common and can be severe from the earliest days, with a risk of aspiration pneumonia; furthermore, the reflux can cause sleep disorders to request the head end of the mattress raised or adding a pillow. Concerning nocturnal respiratory pathology, few children have been diagnosed with sleep apnea and in some of these children removing enlarged tonsils and/or adenoids proved helpful[10] .

In around two babies out of three, the hands are unusual with often just cosmetic complications like a single crease across the palm, and incurving fifth finger, or alternatively syndactyly and missing bones in either the fingers, the hand, or even the lower arm[4,6,9,11,5].

Last but not least, regarding cardiac pathology one third to a half of babies are born with a structural problem with their heart. There is a variety of problems, of which some need minimally invasive surgical correction, while others resolve spontaneously and do not affect the child's overall functioning[5,6,2]. The most common type of issue is either a hole between the upper chambers of the heart (atrial septal defect) or between the lower pumping chambers (ventricular septal defect) or holes between both chambers. The holes may be isolated or may be found with other concerns, including persistent ductus arteriosus (PDA) after birth supplying the lungs with more blood than they should have, and making the heart work too hard[2,5,6].

2.2 Cognitive and psychiatric features in 2q24.3DS

As regards the neurocognitive profile, individuals with 2q24.3 deletion present with significant functional deficits. Evaluating the clinical phenotype, **Table 1** shows that the developmental delay and autistic features are the two most common presentations[12]. Psychomotor development is initially normal and usually, the delay becomes evident from the second year of life and involves both motor and linguistic activities.

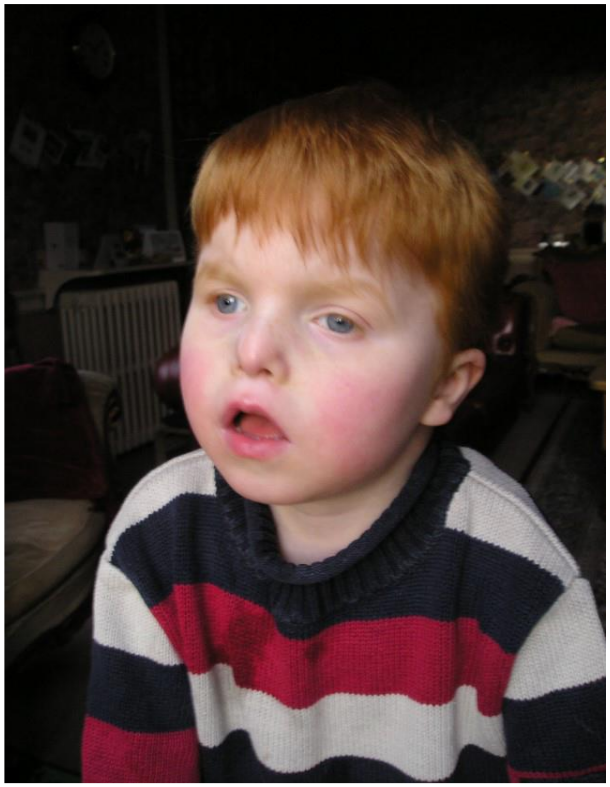

Fig. 2: child with eye contact delayed (Unique, 2014)

Specifically, eye contact is typically delayed so babies do not always look into their mother's eyes and first smiles arrive late (**Fig. 2**). Communication is supported by children's generally sociable temperament, but frequently they communicate their needs by crying and facial expression and, as they mature, by body language and gestures[4,9,13]. Children usually understand more than they can express, especially when they are given maximum help using focused attention, and short phrases and words are supplemented by body language and physical manipulation. Comparing these patients with others with a similar level of learning ability, they are commonly late in showing interest or curiosity in their surroundings, have a short attention span and require longer time than normal to process information and project a response; families usually say that their children learn best by repetition and individualized teaching[14].

Fig. 3: Child with an atypical smiling and sociable attitude (Unique, 2014)

It is not yet known whether there is a typical behavior pattern but often children are happy, laughing, smiling, and most sociable (**fig. 3**), particularly with familiar people, and usually prefer to relate to adults who can meet their needs more than to other children[14]. Stereotypic behavior like self-stimulating, self-talking, hand-washing can be observed and episodes of agitation can be related to discomfort or pain[14].

Table 1: Developmental course, neurological symptoms, and dysmorphic features in patients with 2q24.3 deletion syndrome (Akihisa Okumura, 2015)

Patient	**Reference**	**Development**	**Other neurological symptoms**	**Facial dysmorphisms**	**Other symptoms**
1	Madia et al10 Patient 1	Delayed	Not mentioned	Bulbous nose, bow-shaped mouth, hypotonic face	Not mentioned
2	Patient 2	Delayed	Mild	Down-slanting	Not mentioned

			generalized hypotonia, uncoordinated gait	palpebral fissures, low-implanted ears, broad nasal bridge	
3	Patient 3	Severely delayed, autistic features	Clumsy gait, mild generalized hypotonia	Bitemporal narrowing, frontal bossing, tubular nose, anterior open bite	Cleft palate, central precocious puberty
4	Pescucci et al. [11]	Severely delayed, autistic features	Sleep disturbance with breath holding	Hypotelorism, down-slanting palpebral fissures, long eye lashes, ptosis, high nasal bridge with large nose, thick helices and ear lobule, mild micrognathia, cupid bow mouth	Cleft palate, gastroesophageal reflux, hand and foot anomalies, failure to thrive
5	Davidsson et al. [12]	Severely delayed, autistic features	Hypotonia, brisk tendon reflexes	Small eyes, slight bilateral ptosis, micrognathia, low set small ears	Cleft palate, high anal atresia, atrial septal defect, pansynostosis, syndactyly
7	Krepischi et al. [14] Patient 3	Severely delayed	Swallowing difficulty	Micro/brachycephaly, thin nose with depressed and broad nasal bridge, anteverted nares, short philtrum	Hypothyroidism, tapered fingers and anteriorly displaced anus, left eye iris coloboma, right eye choroid, and retina coloboma
8	Takatsuki et al. [15]	Delayed	Generalized hypotonia	Thick arched eyebrows, upslanting palpebral fissures, long eyelashes, flat nasal bridge, short nose, long philtrum, small mouth, micrognathia, low-set ears	Pulmonary emphysema, fetal growth retardation
10	Bartnik et al.	Severely	Hypotonia,	Short palpebral	Central obesity

	[17]	delayed, autistic features	bipolar disorder, ocular motor apraxia	fissures, mild dental crowding, short neck	
11	Nimmakayalu et al. [18] Patient 1	Severely delayed	Hypotonia, microcephaly, feeding difficulty, irritability, hypoplastic optic nerves	Narrow palpebral fissures, deep set eyes, full cheeks, small mandible, prominent lateral palatine ridges, prominent frenulum between upper central incisors, tented upper lip with downturned corners of mouth, bitemporal narrowing, short nose with bulbous tip, dimple at the end of the nose	Fetal growth retardation, gastroesophageal reflux, poor growth, short sternum, small hands short slender tapered fingers
15	Traylor et al. [19] Patient 4	Severely delayed	Chiari I malformation	Small downslanting palpebral fissures, posteriorly rotated ears, uveal coloboma, coloboma of the choroid and retina	Fetal growth retardation, wide-spaced nipples, chordee of penis, brachydactyly, single transverse palmar crease, bridged palmar crease, craniosynostosis, failure to thrive

In 2010, Bartnik M et al. published a short report where they described the clinical course of a female proband born at term. At 1 year of age, she developed generalized, not clearly epileptiform, abnormalities at the EEG without any psychiatric symptom, which instead

appeared associated with global developmental delay at 3-4 years of age[8]. Afterwards, at the age of 25 years, the girl showed mild mental retardation, manic depression (bipolar disorder, quite rarely observed in 2q24.3DS), auditory hallucination, sleep disturbances, lack of coordination, behavioral concerns with impulsivity and exacerbation of outbursts and aggression She was unable to live independently[8]. Evaluating the girl's medical history, it was possible to conclude that growth leads to an increased incidence and severity of psychiatric problems, which can can persist throughout adult life.

Reviewing the international literature previous reports mainly described deletions on chromosome 2q24.3 being associated with seizures complicated by neurobehavioral comorbidities such as cognitive impairment, psychiatric disorders, and social problems[1]. To date, there are only two published case with a deletion on chromosome 2q24.3 displaying a phenotype with autistic features and developmental delay, but no seizures: Celle et al.[15] described the case of a 3-years-old boy with autism and developmental delay, carrying an interstitial deletion at band 2q24.3 with loss of the entire *SCN2A* gene and part of the *SCN3A* gene.

Kathrin Nickel et al.[1] presented the case of a 28-years-old male patient with symptoms of early infantile autism and Tourette syndrome.

The patient showed psychomotor retardation: he walked independently at 26 months and was not able to sit alone without falling over to one side even at the age of 2 years. The patient's parents reported early autistic features such as difficulties in social communication and interaction with avoiding eye contact and poor interest in social interaction. Development of speech was delayed, he refused body contact and demonstrated stereotypic patterns of behavior. Since the age of 2 years, the patient showed relevant aggressive symptoms such as

throwing his head on the floor or biting into items; during nursery school, auto-aggressive symptoms were exacerbated in terms of head-banging behavior injuring his jaw and ears. Later, he presented head throwing movements against his left shoulder and banging of one row of his teeth against the other. There was a pattern of aggravation of the tic symptoms during stressful situations. To date, no similar case has been described in the literature concerning the phenotype of autism spectrum disorder plus Tourette syndrome but without epilepsy[1].

2.3 Seizures and epilepsy in 2q24.3DS

The deletion of a sodium channel gene cluster located on chromosome 2q24.3 is associated with variable epilepsy phenotypes, including Dravet syndrome and migrating partial seizures of infancy (MPSI)[3].

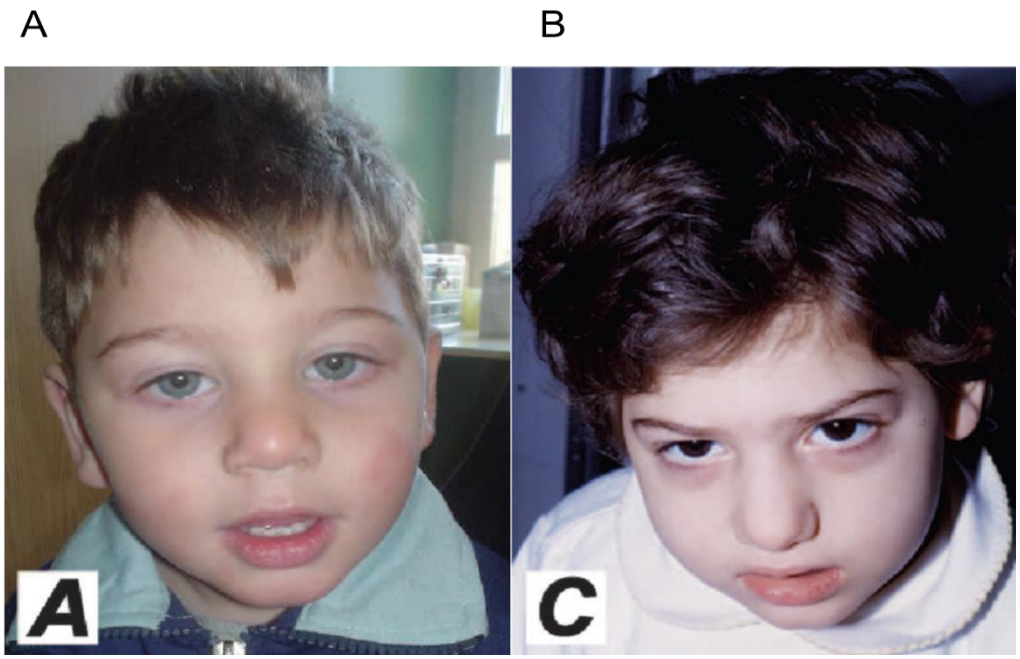

Fig. 4 A; B: Facial phenotypes of the two patients with severe myoclonic epilepsy of infancy (Madia F. et all, 2006)

In most Dravet syndrome cases, such as those described by Madia et al.[16] in 2006 (**Fig. 4. A; B**), the *SCN1A* gene is considered as the major contributor to the epilepsy phenotype; however, other cases with whole sodium channel cluster deletions have been described as rather atypical DS. Dravet syndrome typically presents in the first year of life in a normal child with prolonged, febrile and afebrile, focal (usually hemiclonic), and generalized tonic-clonic seizures. Other seizure types including myoclonic and atypical absence seizures appear between the age of 1 and 4 years. Most patients show seizure onset at less than 15 months of age, however a small minority of cases have onset in the second year of life. The first seizure is associated with a fever in about 60% of cases, and sensitivity of seizures to fever may persist throughout life[17].

The MPSI usually begins in the first 6 months of life (mean 3 months), but later onset in the first year of life has been also described. Both sexes are equally affected. Prenatal and birth history is typically normal. This syndrome is characterized by the onset of refractory focal seizures which arise independently in both hemispheres and can migrate from one cortical region to another randomly but consecutively in the same seizure. The paroxysms are often prolonged with episodes of status epilepticus[18].

In 2014, Byung C. L. et al.[3] described the clinical features of five patients (**Table 2**) to delineate the different epilepsy phenotypes associated with whole sodium channel gene cluster deletion on chromosome 2q24.3 deletion. Although *SCN1A* is considered as the major contributor to epilepsy, the lack of the other sodium channel genes is important to determine complex epilepsy phenotypes including atypical DS and MPSI, together with psychiatric comorbidities[3]. Among the 5 cases described, three cases (Cases 1-3) presented the deletion of the whole *SCN* cluster genes (*SCN3A*, *SCN2A*, *SCN1A*, *SCN9A*, and *SCN7A*) and showing

an intractable infantile-onset epileptic encephalopathy that differed from DS by severe developmental delay, earlier seizures onset and polymorphic focal seizures. These results suggesed that the contiguous deletion of sodium channel genes within the cluster, especially extending to the centromere involving *SCN2A* and *SCN3A*, might be responsible for a complex epilepsy phenotype that is atypical of DS. Some of the cases might also met the clinical features of MPSI, suggesting an independent or additive contribution of sodium channel genes located within the cluster to the epilepsy phenotype. Conversely, the remaining two cases exhibited a deletion of only some of the *SCN* cluster gene, and their epilepsy phenotypes could be classified as DS. Particularly, Case 4, had a whole *SCN1A* deletion and a partial *TTC21B* deletion, and showed few afebrile hypomotor and several prolonged febrile seizures until 33 months of age, without regression or delay in psychomotor development[3]. Conversely, Case 5, harbored a partial deletion of *SCN1A* and *SCN9A* and experienced a more severe clinical course. It seems that complete haploinsufficiency of one *SCN1A* copy may result in milder epileptic encephalopathy than does intragenic or partial deletion of *SCN1A*, as the majority of cases with intragenic *SCN1A* deletions are reported as having typical DS[3]. Lastly, *SCN7A* and *SCN9A* do not appear to be associated with the appearance of the epileptic phenotype[16].

Table 2: Clinical features of five cases with MLPA and CGH array results (Bjung Chan Lim et al., 2014)

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**
Age of onset	3 mo	1mo	2 mo	7 mo	4 mo
Age at last follow-up	5 yr 8 mo	2 yr 5 mo	1 y 3 mo	2 yr 9 mo	9 yr 8 mo
Initial seizure	TC	Autonomic	FC fever	FebSE	HC
Seizure type	MF, autonomic		MF, HC, autonomic	TC Fever, HC Fever	HC, TC
Multifocal EEG	+	+	+	-	+

Seizure outcome	refractory to multiple AEDs and KD	refractory to multiple AEDs	refractory to multiple AEDs and KD	fair response to LEV	refractory to multiple AEDs
Cognitive outcome	severe MR	severe DD	severe DD, possible SUDEP	normal	developmental regression after 12 mo
Acquired microcephaly	+	+	Na	-	Na
Brain MRI	Progressive diffuse atrophy	mild diffuse atrophy	mild diffuse atrophy	normal	mild diffuse atrophy
SCN1A MLPA	whole exon deletion	whole exon deletion	whole exon deletion	whole exon deletion	Exon 1—20 deletion
Deletion size by CGH array	8.4 Mb	4.3 Mb	1.5 Mb	0.2 Mb	0.2 Mb
Deleted sodium channel genes	SCN3A, SCN2A, SCN1A, SCN9A, SCN7A	SCN3A, SCN2A, SCN1A, SCN9A, SCN7A	SCN3A, SCN2A, SCN1A, SCN9A, SCN7A	SCN1A	SCN1A, SCN9A

Legend:

AED: antiepileptic drug; CGH: comparative genomic hybridization; DD: developmental delay; FC: focal clonic seizure; FebSE: febrile status epilepticus; HC: hemiclonic seizure; KD: ketogenic diet; LEV: levetiracetam; MF: multifocal seizures; My: myoclonic seizure; MF: multifocal seizure; MLPA: multiple ligation-dependent probe amplification; MO: months; MR: mental retardation; NA: not avaliable; SUDEP: sudden unexpected death in epilepsy; TC: tonic-clonic seizure; YR: years

Another epileptic syndrome of the neonatal and infantile period is West syndrome (WS). It is characterized by the onset of epileptic spams between 3 and 12 months of age, although later onset may occur. In some cases, infants with Ohtahara syndrome or other early-onset epilepsies (typically with focal seizures) may evolve to have clinical and EEG features of WS

after 3-4 months of age. Both sexes are affected, with a higher incidence in males. Global developmental impairment is typically seen at the onset of epileptic spasms[20].

In 2018 Pin F.C. et al.[21] published the first report a deletion in the chromosome 2q24.3 region including the *SCN2A* and *SCN3A* genes without *SCN1A* involvement as a probable genetic cause in a clearly defined WS case associated with autistic features and developmental problems.

Table 3: Clinical phenotypes of SCN2A and SCN3A deletion cases without SCN1A involvement (Pin Fee Chong et all, 2018)

	Patient 1	**Patient 2**	**Patient 3**	**Present Case**
Sex	Female	Female	Male	Male
Age at report	40 mo	25 yr	3 yr	21 mo
Development	Delayed	Mildly delayed	Delayed	Delayed
Autistic features	Yes	Yes	Yes	Yes
Other neurological symptoms	Not mentioned	Mild hypotonia, Ocular motor apraxia, Bipolar disorder	Microcephaly	Generalized hypotonia, dystonia-like movement
Facial dysmorphism	Prominent nasal bridge, down slanting palpebral fissure, low-set ears, micrognathia	Short palpebral fissures, short neck	Not mentioned	Upslanted palpebral fissure, hypertelorism, cupid's bow mouth
Epilepsy	No	Yes	No	Yes
Seizure types/Diagnosis	-	'shiver-like' episodes/unknown	-	Infantile spasms/West syndrome
Brain MRI	Immature myelination at periventricular areas	Not mentioned	Not mentioned	Normal

Electroencephalogram	No epileptiform disharges	No epileptiform disharges	Not mentioned	Hypsarrhythmia
Deletion size	2.8 Mb	112 kb	230 kb	1,102 kb
Involved genes	FIGN, GRB14, COBLL1, SLC38A11, SCN3A, SCN2A, part of CSRNP3	Part of SCN2A, part of SCN3A	SCN2A, part of SCN3A	FIGN, GRB14, COBLL1, SLC38A11, SCN3A, SCN2A, part of CSRNP3

Legend:

MO: months; YR: years

The proband (**Table 3, present case**) was born at 39 weeks of gestation to healthy non-consanguineous parents. He developed normally until the first 4 months when, eye contact and facial expression disappeared. Then, the boy came to medical attention at 10 months of age due to recurrent episodes of head nodding and upward eye deviation occurring in clusters. At the clinical examination, he presented facial dysmorphisms, poor head control, generalized muscular hypotonia, and stereotypic behavior[21]. Interictal electroencephalogram demonstrated a typical hypsarrhythmic pattern during wakefulness and sleep (**Fig. 5 A)** point towards the diagnosing of WS[21]. The Authors demonstrated that the *SCN2A* deletion might be sufficient to present a severe epileptic phenotype, while haploinsufficiency of *SCN3A* or dual haploinsufficiency of *SCN2A* and *SCN3A* may lead to the syndromic phenotypes of WS, ASD, and dysmorphisms[21].

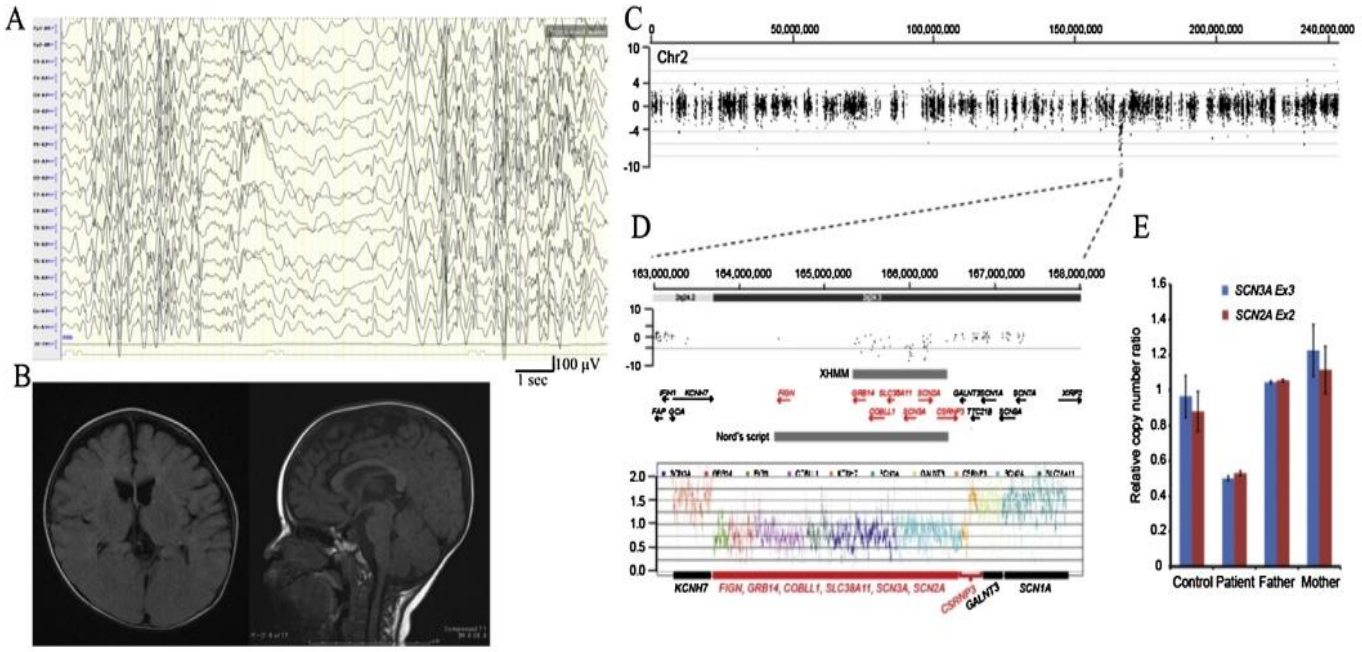

Fig. 5. (A) Interictal electroencephalogram (EEG) at 10 months of age revealed the characteristic random high-voltage slow waves with spikes and polyspikes activity. Fragmentation of the hypsarrhythmic activity was noted in this sleep EEG recording. (B) Brain magnetic resonance imaging on admission at 10 monthsrevealed no pathological findings. Axial T2-weighted FLAIR image (left) and sagittal T1-weighted image (right). (C) XHMM analysis of the whole of chromosome 2. X- and Y-axes show the physical position and read-depth z-score, respectively. XHMM automatically called a deletion (gray box). (D) High-magnification view of the deleted interval. Deletion interval called by XHMM (upper panel) contained 6 genes including SCN3A and SCN2A (middle panel), and Nord's script analysis could detect additional FIGN deletion (lower panel). Deleted genes are highlighted in red. (E) Quantitative real-time PCR analysis confirmed the heterozygous deletion of SCN3A and SCN2A in the patient but not in his parents, indicating that the microdeletion occurred de novo (Pin Fee Chong et al, 2018).

2.4 Motor disorders in 2q24.3DS

One of the main clinical features that distinguish babies with 2q24.3DS is the hypotonia (**Fig. 6**), indeed they are typically unusually "floppy" children and acquire head control late. To this in addition to regular physiotherapy, special clothing to support the upper body may be needed taping the trunk to promote stability[4,9,13].

Fig. 6: Hypotonic child supported to increase stability (Unique, 2014)

Early developers may learn to roll around 9 months of age, but others are unable to roll over until their third year or even later. Children may become mobile, by conventional crawling, but others using ingenious alternatives including spinning or continuous rolling in their second or third year, yet this is not possible for all[4,9,13]. First supported or unsupported steps may be possible in few children as early as 18 months but usually emerge much later, typically between 4 and 8 years, and may follow years of persistent practice[4,9,13]. Problems

with balance can persist, although climbing stairs may be possible by 4 years. In general, most children will use a wheelchair outdoors and may need one indoors as well[4,9,13].

Weakness, low muscle tone, and coordination difficulties mean that children are likely to experience a very considerable delay in learning to use their hands[14]. The extent of the delay varies between individuals: some children can hold a spoon or favorite toys by the age of 4 or 5 years, while others do not consistently acquire this ability[14]. Others can pick objects up but not hold them for long. This means that those children who take solid foods are likely to need to be fed and it seems that they are also likely to need a lot of help with other personal care skills such as dressing and undressing, although they can be cooperative by holding out an arm for a sleeve, for example[14]. Motivation is the key to acquiring personal care skills which generally go hand in hand with the ability to grasp, hold onto and manipulate objects and toys and again there is some variation but children generally depend entirely on their family and caregivers[14].

2.5 Brain neuroimaging features in 2q24.3DS

Neuroimaging studies in 2q24.3DS are of utmost importance since they can help to delineate the brain alterations typically associated with the deletions. Analyzing the MRI reports the most frequent finding was the diffuse cerebral atrophy with mild or progressive grade described in cases 1-3 and 5 by byung C.L.[3]. Moreover, mild ventricular dilatation of lateral ventricles has been highlighted in one 4-years-old patient by Madia F. et al.[16], while in 1996 Chinen et al. described a female infant with an occipital encephalocele[22].

Evaluating the literature, the major morphological alterations include microcephaly, also detectable by MRI, which has been found in up to 50% of the described clinical cases[10,22,7,15,3,1]. In the remaining patients with the same deletion, the neuroradiological investigation showed a normal presentation.

2.6 2q24.3DS: molecular bases

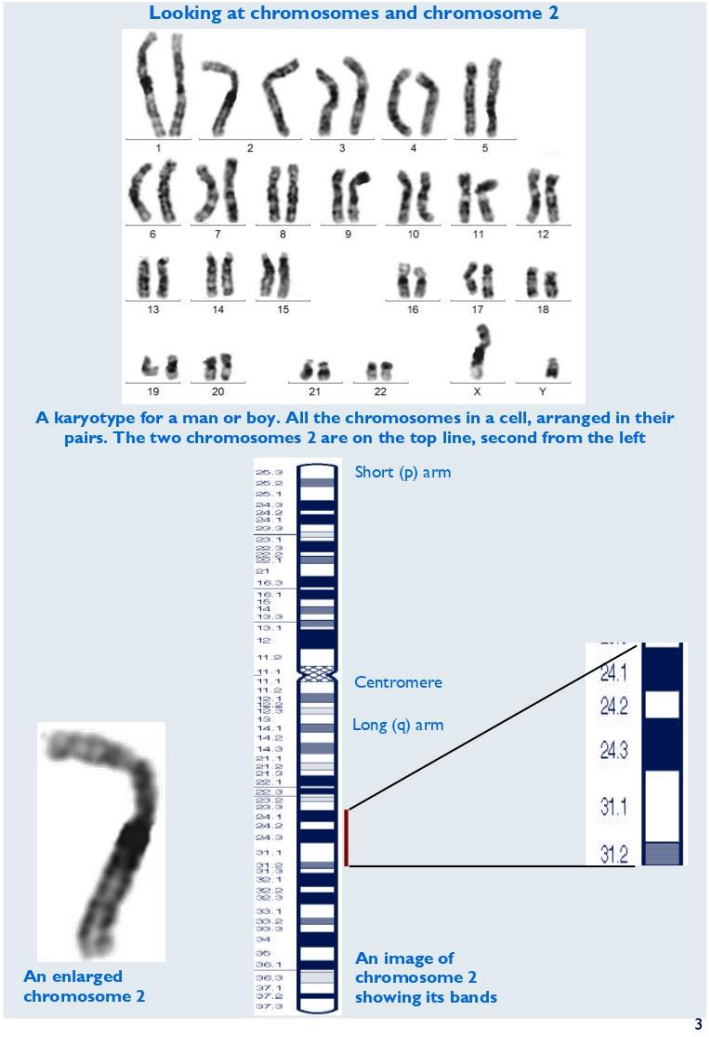

Fig. 7: 2q24.3 band on the long arm of the chromosome 2 (Unique, 2014)

The 2q24.3 band is located on the long arm of chromosome 2 between the 2q24.2 and 2q31.1 bands, and involves approximately 6 million base pairs (6 Mb) in length (**Fig. 7**). The region contains 52 genes of which 20 are protein-coding: the most representative genes in determining clinical phenotypes in 2q24.3DS encode for sodium channels (*SCN1A*, *SCN2A*, *SCN3A*, *SCN7A*, and *SCN9A*) (**Fig. 9**). The *SCN1A* has four homologous domains, each of which contains six transmembrane regions and encodes for the alpha-1 subunit of the voltage-

gated sodium channel Na(V)1.1. The transmembrane alpha subunit forms the central pore of the channel. This ion channel is critical to the generation and propagation of action potentials. The channel responds to the voltage difference across the cell membrane to create a pore that allows sodium ions passage through the membrane. The influx of sodium creates an action potential, which is critical to signaling within the brain[23].

SCN2A (**Fig. 8**) encodes the alpha-subunit of voltage gated channels (VGCs) Na(V)1.2, which are highly expressed in the brain at an early postnatal stage predominantly at terminals and initial segments of axons, playing essential roles in regulating both neuronal firing and inter-neuronal connectivity[21]. Likewise, SCN3A (**Fig. 8**), is a protein encoded by the Na(V)1.3 gene that, similarly to SCN2A, is responsible for the generation and propagation of action potentials in neurons and muscle; moreover, it is involved in folding of the human cerebral cortex, a process called "gyrification", and affects speech production brain areas[21].

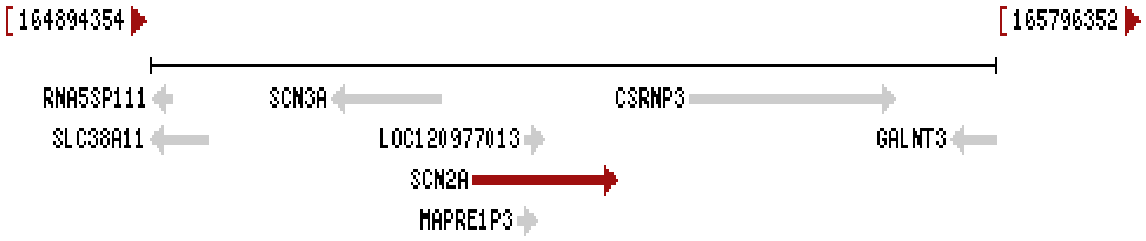

Fig. 8: SCN2A and SCN3A location on 2q24.3 band (NCBI)

The SCN7A is essential for the proper functioning of neurons and muscles during action potentials and directs sodium ion diffusion for membrane depolarization. Although, this sodium channel protein has some atypical characteristics; the similarity between the human and mouse proteins is lower as compared to other orthologous sodium channel pairs. Also,

the S4 segments, which sense voltage changes, have fewer positive charged residues than in other sodium channels. Particularly, it has fewer arginine and lysine residues as compared to other sodium channel proteins[24]. The SCN7A is also involved in CNS sodium-level sensing[25], and impaired salt intake occurs in homozygous *SCN7A* knock-out mice[26]. Lastly, *SCN9A* encodes a voltage-gated sodium channel which plays a significant role in nociception signaling. Mutations in this gene have been associated with primary erythermalgia, which is an early-onset neuropathy with burning pain and redness of the skin of the extremities in response to warm stimuli or exercise, channelopathy-associated insensitivity to pain, and paroxysmal extreme pain disorder[27]. However, *SCN7A* and *SCN9A* haploinsufficiency does not appear to have phenotypic correlates[16].

In addition to the *SCN* cluster, deletions found in the literature also involved *COBLL1, SCL38A11, CSRNP3, GALNT3, GRB14, TTC21B, STK39,* and *NOSTRIN* (**Fig. 10: NCBI**). *COBLL1* is a highly conserved gene that is implicated in the early development of the neural tube and it encodes the Cordon-Bleu WH2 Repeat Protein Like 1, hence, being suggested as a negative regulator of apoptosis, and associated with lower insulin resistance[28,29]. *GRB14* encodes a grow factor receptor-binding protein acting as an inhibitor of intracellular signaling pathways and regulating growth and metabolism[30], while *SCL38A11* is a novel putative protein with unknown function that harbors a transmembrane domain found in various amino acid transporters (e.g., γ-aminobutyric acid transporter and transport system N vesicular protein) and implicated in synaptic transmission[16].

TTC21B, located in the central region of the 2q24.3 band, together with *CSRNP3* and *GALNT3* (**Fig. 9**), contains several tetratricopeptide repeat (TPR) domains. The encoded protein is localized to the cilium axoneme and may play a role in retrograde intraflagellar

transport in cilia. Mutations in this gene are associated with various ciliopathies, nephronophthisis 12, and asphyxiating thoracic dystrophy 4[31]. *GALNT3*, instead, encodes for the UDP-GalNAc transferase 3, a member of the GalNAc-transferases family. This family transfers an N-acetyl galactosamine to the hydroxyl group of a serine or threonine residue in the first step of O-linked oligosaccharide biosynthesis[32].

At the end of the band (**Fig. 9**) *STK39* is thought to function in the cellular stress response pathway. The encoded kinase is activated in response to hypotonic stress, leading to phosphorylation of several cation-chloride-coupled cotransporters. The catalytically active kinase specifically activates the p38 MAP kinase pathway, and its interaction with p38 decreases upon cellular stress, suggesting that this kinase may serve as an intermediate in the response to cellular stress[33]. Finally (**Fig. 9**) *NOSTRIN* binds the enzyme responsible for nitric oxide (NO) production, endothelial NO synthase, and triggers the translocation of endothelial nitric oxide synthase (ENOS) from the plasma membrane to vesicle-like subcellular structures, thereby attenuating ENOS-dependent NO production; indeed, NO is a potent mediator in biologic processes such as neurotransmission, inflammatory response, and vascular homeostasis[34].

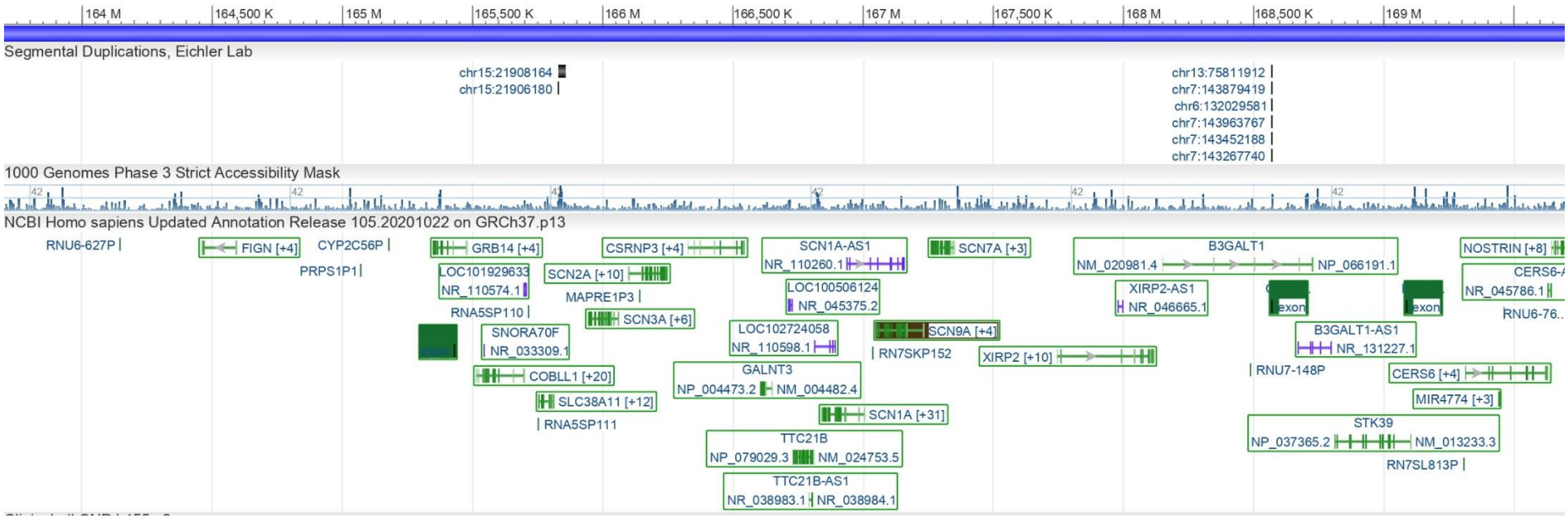

Fig. 9: Representation of genes in the 2q24.3 band (NCBI)

2.7 Role of 2q24.3 deletion in neuropsychiatric phenotypes

A pivotal role in determining the behavioral, neurocognitive, and psychopathological phenotype is certainly played by the reduced dose of 2q24.3 genes normally expressed in the brain from early development through maturity. As previously described, among the genes located in this chromosome section, disease causative genes are those of the sodium channels cluster (*SCN3A*, *SCN2A*, *SCN1A*, *SCN9A*, and *SCN7A*); their absence is associated with variable epilepsy phenotypes, including DS[3].

In a study, published in 2010, Bartnik et al.[8] described a 25-years-old girl with a history of infantile seizures, developmental delay, and psychiatric abnormalities including bipolar disorder and auditory hallucinations. Also, in this case, the genetic analysis pointed out the haploinsufficiency of *SCN2A* without the involvement of *SCN1A,* further confirming that the *SCN2A* plays an important role in the genetic basis of neurodevelopmental and neurobehavioral disorders[8]. The same conclusion was reached by Celle et al.[15] in 2013 describing the first case, at that time, of an *SCN2A* and *SCN3A* deletion (**Fig. 10**) in a patient with autistic features, language delay, but without a history of seizures emphasizing that *SCN2A* can be associated not only with epilepsy but also with autistic features.

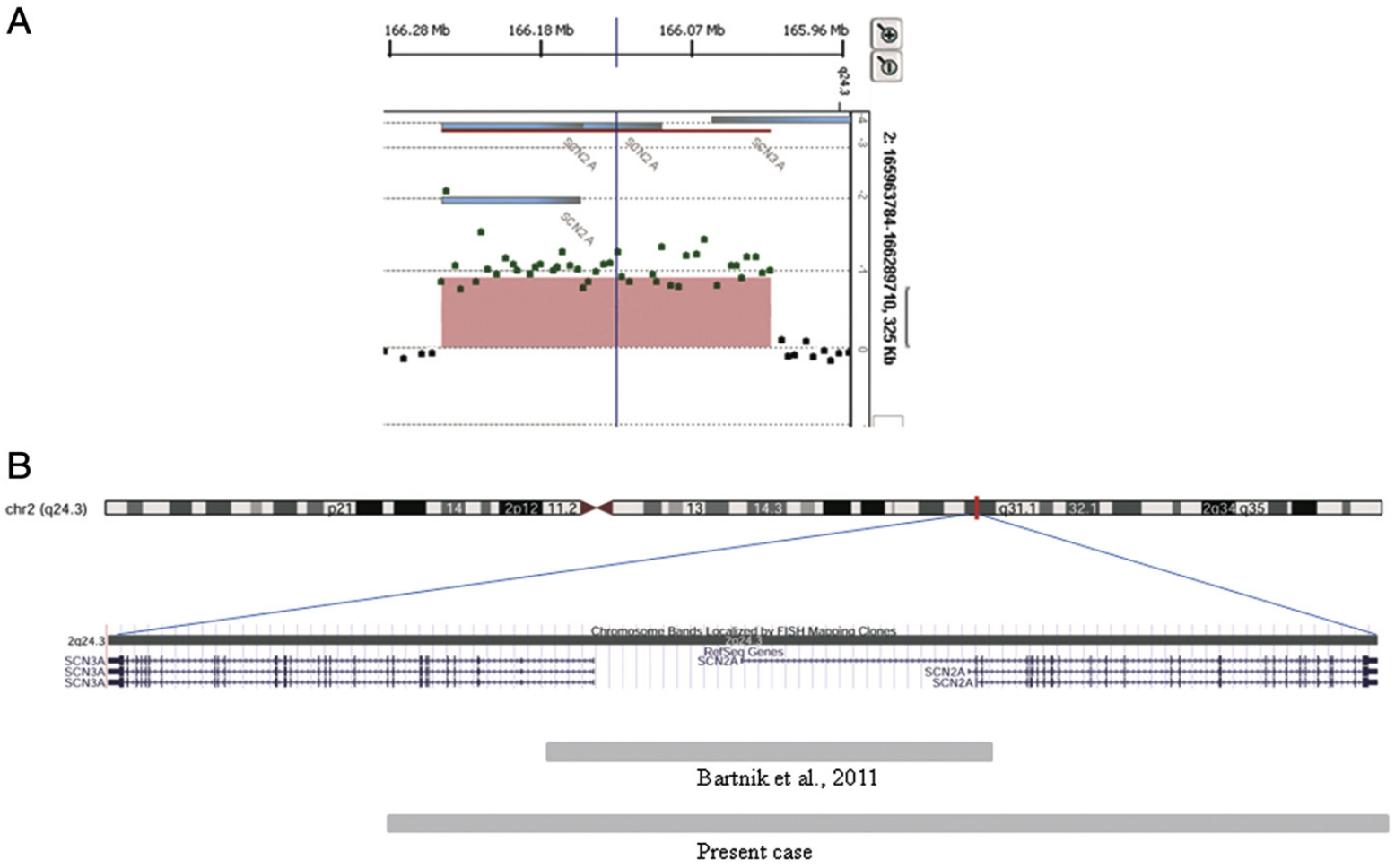

Fig. 10: Results of array-CGH analysis: A) Array-CGH analysis shows an interstitial deletion of ~230.1 kB at band 2q24.3 of chromosome 2. The deletion spanned from probe A_14_P131894 (166,019,786) to probe A_16_P00519262 (166,249,879) flanked by probe A_16_P00518842 (166,011,697) and probe A_16_P15912639 (166,256,414). B) Shows the deleted region of the prrsent case and its gene content, including the SCN2A and SCN3A loci, compared to the deletion identified by Bartnik et al. (2011) (Celle et all, 2013)

Furthermore, Bartnik et al.[8], supported that, although the non-coding exon 1a of *SCN3A* was deleted, it is not excludable that its function is also disrupted thus also having a role in the appearance of the disease. Bartnik et al.[8] lastly proposed that missense mutations of *SCN2A* are responsible only for isolated epilepsy.

Regarding the psychiatric comorbidities, in 2018 Kathrin Nickel et al.[1] presented the first case of a patient affected by ASD and Tourette syndrome associated with *SCN2A* and *SCN3A* deletion.

Since sodium channels are critical for action potential generation and propagation, a causative association between seizures and sodium channel dysfunction is plausible, whereas it is more difficult to understand this link for ASD and tics[35]. It has been proposed that the reduction of gamma-aminobutyric acid (GABA) release leades to an inappropriate inhibition in related neuronal networks[36]. Parvalbumin neurons represent inhibitory GABAergic cells that are involved in various forms of feed-forward inhibition within the striatum[37]. Postmortem studies in patients with Tourette syndrome demonstrated a consistent and profound imbalance of parvalbumin-positive neuronal distribution in the basal ganglia[38]. The selective deficit of parvalbumin-positive and cholinergic striatal interneurons in Tourette syndrome was supposed to result in an impaired cortical/thalamic control of striatal neurons[39]. Evidence from studies demonstrated that Na(v)1.2 (the protein encoded by *SCN2A*) is abundant in parvalbumin-positive GABAergic inhibitory interneurons, at least in the hippocampus and the temporal lobe[40]. Apart from *SCN2A* and *SCN3A* genes being affected by the presently detected deletion, *GRB14* (exon 1 to intron 2–3), *COBLL1* and *SLC38A11* were also deleted in the 28-years-old male. Thus, heterozygous deletion of these genes might also play a pivotal role in conferring ASD symptoms and could even be suggested to confer symptoms of

Tourette syndrome in the present case: loss of *SLC38A11*, a putative sodium-coupled neutral amino acid transporter[41] might enhance the effects of *SCN2A* and *SCN3A* deletion. One previous investigation reported a deletion on chromosome 2q comprising *GRB14* and *COBLL1* in a patient with autistic features, developmental delay, mental retardation, language impairment, and dysmorphic features[1]. In this case, additional repetitive hand movements have been described[15,42]. Therefore, deletions of *GRB14* and *COBLL1* may contribute to tic symptoms[1]. *SLC38A11* and *COBLL1* deletion was identified also in the third of 3 patients (**Fig. 4 B**) reported by Madia F. et al. in 2006[16]. The boy was affected by DS, autistim spectrum disorder, behavior, central precocious puberty and palatoschisis[16]. Central precocious puberty and palatoschisis were not previously associated with DS. This observation suggests that one or more genes centromeric to *CSRNP3*, namely, *SCN2A*, *SCN3A*, *SLC38A11*, and *COBLL1*, may be involved in the pathogenesis of these features[16]. Central precocious puberty is defined as the early onset of secondary sexual characteristics due to increased activity of hypothalamic gonadotrophin-releasing hormone (GnRH)[16]. This condition is frequent in girls and is often associated with midline structural abnormalities[43]. However, it is not known how the haploinsufficiency of one or more voltage-gated sodium channels would increase GnRH levels and so lead to central precocious puberty[16]. Specifically, *COBLL1* is expressed in the medial part of the developing branchial arch 1 and is also found in the nasal placodes, from which GnRH neurons originate[44]. It remains to be established whether these genes play a role in the development of central precocious puberty and palatoschisis[16].

3. Aims of the study

The aim of the current study was to outline the neurological and psychiatric features of a cohort of patients with 2q24.3DS. Particularly, we focused on dissecting genotype-phenotype correlation, trying to infer the contribution of specific genes deletion on the final phenotypic expression of the patients, with a special focus on the epileptic phenotype.

4. Methods

4.1 CGH-array

DNA was analyzed by Comparative Genome Hybridization, CGH-array, using the Human Genome CGH Microarray 4×180K Kit, probe design 086332 (Agilent Technologies, Santa Clara, CA), according to the manufacturer's instructions. The Agilent platform is an oligonucleotide-based microarray with an average resolution of about 25 kb to detect copy number variations, and loss of heterozygosity (LOH) of 4 Mb. Raw data were analyzed using the Genomic Workbench 7.0.40 software (Agilent). Altered chromosomal regions and breakpoints and LOH events were detected using ADM-1 (threshold 10) with 0.5 Mb window size to reduce false positives. For aberration detection, the diploid peak centralization algorithm and the legacy centralization algorithm were applied to set the most common ploidy to zero. This is needed to ensure that the zero point reflects the most common ploidy state. Chromosome positions were determined using GRCh37/hg19 (UCSC Genome Browser, http://genome.ucsc.edu, release 7 July 2000).

4.2 Patients selection

Patients with a clinical picture of DS and lack of point mutation in SCN1A undertook CGH-array analyses. The presence of symptoms other than epilepsy was considered suggestive of chromosomal microdeletions and was not an exclusion criterion. Clinical data were collected by face-to-face interview and structured spreadsheet. EEG and brain MRI reports were reviewed. Epilepsy syndromes were classified according to the International League Against Epilepsy. A PubMed search was performed using the terms “2q24.3 deletion syndrome”, “SCN channel deletion”, “2q24.3 deletion AND clinical phenotypes”, “2q24.3 deletion AND

epilepsy AND developmental delay" (last search August 2021). Multiple descriptions of the same individual in different publication were identified by extension of the sex, age at study, age at seizure onset, seizure types at onset, neurological examination, developmental delay, and psychiatric comorbidities, acquired microcephaly and other dysmorphisms, deletion size detected by CGH-array, deleted sodium channels, and lastly anti-seizure medications.

5. Results

We studied 2 novel probands and reviewed the available electro-clinical information for the 12 patients already published in the literature. Two (#6; #14) patients were excluded as aged above 18 years at the time of the last follow-up while 1 (#12) patient was over the age of 9. 14 patients (6 females) aged between 1 and 5 years of age were investigated. The mean age at randomization was 6.93 years (age range, 2.6 - 28 years). The age at seizure onset ranged from 2 to 12 months (mean 4.75 months) with no differences between males and females. Only two (#7; #14) (14%) patients, did not develop seizures. Tonic-clonic (#1; #5; #8) (21%) and focal clonic (#1; #2; #10) (21%) seizures, 3 (#1; #5; #10) of whom with fever as a trigger, were the most frequent seizures types at onset, followed by myoclonic seizures in 2 (#1; #4) (14%) patients, in one both febrile and non-febrile), autonomic seizures (#3; #9) (14%), absences (#6) (7%), hemiclonic seizures (#12) (7%), and febrile status epilepticus (#11) (7%). Lastly, one patient reported epileptic spasms (7%). The other types of seizures developed at follow-up were: myoclonic in 7 (#1; #2; #3; #5; #8; #9; #10) (50%) cases (5 of whom with fever as a trigger), tonic-clonic in 6 (#1; #2; #3; #9; #11; #12) (42%) cases (two of whom with fever as a trigger), autonomic (#8; #10) (14%) and absences (#2; #4) (14%) in 2 cases each. Hemiclonic seizures (#10; #11) (14%), status epilepticus (#3; #4) (14%), and gelastic seizures (#1) (7%) were also reported. Considering both seizures at onset and those that developed subsequently, were found: 8 (57%) cases with tonic-clonic seizures; 5 (35%) cases with myoclonic seizures; 4 (28%) cases with multifocal and autonomic seizures; 3 (21%) cases each with hemiclonic seizures, focal clonic seizures, absences, and status epilepticus; and 1 (7%) case each with gelastic seizures, and epileptic spams.

Neurological examination At the neurological examination, hypotonia was the most frequent feature, presenting in 8 (57%) patients. Hypotonia could be either generalized or localized and manifesting as reduced head control. Together with the loss of muscle tone, patients often exhibited hyperkinesia and afinalistic and stereotyped movements, especially involving the hands and the head. Patient #1 also presented oro-buccal automatisms. Other evident clinical signs were the poor visual contact, the lack of pursuit and fixation of objects, delayed language, and in one case (#5) delay in the acquisition of eye-hand coordination. Moreover, performing the objective examination of these patients several dysmorphisms were found, among which the most frequent was the microcephaly; specifically, in 5 cases out of 14 (35%). Finally, all patients showed a mild-to-moderate intellectual disability.

Neuropsychiatric comorbidities Simple vocal tics were found in one child (7%) (#14) with a clear diagnosis of Tourette syndrome. To date, this is the only case reported in the literature showing Tourette syndrome associated with the 2q24.3DS. Dissecting the neuropsychiatric features of one of our proband (#1), a diagnosis of autism spectrum disorder (ASD) was made on the bases of typical attitudes patterns. ASD was also diagnosed in 4 other patients (#5; #7; #13; #14) reported in the literature for a total of 5 cases (35%). In 6 (#3; #8; #9; #10; #11; #12) (42%) patients, psychiatric comorbidities were unknown. In addition, analyzing the cohort, one patient (#6) developed bipolar disorder, anxiety disorder, and auditory hallucinations.

EEG findings The first electroencephalographic (EEG) recording of the patients in our cohort was not altered in 3 (#5; #7; #11) (21%) cases or was not clearly epileptiform in 3 (21%) others case, including one of our two probands (#2; #6; #14), whose pattern was artefactual. In the remaining cohort, the EEGs revealed mainly two types of pathological changes:

multifocal epileptic discharges in 4 (28%) cases, and slow background activity in 5 (35%) others. In only one child (#13) (7%) an hypsarrhythmic pattern was found (**Fig. 5 A**), consistent with frequent infantile spasms and the diagnosis of WS.

Genetic testing CGH-array of the 2q24.3 band showed all patients to carry a deletion sized between 112 and 4.800 Kb (85%), excluding two cases (#8; #13) with a deletion of 8.400 Kb and 1,102 Kb, respectively. The absence of the chromosomal band determined a deletion, among others, of the genes encoding for sodium channels (SCN), and, particularly, 5 (35%) patients carried a deletion involving all five sodium channels genes (i.e, *SCN1A, SCN2A, SCN3A, SCN7A* and *SCN9A*) while 4 (28%) (#6; #7; #13; #14) patients showed haploinsufficiency of the *SCN2A* and *SCN3A* genes, 2 (14%) (#1; #2) cases showed deletion of the *SCN1A*, *SCN2A,* and *SCN9A* genes, and, finally, 3 cases carried a deletion of *SCN1A* (#11)*; SCN1A, SCN7A*, and *SCN9A* (#4); and *SCN1A, SCN9A* (#12), respectively. Comparing the array CGH analysis with sodium channels genes (SCN) haploinsufficiency, the 5 (35%) cases with absence of all five sodium channels showed a deletion size greater than 1.500 Kb, whereas the remaining cases with partial channel absences showed deletions ranging between 719 Kb and 1,102 Kb. The only exception was represented by one of our two probands (#1), which presented a deletion size of 1.800 Kb and the absence of *SCN1A, SCN2A,* and *SCN9A* genes.

Anti-seizure medications: Excluding 2 (#7; #14) cases that did not present seizures, and 1 (#6) case that did not require treatment, the remaining 11 (78%) cases underwent polypharmacological treatment with several anti-seizure medications (ASM). The most common ASM were valproic acid (VPA), phenobarbital (PHB), topiramate (TPM), levetiracetam (LEV), clobazam (CLB), pyridoxine (PDX), and clonazepam (CLZ). In 1 (#13)

(7%) case, administration of ACTH along with pyridoxine and topiramate was also tried while in 2 (14%) (#8; #10) patients the medical team also experimented the ketogenetic diet. Finally, in one of our probands (#1), along with valproate acid and clobazam, stiripentol was added at the last follow up, with poor seizures control.

5.1 Novel cases description

5.2 Case #1

The patient was a 3-years-old girl with a mild congenital ptosis. At age of 2 months, she presented focal clonic seizures with alternating hemisphere of onset. About 10 episodes occurred over a relatively short period time, being never triggered by fever. In the following months, the girl also showed primarily generalized tonic-clonic seizures, myoclonic seizures, and absences. Developmental stagnation was noticed at this point, followed soon after by clear developmental delay. Subsequently, the patient presented cortical visual impairment and communication difficulties, using only few words to express her ideas. At the most recent follow-up, very slow gains in cortical functions were noticed.

Hyperkinesia associated with stereotyped movements, especially involving the hands, was also evident: the girl wrung her hands, brought them together, and flicked the fingers. She also moved her head from side to side in repeated horizontal movements. The joints were mildly hypermobile, deep tendon reflexes (DTs) were brisk with, at times, a sustained clonus. Laboratory tests showed normal blood glucose, and plasma amino acid, while plasma lactate was mildly up (2.9 mmol/L). Tests on cerebro spinal fluid (CSF) also come out as normal.

The first EEG, performed at 2 months of age, showed only some probable artefactual sharps. Conversely, subsequent EEGs performed over time (**Fig. 11**), showed an abnormal background with epileptic discharges. Brain MRI was normal.

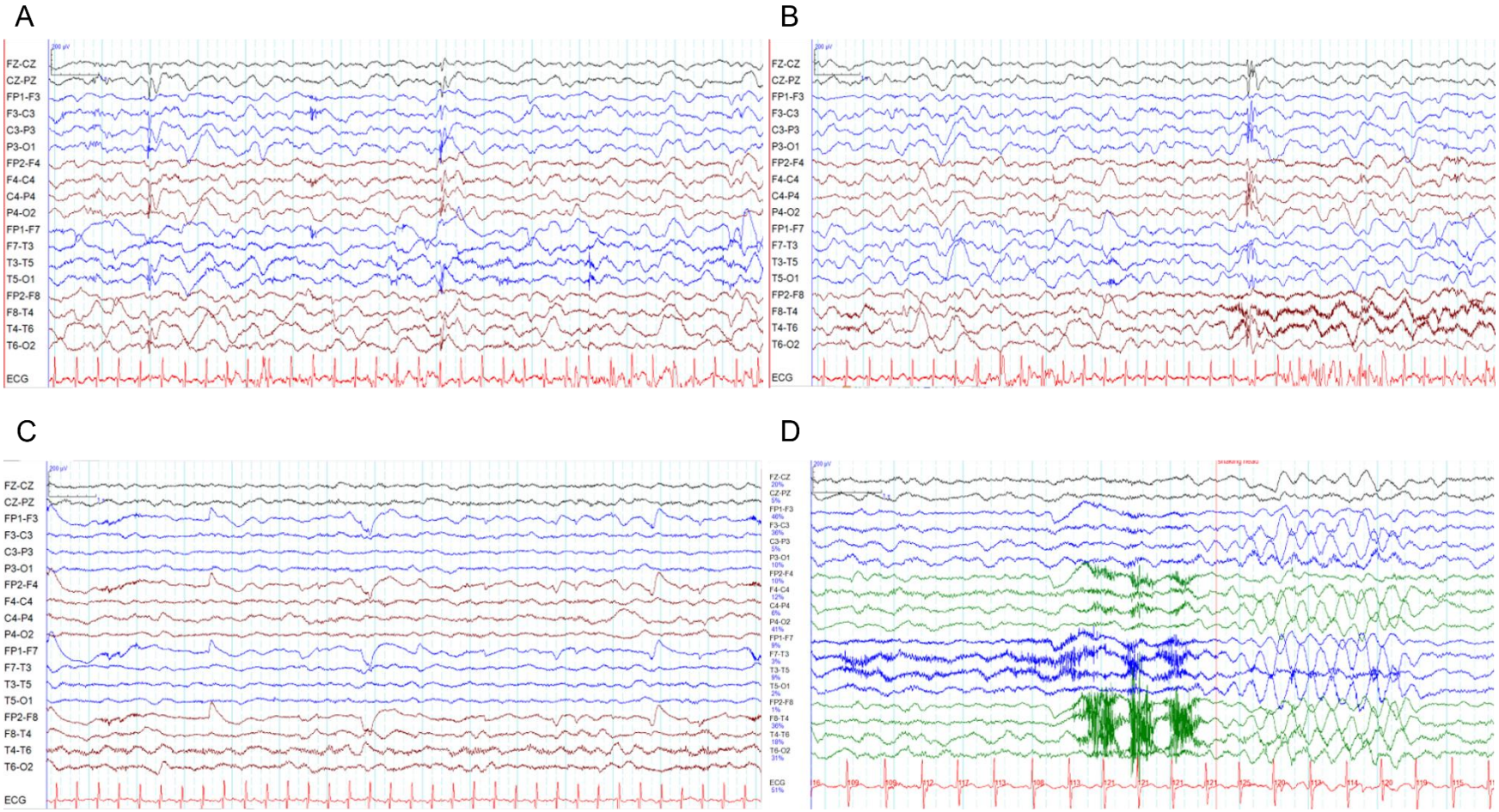

Fig. 11. A,B,C) Interictal EEG performed at 1 year and 9 months of age (A,B) and at 3 years and 1 months (C) showing slowed background activity, poorly reactive to eyes closure, with multifocal spikes-and-sharp waves complexes or polyspikes; D) Ictal EEG performed at 3 years and 1 months showing a burst of slow, high-amplitude, waves over the vertex and the temporo-parietal lobes of both hemispheres.

Microarray analysis revealed a deletion of the 2q24.3 band that included the *SCN1A*, *SCN2A*, and *SCN9A* genes.

ASMs tried over time included LEV, CLZ, CLB, TPM, and parathyroid hormone (PTH). The patient was treated with TPM and LEV up to 2 years and 1 month of age. CLZ was started to 1 year and 9 months as adjunctive treatment. Subsequently, TPM was gradually decreased and STP considered as alternative treatment during the first year of life. Although, at that age of 1 year seizures became almost totally controlled with low doses of TPM and LEV. No defined events were reported up to 4 years and 2 months of age, except for one brief cluster of seizures when TPM was almost fully weaned off. Hence, physicians went back to increase TPM, still present in the background therapy together with LEV and CLZ. However, the patient had a brief episode 2 weeks later. As a result, alternatively, the dosage of LEV was increased and TPM was again gradually reduced. At last follow-up, the girl showed occasional (every 6 months) blinking in front of the sun, but it is uncertain whether they are epileptic relapse, or not.

5.3 Case #2

A male infant born at 36 weeks of gestation after a pregnancy complicated by threats of abortion and fetal distress, ending up to the need of a programmed cesarean section. The boy was the second child to healthy non-consanguineous parents. Family history was unremarkable for neurological disorders. Birth weight, length, occipital frontal circumference (OCF) was within the normal range. Psychomotor development was initially normal. At the age of 2 months he was vaccinated and after 12 hours he experienced his first prolonged unilateral clonic febrile seizure, followed by a generalized tonic-clonic seizure. The EEG showed slowed background activity, while brain MRI revealed enlargement of the

subarachnoid spaces. Delayed psychomotor development became evident starting from 6 months of life. At age 7 months, the boy was admitted to the 'G. Martino' Hospital of Messina, Italy. On examination, the child showed poor visual contact, generalized hypotonia, and stereotyped afinalistic movements. In the following weeks, he had mainly febrile seizures, either myoclonic seizures, focal or massive, and complex partial seizures, isolated or in clusters, sometimes followed by prolonged generalized tonic-clonic seizures. Convulsive status epilepticus during fever also occurred. Interictal sleep EEGs showed focal paroxysmal activity, localized over the central and temporal areas of both hemispheres, sometimes generalized or predominant on the right (**Fig. 12**). An ictal EEG revealed predominantly central and fronto-temporal paroxysmal activities, followed by generalized slow activity. Clinically, the patient experienced a prolonged left hemi-tonic seizure with flushing and sialorrhea (**Fig. 13. A; B; C**)

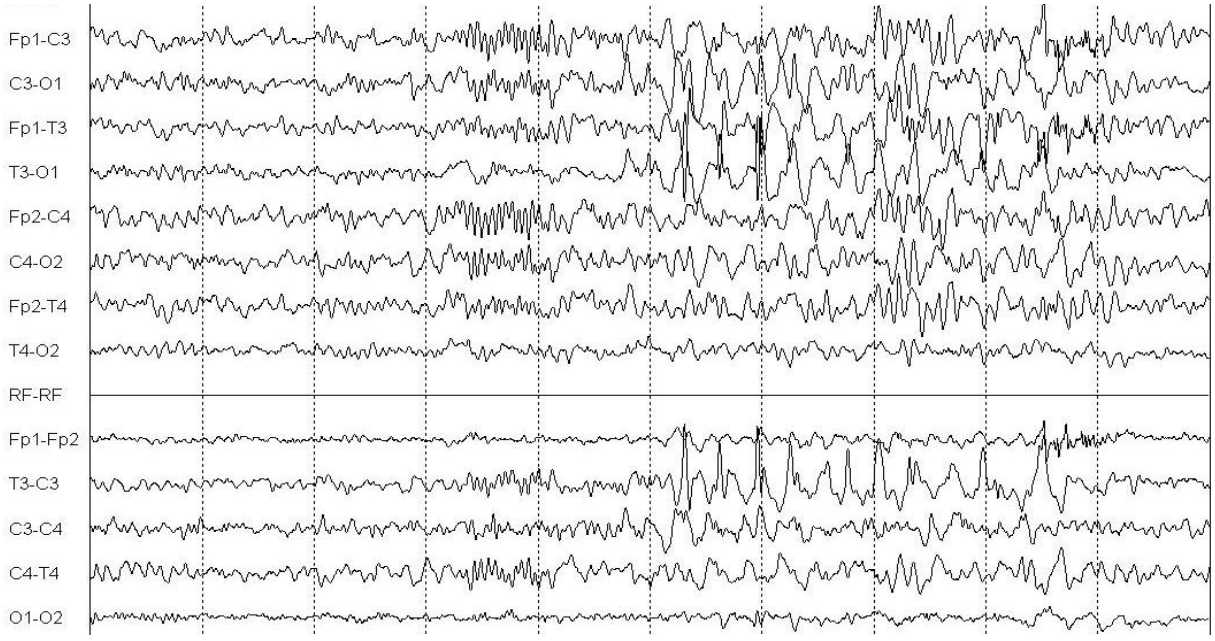

Fig.12: Interictal sleep EEG showing focal paroxysmal activity, localized over the central and temporal bilateral areas, sometimes generalized.

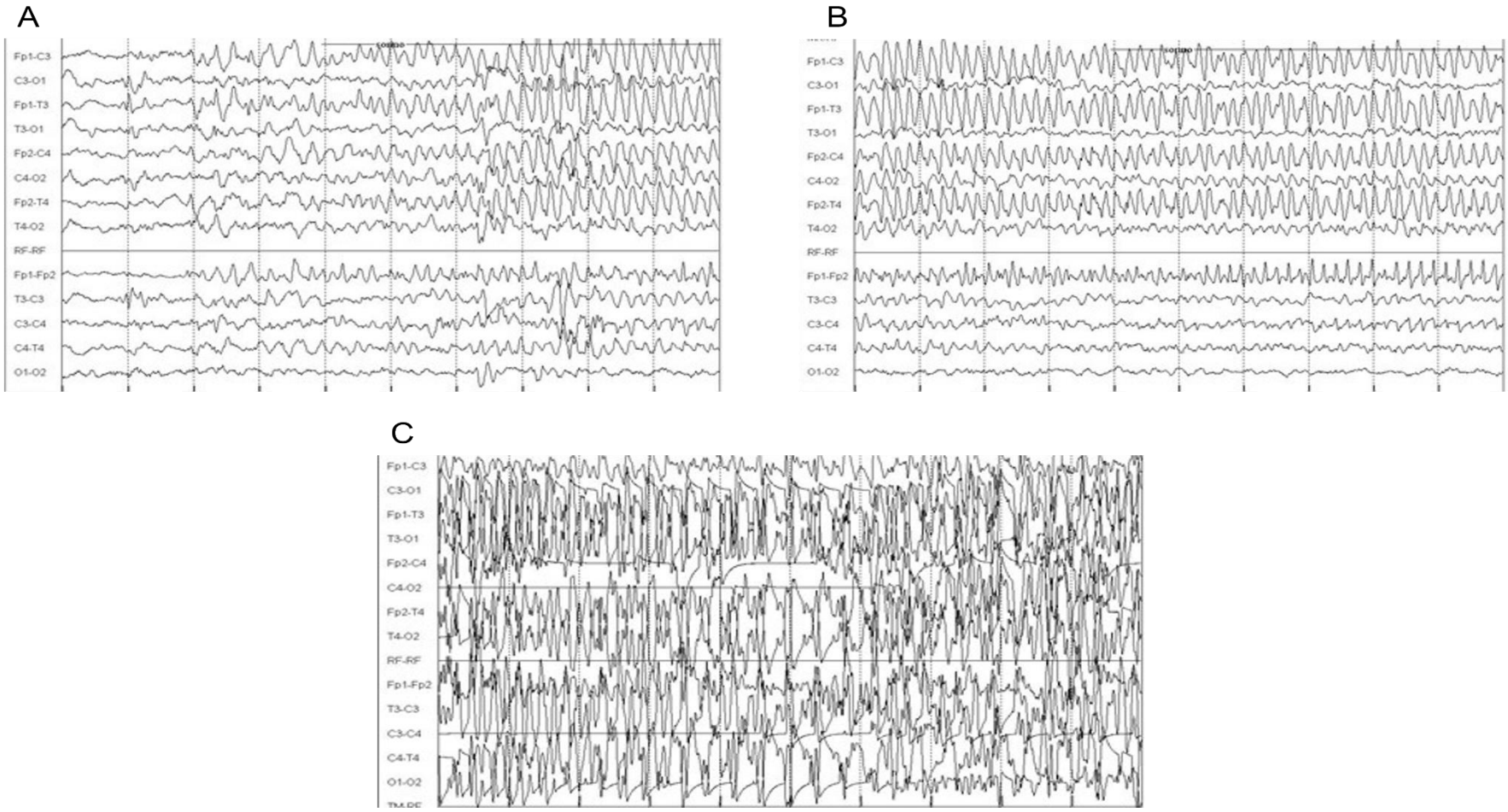

Fig. 13. A, B): Sleep EEGs showing predominantly central and fronto-temporal paroxysmal activities with tendency to diffusion; C) Ictal sleep EEG showing a burst of spike-sharp waves complexes predominant over the centro-temporal areas of the right hemisphere, but with tendency to diffusion.

Despite treatment with LEV (200 mg/day) and PHB (45 mg/day), prolonged febrile seizures recurred needing treatment with carbamazepine (CBZ), sodium and magnesium valproate, benzodiazepine (CLZ 0.5mg/day, CLB), TPM 25 mg/day, vigabatrin (VGB), tiagabine (TGB), ACTH, corticosteroid, was administered with poor success. At the age of 2 years and 1 month, the child presented a critical convulsive episode with fever (38°C). Therefore, the child was taken to the emergency department where diazepam (DZP) 5 mg was administered endorectally without resolution of the seizures. Given the sub-continuous coma symptoms, infusion therapy was started with DZP 1 vial of 10mg in 500ml of saline at a rate of 30ml/h. Due to the lack of critical control the patient was transferred to the intensive care unit where he continued infusion therapy with DZP. The infusion rate of which was gradually decreased, due to the progressive improvement of the general conditions of the child.

Neurological examination at 2 years of age showed mild generalized hypotonia, diskinetic and afinalistic moviments, oro-buccal automatisms, and autistic tracts (i.e gestual stereotypies and inconstant visual contact). Mild muscle hypotonia was present and autonomic gait was not possible, no tendon reflexes were observed. The face was not clearly dysmorphic, left divergent strabismus was evident, OCF was 45 cm (<3 SD). The child had feeding difficulties with poor growth.

The ophthalmologic examination was normal, while an abdominal sonogram showed mild hepatic steatosis and small left renal stone (2 mm on diameter).

At follow-up, seizures were mainly complex partial seizures triggered by fever, with the rare occurrence of gelastic seizures. The EEGs performed confirmed focal paroxysmal activity.

Neurometabolic investigations showed normal organic and amino acids in both urine and plasma. Also, carnitine, lactate, piruvate, routine blood, AGA, EMA, TGA dosage, and urine sampling showed normal results.

Considering the electro-clinical picture, *MECP2, CDKL5*, and *SCN1A* genes screening was performed, with negative results for point mutations. Karyotype was normal in both the patient and his parent. Array-CGH showed a del (2) (q24.3) spanning a 1.8 Mb between 165.159 and 167.008 that included haploinsufficiency of *SCN1A, SCN2A*, and *SCN9A*. Both parents displayed a normal hybridation pattern, which confirms the de novo origin of the deletion.

The patient is currently under treatment with VPA (300 mg/die), CLB (15 mg/die), STP (500 mg/die) with poor seizures control.

6. Discussion and conclusions

Most of the studies[1,7,16,21,15,3,8] in the literature reportes on the clinical picture of patients with the 2q24.3DS. An additional study[12] reviewed the previous publications, evaluating the possible different clinical and genetic presentations associated with the above-mentioned syndrome. Basing on these findings, we integrated the casuistry from the literature with the clinical descriptionof 2 novel probands with 2q24.3DS. Reviewing the different clinical histories, haploinsufficiency of SCN1A was found in all patients who presented with seizures, confirming the importance of this sodium channel in the genesis of the seizures themselves. The only exception to this is the case described by Pin F.C.[21], in which only the absence of *SCN2A* and *SCN3A* was found, and led to the development of infantile spasms. Of the entire cohort, 6 (54%) (#1; #4; #8; #9; #10; #12) out of 11 epileptic patients were resistant to multiple ASM; one patient (#11), described by Byung C.L. et al.[3], is under therapeutic control, while in patient #5, described by Madia et al.[16] TPM determined the disappearance of myoclonic jerks and the reduction in clonic seizures The patient (#13) reported by Pin F.C.[21] is effectively treated with TPM. Lastly, one of our two (#2) probands has no clear pathological recurrences and, hence, is not currently under treatment, and the patient (#3) described by Pereira S. et al.[7] was lost at follow-up. These data show that the epileptic outcome of these patients is negative in more than 50% of the cases with no response to multiple treatments, underling the difficulty in treating the various types of seizures associated with the 2q24.3DS.

As concerns with developmental delay, the outcome is unfavorable too. Twelve out of 14 patients present a severe grade developmental delay, confirming that poor seizure control almost invariably impacts over the developmental trajectories of these children. Within our

cohort, five cases of ASD, one of which is patient #1, are confirmed with an additional case (#14) of Tourette's syndrome. It should be stressed that in 6 cases psychiatric comorbidities were not known, so patients with autistic traits could be potentially even more. Three (#7; #13; #14) of the cases of ASD described, present deletion of both *SCN2A* and *SCN3A* without the involvement of *SCN1A*, and in two (#7; #14) of these patients, there is no epileptic pathology, demonstrating the importance of the SCN2A and SCN3A in the genesis of psychiatric comorbidities, and confirming again the role of *SCN1A* in the genesis of coma seizures. In the remaining two (#1; #5) cases of ASD deletions of *SCN1A*, *SCN2A*, and *SCN9A*, patient #1, and of the entire SCN cluster in patient #5 (described by Madia et al.[16]) were described. We can conclude that the only sodium channel always deleted in the 5 cases with ASD, is SCN2A, which consequently plays a key role in the development of autistic traits in the 2q24.3DS. This also points towards the need to evaluate patients with 2q24.3DS methodically and analytically for the presence of ASD, particularly when it is ascertained that the deletion involves the *SCN2A* gene. To date, SCN3A has not yet been associated with human phenotypes, but it is not excluded that its deletion may also be responsible for the appearance of neurodevelopmental and/or neurobehavioral disorders.

Finally, evaluating the other genes involved in the 2q24.3DS, we can highlight how SCL38A11, COBLL1, and GRB14 are deleted in the patient (#14) affected by Tourette syndrome and ASD. Kathrine et al.[1], in their publication, have hypothesized, based on previous studies[15,42], that these 3 genes in conjunction with the deletion of SCN2A and, SCN3A could contribute both to the development of tic symptoms and autistic traits. Interestingly, analyzing the results of the cohort, a total of 3 (#5; #13; #14) (60%) out of 5 patients with ASD showed deletion of SCL38A11 and COBLL1, and 2 (#13; #14) (40%) out

of 5 patients showed deletion of GRB14, further highlighting a possible involvement of these in the phenotypic psychiatric appearance and the need of much more studies to understand the impact of "genetic predisposition" also in complex psychiatric diseases.

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8. Supplementary materials

Supplementary Table 1: Electro-clinical features of the patients of our cohort.

	Our cases		Pereira et al., 2004	Madia et al., 2006		Bartnik et al., 2010	Celle et al., 2013
	Case 1	Case 2		Case 1	Case 2		
N° of pts	#1	#2	#3	#4	#5	#6	#7
Sex (M/F)	M	F	F	M	F	F	M
Age at last FU (y, mo)	3 y 1 mo	3 y 3 mo	2 y 6 mo	4 y	4 y	25 y	3 y
Age at sz onset (mo)	2 mo	2 mo	2 mo	8 mo	4 mo	12 mo	No
Sz type at onset	unilateral clonic febrile sz followed by GTCS; myoclonic sz;	Focal clonic sz	Autonomic sz	Febril and afebrile sz; myoclonic sz sometimes followed by	Unilateral febrile clonic sz	Absences, zone out and shiver-like episodes	No

	focal sz			GTCS			
Other sz types	Myoclonic; complex partial szs; TC sz; rare gelastic sz	GTCS; myoclonic sz; TC sz in fever and absences	TC sz; SE; myoclonic sz in fever	Absences, febSE	Febrile and afebrile myoclonic sz either focal or massive; complex partial sz	No	No
Neurological examination	mild generalized hypotonia in all 4 limbs; diskinetic and afinalistic movements, oro-buccal automatisms; gestual stereotypies; vague and inconstant visual contact	Hyperkinesia and stereotypical head and hands movements (hand washing)	abnormal behavior with no consistent fixation; poor suction and poor muscle tone	Eye-hand coordination at 20 months and expressive language abilities at 18 months; hypotonic face	stereotypical hand movement; mild generalized hypotonia; poor expressive language and eye-hand coordination	Hypotonia, ocular motor apraxia	Stereotyped sensorimotor activities; language delay and irritability
EEG findings at first visit	slowed background activity	Left temporal epileptiform abnormalities (artefactual)	Slight diffuse slowing and interictal focal changes	Slowed background activity; high-voltage generalized spike and waves; photosensitivity	Normal	Irritative abnormalities without clear epileptiform activity	Normal

EEG findings at FU	Slowed background activity with multifocal spikes-and-sharp waves complexes or polyspikes	Central and fronto-temporal paroxysmal activities with tendency to diffusion	Na	Na	Slowed background activity; focal or generalized spike and waves. The last EEG showed interictal left fronto temporal sharp and slow waves and right or left temporal sz onset	Na	Na
ASM	VPA; CLB; STP	CLZ; LEV; TPM	VGB; LTG; CLZ; PHB; PDX	VPA; TPM	VPA; PHB; TPM	No	No
DD (grade)	Yes, severe	Yes, severe	Yes, severe	Yes, severe	Yes, severe	Yes, mild	Yes, severe
Psychiatric comorbidities	ASD	No	Na	No	ASD	Bipolar and anxiety disorder; auditory hallucinations	ASD
Acquired microcephaly	No	No	Yes	No	No	No	Yes

dysmorphisms	No	Na	Cleft lip and palate; downslanting and small palpebral fissures; abnormal external ears and partially syndactyly between the second and third toes	Bulbous nose; bow-shaped mouth	Bitemporal narrowing; frontal bossing; tubular nose; anterior open bite, and palatoschisis	Short palpebral fissures; mild dental crowding, and short neck	No
Brain MRI	enlargement of subarachnoid space	Normal	Normal	Normal	Mild ventricular dilatation of lateral ventricles	Na	Na
Deletion size by CGH array	1800kb	Na	2900Kb	607Kb	3100Kb	112 Kb	230Kb
Deleted Na channels	SCN1A; SCN2A; SCN9A	SCN1A; SCN2A; SCN9A	SCN3A; SCN2A; SCN1A; SCN9A; SCN7A	SCN1A; SCN7A; SCN9A	SCN3A; SCN2A; SCN1A; SCN9A; SCN7A	SCN2A; SCN3A	SCN3A; SCN2A
Outcome	VPA, CLB, STP with poor sz control; severe DD, ASD	Sz well controlled with LEV, TPM, and CLZ; severe DD	Sz outcome NA; severe DD	VPA and TPM not resolving on myoclonic and absence sz	TPM resulted in disappearance of myoclonic jerks and reduction in clonic sz frequency	Mild DD, bipolar disorder, auditory hallucinations, no sz, unable to live independently	ASD, language delay

	Byung et al., 2014					Pin et al., 2018	Kathrin et al., 2018
	Case 1	Case 2	Case 3	Case 4	Case 5		
N° of pts	#8	#9	#10	#11	#12	#13	#14
Sex (M/F)	F	M	M	M	F	M	M
Age at last FU (y, mo)	5 y 8 mo	2 y 5 mo	1 y 3 mo	2 y 9 mo	9 y 8 mo	1 y 8 mo	28 y
Age at sz onset (mo)	3 mo	1 mo	2 mo	7 mo	4 mo	9/10 mo	No seizure
Sz type at onset	TC sz	Autonomic sz	Focal febrile sz	FebSE	HC sz	Infantile spams;WS	No sz

Other sz types	Myoclonic sz in fever; autonomic sz	Myoclonic febrile and afebrile sz; TC sz	Myoclonic febrile sz; HC and autonomic sz	TC sz in fever; HC sz in fever	TC sz	Na	No sz
Neurological examination	Na	Poor eye contact and difficult head control	No head control and fixation	Na	Independent walking at the age of 18 mo but severe language delay	Generalized hypotonia, head nodding and poor head control; upward eye deviations; dystonia-like movements; absence of eye contact and smiles, stereotypic behavior	hyperkinesia with auto-aggressive beahvior; stereotypical hand movement and simple vocal tics
EEG findings at first visit	Multifocal epileptiform discharges	Multifocal epileptiform discharges with diffuse theta to delta slowings	Multifocal epileptiform discharges from the left or right temporal and frontal areas	Normal	High-amplitude delta activity on whole background and frequent multifocal spike or polispike discharges from the right or left frontal or occipital areas	Hypsarrhythmic pattern when sleeping and awake	Slow background activity without superimposed clear epileptiform abnormalities
EEG findings at FU	Na	Na	Na	Normal	Na	Na	Na

ASM	LEV; TPM; CLB; KD	Refractory to multiple ASM	VPA; LEV; TPM; KD	VPA	VPA; PHB; TPM	PDX; VPA; ACTH; TPM	No
DD (grade)	Yes, severe	Yes, severe	Yes, severe	No	Yes, severe	Yes, severe	Yes, severe
Psychiatric comorbidities	Na	Na	Na	Na	Na	ASD	ASD and Tourette syndrome
Acquired microcephaly	Yes	Yes	Na	No	Na	No	Yes
dysmorphisms	Na	Na	Na	Na	Na	Upslanted palpebral fissure, hypertelorism and cupid's bow mouth	Synophrys, epicanthus, modelled ears and deep joined thumb
Brain MRI	Progressive diffuse atrophy	Mild diffuse atrophy	Mild diffuse atrophy	Normal	Mild diffuse atrophy	Normal	Normal
Deletion size by CGH array	8400 Kb	4300 Kb	1500 Kb	200 Kb	200 Kb	1,102Kb	719 Kb

Deleted Na chennels	SCN3A; SCN2A; SCN1A; SCN9A; SCN7A	SCN3A; SCN2A; SCN1A; SCN9A; SCN7A	SCN3A; SCN2A; SCN1A; SCN9A; SCN7A	SCN1A	SCN1A; SCN9A	SCN2A; SCN3A	SCN2A; SCN3A
Outcome	Refractoty to TPM, LEV, CLB and KD; severe DD	Refractory to multiple ASM; severe DD	Refractory to VPA, LEV, TPM, and KD; severe DD	Fair response to LEV; normal cognitive outcome	Refractory to PHB, VPA and TPM; severe DD	PDX and VPA did not improve, ACTH was effective, control with TPM; ASD and DD	ASD and Tourette syndrome

Legend pts:

ACTH: adrenocorticotropic hormone; ASD: autistic spectrum disorder; ASM: anti-seizure medication; CGH: comparative genomic hybridization; CLB: clobazam; CLZ: clonazepam; DD: developmental delay; EEG: electroencephalogram; FC: focal clonic seizure; FebSE: febrile status epilepticus; FU: follow up; GTCS: generalized tonic-clonic seizures; HC: hemiclonic seizure; LEV: levetiracetam; LTG: lamotrigine; KD: ketogenic diet; MO: month; PHB: phenobarbital; PDX: pyridoxine; PTS: patients; SE: Status epilepticus; SZ: seizure; TC: tonic-clonic seizure; TPM: topiramate; VGB: vigabatrin; VPA: valproic acid; Y: year

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