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CLINICAL REPORT

Two new cases of interstitial 7q35q36.1 deletion including *CNTNAP2* and *KMT2C*

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Abstract

Background: Terminal deletions of the long arm of chromosome 7 are well known and frequently associated with syndromic holoprosencephaly due to the involvement of the *SHH* (aliases *HHG1*, *SMMCI*, *TPT*, *TPTPS*, and *MCOPCB5*) gene region. However, interstitial deletions including *CNTNAP2* (aliases *Caspr2*, *KIAA0868*, and *NRXN4*) and excluding the *SHH* region are less common.

Methods: We report the clinical and molecular characterization associated with pure 7q35 and 7q35q36.1 deletion in two unrelated patients as detected by oligonucleotide-based array-CGH analysis.

Results: The common clinical features were abnormal maternal serum screening during first-trimester pregnancy, low occipitofrontal circumference at birth, hypotonia, abnormal feet, developmental delay, impaired language development, generalized seizures, hyperactive behavior, friendly personality, and cranio-facial dysmorphism. Both deletions occurred de novo and sequencing of *CNTNAP2*, a candidate gene for epilepsy and autism showed absence of mutation on the contralateral allele.

Conclusion: Combined haploinsufficiency of *GALNTL5* (alias *GalNAc-T5L*), *CUL1*, *SSPO* (aliases *SCO-spondin*, *KIAA0543*, and *FLJ36112*), *AOC1 (*alias *DAO*), *RHEB*, and especially *KMT2C* (alias *KIAA1506* and *HALR*) with monoallelic disruption of *CNTNAP2* may explain neurologic abnormalities, hypotonia, and exostoses. Haploinsufficiency of *PRKAG2* (aliases *AAKG*, *AAKG2*, *H91620p*, *WPWS*, and *CMH6*) and *KCNH2* (aliases *Kv11.1*, *HERG*, and *erg1*) genes may be responsible of long QT syndrome observed for one patient.

KEYWORDS
7q35q36.1, array-CGH, *CNTNAP2* disruption, interstitial deletion, *KMT2C* haploinsufficiency

1 | INTRODUCTION

Genomic rearrangements contribute to a substantial fraction of human genetic variation and are responsible for a wide variety of congenital malformations and intellectual disability (ID). The deletion of the distal 7q region is a rare chromosomal anomaly associated with multisystemic involvement and cardiac implications (Ayub et al., 2016). Terminal deletions of the long arm of chromosome 7 including 7q35 and/or 7q36 region are well known. They are frequently associated with growth retardation, microcephaly, large malformed ears, cleft lip and palate, sacral malformations, or agenesis, and holoprosencephaly (with involvement of the *SHH* [OMIM#600725], *En2* [OMIM#131310], and *HTR5A* [OMIM#601305, alias *5-HT5A*]) genes located in 7q36.3; Ayub et al., 2016). However, the report of interstitial deletion that does not implicate the *SHH* region is missing. Indeed, deletions affecting a more proximal part, namely, bands 7q35q36.1, are less common (Fagan et al., 1994; Friedman et al., 2008; Smogavec et al., 2016; Suri & Dixit, 2017). We report two patients with a 4.4-Mb interstitial deletion in 7q36.1 and a 4.8-Mb interstitial deletion in 7q35q36.1 detected by array-comparative genomic hybridization. We compared both deletions to previously reported patients and proposed a phenotype characterized by ID, unusual facial features, and prolonged QT interval due to loss and as a result of the haploinsufficiency of *GALNTL5* (OMIM#615133), *CUL1* (OMIM#603134), *SSPO* (OMIM#617356), *KMT2C* (OMIM#606833), *AOC1* (OMIM#104610), *RHEB* (OMIM#601293), *PRKAG2* (OMIM#602743), *KCNH2* (OMIM#152427) combined with monoallelic disruption of *CNTNAP2* (OMIM#604569).

2 | MATERIAL AND METHODS

2.1 | Ethical compliance

The study was approved by an ethics committee. Informed consent for genetic analyses was obtained from the parents according to local ethical guidelines.

2.2 | Clinical presentation and family history

Patient 1 was born to unrelated Caucasian parents aged 27 years and 34 years at the time of conception. Indicated by abnormal maternal serum screening for the risk of aneuploidy at the end of first trimester of pregnancy, fetal karyotype was established on amniotic fluid, yielding a 46,XX complement. Her birth parameters at 39 weeks of amenorrhea were within the normal range for weight and height (2960 g; 49 cm) whereas occipitofrontal circumference (OFC) was at −2.2 SD (31 cm). Hypotonia was noted. She showed an abnormality of the talus. Her developmental milestones were delayed: walking without assistance was obtained at 22 months, and no language was recorded at 3 years, at the time of her first generalized seizures that occurred without fever. Brain magnetic resonance imaging (MRI) was normal. Absences and complex partial crises occurred thereafter, prompting a treatment with valproate. A short attention span, poor motor coordination, and stereotypy were noted. At the age of 18, the patient biometric parameters were 44 kg for weight, 153 cm for height, and 50 cm for OFC (−4 SD). She presented with hirsutism, retrognathia, synophrys, equinovarus,deformity and moderate to severe intellectual disability. Cardiac examination revealed a prolonged QT interval. She had about one seizure crisis per year and was treated with lamotrigin. At the age of 18, she could not read or write. She needed supervision to wash and dress herself.

Patient 2 is the only child of unrelated Caucasian parents. Pregnancy was marked by maternal diabetes and abnormal maternal serum screening at the end of first trimester. Noninvasive prenatal testing was negative for trisomy 13, 18, and 21. The boy was born at 38 weeks of amenorrhea, and fetal cardiac arrhythmia was observed. Birth parameters were within the lower range for weight, height, and OFC (2600 g, 46 cm, and 34.5 cm, respectively). He presented with moderate axial and peripheral hypotonia, cryptorchidism, flexion contracture of the four limbs, and wide intermammillary distance. Clinical findings were a skin excess, equinovarus deformity, and a cranio-facial dysmorphism with low-set ears, prominent forehead, retrognathia, hypertelorism, anteverted nares, scaphocephaly, and horizontal palpebral fissures. Brain MRI showed partial and posterior agenesis of the corpus callosum and absence of irregularity of the white matter. Skeletal X-ray radiograph highlighted hypertelorism and prominent forehead. Ophthalmologic examination, electroencephalogram, as well as abdominal, cardiac, and renal ultrasound were otherwise unremarkable. At the age of 1 month, eye monitoring was slight, axial hypotonia was still present, but peripheral tone was normal. External genitalia were normal. The patient showed orality troubles, and seizures were suspected on electroencephalogram. His developmental milestones were delayed: walking without assistance was obtained at 24 months but remained uncertain, and language was limited to few words. Hyperactive behavior and friendly personality were noted. Global comprehension was good, and the sleep as well as the feeding was satisfactory. Since the age of 30 months, he presented three generalized seizures without fever. At 3 years of age, height was 86 cm (−2.14 SD), OFC was 49.5 cm (normal range), and weight was 12.3 kg (−0.9 SD). Dysmorphic features at the age of 3 years are showed in Figure 1.

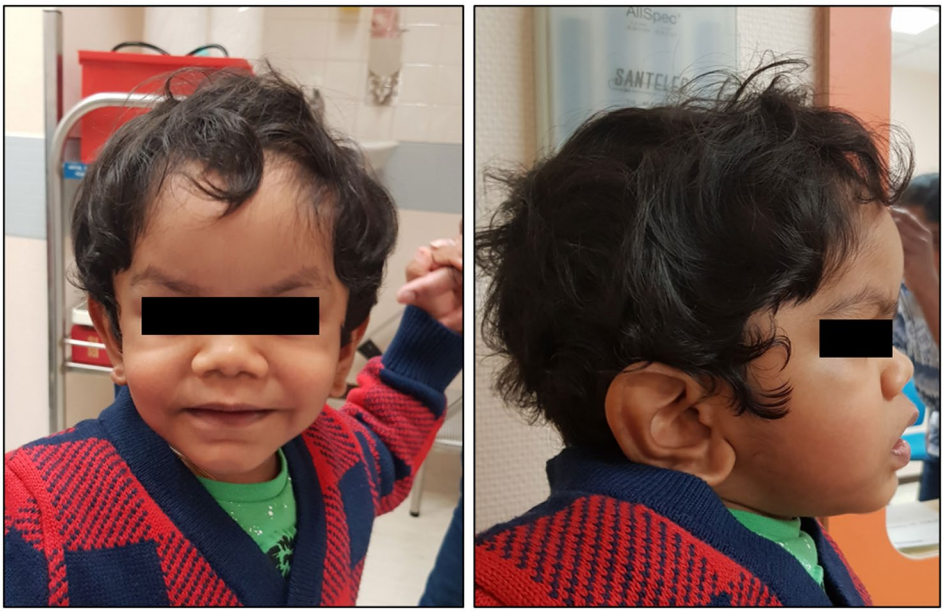

FIGURE 1 Photographs of the face and profile of patient 2 at 3 years of age demonstrating facial dysmorphism including low-set ears, prominent forehead, retrognathia, hypertelorism, anteverted nares, scaphocephaly, and horizontal palpebral fissures

2.3 | Conventional cytogenetic analysis

Chromosome analysis was carried out for patient 1 using conventional cytogenetic techniques from synchronized peripheral blood lymphocytes and amniotic fluid cultures and stained by RHG banding and GTG banding.

2.4 | Array-comparative genome hybridization

Genomic DNA was isolated from probands peripheral blood. Agilent oligonucleotide human 180 K array for both patients was used to detect chromosomal abnormalities. Comparison was to sex-matched blood donor DNA. Hybridization was performed according to the manufacturer's protocol. The slides were scanned on an Agilent Microarray Scanner. Images processing and data analysis were performed with CytoGenomics software 4.0.3.12 (Agilent Technologies). ADM2 algorithm was used for statistical analysis. Copy number variations were considered significant if they could be defined by 4 or more oligonucleotides spanning at least 52 Kb, contained at least one gene, and were not identified in the Database of Genomic Variants (http://projects.tcag.ca/cgi-bin/variation/gbrowse/hg19). Results were also checked against the Cartagenia database of the French Copy Number Variation Consortium (https://abc.cnv.cartagenia.com). The Genome Browser used to analyze genes content was hg19, Build37 (http://genome.ucsc.edu/).

2.5 | Fluorescence in situ hybridization

FISH analysis was performed on metaphase spreads from lymphocytes. BACs clones specific for the 7q36.1 region were used: RP11-445N20 (Bluegnome, France) for patient 1 and her parents, and RP11-933K14 (RainbowFISH, Amplitech, France) for patient 2 and his parents. Probes targeting 7pter and 7qter subtelomeres regions were used as a control (Vysis Downers Grove, IL, USA).

2.6 | *CNTNAP2* sequencing

Genomic DNA was isolated from probands peripheral blood. Next generation sequencing (NGS) was performed using a custom sequence capture (Nimblegen SeqCap EZ Choice system, Roche) of the exons and the flanking intronic sequences of the *CNTNAP2* gene. Sequencing was performed on MiSeq (Illumina) platforms according to the manufacturer's instructions. The conventional bioinformatics pipeline, previously described, was used to study germline mutations.

3 | RESULTS

Cytogenetic analysis of the amniotic cells showed a normal female karyotype 46,XX for proband 1 that was confirmed in post-natal period. For proband 2, conventional karyotype was not realized because array-CGH was the first intention technic.

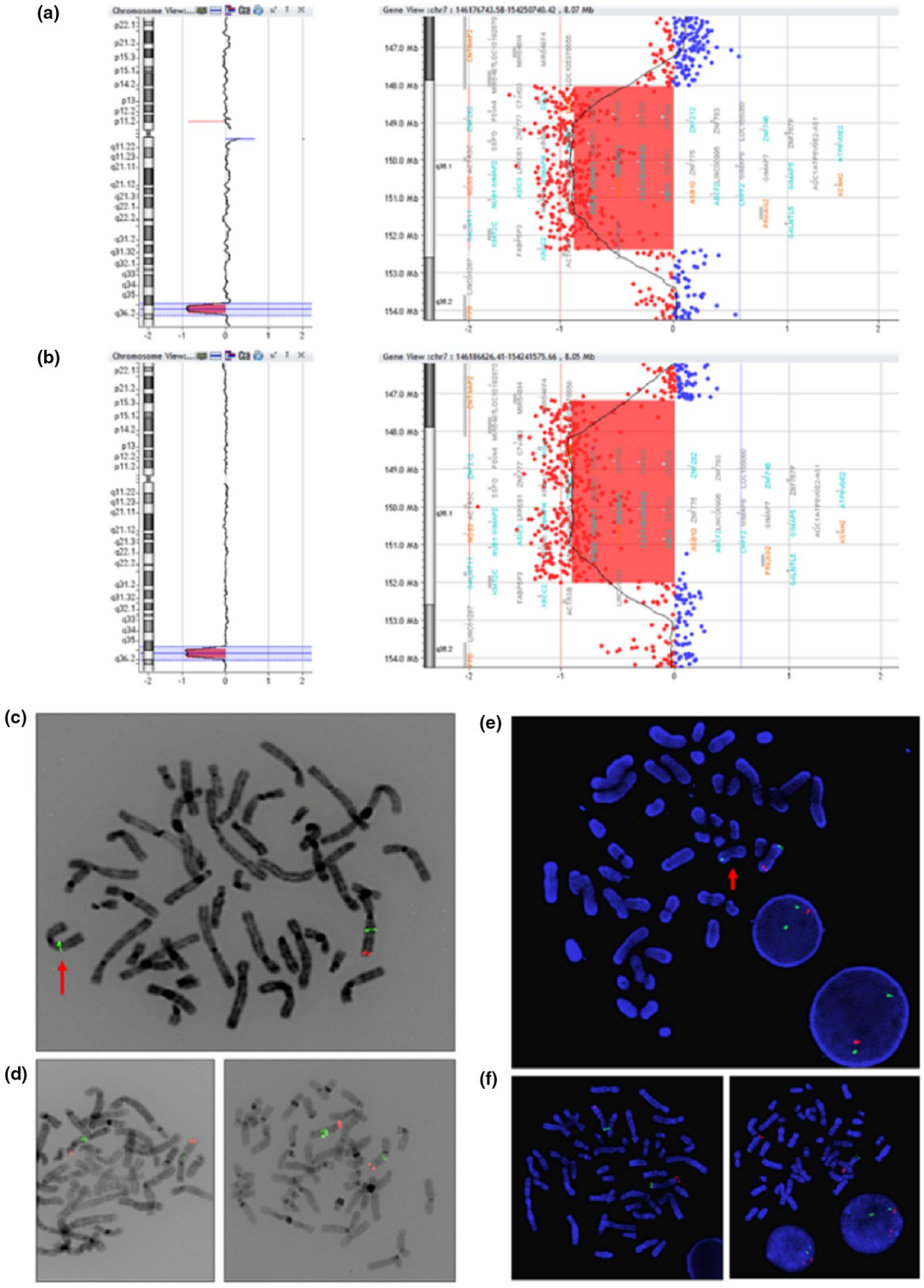

FIGURE 2 Array CGH results. (a and b) Chromosome 7 profile with interstitial deletion 7q36.1 of 4.3 Mb in patient 1 and interstitial deletion 7q35 of 4.8 Mb in patient 2. (c) FISH results for patient 1 using 7q36.1 targeted probe (RP11-445N20, red) and control probe on 7q21.11 (RP4-560O14, green). (d) FISH results for patient 1 parents (mother, left panel; father, right panel) using 7q36.1 targeted probe (RP11-445N20, red) and control probe on 7q21.11 (RP4-560O14, green). (e) FISH results for patient 2: 7q36.1 targeted probe (RP11-933K14, red) and a control probe on 7qter (RP4-560O14, green). (f) FISH results for patient two parents (mother, left panel; father, right panel) with 7q36.1 targeted probe (RP11-933K14, red) and on 7qter (RP4-560O14, green)

180 K array-CGH on genomic DNA revealed an interstitial 7q36.1 and 7q35q36.1 deletion sizing 4.3 Mb in patient 1 and 4.8 Mb in patient 2, respectively (Figure 2). The imbalances were reported according to ISCN 2016: Patient 1: arr[GRCh37] 7q36.1(148,039,951x2,148,047,494–152,379,990x1,152,397,173x2) (Figure 2a); Patient 2:

arr[GRCh37] 7q35q36.1(147,157,477x2,147,180,572–152,004,588x1,152,012,189x2) (Figure 2b). Common genes, pseudogenes, or open reading frames included in the deleted region were: *CNTNAP2*, *C7orf33*, *CUL1*, *EZH2* (aliases *EZH1*, *ENX-1*, *KMT6*, and *KMT6A*), *RNY5* (alias *hY5*), *RNY4* (alias *hY4*), *RNY3* (alias *hY3*), *RNY1* (alias *hY1*), *GHET1* (alias *lncRNA-GHET1*), *PDIA4* (aliases *ERP70* and *ERP72*), *ZNF786* (alias *DKFZp762I137*), *ZNF425*, *ZNF398* (aliases *ZER6*, *KIAA1339*, *P51*, and *P71*), *ZNF282* (alias *HUB1*), *ZNF212* (alias *C2H2-150*), *ZNF783* (alias *DKFZp667J212*), *LOC155060*, *ZNF777* (alias *KIAA1285*), *ZNF746* (aliases *FLJ31413* and *PARIS*), *ZNF767P* (alias *FLJ12700*), *KRBA1* (alias *KIAA1862*), *ZNF467* (aliases *EZI* and *Zfp467*), *SSPO*, *ZNF862*, *ATP6V0E2-AS1*, *ATP6V0E2*, *ACTR3C* (alias *ARP11*), *LRRC61* (aliases *MGC3036*, *FLJ31392*, and *HSPC295*), *ZBED6CL* (alias *C7orf29*), *RARRES2* (aliases *TIG2* and *HP10433*), *REPIN1* (aliases *RIP60*, *AP4*, *H_DJ0584D14.12*, and *Zfp464*), *ZNF775* (alias *MGC33584*), *LOC728743*, *LINC00996*, *GIMAP8* (aliases *DKFZp667I133*, *hIAN6*, and *IAN9*), *GIMAP7* (aliases *MGC27027* and *IAN7*), *GIMAP4* (aliases *HIMAP4*, *FLJ11110*, *IMAP4*, and *IAN1*), *GIMAP6* (aliases *FLJ22690* and *IAN6*), *GIMAP2* (aliases *DKFZp586D0824*, *HIMAP2*, *IMAP2*, and *IAN12*), *GIMAP1* (aliases *HIMAP1*, *IMAP38*, *IMAP1*, and *IAN2*), *GIMAP1-GIMAP5*, *GIMAP5* (aliases *HIMAP3* and *IAN5*), *TMEM176B* (aliases *LR8* and *MS4B2*), *TMEM176A* (aliases *HCA112* and *MS4B1*), *AOC1*, *KCNH2*, *NOS3* (aliases *ECNOS* and *eNOS*), *ATG9B* (aliases *FLJ14885*, *APG9L2*, and *SONE*), *ABCB8* (aliases *EST328128*, *M-ABC1*, *MABC1*, and *MITOSUR*), *ASIC3* (aliases *TNaC1* and *DRASIC*), *CDK5* (alias *PSSALRE*), *SLC4A2* (aliases *HKB3*, *BND3L*, and *NBND3*), *FASTK* (alias *FAST*), *TMUB1* (aliases *SB144* and *HOPS*), *AGAP3*, *GBX1*, *ASB10*, *IQCA1L* (alias *TCAG_9762*), *ABCF2* (aliases *EST133090*, *ABC28*, *M-ABC1*, and *HUSSY-18*), *CHPF2* (aliases *KIAA1402*, *ChSy-3*, and *CSGlcA-T*), *MIR671* (alias *hsa-mir-671*), *SMARCD3* (aliases *BAF60C*, *Rsc6p*, and *CRACD3*), *NUB1* (aliases *BS4*, *NYREN18*, and *NUB1L*), *WDR86*, *WDR86-AS1*, *CRYGN*, *MIR3907* (alias *hsa-mir-3907*), *RHEB*, *PRKAG2*, *PRKAG2-AS1*, *GALNTL5*, *GALNT11* (alias *GalNAc-T11*), *KMT2C*, *FABP5P3* (alias *TCAG_1781704*), and *LINC01003* (Table S1). In patient 1, chromosomal deletion included the additional gene *XRCC2* (alias *FANCU*).

FISH assays were carried out on lymphocytes of the probands and their parents. To target 7q36.1 region, RP11-445N20 and RP11-933K14 BAC probes gave one signal on abnormal chromosome 7 for patients 1 and 2, respectively; and two signals on normal chromosome 7 for both patients (Figure 2c,e). No hybridization signal of these BAC probes was detected on any other chromosome. These results were in accordance with the array-CGH results. In parents of both cases, two signals were observed on both chromosomes 7 indicating the de novo inheritance of the deletion (Figure 2d,f).

NGS of *CNTNAP2* did not show any mutation. However, the 5′ breakpoint of the deletion was specified for both patients using DeCovA tool for displaying gene coverage (Dimassi et al., 2015). Indeed, the 5′ end proximal breakpoint of patient 1 was located on chromosome 7 between 147,964,268 and 148,080,690 positions and the one of patient 2 on chromosome 7 between 147,092,922 and 147,182,976 positions using the hg19 assembly. Combined with array-CGH data patient 1 proximal breakpoint was estimated into intron 21 and for patient 2 into intron 10.

4 | DISCUSSION

Array-CGH is a powerful tool for detecting structural unbalanced chromosomal rearrangements in clinical diagnostics by providing high density coverage of coding and noncoding DNA by oligonucleotide probes and with excellent specificity. It is offered primarily in patients with ID and congenital malformations or for prenatal diagnosis (Ayub et al., 2016; Naud et al., 2017; Szczaluba & Demkow, 2017; van der Zwaag et al., 2009). Interestingly, our patients had in common abnormal maternal serum screening for the risk of aneuploidy at the end of first trimester of the pregnancy. At birth, the OFC was in the lower range and muscular hypotonia and abnormal feet were observed for both patients. Later, they presented with developmental delay, impaired language development, generalized seizures, hyperactive behavior, friendly personality, and cranio-facial features such as anteverted nares and retrognathia. The common part of the deletion (chr7:148,047,494– 152,004,588) includes 75 genes, pseudogenes, or open reading frames (Table S1). In addition, the *XRCC2* gene was deleted in patient 1. Among these genes, *CNTNAP2*, *EZH2*, *KCNH2*, *NOS3*, *CDK5*, *ASB10*, *PRKAG2*, *KMT2C*, and *XRCC2* genes were associated with an OMIM phenotype (Table S1). Through a systematic review of patients carrying a pure heterozygous 7q deletion which boundaries were included in those of our patients, we identified three patients in literature reports (Friedman et al., 2008; Smogavec et al., 2016; Suri & Dixit, 2017), including one case with two intragenic deletions in *CNTNAP2* gene an, four cases in the DECIPHER database (https://decipher.sanger.ac.uk). The clinical and the molecular data from nine patients (two from this study and seven previously reported) carrying a 7q35 and/or 7q36.1 deletion are summarized in Table 1 and Figure 3. Relevant clinical features included learning disability, ID, global developmental delay, behavioral abnormality, delayed speech, and language development and/or autistic behavior in 8/9 of the patients; cardiovascular anomalies (5/9); particular facial features (4/9); seizures (4/9); motor delay (4/9); short stature (3/9); low OFC at birth (2/9); and abnormality affecting skeletal system or limbs (2/9). According to the repartition of the nine cases of

TABLE 1 Genotype–phenotype correlation

		Patient 1	Patient 2	Suri and Dixit (2017)	Patient 2 Friedman et al. (2008)	Patient 3 Smogavec et al. (2016)	Decipher 293275	Decipher 303634	Decipher 357002	Decipher 360726
Patient and genetic characteristics	Gender	F	M	M	F	M	M	M	F	M
	age at last clinical assessment (years)	<1	<1	11	53	4.5	5	12	22	7
	Size of the deletion (Mb)	4.3	4.8	1.2	1.5	1.23	1.09	0.086	0.031	0.937
	Chromosomal region	7q36.1	7q35q36.1	7q36.1	7q35q36.1	7q35q36.1	7q35q36.1	7q36.1	7q36.1	7q36.1
	Deletion boundaries (hg19)	148,047,494–152,379,990	147,180,572–152,004,588	148,456,556–149,666,328	146,670,000–148,500,000 (estimated)	146,730,472–147,928,239	147,369,972–148,464,598	151,884,663–151,971,034	150,643,965–150,674,926	149,280,420–150,218,014
	Inheritance	dn	dn	dn	NR	pat	mat	dn	NR	NR
	Associated CNV on CNTNAP2	–	–	–	–	Del 7q35 145,795,795–145,824,743 mat of 0.056 Mb	–	–	–	–
Birth	Low OFC	+	+	–	NR	–	NR	NR	NR	NR
Growth abnormality	Asymmetric growth	–	+	+	NR	+	NR	+	NR	NR
	Short stature	–	+	–	NR	+	NR	+	NR	NR
	Tall stature	–	–	+	NR	–	NR	NR	NR	NR
Abnormality of the respiratory system	Respiratory distress	–	+	NR	NR	NR	NR	NR	NR	NR
Abnormality of the nervous system	Short attention span	+	NR	NR	NR	NR	NR	NR	NR	NR
	Specific learning disability	+	NR	+	NR	NR	NR	NR	NR	+
	Intellectual disability	+	NR	+	+	+	NR	NR	NR	NR
	Global Developmental Delay	+	+	+	NR	NR	+	NR	NR	NR
	Motor delay	+	+	+	NR	+	NR	NR	NR	NR
	Behavioral abnormality	+	+	–	+	+	NR	NR	NR	+
	Seizures	+	+	–	+	+	NR	NR	NR	NR
	Delayed speech and language development	+	+	+	+	–	+	NR	NR	+
	Autistic behavior	–	–	–	NR	NR	NR	+	NR	NR
	Sleep disturbance	–	–	NR	NR	NR	NR	NR	NR	NR

(Continues)

TABLE 1 (Continued)

		Patient 1	Patient 2	Suri and Dixit (2017)	Patient 2 Friedman et al. (2008)	Patient 3 Smogavec et al. (2016)	Decipher 293275	Decipher 303634	Decipher 357002	Decipher 360726
	Holoprosencephaly	−	−	−	NR	NR	NR	NR	NR	NR
	Abnormality of the corpus callosum	−	+	NR	NR	NR	NR	NR	NR	NR
Abnormality of the musculature	Muscular hypotonia	+	+	+	NR	NR	NR	NR	NR	NR
Facial dysmorphy	Abnormality of the face	+	+	+	NR	−	NR	NR	NR	NR
	Short nose	+	−	NR	NR	NR	NR	NR	NR	NR
	Periorbital edema	+	−	NR	NR	NR	NR	NR	NR	NR
	Anteverted nares	+	+	NR	NR	NR	NR	NR	NR	NR
	Abnormality of the eyebrow	+	−	NR	NR	NR	NR	NR	NR	NR
	Micrognathia/ retrognathia	−	+	+	NR	NR	NR	NR	NR	NR
	Microcephaly	+	+	NR	NR	NR	NR	NR	NR	NR
	Scaphocephaly	−	+	NR	NR	NR	NR	NR	NR	NR
	Synophrys	−	−	NR	NR	NR	NR	+	NR	NR
	Prominent forehead/ Frontal bossing	−	+	NR	NR	NR	NR	NR	NR	NR
	Low−set ears	−	+	NR	NR	NR	NR	NR	NR	NR
	Hypertelorism	−	+	+	NR	NR	NR	NR	NR	NR
	Abnormality of the palpebral fissures	−	+	+	NR	NR	NR	NR	NR	NR
Abnormality of the integument	Elbow hypertrichosis	+	−	NR	NR	NR	NR	NR	NR	NR
	Coarse hair	−	−	+	NR	NR	NR	NR	NR	NR
	Spotty hyperpigmentation	−	−	+	NR	NR	NR	NR	NR	NR
Abnormality of the breast	Wide intermamillary distance	−	+	NR	NR	NR	NR	NR	NR	NR
Abnormality of the genitourinary system	Cryptorchidism	−	+	−	−	NR	NR	NR	−	NR
	Hypospadias	−	−	−	−	NR	NR	NR	−	NR
Neoplasm	Exostoses	+	−	NR	NR	NR	NR	NR	NR	NR
Abnormality of the skeletal system	Scoliosis	−	−	−	NR	NR	NR	NR	NR	NR
	Pectus excavatum	−	−	+	NR	NR	NR	NR	NR	NR
	Sloping shoulders	−	−	+	NR	NR	NR	NR	NR	NR

(Continues)

TABLE 1 (Continued)

		Patient 1	Patient 2	Suri and Dixit (2017)	Patient 2 Friedman et al. (2008)	Patient 3 Smogavec et al. (2016)	Decipher 293275	Decipher 303634	Decipher 357002	Decipher 360726
Abnormality of limbs	Equinovarus	–	+	NR	NR	NR	NR	NR	NR	NR
	Abnormality of the talus	+	–	NR	NR	NR	NR	NR	NR	NR
Abnormality of the cardiovascular system	Arrhythmia	–	+	–	+	NR	NR	+	NR	NR
	Long QT syndrome	+	–	–	NR	NR	NR	NR	+	NR

Note: Summary of clinical and genetic features in present patients and previously reported case of deletion including 7q35 and/or q36.1 region.

Abbreviations: dn, *de novo*; F, female; hmz, homozygous; htz, heterozygous; M, male; mat, maternally inherited; NR, not reported; pat, paternally inherited.

deletion 7q (7q35q36.1 or 7q36.1), we identified five overlapping regions (Figure 3 and Table 2).

CNTNAP2 encodes for a transmembrane protein CASPR2 which is a cell adhesion factor bellowing to the neurexin super-family. It mediates cell–cell interactions in the nervous system. Neurexins have been shown to play a role in the development of the nervous system, synaptic functions, and neurological diseases. Rare and common variations in *CNTNAP2* confer a risk for developmental language and autism spectrum disorders (Peñagarikano & Geschwind, 2012). *CNTNAP2* has also been linked to schizophrenia, epilepsy, ID, learning,disability and attention-deficit/hyperactivity disorder (ADHD; Rodenas-Cuadrado et al., 2016). Monoallelic or biallelic null mutations in *CNTNAP2* have been found in patients with syndromic ID (Gregor et al., 2011; Smogavec et al., 2016; Zweier et al., 2009), and heterozygous rearrangements and CNVs involved *CNTNAP2* in a variety of neurodevelopmental disorders including ID or ADHD (Malhotra & Sebat, 2012; Poot, 2015; Rodenas-Cuadrado et al., 2016). Both of our patients showed developmental delay and epilepsy and carried a deletion which 5' boundary interrupted *CNTNAP2*. To verify if a second genetic event was present, *CNTNAP2* sequencing was realized. No pathogenic variant was highlighted but it is of note that transcripts abnormalities have not been explored and abnormal splicing event could have happened. Because patients carried a *de novo* monoallelic impairment of *CNTNAP2* its incidence on phenotype could not be excluded.

CUL1 (cullin 1) is a negative regulator of the cell cycle as in cul1 mutants, the G1-to-S-phase progression is accelerated, overriding mechanisms for mitotic arrest. As a result, CUL1 is mainly involved in the regulation of proliferation and tumorigenicity. Moreover, Hagens et al. (2006) reported that the predicted human FBP hFBX25 that confer substrate specificity to the SCF-type (Skp1/Cul1/FBP) was found to be disrupted in a translocation carrier suffering from ID and epileptic seizures. The study showed strong transcription in human brain. In mice, mFbx25 shows predominantly neuronal expression in embryos. In adult, the expression is confined to the hippocampus, the cerebral cortex, and the Purkinje cell layer (Hagens et al., 2006). *SSPO* (SCO-spondin) is a large secreted glycoprotein conserved among mammals. It is expressed in developing central nervous system and was shown to increase neurite length and outgrowth in a dose-dependent manner when overexpressed in rat neuroblastoma cells. Thus, even if *CUL1* and *SSPO* had not been related to neurodevelopmental disorders such as ID, we cannot exclude an impact of their haploinsufficiency in view of their expression and function in the brain.

PRKAG2 and *KCNH2* genes are implicated in cardiopathies associated with a dominant inheritance. *KCNH2* encodes for a cardiac potassium channel and was related to long QT syndrome. Point mutation and deletion on *KCNH2* are

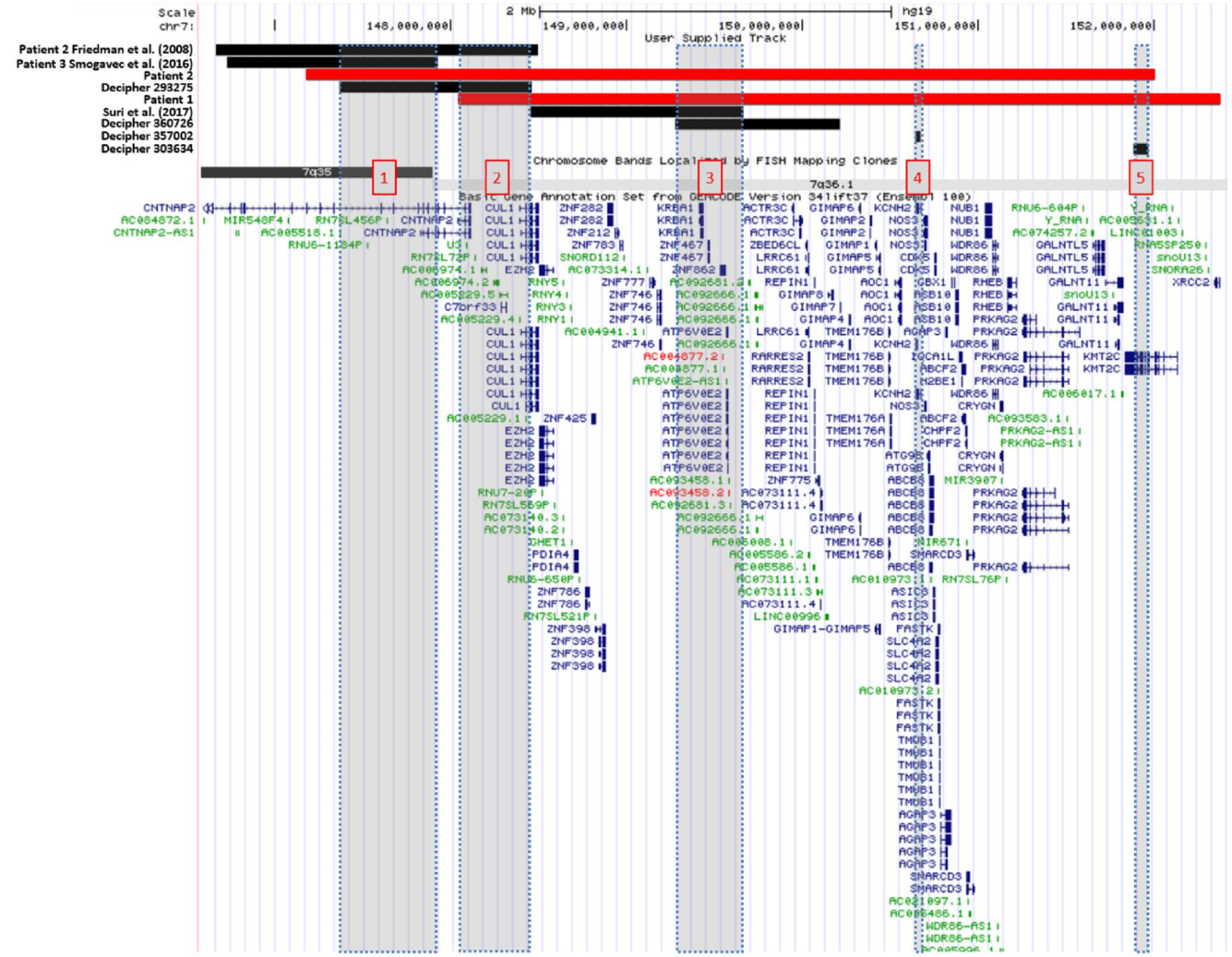

FIGURE 3 Map of the nine deletions 7q35 and/or 7q36.1 including ours according to UCSC February 2009 (hg19)

pathogenic (Huang et al., 2010). Other patients harboring a 7q deletion encompassing *KCNH2* were found to have a long QT syndrome (Ayub et al., 2016; Di Stolfo et al., 2019; Suri & Dixit, 2017). Thus, cardiac monitoring should be implemented in the patients with a defect of these genes. We can already note that patient 1 presented at the last clinical examination a QT interval at the upper limit of normal. *KMT2C (MLL3*, myeloid/lymphoid or mixed-lineage leukemia 3) is included in region 5. Arcipowski et al. (2016) suggested that gene loss may contribute to the progression of myelodysplastic syndrome and acute myeloid leukemia by promoting myelopoiesis (Arcipowski et al., 2016). Among the most frequent chromosomal abnormalities in these disorders are alterations of chromosome 7 including partial deletion of 7q (Arcipowski et al., 2016).

Importantly, recently de novo mutations of *KMT2C* gene were associated with Kleefstra syndrome which include decreased height, microcephaly, dysmorphic facial features, hypotonia and alterations of the central nervous system (such as delayed psychomotor development, ID, speech delay, seizures) and behavioral manifestations (Koemans et al., 2017). Furthermore, as illustrated by Figure 3 and Table 2, the Decipher patient #303634 carrying a *KMT2C* intragenic deletion of 86 Kb showed autistic behavior, short stature, supraventricular arrhythmia, and synophrys. Thus, *KMT2C* haploinsufficiency may have strongly contributed to the neurodevelopmental phenotype of our both patients in addition to their dysmorphic facial features and decreased heights. It is also of note that seizures have been described in patients carrying truncated or frameshift variants in this gene (Koemans et al., 2017).

In addition to the defined regions of overlap, other deleted genes are relevant. *GALNTL5* has been described as a susceptibility gene in autism. Moreover, a deficit in O-glycosylation is associated with muscular dystrophy or failure of bone modelling like exostoses (Jaeken et al., 2008; van der Zwaag et al., 2009). In view of the language delay observed, a change in the ratio of these enzymes in the brain may lead to aberrant sugar chains on their protein substrates disturbing the brain function. F-actin binding protein Abp-1 encoded by the gene

TABLE 2 Genotype–phenotype correlation based on the five small regions of overlap identified

Patients	Region 1 Patient 2, patient 2 Friedman et al. (2008), patient 3 Smogavec et al. (2016), Decipher 293275	Region 2 Patient 1, patient 2, patient 2 Friedman et al. (2008), decipher 293275	Region 3 Patient 1, patient 2, Suri and Dixit (2017), decipher 360726	Region 4 Patient 1, patient 2, decipher 357002	Region 5 Patient 1, patient 2, decipher 303634
Boundaries on chr7 (hg19)	147,369,972–147,928,239	148,047,494–148,464,598	149,280,420–149,666,328	150,643,965–150,674,926	151,884,663–151,971,034
Genes	*CNTNAP2* (OMIM#604569)	*CNTNAP2* (OMIM#604569), *CUL1* (OMIM#603134)	*ZNF767*, *KRBA1*, *ZNF467* (OMIM#614040), *SSPO* (OMIM#617356), *ZNF862*, *ATP6V0E2* (OMIM#611019)	*KCNH2* (OMIM#152427)	*KMT2C* (OMIM#606833)
Low OFC	–	2/4	2/4	2/3	2/3
Asymmetric growth	–	–	2/4	–	–
Short stature	2/4	–	–	–	2/3
Specific learning disability	–	–	3/4	–	–
Intellectual disability	2/4	2/4	2/4	–	–
Global Developmental Delay	3/4	3/4	3/4	2/3	2/3
Motor delay	2/4	2/4	3/4	2/3	2/3
Behavioral abnormality	3/4	3/4	3/4	2/3	2/3
Seizures	3/4	3/4	2/4	2/3	2/3
Delayed speech and language development	3/4	4/4	4/4	2/3	2/3
Muscular hypotonia	–	2/4	3/4	2/3	2/3
Abnormality of the face	–	2/4	3/4	–	–
Anteverted nares	–	2/4	2/4	2/3	2/3
Micrognathia/ retrognathia	–	–	2/4	2/3	2/3
Microcephaly	–	2/4	2/4	2/3	2/3
Hypertelorism	–	–	2/4	–	–

(Continues)

TABLE 2 (Continued)

Patients	Region 1	Region 2	Region 3	Region 4	Region 5
	Patient 2, patient 2 Friedman et al. (2008), patient 3 Smogavec et al. (2016), Decipher 293275	Patient 1, patient 2, patient 2 Friedman et al. (2008), decipher 293275	Patient 1, patient 2, Suri and Dixit (2017), decipher 360726	Patient 1, patient 2, decipher 357002	Patient 1, patient 2, decipher 303634
Abnormality of the thumbs	–	2/4	2/4	2/3	2/3
Cardiac arrhythmia	2/4	2/4	–	–	2/3

Abbreviations: dn, *de novo*; F, female; M, male; mat, maternally inherited; NR, not reported; pat, paternally inherited.

AOC1 has been shown to be expressed in the post-synaptic density and being implicated in endocytosis and synaptic organization (Qualmann et al., 2004). RHEB is enriched in brain and is implicated in the Tsc-Rheb-Tor pathway that is critical for integrating a variety of signals that govern cellular and organismal growth. Inappropriate activation of the pathway also leads to severe neurological and behavioral abnormalities, such as ID, autism, and epilepsy. Studies have shown that decreased levels of Rheb activity compromise synapse development (Knox et al., 2007). As a result, impairment of *AOC1* and *RHEB* may have caused development delay.

Thus, we report a complex syndrome originating from the 7q35q36.1 deletion characterized by multisystemic involvement. In a genetic counselling perspective, it is important to emphasize the relevance of the characterization of a chromosomal abnormality, which has led to the definition of a correct diagnosis and consequently to the establishment of a correct recurrence risk for the couple. Finally, a common phenotype seems to emerge with ID, unusual facial features, and cardiovascular issues. Combined haploinsufficiency of *GALNTL5*, *CUL1*, *SSPO*, *KMT2C*, *AOC1*, *RHEB*, and mainly *KMT2C*, with monoallelic impairment of *CNTNAP2* may explain abnormality of the nervous system, hypotonia and exostoses; and haploinsufficiency of *PRKAG2* and *KCNH2* genes may be responsible of long QT syndrome.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

L.D. and I.G. carried out NGS data analysis and interpretation. L.T. and C.M. wrote the manuscript. L.T., A.M., A.B., L.L., G.T., and C.M. carried out aCGH data analysis and interpretation. L.V.M, Q.L., A.J.K.,and J.C.G. participated in patient care. All the authors were given the opportunity to revise the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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