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Further delineation of auriculocondylar syndrome based on 14 novel cases and reassessment of 25 published cases

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Abstract

Auriculocondylar syndrome (ACS) is a rare craniofacial disorder characterized by mandibular hypoplasia and an auricular defect at the junction between the lobe and helix, known as a "Question Mark Ear" (QME). Several additional features, originating from the first and second branchial arches and other tissues, have also been reported. ACS is genetically heterogeneous with autosomal dominant and recessive modes of inheritance. The mutations identified to date are presumed to dysregulate the endothelin 1 signaling pathway. Here we describe 14 novel cases and reassess 25 published cases of ACS through a questionnaire for systematic data collection. All patients harbor mutation(s) in *PLCB4*, *GNAI3*, or *EDN1*. This series of patients contributes to the characterization of additional features occasionally associated with ACS such as respiratory, costal, neurodevelopmental, and genital anomalies, and provides management and monitoring recommendations.

KEYWORDS
auriculocondylar syndrome, craniofacial anomalies, EDN1, GNAI3, PLCB4, question mark ear

1 | INTRODUCTION

Auriculocondylar syndrome (ACS; MIM#s 602483, 614669, and 615706) is a rare craniofacial disorder affecting the first and second branchial arches. It is mainly characterized by variable micrognathia, hypoplasia of the condyle of the mandible, and an auricular defect at the lobe-helix junction, known as a "Question Mark Ear" (QME) (Cosman, 1984; Cosman et al., 1970). Intra and interfamilial phenotypic variation in ACS has previously been reported (Gerkes et al., 2008; Gordon, Petit, et al., 2013; Gordon, Vuillot, et al., 2013; Kokitsu-Nakata et al., 2012; Masotti et al., 2008; Rieder et al., 2012). The mildest end of the spectrum is an isolated QME (IQME; MIM# 612798). Several studies have described other frequently associated features including microstomia, full cheeks, facial asymmetry, cleft palate, hearing loss, and lingual anomalies (Gerkes et al., 2008; Gordon, Petit, et al., 2013; Gordon, Vuillot, et al., 2013; Kokitsu-Nakata et al., 2012; Masotti et al., 2008; Rieder et al., 2012). Extra-craniofacial manifestations include central sleep apnea, gastrointestinal disorders, and genital anomalies (Gordon, Vuillot, et al., 2013; Kido et al., 2013; Leoni et al., 2016).

ACS is genetically heterogeneous with both autosomal dominant and recessive modes of inheritance. Mutations in the Phospholipase C beta 4 gene (*PLCB4*; MIM# 600810) located on 20p12.3-p12.2 and in the Guanine Nucleotide Binding protein alpha inhibiting activity polypeptide 3 gene (*GNAI3*; MIM# 139370) located on 1p13.3 were identified by whole-exome sequencing and confirmatory studies in affected individuals (Gordon, Vuillot, et al., 2013; Rieder et al., 2012). The Endothelin 1 gene (*EDN1*; MIM# 131240) was subsequently identified as a third locus at 6p24.1 in ACS and IQME patients (Gordon, Petit, et al., 2013). Studies in animal models have highlighted the key role of the EDN1-Endothelin Receptor type A (EDNRA; MIM# 131243) signaling pathway in the development of the jaw (Clouthier et al., 2013; Walker et al., 2007). Mice with targeted deletion of *Edn1* or *Ednra* present with severe defects in jaw development and a homeotic transformation of the lower jaw into an upper jaw (Clouthier et al., 2010, 2013; Ozeki et al., 2004; Ruest et al., 2004). Mutations in GNAI3 and PLCB4 are thought to interfere with cytoplasmic signaling in the EDN1-EDNRA pathway (Marivin et al., 2016). This is supported by the observation of genetic interaction between *edn1* and *plcb3* in zebrafish during craniofacial development (Walker et al., 2007) and by the fusion and hypoplasia of branchial arch cartilage elements observed in zebrafish with *plcb3* missense mutations (Walker et al., 2007).

We herein describe 14 novel patients with ACS/IQME from 10 families harboring *PLCB4*, *GNAI3*, or *EDN1* mutations. We analyzed clinical features of these and previously reported patients in an attempt to establish correlations with the identified disease-causing mutations and to propose recommendations aiming at improving the management and monitoring of patients with ACS.

2 | PATIENTS AND METHODS

PLCB4, *GNAI3*, or *EDN1* Sanger sequencing was performed in most ACS or QME patients, according to standard procedures (Romanelli Tavares et al., 2017). Patients 8 and 9 were submitted to next-generation sequencing of a panel of neurocristopathy-related genes, including the three ACS genes (Gordon et al., 2018). Whole-exome sequencing was performed for Patients 23, 29, and 39. For Patient 23 the exome protocol has been published (Thevenon et al., 2016). For Patient 29, exome enrichment (Agilent SureSelectXT Human All Exon 50Mb), exome sequencing (Illumina HiSeq), read alignment (BWA), and variant calling (GATK) were performed at BGI-Europe, and variant annotation and prioritizing were performed by the Department of Human Genetics, Radboud University Medical Center. For Patient 39, exome enrichment (Agilent Sureselect QXT CREv1 kit), exome sequencing (Illumina instrument), read alignment, and variant calling were performed by the Clinical genomics laboratory, Victorian Clinical Genetics Services, Melbourne, Australia. Molecular data are summarized in Table 1.

Clinical data of patients with ACS were collected through a standardized questionnaire sent to each referring physician requesting personal and familial medical history of affected individuals, clinical and radiological features, treatments, and outcomes. Table 2 indicates the frequency of the major clinical features while Table S1 provides detailed phenotypic information for each patient. Information about some clinical features (full cheeks, microstomia, and postauricular tags) was obtained from photographs. Available radiological exams were reviewed by a pediatric radiologist (Dr Breton) with expertise in craniofacial imaging at the Necker-Enfants Malades Hospital.

The 14 previously unreported patients are described as follows.

Patient 8 from Family 7, A male was born at term from healthy non-consanguineous parents after an uneventful pregnancy with birth parameters in the normal range. At birth, bilateral QME with hypoplastic antihelix and prominent antitragus, micrognathia, and a hypomobile tongue was noted. Swallowing difficulties and poor suck required nasogastric tube feeding for the first 5 months of life. He snored during sleep but polysomnographic recording was not performed. The speech was delayed with dysarthria, due in part to hearing loss and hypomobile tongue. Growth parameters were normal at the last examination at 7 years of age. Cardiovascular examination and skeletal X-rays were normal. Computed tomography (CT)-scan demonstrated mandibular hypoplasia, dysplasia of the condyles and temporomandibular joints (TMJs), and normal inner ears. A right mandibular osteotomy and mandibular distraction were performed at 2.5 years of age and a second mandibular distraction 33 months later. We identified a de novo c.1861C>T; p.(Arg621Cys) mutation in *PLCB4* (RefSeq NM_000933.4) in this patient.

Family 8 includes the proband, a male (**Patient 9**), his mother (**Patient 10**), and maternal grandmother (**Patient 11**). ACS was diagnosed in the proband at 3 years of age based on the association of micro-retrognathia, microstomia, and unilateral QME. He was term-born from nonconsanguineous parents after an uneventful pregnancy with birth parameters in the normal range. He had neither feeding nor breathing difficulties but presented with neonatal hypotonia followed by delayed motor and speech milestones. Craniofacial CT scan and polysomnographic recordings were not performed. The hearing was normal and he had no surgical interventions.

His mother was diagnosed with bilateral QMEs. Since craniofacial CT scan showed dysplasia of the condyles, she was classified as ACS. She presented with ptosis, hypermetropia, and learning difficulties. Clinical examination of the maternal grandmother was normal. The proband, his mother, and maternal grandmother were heterozygous for the *PLCB4* mutation c.1862G>A; p.(Arg621His).

Family 10 consists of an affected female (**Patient 14)** and her mother (**Patient 15)**. The diagnosis of ACS was made at 5 years of age in patient 14. She was born full-term after an uneventful pregnancy with growth parameters in the low-normal range. She presented with bilateral QMEs, a bifid uvula, and protrusion of the tongue. She had no feeding or breathing difficulties. Growth and motor development were normal. Later, orofacial dyspraxia and snoring were noted but polysomnography was not performed. Craniofacial CT scan was not available.

Her mother presented with a mild unilateral QME, diabetes mellitus, a single kidney, and a bifid uterus. Both harbored a heterozygous c.1705A>T; p.(Ile569Phe) mutation in *PLCB4*.

Family 11 consists of a father, **Patient 16**, and his son, **Patient 17**. The father was diagnosed with ACS in his fifties based on the association of microretrognathia and bilateral QME (Figure 1a–c). He snored and experienced obstructive sleep apnea. He has had bilateral mandibular osteotomies and auricular reconstruction. Cardiovascular, ophthalmological, and hearing examinations have been normal. The speech was delayed without hearing loss in childhood and intelligence is within the normal range. A craniofacial CT scan showed a hypoplastic mandible and hypogenesis or agenesis of the TMJs and condyles.

His son, **Patient 17**, presented with bilateral IQMEs (Figure 1d–f) and scoliosis. Speech had been delayed without hearing loss and intelligence was within the normal range. Craniofacial CT scan was not performed. The father and son have no facial hair in a proximal region along the horizontal branch of the mandible reflecting the putative mandibular to maxillary homeotic transformation underlying ACS (Figure 1a–f) (Gordon et al., 2014). Both were heterozygous for a *PLCB4* c.1015G>A; p.(Gly339Arg) mutation.

Patient 18, a female, was term-born after an uneventful pregnancy from healthy consanguineous parents originating from North Africa. She presented with bilateral QME, severe microretrognathia and microstomia. Dental anomalies including agenesis and misalignment were

TABLE 1 Molecular data of the 39 patients

Gene	Type of mutation	cDNA modification	Protein change	Inheritance	Diagnosis	Familial	Patient number	Publication
PLCB4	HTZ	c.983A>G	p.(His328Arg)	AD	ACS	Yes	7	Kokitsu-Nakata et al. (2012) (Case 2). Romanelli Tavares, et al. (2017)
		c.1015G>A	p.(Gly339Arg)	AD	ACS	Yes	16	0
		c.1015G>A	p.(Gly339Arg)	AD	IQME	Yes	17	0
		c.1072G>C	p.(Glu358Gln)	AD	ACS	No	6	Kokitsu-Nakata et al. (2012) (Case 1). Romanelli Tavares, et al. (2017)
		c.1073A>T	p.(Glu358Val)	AD	ACS	Yes	12	Shkalim et al. (2008), (Case III:2). Gordon, Vuillot, et al. (2013), (Case 1-III:2)
		c.1073A>T	p.(Glu358Val)	AD	ACS	Yes	13	Shkalim et al. (2008), (Case II:3). Gordon, Vuillot, et al. (2013), (Case 1-II:1)
		c.1705A>T	p.(Ile569Phe)	AD	ACS	Yes	14	0
		c.1705A>T	p.(Ile569Phe)	AD	ACS	Yes	15	0
		c.1861C>T	p.(Arg621Cys)	AD	ACS	No	4	Gerkes et al. (2008). Gordon, Vuillot, et al. (2013), (Case 4)
		c.1861C>T	p.(Arg621Cys)	AD	ACS	Yes	5	Rieder et al. (2012), (Case M003). Gordon, Vuillot, et al. (2013), (Case 9)
		c.1861C>T	p.(Arg621Cys)	AD	ACS	No	8	0
		c.1861C>T	p.(Arg621Cys)	AD	ACS	No	18	0
		c.1862G>A	p.(Arg621His)	AD	ACS	No	1	Gordon, Vuillot, et al. (2013) (Case 6)
		c.1862G>A	p.(Arg621His)	AD	ACS	Yes	9	0
		c.1862G>A	p.(Arg621His)	AD	ACS	Yes	10	0
		c.1862G>A	p.(Arg621His)	AD	ACS	Yes	11	0
		c.1862G>A	p.(Arg621His)	AD	ACS	No	19	0
		c.1862 G>T	p.(Arg621Leu)	AD	ACS	Yes	2	Gordon, Vuillot, et al. (2013) (Case 5, I:1)
		c.1862 G>T	p.(Arg621Leu)	AD	ACS	Yes	3	Gordon, Vuillot, et al. (2013) (Case 5, II:1)
	HMZ	c.624delG	p.(Lys208Asnfs*5)	AR	ACS	No	22	Leoni et al. (2016)
		c.1620dup	p.(Thr541Hisfs*5)	AR	ACS	No	23	0
	HTZ compound	c.854-1G>A, c.1238+1G>C		AR	ACS	Yes	20	Kido et al. (2013) (Case 1)
		c.854-1G>A, c.1238+1G>C		AR	ACS	Yes	21	Kido et al. (2013) (Case 2)

(Continues)

TABLE 1 (Continued)

Gene	Type of mutation	cDNA modification	Protein change	Inheritance	Diagnosis	Familial	Patient number	Publication
GNAI3	HTZ	c.133G>A	p.(Gly45Ser)	AD	ACS	No	28	0
		c.134G>T	p.(Gly45Val)	AD	ACS	No	26	Propst et al. (2013). Romanelli Tavares et al. (2015) (Case Sp1)
		c.140G>A	p.(Ser47Asn)	AD	ACS	Yes	29	0
		c.141C>A	p.(Ser47Arg)	AD	ACS	Yes	24	Gordon, Vuillot, et al. (2013) (Case 7)
		c.143C>A	p.(Thr48Asn)	AD	ACS	No	27	Romanelli Tavares et al. (2015) (Case Sp2)
		c.805A>T	p.(Asn269Tyr)	AD	ACS	Yes	25	Guion-Almeida et al. (2002) (Case 1). Masotti et al. (2008) (F2: III-20). Romanelli Tavares et al. (2015) (F1: III-20)
EDN1	HTZ	c.3G>C	p.?	AD	ACS	No	39	0
		c.191T>A	p.(Val64Asp)	AD	IQME	Yes	33	Gordon, Vuillot, et al. (2013) (Case 11). Gordon, Petit, et al. (2013) (Case F2-I:2)
		c.191T>A	p.(Val64Asp)	AD	IQME	Yes	34	Gordon, Vuillot, et al. (2013) (Case 11). Gordon, Petit, et al. (2013) (Case F2-II:2)
		c.249T>G	p.(Tyr83*)	AD	IQME	Yes	30	Gordon, Vuillot, et al. (2013) (Case 12). Gordon, Petit, et al. (2013) (Case F3-III:2)
		c.249T>G	p.(Tyr83*)	AD	IQME	Yes	32	Gordon, Vuillot, et al. (2013) (Case 12). Gordon, Petit, et al. (2013) (Case F3-IV:2)
	HMZ	c.230C>A	p.(Pro77His)	AR	ACS	No	38	Guion-Almeida et al. (2002) (Case 2). Gordon, Petit, et al. (2013) (Case S1-II:1)
		c.271A>G	p.(Lys91Glu)	AR	ACS	Yes	35	Gordon, Vuillot, et al. (2013) (Case 10). Gordon, Petit, et al. (2013) (Case F1-II:1)
		c.271A>G	p.(Lys91Glu)	AR	ACS	Yes	36	Gordon, Vuillot, et al. (2013) (Case 10). Gordon, Petit, et al. (2013) (Case F1-II:3)
		c.271A>G	p.(Lys91Glu)	AR	ACS	Yes	37	Gordon, Petit, et al. (2013) (Case F1-II:4)
	(not tested)	(not tested)	(not tested)	AD	IQME	Yes	31	Gordon, Vuillot, et al. (2013) (Case 12). Gordon, Petit, et al. (2013) (Case F3-IV:1)

Note: Variants are ordered from 5′–3′, in separate blocks for each gene and mode of inheritance. Patient 31, although not tested, is the affected brother of Patient 32, harboring a heterozygous EDN1 mutation.
Abbreviations: ACS, auriculocondylar syndrome; cDNA, complementary DNA; HMZ, homozygous; HTZ, heterozygous; IQME, Isolated question mark ear.

TABLE 2 Frequencies of major phenotypes in the 39 patients

Question mark ear		
	Total	**37/38**
	Bilateral	**24/31**
	Unilateral	**7/31**
Postauricular tag	6/37	16%
Hearing loss	8/36	22%
Mandibular hypoplasia (clinical diagnosis)	31/35	89%
Full Cheeks	18/31	58%
Microstomia	16/39	41%
Dental anomalies	12/35	3%
Eye anomalies	5/36	14%
Neuro-developmental defects	14/38	37%
Intellectual Disability	3/23	13%
Maxillo-facial imaging abnormalities	22/24	92%
Mandibular hypoplasia (radiology)	19/24	79%
Condyle anomalies	15/24	63%
Condyle hypoplasia	13/15	87%
TMJ anomalies	14/24	58%
Bone abnormalities	6/32	19%
Sleep disorder	18/21	86%
Snoring	23/36	64%
Obstructive apnea	8/21	38%
Mixed apnea	8/21	38%
Gastrointestinal disorder	15/38	39%
Feeding difficulties	12/38	31%

Abbreviation: TMJ, temporomandibular joint.

subsequently observed. Cardiovascular and hearing tests have been normal. She snores and sleeps in a sitting position. A sleep study performed before distraction at 7 years of age demonstrated both obstructive and central apnea (apnea-hypopnea index (AHI) of 86 events per hour) with hypercapnia. Unfortunately, polysomnography was not repeated after mandibular distraction. Growth and psychomotor development were normal. CT scan confirmed mandibular and condylar dysplasia. Inner ears appeared normal. She harbors the heterozygous mutation c.1861C>T; p.(Arg621Cys) in *PLCB4*.

Patient 19, a female, was term-born in Algeria after an uneventful pregnancy with normal birth parameters. Her parents are not consanguineous and have no relevant medical history. She presented with bilateral QME and retrognathia (Figure 1g–i). No feeding or breathing difficulties were noted. She developed post-natal growth retardation (weight far below the third centile and height below the fifth centile at 15 years old), delayed puberty, intellectual deficiency, and motor dyspraxia. Bilateral otoplasty was performed at 13 years of age and the diagnosis of ACS was made at that time. She reported neither sleeping difficulties nor snoring. Cardiovascular, ophthalmologic, and hearing examinations were normal. She harbors a de novo heterozygous c.1862G>A; p.(Arg621His) mutation in *PLCB4*.

Patient 23, a female, was born from consanguineous parents originating from Turkey and has three healthy siblings. A club foot and unilateral ear dysplasia were noted on ultrasound at 23 weeks of gestation (WG) and 36 WG, respectively. She was term-born with growth parameters in the normal range. She presented with a right QME and a slight notch on the left ear and mild retrognathia without palate or tongue anomalies (Figure 1j–l). She had severe mixed sleep apnea on polysomnography at 2 months of age, requiring nocturnal noninvasive ventilation. Feeding difficulties necessitated tube feeding for 3 months. She was hypotonic and had several acute episodes of hypertonia, dystonia, swallowing difficulties, irregular breathing, and nystagmus. External genitalia was normal. She walked unaided at 2.5 years of age and speaks in short sentences at 4.5 years of age but is still not toilet trained. She integrated into kindergarten with a school life assistant. She has a homozygous c.1620dup; p.(Thr541Hisfs*5) mutation in *PLCB4* identified through exome sequencing. The heterozygous parents do not have craniofacial anomalies. The father has unilateral hearing loss of unknown origin, the mother has bilateral iris coloboma and a sister has unilateral iris coloboma.

Patient 28, a male, was term-born after an uneventful pregnancy with normal birth parameters. Parents had no relevant family history. The diagnosis of ACS was made at birth with the association of QME, severe micrognathia, full cheeks, and microstomia requiring gastrostomy and tracheostomy. He presented with failure to thrive, but with normal height. Psychomotor development was in the normal range at 12 months of age. The hearing was normal. Craniofacial CT-scan showed mandibular hypoplasia, abnormal condyles, and TMJs, and unspecified inner ear anomalies. He harbors a de novo heterozygous c.133G>A; p.(Gly45Ser) mutation in *GNAI3* (RefSeq NM_006496.4).

Patient 29, a female, is the only child of nonconsanguineous parents originating from China. Retrognathia was noted on ultrasound at 33 WG. She was born at 35 WG with parameters in the normal range. She presented with severe ACS, characterized by bilateral QME with thick, overfolded, and dysplastic helices, severe microretrognathia, full cheeks, microstomia, and cleft palate (Figure 1m–o) leading to severe upper airway obstruction and feeding difficulties. A patent ductus arteriosus closed on the 9th day of life. She underwent tracheostomy at birth and gastrostomy at 16 months of age. Craniofacial CT imaging demonstrated a small mandible and bilateral TMJ ankylosis that benefited from arthroplasty and costochondral grafts at 4 years of age, followed by reconstructive surgery of the ears at 5 years of age and external mandibular distraction at 6 years of age. Psychomotor development and growth were normal with OFC on the third centile. She had profound bilateral hearing loss, a speech disorder, and orofacial dyspraxia. The tongue was normal and gum hypertrophy was noted. She harbors the heterozygous mutation c.140G>A; p.(Ser47Asn) in *GNAI3*. The mutation was inherited from her mother who presented with mild retrognathia and no ear anomalies.

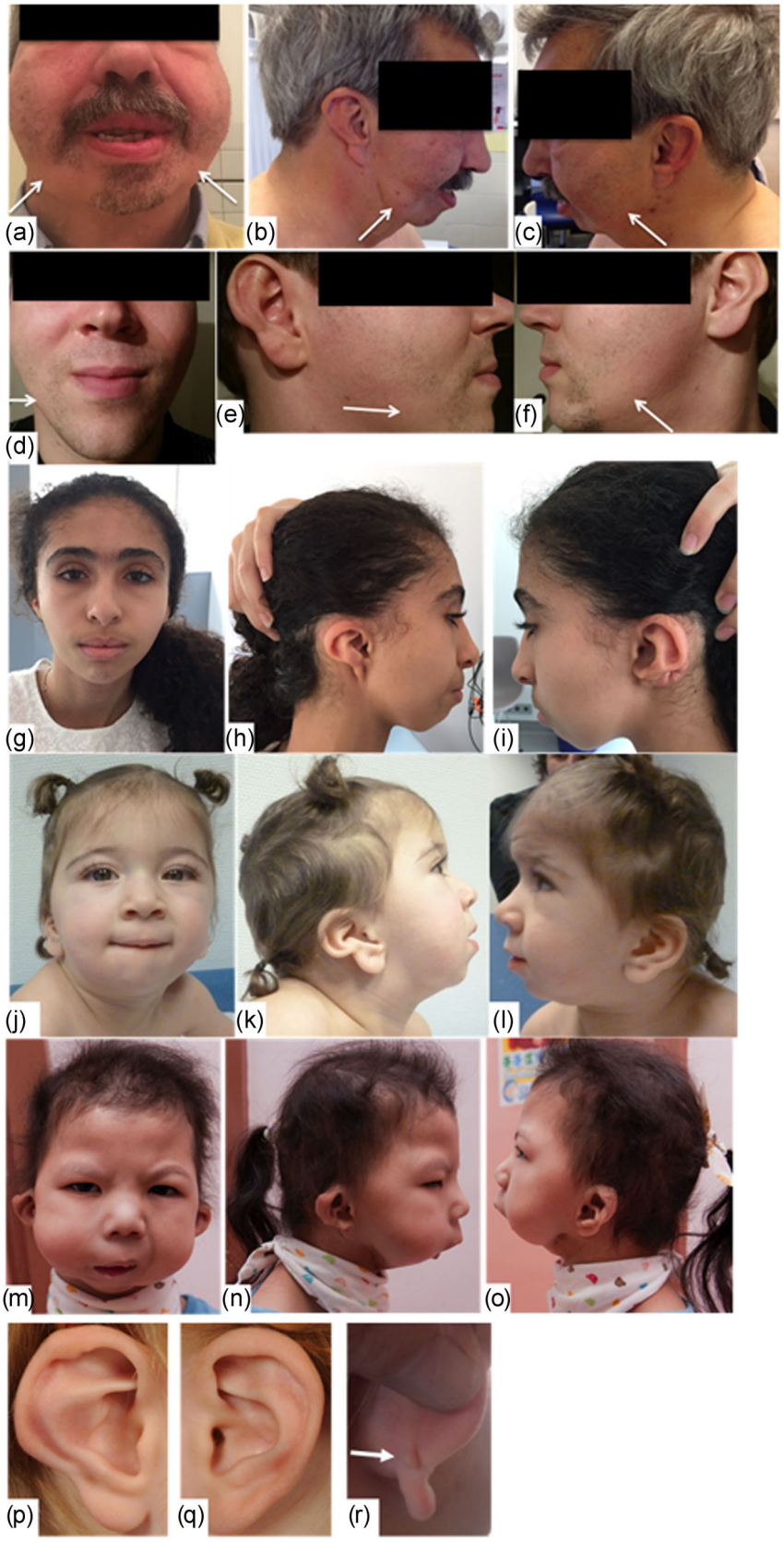

FIGURE 1 Photographs of novel auriculocondylar syndrome (ACS) cases. In Family 11, the father (Patient 16) presents ACS (a–c) and his son (Patient 17) presents isolated Question Mark Ear (IQME) (d–f). Note the facial hair growth pattern in both patients (arrows), showing no hair along the proximal region of the horizontal branch of the mandible. Patient 19 presents retrognathia, bilateral QME with dysplastic and protruding antihelix and ear lobe hypoplasia (g–i; the photographs were taken after otoplasty). Patient 23 presents bilateral QME, retrognathia, and full cheeks (j–l). Patient 29 presents severe ACS with micro-retrognathia, full cheeks, microstomia, and QMEs with dysplastic and over folded helices (m–o). Patient 39 presents a unilateral QME (p and q) with postauricular tag (r)

Patient 39, a male, was term-born with normal birth parameters from healthy nonconsanguineous parents originating from Australia. Unilateral hydronephrosis had been noted on ultrasound at 32 WG, and a scarred and the small left kidney was noted on subsequent postnatal imaging. At birth, he presented with a right QME with a postauricular tag (Figure 1p–r), microstomia, submucous cleft palate, and mild micrognathia. Growth and psychomotor development were normal. Trigger thumbs were noted. Conductive hearing loss necessitated tympanostomy tube insertion. Sleep apnea with snoring was treated by tonsillectomy at 2 years of age. Craniofacial CT scan was not available. He harbors a heterozygous c.3G>C; p.? mutation in *EDN1* (RefSeq NM_001955.5), inherited from his father, who has normal ears and no micrognathia or abnormal distribution of his facial hair.

3 | RESULTS

Of 44 questionnaires sent to the referring doctors, 39 patients from 27 families with a completed questionnaire were included, comprising 33 ACS and six IQME cases.

A mutation in one of the three known genes was previously reported or identified in this study in all patients except patient 31, an affected member of a family harboring an *EDN1* mutation (Table 1). We identified six previously undescribed mutations; heterozygous p.(Ile569Phe) and p.(Gly339Arg) in *PLCB4* (Families 10 and 11, respectively), homozygous p.(Thr541Hisfs*5) in *PLCB4* (Patient 23), heterozygous p.(Gly45Ser) and p.(Ser47Asn) in *GNAI3* (Patients 28 and 29, respectively) and heterozygous p.? in *EDN1* (Patient 39). When considering index cases from each family, *PLCB4* is the most common gene associated with ACS (16/27, 59%). Of the 16 families with ACS-associated *PLCB4* mutations, 13 harbored heterozygous missense variants clustered in the catalytic domain of the protein with one hotspot at codon Arg621, which is replaced by a histidine, a cysteine or leucine in 3, 4, and 1 cases, respectively (Table 1). Gly339 and Ile569 are conserved amino acids of the PLCB4 catalytic X and Y domains respectively, mutations of which are not reported in the gnomAD database. In the remaining three families with *PLCB4* mutations, the variants were homozygous truncating or compound heterozygous affecting essential splice sites (Patients 20–23). *GNAI3* missense heterozygous mutations were less frequent (6/27, 22%). All mutations clustered in the G1 box of the catalytic domain except the previously published mutation p.(Asn269Tyr) (located in the G4 box; patient 25). Concerning *GNAI3* codons Gly45 and Ser47 in the G1 motif, mutations at these positions have already been reported (Gordon, Vuillot, et al., 2013; Romanelli Tavares et al., 2015) but the amino acid substitutions are different for the two novel cases we report (Patients 28 and 29). The novel *EDN1* mutation we identified (c.3G>C, p.?, Patient 39) is not found in gnomAD and is predicted to alter the initiation codon of translation and result in loss-of-function, considering that methionine residues at position 6 and 59 of EDN1 are not in a consensus Kozak configuration.

As previously mentioned in the literature, incomplete penetrance and variable expressivity are observed within families segregating heterozygous mutations in either *PLCB4* (Families 8, 10, and 11) or *GNAI3* (Family 18; (Romanelli Tavares et al., 2015)). Variable expressivity among unrelated patients harboring *PLCB4* recurrent heterozygous mutations is also observed (e.g., Patients 4, 5, 8, and 18, each with p.(Arg621Cys)).

The core craniofacial features of the 39 patients are described below, followed by extra-craniofacial features.

3.1 | Craniofacial anomalies

A small mandible was noted at examination in 31 patients (31/35, 89%). Craniofacial imaging, which was available for 24/39 (62%) patients, demonstrated mandibular hypoplasia in 19/24 (79%), condyle anomalies in 15/24 (63%) (the latter consistingof hypoplasia in 13 out of 15 cases (87%)), and TMJ anomalies in 14/24 (58%) (Figure 2). Interestingly, we observed hypoplasia of the hyoid bone in two patients (Patients 5 and 36, Figure 2), while medial and lateral pterygoid muscles could not be distinguished from one another on imaging in Patient 5. From photographs, full cheeks were observed in 18/31 (58%) patients and microstomia in 16/39 (41%). Tongue anomalies were noted in 11/33 (33%), the majority being glossoptosis, while orthodontic anomalies were noted in 12/35 (34%).

External ear anomalies ranged from normal to severe microtia (Figure 3), and were frequently asymmetric. QMEs were present in most cases (37/38, 97%) and were predominantly bilateral (24/31, 77%). Postauricular tags were reported in six patients with ACS (6/37, 16%), four of them harboring a *PLCB4* mutation at codon Arg621 (Patients 1, 3, 4, and 5), one being homozygous for a frameshift mutation of *PLCB4* (Patient 22) and one carrying a heterozygous mutation of *EDN1* (Patient 39; Figure 1). As the presence of ear tags was not specifically elucidated in the questionnaire, the frequency of this feature may be underestimated.

3.2 | Hearing loss

Hearing loss was noted in eight patients with ACS. Conductive hearing loss (CHL) was reported in five patients (Patient 3 with a heterozygous *PLCB4* mutation, Patients 26 and 27 with a *GNAI3* mutation, Patient 36 with a homozygous *EDN1* mutation, and Patient 39 with a heterozygous *EDN1* mutation). The type of hearing loss was unspecified in three patients (Patients 8 with a heterozygous *PLCB4* mutation and 25 and 29 with a *GNAI3* mutation). Auditory canal stenosis was reported once in Patient 36.

3.3 | Costal anomalies

Rib defects were reported in two patients harboring a *GNAI3* mutation (Patients 24 and 26; Figure 4). Case 24 had hypoplastic first ribs and Case 26 had a unilateral fusion of the first and second ribs.

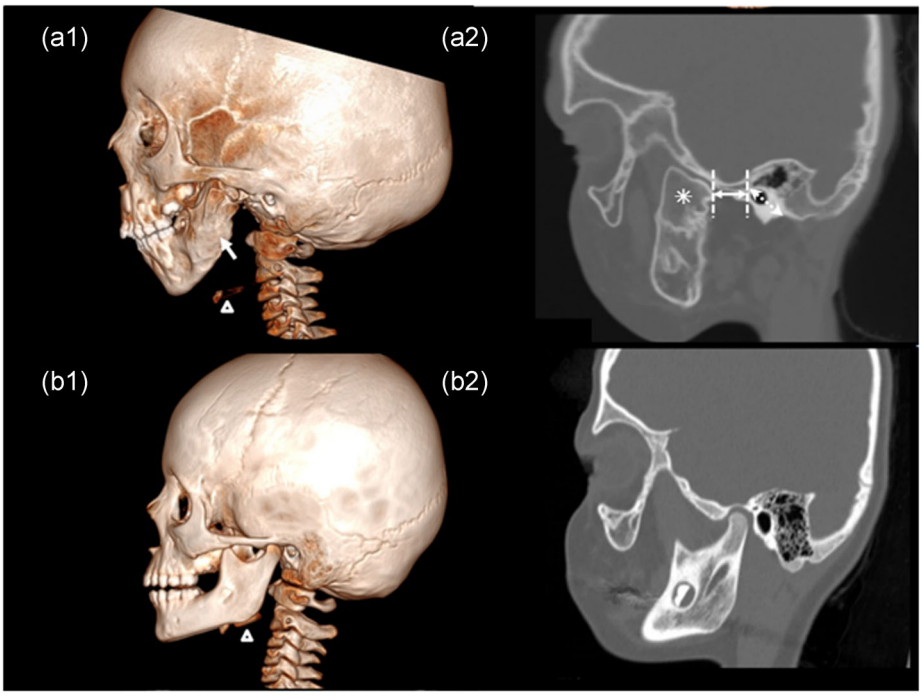

FIGURE 2 Craniofacial computed tomography (CT) images demonstrating lower jaw anomalies in auriculocondylar syndrome (ACS). For Patient 5 (a1 and a2) and a sex- and age-matched control (b1 and b2), three-dimensional surface reconstructions (a1 and b1) and oblique sagittal images through the temporomandibular joint (a2 and b2) are shown. In panel (a1), full arrow indicates mandibular dysplasia (micrognathia with hypoplasia and shortening of the ascending branch), and arrowhead indicates dysplasia of the hyoid bone, with horizontalization and hypoplasia of its major branches compared to control (b1). Double full arrow in (a2) indicates increased distance between external auditory canal and glenoid fossa while double dotted arrow indicates dysplastic tympanic bone (oblique and thickened). Asterisk shows dysplastic temporomandibular joint (condylar articular facet flattened and tilted backwards) compare to control (b2)

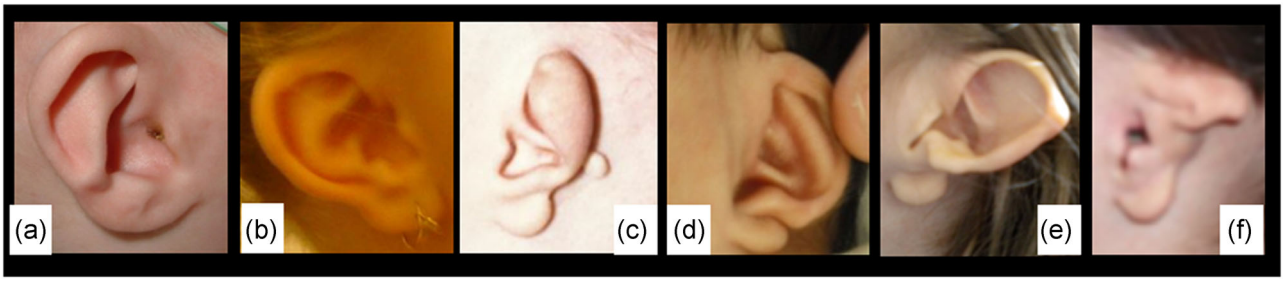

FIGURE 3 Question mark ears with increasing severity of malformation. (a) protruding antihelix without notch of the pinna, (b) notch of the pinna, (c) notch of the pinna, over-folded helix and postauricular tag, (d) cleft between lobe and helix, protruding antihelix and hypoplastic helix, (e) cleft between lobe and helix, and Darwin tubercle, (f) microtia with thick and overfolded pinna

3.4 | Vascular anomalies

Cerebrovascular malformations were detected by brain MRI in two individuals with no neurological symptoms. Patient 35, carrying a homozygous *EDN1* gene mutation, has an aneurysmal dilatation of the vein of Galen associated with two arteriovenous fistulae (Gordon, Petit, et al., 2013; Gordon, Vuillot, et al., 2013). Patient 26, carrying a *GNAI3* mutation, presented with a left parieto-occipital arteriovenous malformation.

A patent ductus arteriosus, closed by fluid restriction by Day 9 (Patient 29), is the only congenital heart defect identified upon echocardiography performed in 17 individuals.

3.5 | Neurological anomalies

Psychomotor developmental delay was reported in 12/27 (44%) patients, and includes neonatal hypotonia, walking unaided after

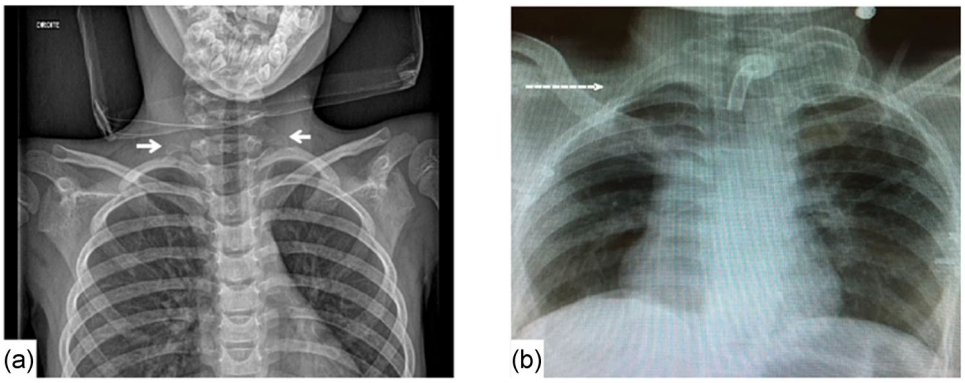

FIGURE 4 Antero-posterior X-ray of cervical and thoracic spine of Patients 24 and 26. Arrows show the hypoplastic first ribs in Patient 24 (a), dashed arrow shows the fusion of the first and second ribs on the right in Case 26 (b)

18 months of age, and speech delay. However intellectual disability was rare (3/23 patients, 13%) and autistic spectrum disorder was reportedin one individual. Among the 12 patients with psychomotor delay, seven harbored heterozygous *PLCB4* mutations, two had biallelic *PLCB4* mutations, one had a *GNAI3* mutation and two had homozygous *EDN1* mutations. Neuroimaging revealed nonspecific abnormalities in two patients (22 and 38).

Finally, sleep disorders (as assessed by sleep study) were common (18/21, 86%), and consisted of obstructive (8/21 patients, 38%) or mixed apnea (8/21 patients, 38%), with snoring reported in many patients (23/36, 64%). Feeding difficulties and/or gastroesophageal reflux (15/38, 39%) were also frequent.

4 | DISCUSSION

This study describes 39 patients with the phenotypic spectrum of IQME-ACS, and has identified several novel variants in the three causal genes.

PLCB4 and *GNAI3* mutations are associated with an ACS phenotype. With respect to *PLCB4*, two modes of inheritance (autosomal dominant or recessive) are observed. All heterozygous missense mutations affect the catalytic domain of the protein and are predicted to act via a dominant-negative mechanism. We observed incomplete penetrance and/or variable expression within families. Conversely, while individuals with heterozygous loss-of-function mutations in *PLCB4* are asymptomatic, craniofacial phenotypes are highly penetrant in patients with homozygous or compound heterozygous loss-of-function alleles and they can present with additional features, including central sleep apnea, constipation, and genitourinary anomalies (Leoni et al., 2016). With respect to *EDN1*, we previously observed that patients heterozygous for predicted loss-of-function mutations present with IQME, however patient 39 reported here, with a heterozygous *EDN1* start site mutation, has QME plus mild mandibular hypoplasia. Predicted hypomorphic *EDN1* mutations result in an ACS phenotype in homozygotes and no phenotype in heterozygotes (Gordon, Petit, et al., 2013). With respect to *GNAI3*, only heterozygous mutations have been identified thus far. Expressivity is highly variable within families and craniofacial anomalies can be severe, requiring maxillofacial surgery, tracheostomy, and gastrostomy.

It remains unclear whether some associated features of ACS are suggestive of mutation in a specific gene. Postauricular tags have been observed in a few patients with *PLCB4* mutations but were also observed here in Patient 39 with an *EDN1* mutation. Genital anomalies can occur in association with a biallelic mutation in *PLCB4* (Leoni et al., 2016) and rib anomalies may suggest a *GNAI3* mutation (described here for Patients 24 and 26). Interestingly, rib and vertebral fusions are observed in *Gnai3* null mice (Plummer et al., 2012). The possibility of vertebral fusion in patients with ACS remains inconclusive and we recommend cervico-thoraco-lumbar X-rays in all patients with a *GNAI3* mutation. However, all of the above-proposed associations are based on relatively few patients, and a larger number of patients will be required to confirm these associations.

Previous studies have suggested a homeotic transformation of the mandible into an upper jaw-like structure in patients with ACS and *PLCB4*/*GNAI3* mutations (Clouthier et al., 2013; Rieder et al., 2012). Other clinical manifestations of a homeotic transformation within the first branchial arch are palate-like soft tissue on the floor of the mouth in some patients (Clouthier et al., 2013; Rieder et al., 2012) and an abnormal pattern of facial hair in adult males (Gordon et al., 2014; and Family 11 reported here). Possible homeotic transformation of the ossicles, as well as permanent teeth, remains to be investigated. Considering second and third branchial arch development, we report hyoid bone anomalies in Patients 5 and 36. Hyoid defects have previously been described in *Edn1*$^{-/-}$ and *Ednra*$^{-/-}$ mice (Clouthier et al., 1998; Ozeki et al., 2004).

Hearing loss in ACS patients has not been thoroughly described. As expected, conductive hearing loss seems to be predominant and has been attributed to fusion of the malleus and incus in a patient with a *GNAI3* mutation (Patient 26) (Propst et al., 2013). Interestingly, *Gnai3*$^{-/-}$ mice have cochlear hair cell anomalies (Ezan et al., 2013) and Gαi isoforms have been shown to play essential and redundant roles in the development and maturation of various cell types of the

cochlea, auditory nerve, and central auditory neurons (Beer-Hammer et al., 2018). Attention needs to be paid to middle and inner ear function by way of an audiogram in all patients with ACS to evaluate for hearing loss.

Central sleep apnea has previously been reported in patients with ACS. Although it could be the consequence of severe obstructive apnea (Eckert et al., 2009) it persisted after treatment of the obstruction by distraction surgery for some patients (Gordon, Vuillot, et al., 2013). Therefore monitoring for both obstructive and central apnea should be considered in all patients with ACS. Of note, the Endothelin 1 signaling pathway is involved in the regulation of ventilation in mice (Gaultier, Amiel, et al., 2004; Gaultier, 2004).

Motor and intellectual development are in the normal range in the vast majority of patients with ACS. Although speech delay was reported in several patients, it is unclear to what extent this phenotype may be due to oral motor issues. However, patients with biallelic *PLCB4* mutations may be at higher risk of developmental delay. Recent reports have highlighted that heterozygous myocyte enhancer factor 2C (*MEF2C*; MIM# 600662) loss-of-function mutations should be considered as a possible cause of QME associated with severe intellectual deficiency and/or epilepsy (Gordon et al.,2018; Le Meur et al., 2010). *Mef2c* is essential for the development of the craniofacial skeleton in mice and zebrafish (Miller et al., 2007; Verzi et al., 2007), and is a transcriptional target of endothelin signaling in cranial neural crest cells (Hu et al., 2015). Finally, brain MRI may be worthwhile, considering the possible risk of neurovascular malformations, even if developmental milestones are within the normal range.

In conclusion, through systematic analysis of a large cohort of patients with ACS, we have highlighted how the core craniofacial features can be associated with rare extra-branchial arch manifestations such as ventilatory, neurodevelopmental, vascular, genital, and skeletal anomalies. Additional anomalies of the middle ear, facial musculature, and teeth still need to be better elucidated. Taking into account the frequent and rare features of this condition, this study allows us to make recommendations for the assessment and follow-up of patients with ACS.

WEB RESOURCES

OMIM: https://www.omim.org

GnomAD: https://gnomad.broadinstitute.org

LOVD: http://www.lovd.nl

Mutalyzer: https://mutalyzer.nl/

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

Informed consent for genetic testing and for publication of images was obtained for all patients. Human genetic research in this study was performed with approval from the institutional review board "Comité de Protection des Personnes Ile-de-France II" (Necker Hospital); approval was received June 10, 2015.

DATA AVAILABILITY STATEMENT

All available clinical and sequencing data are provided in the manuscript and supplementary information. Previously unreported variants have been submitted to http://www.lovd.nl.

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SUPPORTING INFORMATION

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