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Clinical and molecular cytogenetic studies of five new patients with 20q11q12 deletion and review of the literature: Proposition of two critical regions

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Abstract

Deletions of the long arm of chromosome 20 (20q) are rare, with only 16 reported patients displaying a proximal interstitial 20q deletion. A 1.62 Mb minimal critical region at 20q11.2, encompassing three genes *GDF5*, *EPB41L1*, and *SAMHD1*, is proposed to be responsible for this syndrome. The leading clinical features include growth retardation, intractable feeding difficulties with gastroesophageal reflux, hypotonia and psychomotor developmental delay. Common facial dysmorphisms including triangular face, hypertelorism, and hypoplastic alae nasi were additionally reported. Here, we present the clinical and molecular findings of five new patients with proximal interstitial 20q deletions. We analyzed the phenotype and molecular data of all previously reported patients with 20q11.2q12 microdeletions, along with our five new cases. Copy number variation analysis of patients in our cohort has enabled us to identify the second critical region in the 20q11.2q12 region and redefine the first region that is initially identified. The first critical region spans 359 kb at 20q11.2, containing six MIM genes, including two disease-causing genes, *GDF5* and *CEP250*. The second critical region spans 706 kb at 20q12, encompassing four MIM genes, including two disease-causing genes, *MAFB* and *TOP1*. We propose *GDF5* to be the primary candidate gene generating the phenotype of patients with 20q11.2 deletions. Moreover, we hypothesize *TOP1* as a potential candidate gene for the second critical region at 20q12. Of note, we cannot exclude the possibility of a synergistic role of other genes involved in the deletion, including a contiguous gene deletion syndrome or position effect affecting both critical regions. Further studies focusing on patients with proximal 20q deletions are required to support our hypothesis.

KEYWORDS
20q11.2q12 deletion, chromosomal microarray analysis, development delay, intellectual disability

1 | INTRODUCTION

Interstitial proximal deletions of the long arm of chromosome 20 (20q11.2q12) are rare, with 16 unrelated patients exhibiting a proximal interstitial 20q deletion reported so far (Callier et al., 2006; Gervasini et al., 2013; Hiraki et al., 2011; Iourov et al., 2013; Iqbal & Al-Owain, 2007; Jedraszak et al., 2015; Petersen et al., 1987; Posmyk et al., 2014; Santoro et al., 2013; Shabtai et al., 1993). The clinical phenotype of patients with proximal 20q deletions comprises prenatal and postnatal growth retardation, feeding difficulties, psychomotor retardation, intellectual disability, and craniofacial dysmorphisms (Loddo et al., 2018). Anomalies of the extremities like brachydactyly, clinodactyly, and polydactyly have also been reported in patients with 20q proximal deletion syndrome (Hiraki et al., 2011; Jedraszak et al., 2015; Posmyk et al., 2014). Jedraszak et al. (2015) proposed a 1.62 Mb region at 20q11.2 to be responsible for the genotype–phenotype association, involving the three genes *GDF5*, *EPB41L1*, and *SAMHD1*.

Chromosomal microarray analysis (CMA) significantly increases the diagnostic yield in neurodevelopmental diseases, resulting in CMA being the first-tier test in these indications (Battaglia et al., 2013; Jouan-Flahault et al., 2018). We herein present five new unrelated patients with proximal 20q deletion characterized by CMA, and we propose two critical regions as being causative for this syndrome.

2 | CLINICAL REPORT

2.1 | Patient #1 (decipher number 280177)

This male patient was the child of non-consanguineous parents, born at term, delivery being uncomplicated after an uneventful pregnancy. His birth weight was 3.16 kg (−0.39) standard deviation (*SD*), length 49.5 cm (−0.2 *SD*), and occipital frontal circumference (OFC) 34.5 cm (mean). Febrile seizures began when he was 6 months old, followed by generalized tonic–clonic seizures, occurring at night, treated with phenytoin and valproate. A global developmental delay was noted: the boy did not walk until 20 months, and he only used two-word phrases at 4 years. He tended to gain weight easily from mid-childhood on, and he was myopic (−3 diopters), with normal fundoscopy. At 10 years old, he underwent surgery for bilateral spastic equinovarus. At 14 years old, he was diagnosed with type 2 diabetes. In his twenties, he developed motor tics like finger flicking and blinking, along with a moderate intellectual disability. He exhibited facial dysmorphisms including epicanthic folds, upslanting palpebral fissures, anteverted nares, fleshy earlobes, and a low hairline (Figure 1A, a–d). He had bilateral single palmar creases and small hands and feet (Figure 1B,C). Adult height was 160 cm (−2.2 *SD*) and OFC 57 cm (+0.1 *SD*). A renal ultrasound, echocardiogram, and brain computed tomography (CT) were normal.

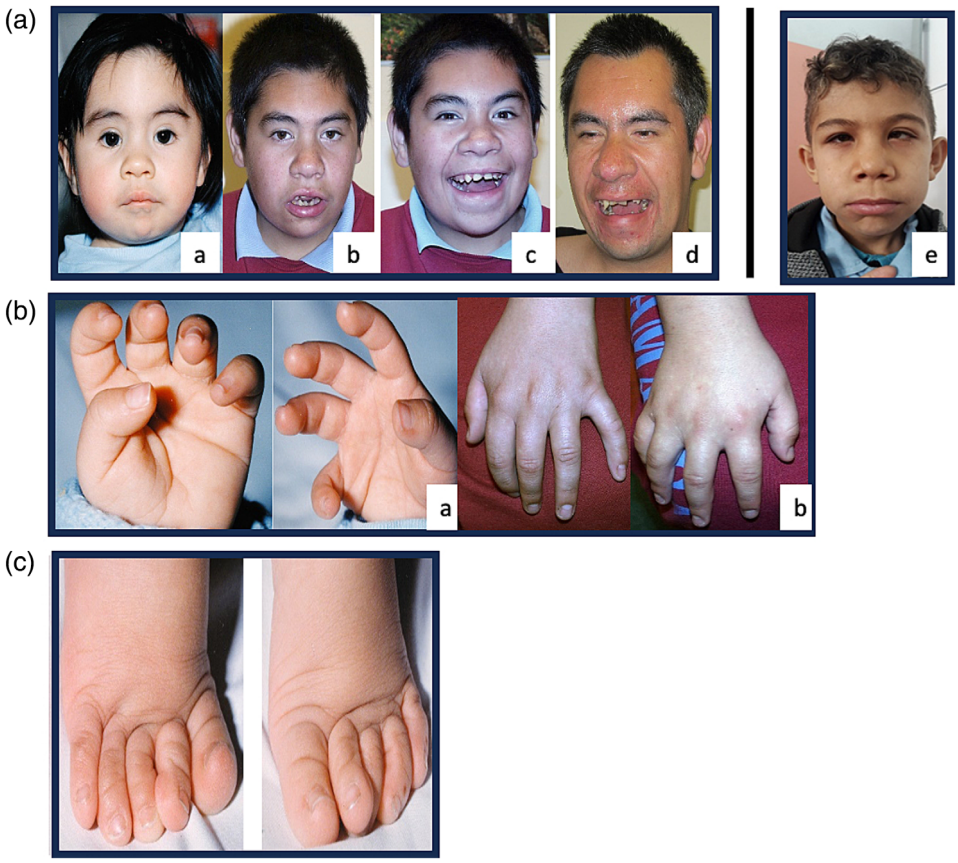

FIGURE 1 Clinical characteristics of the patients. (A) Patient #1 at several ages, including (a) 1 years, (b and c) 14 years, and (d) 26 years, showing evolving facial dysmorphisms including fleshy earlobes (a–d), low anterior hairline (b,c), wide mouth (c,d), and flared lateral eyebrows (b–d). (e) Patient #5 at 13 years with strabismus, bulbous nose, large mouth, fine upper lip, triangular face, and ears anomalies. (B) Hand anomalies in Patient #1 displaying (a) short hands and fetal pads at 1 year and (b) short hands and 5th finger clinodactyly at 14 years. (C) Feet anomalies in Patient #1 with long and overlapping toes.

2.2 | Patient #2 (decipher number 306791)

This female patient was the child of non-consanguineous parents. Her family history disclosed hypotonia and psychomotor delay in a maternal cousin, and growth retardation and autism in another maternal cousin. She was born at term with an uncomplicated delivery after an uneventful pregnancy. Apgar scores were 8 and 9 at 1 and 5 min, respectively. A few days after birth, an audiological examination disclosed bilateral sensorineural hearing loss.

At 2 months, a renal ultrasound displayed a dilation of the pyelocaliceal cavities. Developmental milestones were delayed: at 7 months, her weight was 5.43 kg (<−3 *SD*), and her height was 61 cm (−3 *SD*). She never experienced epileptic seizures, and an electroencephalogram (EEG) performed at 2 years was normal. Brain magnetic resonance imaging (MRI) at 2 years disclosed an abnormal morphology of corpus callosum with squat isthmus and cystic cochleovestibular malformation. The girl was then hospitalized for skin pallor, with an iron-deficiency anemia diagnosed. At the last examination, at 2 years, auxological parameters were under normal range for weight at 9.30 kg (−3 *SD*) and length at 95 cm (+2 *SD*), with OFC within the normal range at 47.50 cm (−0.2 *SD*). Physical examination disclosed craniofacial dysmorphism with hypertelorism, short palpebral fissures, and cupped ears. Chromosomal analysis, telomeric sequence, and molecular analysis of *CX26* gene were normal, with *CHD7* analysis excluding the CHARGE syndrome.

2.3 | Patient #3 (decipher number 267065)

This female patient was adopted from Brazil at 6 years of age, without any information available concerning her birth or early infancy. In the medical file transmitted to her foster parents, her psychomotor development was described as normal. At 7 years old, she could walk properly, but she presented a speech delay, as she could only say a few words, with impaired fine motor skills. A neuropsychological evaluation concluded that she displayed a global development delay. She was able to attend school until she was 12 years old with the help of a school life assistant; she became homeschooled thereafter. The girl displayed behavioral disorders including anxiety and episodes of clashes, for which she was treated with risperidone and sometimes loxapine. She had difficulty falling asleep. She had no eating disorders and never had any seizure. When she was 14 years old, a sound clinical examination was performed by both a neuropediatrician and a geneticist. Her height was 1.59 m (−0.1 *SD*), while her weight was 67 kg (+1.4 *SD*) and OFC 55 cm (+0.8 *SD*). The adolescent exhibited three-to-four café-au-lait spots, but her neurological examination was normal. On physical examination, she had horizontal eyebrows, broad nasal bridge, short philtrum, and thick lips, while several morphological features may be accounted for by her ethnicity. Puberty was complete, and menarche occurred when she was 12 years old. A metabolic assessment was conducted, which was normal; MRI could not be performed on account of her agitation. The fragile X syndrome was excluded based on the molecular analysis of the *FMR1* gene's first exon.

2.4 | Patient #4 (decipher number 422691)

This patient was the daughter of non-consanguineous healthy parents with unremarkable family history. She was born at term via an uncomplicated delivery after an uneventful pregnancy.

A few days after birth, she underwent surgery for meconium ileus. The newborn displayed psychomotor and language delays, and she experienced generalized epileptic seizures. The EEG performed at 15 years of age demonstrated generalized spike-waves. Brain MRI and CT were normal, whereas the neurological examination revealed motor dyspraxia. The ophthalmologic evaluation revealed Duane retraction syndrome, a congenital form of strabismus. At that time, her clinical evaluation disclosed a weight of 58 kg (+1 *SD*) and length of 164 cm (mean).

2.5 | Patient #5

The patient was a 13-year-old boy, being the child of healthy but consanguineous parents, with four other healthy brothers and a healthy sister. He was born at 38 weeks of gestation by spontaneous vaginal delivery. Birth weight was 2.5 kg (−2 *SD*), and his psychomotor development was delayed. He sat at 2 years, walked at 3 years, and pronounced his first words at 5 years only. At the last clinical evaluation conducted when he was 13 years old, his weight was 24 kg (<−3 *SD*), height 123 cm (<−3 *SD*), and head circumference 55 cm (+0.5 *SD*). A clinical examination disclosed hypertelorism, strabismus, broad nasal bridge, anteverted nares, and low-set ears (Figure 1A[e]). In adolescence, he experienced epileptic seizures, and his feeding difficulties were accompanied by weight loss. Complementary studies were conducted, including MRI, disclosing brain malformation.

3 | MOLECULAR CYTOGENETIC CHARACTERIZATION

CMAs were performed after obtaining informed consent from all patients. It was conducted using oligo 60k Agilent® array for Patients #1, #2, and #3, and oligo 180k Agilent® array (Agilent technologies, Santa, Clara, CA, USA) for Patient #5. Data were analyzed using Agilent CytoGenomics Software. CMA was performed using Infinium CytoSNP-850K BeadChip (Illumina, San Diego, CA, USA) for Patient #4, with DNA obtained from peripheral blood. Copy number variation (CNV) analysis was carried out using BlueGnome BlueFuse Multi software. Confirmation and segregation tests were performed by means of FISH using locus-specific BAC clone RP11-469I22 (20q12) on metaphases of Patients #1, #2, and #4. Deletions were examined using the UCSC genome browser (http://genome.ucsc.edu/; February 2009 Assembly, hg19), as shown in Figure 2.

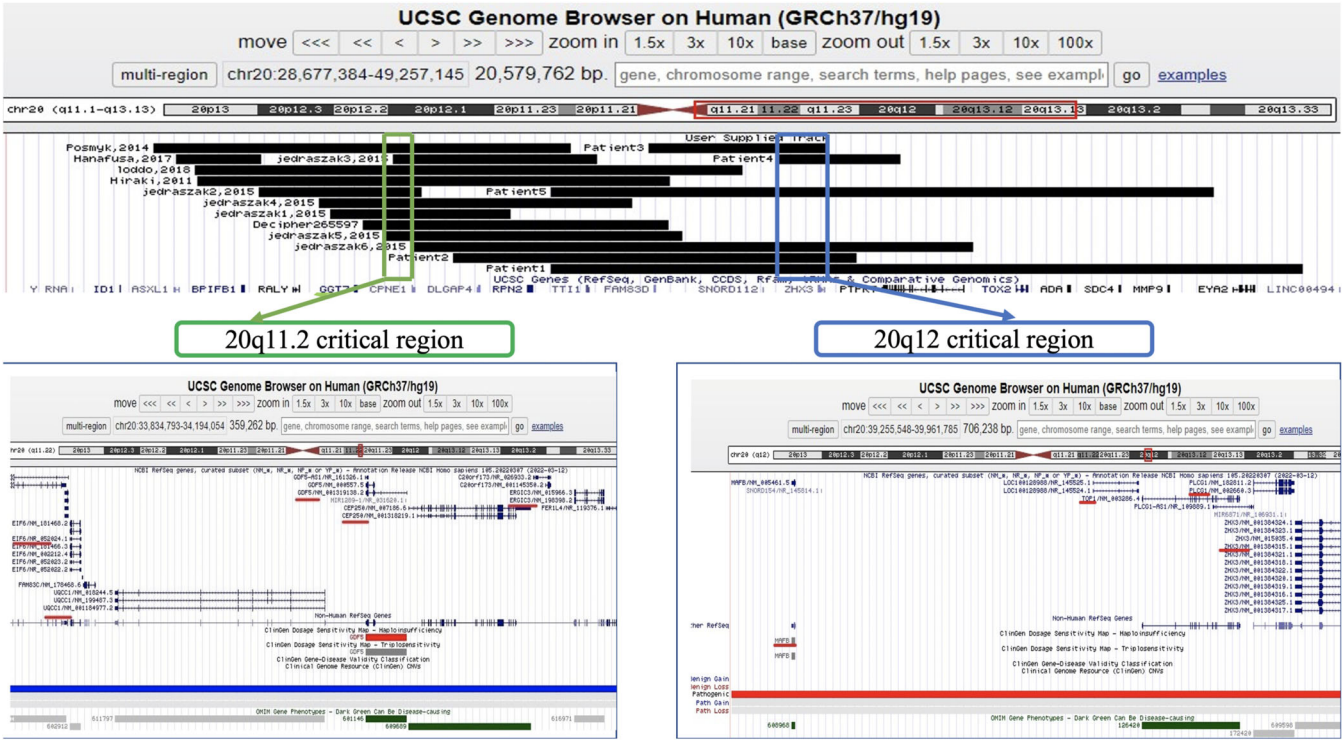

FIGURE 2 Comparison of deleted 20q proximal region. UCSC genome browser on human (GRCh37/hg19) capture refined showing two minimal critical regions. The 20q11.2 critical region extends over 350 kb from 33,834,793 to 34,194,054 (hg19), and the 20q12 critical region extends over 706 kb from 39,255,548 to 39,961,785 (hg 19).

4 | RESULTS

CMA analyses revealed an interstitial 20q microdeletion in all patients, with sizes ranging from 1.7 to 10.35 Mb (Supplemental Table 2). The de novo occurrence of the deletions was confirmed for Patients #1, #2, and #4 using cytogenetic analysis and FISH. As Patient #3 was adopted, whether this deletion occurred de novo or was inherited could not be proven. Blood samples of Patient #5's parents were unavailable.

The maximal region encompasses 171 genes including several MIM genes. The minimal region encompasses 17 genes with three MIM genes, including *MAFB*, *TOP1*, and *PLCG1* genes. No other unbalanced pathogenic variation was identified by CMA.

Molecular analyses including our own cases and previously overlapping published cases enabled us to propose two critical regions, namely 20q11.22 and 20q12. The 20q11.22 critical region overlaps the critical region proposed by Jedraszak et al. Molecular analysis of CNVs from patients in our cohort enabled us to reduce this critical region to 350 kb from 33,834,793 to 34,194,054 (hg19). The 20q12 critical region extends over 706 kb from 39,255,548 to 39,961,785 (hg19).

5 | DISCUSSION

We described five new patients with an interstitial 20q11q12 deletion, comparing these cases with previous literature reports. The phenotypic features of patients with previously reported 20q11.2 deletion as compared with our patients with 20q12 deletion were summarized (Supplemental Table 1).

Jedraszak et al. (2015) reported six new patients with 20q11.2 microdeletions, with clinical phenotypes including craniofacial dysmorphism, intellectual disability, anomalies of the extremities, feeding difficulties, and variable malformations. These authors proposed the 20q11.2 microdeletion syndrome to be a new contiguous gene deletion syndrome with a recognizable phenotype; the researchers identified a 1.62 Mb minimal critical region, encompassing three genes (*GDF5*, *EPB41L1*, and *SAMHD1*), as strong candidates to account for several phenotypic features. Hanafusa et al. (2017) reported the smallest de novo 20q11.2 microdeletion (1.2 Mb) in 2-year- and 6-month-old Japanese girls with intellectual disability, motor developmental delay, and facial features without feeding problems. This deletion did not overlap the minimal critical genomic region described by Jedraszak et al., encompassing the *GDF5*, *EPB41L1*, and *SAMHD1* candidate genes. The Hanafusa deletion does not overlap the minimal 20q11.2 critical region defined by Jedrazak. A positional effect could be discussed. The cases we have reported herein enable us to propose a second critical region at 20q12.

5.1 | Clinical discussion

Table 1 comprehensively summarizes the clinical phenotypes of patients harboring deletions within the critical region of either

TABLE 1 Comparison of clinical phenotypes observed in patients with deletions within the critical region of either 20q11.2 or 20q12.

20q11.2 critical region											20q12 critical region						
			Jedraszak, 2015														
	Posmyk	Hiraki	Jedraszak1	Jedraszak2	Jedraszak3	Jedraszak4	Jedraszak5	Loddo	Decipher (265597)	Total	Patient #4	Patient #3	Patient #5	Jedraszak6	Patient #2	Patient1	Total
Age Gender	2y Female	18 m Male	15y Male	5.5y Male	6y Male	2.5y Male	20y Female	8y Female	male	3 females, 6 males	18y Female	14y Female	13y Male	2 m Female	7y Female	26y Male	4 females, 2 males
IUGR	+	+	−	−	−	+	−	+		4/8	−			+	−		1/3
Craniofacial dysmorphism																	
Frontal bossing			+	+	+	−	+	+	+	6/7	−	−	−	−	−	−	0/6
High forehead	+	−	+	+	+	+	+	−		6/8	−	−	−	+	−	−	1/6
Low hairline								−			−	−	−		−	+	1/5
Deep set eyes	+	+	+	+	+	+	+	+	+	9/9	−	−	−	+	−	−	1/6
Strabism	+	−	+	−	−	+	−	+		5/8	+	−	+	−	−	−	2/6
Hypertelorism											−		+		+		2/4
Short philtrum	−	+	+	+	−	−	+	−		4/8	−	−	−	−	−	−	0/6
Triangular face/midface hypoplasia	+	+	+	+	+	+	+	−		7/8	−	−	+	−	−	−	1/6
Anterverted nares/hypoplastic alae nasi	+	+						−		2/3	−	−	+		−	+	2/5
Arched eyebrows	+							+		2/2	−	−	−		−	−	0/5
Low set ears	+	+						−		2/3	−	−	+		−	−	1/5
Ears anomaly	+	+	−	−	+	−	+			4/7			+				1/1
Neurology																	
Microcephaly								+		1/1	−	−	−		−	−	0/5
Hypotonia	+	−	+	−	+	+	−	−		4/8	−	−	+	+	+	−	3/6
Developmental delay	+	+	+	+	+	+	+	+	+	9/9	+	+	+	+	+	+	6/6
ID								−			−	+	+		−		2/4
Epilepsy	−	+						−		1/3	+	−	+		−	+	3/5
Independent walk								15 m		1/1					>2y	20 m	1/2
Language delay	+	+						+		3/3	+	+	+		+	+	5/5
Behavioral disorder	−	+	+		−	−	−	+		3/7	+	+	−	−	−	−	2/6
Brain malformation		+	−	−	−	+	−	−		2/7	−		+	+	+	−	3/5
Ocular anomaly		+	−	+	−	−	+	−		3/8	+	−	+	−	−	+	3/6
Hearing impairment	−	+	+	−	−	−	+	−		3/8	−	−	−	−	+	−	1/6
Skeletal																	
Brachydactyly	−	−	+	−	+	−	+	+		4/8	−	−	+	−	−		1/5
Finger clinodactyly	+	+	+	+	+	−	−	+	+	7/9	−	−	+	+	−	−	2/6
Polydactyly	+	+								2/2				+			1/1
Other features																	
Feedings difficulties	+	+	+	+	−	+	+	+		7/8	−		+	+	−	−	2/5
Cardiomyopathy	+	+	−	−	−	−	+	−		3/8	−	−	−	+	−	−	1/6

Abbreviations: DD, developmental delay; ID, intellectual disability; IUGR, intrauterine growth retardation; m, month; y, years; +, yes; −, no.

Note: Unfilled box means that the feature has not been explored.

20q11.2 or 20q12. For both critical regions, patients exhibited developmental and language delays, hypotonia, and ocular anomaly.

Despite the small number of previously reported patients, we tried to differentiate the phenotype of patients with deletions in the 20q11.2 critical region (first group) from that of patients with deletions in the 20q12 critical region (second group). Indeed, the number of patients so far reported turned out to be small, preventing us from performing reliable statistical tests, though strong trends were observed.

Patients from the first group tended to display more dysmorphic facial features (Table 1), with frontal bossing observed in 6/7 patients, whereas none of the second group patients presented this sign. Moreover, 9/9 of the first-group patients displayed deep set eyes versus 1/6 of the second group; additionally, 7/8 of the first-group patients displayed a triangular face versus 1/6 of the second group.

Anomalies of the extremities were more common in first-group patients, with 4/8 first-group patients displaying brachydactyly versus 1/5 second-group and 7/9 first-group patients featured finger clinodactyly versus 2/6 second-group. Polydactyly was reported twice in the first group and once in the second group. For other classical clinical features described in patients with a 20q11q12 microdeletion, first-group patients exhibited more comorbidities, including hearing impairment (3/8 vs. 1/6), neonatal feeding difficulties (7/8 vs. 2/5), and cardiopathy (3/8 vs. 1/6), whereas second-group patients exhibited more neurological features, including brain malformations (2/7 vs. 3/5) and epilepsy (1/3 vs. 3/5).

From a clinical perspective, patients with a deletion of the 20q11.2 critical region (first group) displayed characteristic facial dysmorphisms, described by Jedraszak et al., along with developmental delay, comorbidities including anomalies of the extremities, neonatal feeding difficulties, and cardiopathy. Patients with a deletion of the 20q12 critical region (second group) exhibited mild craniofacial dysmorphism, fewer comorbidities, yet highly prevalent neurological features including brain malformations and epilepsy. Based on these features, we now recommend specific clinical follow-up for 20q12 deleted patients to obtain an early diagnosis and to promptly initiate epileptic treatment if required.

Despite the limited number of described patients, these findings suggest that the severity and spectrum of clinical manifestations may vary significantly between 20q11.2 and 20q12 deletions, indicating the potential existence of distinct disorders within these genomic regions.

5.2 | Molecular discussion

The breakpoints of the deletions are distinct, with the underlying cytogenetic mechanisms being probably not mediated by NAHR. Considering the first critical region, six genes were deleted, including *GDF5*, *CEP250*, *MMP24*, *EIF6*, *UQCC1*, and *ERGIC3*. Only the *GDF5* gene was among the candidate genes proposed by Jedraszak et al.

GDF5 (growth differentiation factor 5) encodes a secreted growth factor with a regulatory role in embryonic skeletal and joint development in mice and chicks by modulating the BMP pathway (Kania et al., 2020; Merino et al., 1999; Storm & Kingsley, 1996; Waldmann et al., 2022). *GDF5* is an MIM gene responsible for dominantly- and recessively-transmitted bone malformations, including brachydactyly A1 (MIM 615072), A2 (112600), and C (MIM113100). This gene is sensitive to haplo-insufficiency with a pLI score of 0.67 and a LOEUF score of 0.47. *GDF5* haploinsufficiency could explain skeletal abnormalities like brachydactyly. Moreover, *GDF5* mutations were associated with hearing impairment, probably due to middle ear bone anomalies, explaining the hearing impairment noted in these patients. In a murine model, Osório et al. (2013) demonstrated that *GDF5* influenced both morphology and dendrite size, which could partly account for the neurological phenotype of these patients.

The *MMP24* gene (MIM 604871) encodes a matrix metalloproteinase. MMP24 is a zinc-dependent endopeptidase that degrades the different protein components of the extracellular matrix and basement membranes. *MMP24* is abundantly expressed throughout the nervous system; it could possibly degrade several extracellular matrix components, including cell-adhesion molecule N-cadherin known to promote neurite outgrowth in cultured cells (Hayashita-Kinoh et al., 2001). Haploinsufficiency of the *MMP24* gene could plausibly contribute to intellectual disability. However, this gene has not been documented in patients with neurodevelopmental disorders.

The *EIF6* gene encodes the integrin beta-4 subunit. EIF6 is a highly conserved protein of 245 amino acids, localized in the nucleolus and cytoplasm. EIF6's presence in the nucleolus supports its role in ribosome biogenesis. In 2008, Gandin et al. explored *EIF6*'s function in regulating in vivo translation using eif6± mice. The authors revealed eif6 knockout mice to display a lethal phenotype with defects in ribosome biogenesis (Gandin et al., 2008). Koh et al. (2020) proposed EIF6 depletion to disrupt ribosome biogenesis and ribosomal translation, postulating that a de novo heterozygous variant in EIF6 was a possible molecular etiology for Schwachman–Diamond syndrome (SDS). SDS is a rare disorder characterized by exocrine pancreatic insufficiency, hematologic abnormalities, and bone abnormalities. To the best of our knowledge, no evidence exists suggesting exocrine pancreatic insufficiency or hematologic abnormalities in patients with a 20q11q12 deletion encompassing the *EIF6* gene. As the pLI for this gene is low (0.24), it could be interesting to search for such an anomaly in these patients. Lastly, De Marco et al. (2017) studied *EIF6* in *Xenopus*, and the authors reported that EIF6 overexpression delayed *Xenopus* eye development. Indeed, ocular anomalies were observed in patients with a 20q11q12 microdeletion, suggesting a possible link with *EIF6* haploinsufficiency.

The other genes, including *CEP250* (associated with recessive cone-rod dystrophy and hearing loss 2—MIM 618358), *ERGIC3*, and *UQCC1*, are unlikely to be susceptible to haploinsufficiency, as indicated by their low or null pLI values. Consequently, these genes were not considered suitable candidates for our observed phenotypes.

Considering the 20q11.2 critical region, in agreement with Jedraszak et al., we confirm that 20q11.22 is a contiguous gene deletion syndrome with a recognizable phenotype. We have proposed to

reduce this critical region to 350 kb excluding *EPB41L1*, previously proposed to explain intellectual disability, as well as *SAMHD1*, proposed to account for cerebral malformation and retinal dysplasia. Nevertheless, a positional effect could deregulate these genes.

In our view, the second 20q12 critical deleted region extends from 39,255,548 to 39,961,785 and spans over 706 kb, encompassing four genes including *TOP1*, *MAFB*, *PLCG1*, and *ZHX3*.

TOP1 (MIM 126420) codes for a topoisomerase, which catalyzes DNA relaxation and supercoiling so as to enable processes like replication, transcription, and recombination to occur by reversibly nicking one strand and forming transient DNA cleavage complexes (Wang, 2002). Topoisomerases are expressed throughout the developing and adult brain. King et al. investigated the biological consequences of TOP1 inhibition. The authors found that topotecan, a topoisomerase 1 (TOP1) inhibitor, could significantly downregulate 155 genes, while significantly upregulating 28 other genes in neurons, particularly cortical neurons (King et al., 2013). Therefore, the authors proposed that mutations altering topoisomerase activity were likely to reduce the expression of numerous autism spectrum disorder genes. Of note is that rare de novo missense mutations were found in autism patients (Neale et al., 2012). *TOP1* was associated with a pLI of 1 with a LOEUF score of 0.06, suggesting a contribution to the neurodevelopmental phenotype of chromosome 20q12 deletions.

The *MAFB* gene (MIM 608968) was identified by Wang et al. (1999). The transcription factor MAFB enables macrophages to be distinguished from dendritic cells. Kong et al. (2021) postulated that lower *MAFB* gene expression might be related to hypospadias, and that this abnormal expression was likely to affect the urethral development during the embryonic period. Yet, no hypospadias was identified in our patients. Park et al. (2016) reported on three heterozygous loss-of-function *MAFB* mutations causing Duane retraction syndrome, as well as a dominant-negative *MAFB* mutation causing Duane retraction syndrome and deafness. Duane retraction syndrome has been recognized as a cranial dysinnervation disorder, featuring limited unilateral or bilateral horizontal eye movement, accompanied by globe retraction and palpebral fissure narrowing on attempted adduction. Hearing impairment was reported in several patients with a 20q11.2 deletion, but none had Duane retraction syndrome.

The *PLCG1* gene (MIM 172420) is involved in cancer development, though constitutional variants have not been linked to human disease.

The *ZHX3* gene (MIM 609598), encoding zinc finger and homeodomain protein 3, is a ubiquitously expressed transcription repressor involved in pathogenesis of cancer. Recently, Igata et al. (2022) showed *ZHX3* to be an essential transcription factor involved in mitochondria morphology. These authors found that loss of *ZHX3* induced mitochondrial–nucleolar activation and cellular senescence in human fibroblasts. It might thus be interesting to search for signs of early senescence in patients with 20q12 deletions encompassing the *ZHX3* gene.

Considering the 20q12 critical region, the *TOP1* gene is most likely the best candidate to explain much of the observed neurodevelopmental phenotype. We do not have a hypothesis to account for the high frequency of epilepsy in patients with a deletion of the second critical region.

In conclusion, we have proposed two 20q proximal critical deletion regions. On one hand, patients with a deletion of the 20q11.2 critical region display characteristic facial dysmorphisms, previously described by Jedraszak, along with the developmental delay, anomalies of the extremities, and neonatal feeding difficulties. On the other hand, patients with a 20q12 deletion exhibit only mild craniofacial dysmorphisms, but a high prevalence of neurological features including brain malformations and epilepsy. Further studies are necessary to confirm these results.

AUTHOR CONTRIBUTIONS

Souad Bensaid: Investigation; writing—original draft and review. **Malika Bendahmane**: Investigation; writing review. **Sara Loddo**: Investigation; writing review. **Gemma Poke**: Investigation; writing review. **Louis Januel**: Writing original draft and review. **Romain Nicolle**: Investigation; writing review. **Valérie Malan**: Investigation; writing review. **Nicolas Chatron**: Writing review. **Silvia Ottombrino**: Investigation; writing review. **Maria Lisa Dentici**: Investigation; writing review. **Antonio Novelli**: Investigation; writing review. **Maria Cristina Digilio**: Investigation; writing review. **Damien Sanlaville**: Supervision; writing original draft; writing review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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