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Phenotype and genotype analysis of a French cohort of 119 patients with CHARGE syndrome

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CHARGE syndrome (CS) is a genetic disorder whose first description included Coloboma, Heart disease, Atresia of choanae, Retarded growth and development, Genital hypoplasia, and Ear anomalies and deafness, most often caused by a genetic mutation in the *CHD7* gene. Two features were then added: semicircular canal anomalies and arhinencephaly/olfactory bulb agenesis, with classification of typical, partial, or atypical forms on the basis of major and minor clinical criteria. The detection rate of a pathogenic variant in the *CHD7* gene varies from 67% to 90%. To try to have an overview of this heterogenous clinical condition and specify a genotype–phenotype relation, we conducted a national study of phenotype and genotype in 119 patients with CS. Selected clinical diagnostic criteria were from Verloes (2005), updated by Blake & Prasad (2006). Besides obtaining a detailed clinical description, when possible, patients underwent a full ophthalmologic examination, audiometry, temporal bone CT scan, gonadotropin analysis, and olfactory-bulb MRI. All patients underwent *CHD7* sequencing and MLPA analysis. We found a pathogenic *CHD7* variant in 83% of typical CS cases and 58% of atypical cases. Pathogenic variants in the *CHD7* gene were classified by the expected impact on the protein. In all, 90% of patients had a typical form of CS and 10% an atypical form. The most frequent features were deafness/semicircular canal hypoplasia (94%), pituitary defect/hypogonadism (89%), external ear anomalies (87%), square-shaped face (81%), and arhinencephaly/anosmia (80%). Coloboma (73%), heart defects (65%), and choanal atresia (43%) were less frequent.

KEYWORDS
CHARGE syndrome, *CHD7* gene, genotype, phenotype

1 | INTRODUCTION

CHARGE syndrome (CS) is a rare genetic disorder with variable occurrence of (C) coloboma, (H) heart defects, (A) atresia of choanae, (R) retardation of growth or development, (G) genital hypoplasia, and (E) ear abnormalities and deafness. The acronym was coined by Pagon, Zonana, and Graham (1982) but the syndrome was first reported by Hall (1979) and Hittner, Hirsch, Kreh, and Rudolph (1979). Later, semicircular canal (SCC) anomalies, and arhinencephaly were found to be part of CS (Tellier et al. 1998; Amiel et al., 2001; Morimoto et al., 2006). Clinical diagnostic criteria for CS were proposed in 1998 (Blake et al., 1998) and revised in 2005: Verloes (2005) added SCC hypoplasia to the major criteria and proposed a classification of typical, partial, and atypical forms. Then, Blake and Prasad suggested that cleft palate may be used to replace choanal atresia when absent (Blake & Prasad, 2006). Finally, in 2007 arhinencephaly or anosmia was added to the major features (Sanlaville & Verloes, 2007) (Table 1).

In 2004, the chromodomain helicase DNA binding protein 7 (*CHD7*) gene [OMIM *608892] was identified as the major gene involved in CS (Vissers et al., 2004). In the literature, the detection rate of a pathogenic variant in the *CHD7* gene varies from 67% to 90% (Janssen et al., 2012; Jongmans et al., 2006; Zentner, Layman, Martin, & Scacheri, 2010). The elongation factor Tu GTP binding domain-containing 2 (*EFTUD2*) gene has been involved in some cases (Lehalle et al., 2014).

*In 2004, the chromodomain helicase DNA binding protein 7 (CHD7) gene [OMIM *608892] was identified as the major gene involved in CS. In the literature, the detection rate of a pathogenic variant in the CHD7 gene varies from 67% to 90%.*

The aim of this study was first to describe phenotype and genotype of a large cohort of 119 French patients with CS, selected on the basis of their phenotype. The second aim was to search for correlations between the three clinical phenotypes (typical, partial and atypical, based on the Verloes criteria, updated by Blake and Sanlaville), and the molecular anomalies of *CHD7*, mainly to search for differences between patients with or without *CHD7* mutation in these different groups.

2 | PATIENTS AND METHODS

We conducted a national study of the phenotypes and genotypes of individuals with CS of all ages who were patients of developmental anomaly expert centers in France. Selected clinical diagnostic criteria were from Verloes (2005) updated by Blake & Prasad (2006) and Sanlaville & Verloes (2007) (Table 1). Inclusion criteria of the study were a clinical CS according to our criteria, the patient's or his parents' consents for the study and genetic analyses. As our study took place before the publication of Hale, Niederriter, Green, and Martin in 2016, which introduced pathogenic *CHD7* variants as a diagnosis criteria, we did not take this criteria into account for inclusion. Patients without selected clinical criteria of CS, those who refused to participate in the study, or whose parents refused genetic analyses were excluded. We asked all geneticists of the designated developmental anomaly centers to include as many patients fulfilling these criteria as possible, whatever their age. These could be individuals who presented to the center as new patients or existing patients who were contacted back in the context of the study. In total, 125 patients were included from 33 French university hospitals after medical examination by a clinical geneticist between February 2012 and April 2015. Clinical and molecular data were collected by online web-based questionnaire. All information required by the protocol was recorded by investigators in an electronic case report form developed for the study using Captur System software (Clinsight). The questionnaire was developed by a group of geneticists and pediatricians with special concerns in the syndrome. It consisted of multiple choice questions and clarifications could be provided through a written text. Answers to each item were not mandatory. Geneticists described their detailed clinical examination and, when possible, patients underwent full ophthalmologic examination including funduscopy, audiometry, temporal bone CT scan, gonadotropin and thyroid-stimulating hormone analysis, stimulation tests of growth hormone (GH), follow-up of pubertal development when relevant, and brain and olfactory-bulb MRI. The data could be extracted from the patient's file if the patient had had these exams before the time of inclusion.

Results of karyotype, comparative genomic hybridization (CGH) array, and fluorescent in situ hybridization for the 22q11.2 deletion were recorded. All patients underwent *CHD7* sequencing as previously described (Bilan et al., 2012; Sanlaville et al., 2006) and MLPA was performed to exclude exon deletions. Genetic analyses could have been performed before the study in some cases and completed after inclusion if necessary. In total, 125 patients were screened. CHD7 NM_017780.2 and NG_007009.1 were used for nucleotide reference and exon numbering, respectively. Pathogenic *CHD7* variants were classified by their expected impact on the protein. To investigate the potential impact on the splicing mechanism, all intronic and missense pathogenic variants were studied by using Human splicing finder software (http://www.umd.be/HSF3/). In some cases, minigene assays were used to clarify the impact on splicing efficiency. We used Polyphen 2.0 (http://genetics.bwh.harvard.edu/pph2/) to study the pathogenicity of variants, and the de novo origin was taken into account. We systematically queried the *CHD7* database (https://molgenis51.gcc.rug.nl/) to determine whether variants had been described and predicted as pathogenic. CS patients described in this study and their corresponding variants were submitted in the CHD7 database (https://molgenis51.gcc.rug.nl/). Samples for patients with no identified pathogenic *CHD7* variant underwent sequencing for *EFTUD2* (and eventually *HOXA1*, *TBX22*, *FOXE1*, *TXNL4A*).

Statistical analyses involved use of StatView software (SAS, Inc., Cary, NC) and because we could not obtain information for all items for each patient, the given percentages are the number of patients with a particular feature divided by the number of patients with information about the feature. For example, it was not ethically acceptable to

TABLE 1 Comparison of diagnostic criteria for CHARGE syndrome (CS) by Verloes (2005) updated by Blake (2006) and Sanlaville & Verloes (2007) and by Hale (2016)

	Verloes (2005) updated by Blake (2006) and Sanlaville (2007)	Hale (2016)
Major criteria	- Coloboma; - Choanal atresia and/or cleft lip or palate; - Semicircular canals agenesis/hypoplasia; - Arhinencephaly and/or anosmia	- Coloboma; - Choanal atresia or cleft lip or palate; - Abnormal external, middle or inner ears, including hypoplastic semicircular canals; - Pathogenic CHD7 variant
Minor criteria	- Cranial nerves VII to XII palsy; - Hypothalamo-hypophyseal dysfunction; - External- or middle-ear anomalies; - Heart defects or esophageal anomalies; - Intellectual disability	- Cranial nerve dysfunction including hearing loss; - Dysphagia/feeding difficulties; - Structural brain anomalies; - Developmental delay/intellectual disabilities/autism; - Hypothalamo-hypophyseal dysfunction (gonadotropin or growth hormone deficiency) and genital anomalies; - Heart or esophagus malformation; - Renal anomalies; - Skeletal/limb anomalies
	Typical: 3 major or 2 major + 2 minor criteria; Partial: 2 major + 1 minor criteria; Atypical: 2 major + 0 minor or 1 major + 3 minor criteria	2 major + any number of minor criteria

perform brain MRI without direct medical benefit and that sometimes requires general anesthesia for adult patients with a long-term diagnosis of CS.

The persons legally in charge of each patient gave their informed consent for the research. The ethical committee of our institution approved the project.

3 | RESULTS

In total, 125 patients were submitted for the study and screened for *CHD7* mutations. Six patients were excluded because they did not fulfill the inclusion criteria (i.e., less than 2 major criteria or less than 1 major and 3 minor criteria), although three had a pathogenic *CHD7* variant. One young female with intellectual disability, hypoplastic corpus callosum, aortic valve dysplasia, retarded growth, and facial palsy was suspected to have CS because of evocative dysmorphy, but she had neither coloboma, nor choanal atresia, nor olfactory-bulb defect nor hypoplasia of the SCCs. Another newborn did not undergo all the necessary exams because of his young age. Information regarding phenotype of the last case with a *CHD7* mutation, and the three others (one with a pathogenic *EFTUD2* variant) were unavailable.

Finally, we describe a series of 119 patients with CS (62 females; mean age 11 ± 10 years) in terms of the diagnostic criteria we defined. Data for three patients were included after they died. Clinical and genetic data are reported in Table 2. A pathogenic *CHD7* variant was identified in 93/118 patients (79%).

Overall, 107/119 individuals (90%) had typical CS, and 12 (10%) had atypical CS. No partial form was identified. All cases were sporadic except for three familial cases: two cases of transmission from a parent with typical CS to a child, with a pathogenic *CHD7* variant in one, and one case of suspected germ line mosaicism in one parent of two affected children (both parents were healthy and did not bear the pathogenic variant of their children).

3.1 | Monitoring of pregnancies and data at birth

During pregnancy, anomalies were detected in 46.6% (55/118) of cases. Nuchal translucency measurement was increased in four cases with a pathogenic *CHD7* variant. Most anomalies were detected during the second trimester and were isolated in 21/35 cases. The most frequent features were intra-uterine growth retardation (IUGR) (fetal biometry <3th percentile) (13 cases), heart defects (12 cases), cleft lip and/or palate (7 cases), and polyhydramnios (6 cases). For 15 cases, an anomaly was identified during the third trimester and was a hydramnios in 11 cases, a heart defect in 2 cases, an IUGR in 1 case, and a urogenital malformation in one case.

The mean weeks' gestation at birth was 36.6 ± 2.2. The mean weight at birth was 0.6 SD (±1.1 SD) below the mean and mean length 1.3 SD (±1.2 SD) below the mean for the term of delivery. Overall, 29 patients (26%) had IUGR, and 4 (4%) had a head circumference below −2 SD of normal and 7 (7%) above +2 SD of normal. The mean Apgar score at 10 min was 7.7 for patients with a truncating pathogenic variant and 10 for patients with a non-truncating pathogenic variant ($p = 0.036$).

3.2 | Clinical results

Results are detailed in Table 2 and summarized in Table 3. Differences between patients with or without variant in *CHD7* gene are given in Table 4.

3.2.1 | Ear defects

SCC hypoplasia/agenesis was consistent (95% [107/113] of patients; 99% [87/88] of *CHD7*-positive patients) and was bilateral in all but one case. Hearing loss was present in 93% (105/

113) of patients, 97% (86/89) of *CHD7*-positive patients, mostly bilateral (77/82 cases; 94%), of variable severity. Bilateral and asymmetric external-ear abnormalities were almost always observed (87% [100/115] of patients; 86% [78/91] of *CHD7*-positive patients). Almost all patients (96% [109/113]) had a defect of the inner or external ear. Only inner-ear defect was associated with deafness ($p = 0.0192$).

3.2.2 | Craniofacial dysmorphism

The typical shape of ears (asymmetric square ears with a triangular concha and without an earlobe) was the most frequent feature (87% [100/115] of patients; 86% [78/91] of *CHD7*-positive patients), followed by a square-shaped face (81%, 92/113), broad nasal bridge (71%,79/111), and facial asymmetry (64%,71/111) (Figures 1 and S1).

TABLE 2 Clinical features and mutation of patients with mutation in the *CHD7* gene

Patient no.	Sex	Age	Anosmia or olfactory-bulb agenesis	SCC hypoplasia	Coloboma	Choanal atresia	Cleft lip and/or palate	Heart defect	Esophageal atresia	Intellectual disability	Pituitary defect	Hypogonadism	Genital anomalies	Growth retardation	Cranial nerve defects	Deafness	Ear anomalies	Kidney anomalies	CNS anomalies	IUGR	Microcephaly	Classification	Mutation	
1	M	53		+	+	-	+	-	+		+	+	+	-	-	-	+	+			-	Typical	c.5945G>T	p.Gly1982Trp
2	M	29	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	Typical	c.2504_2508delATCTT	p.Tyr835fs
3	F	6		+	+	-	-	-	-	+	+		-	+	+	+	+	+	-	-	+	Typical	c.6165_6166delGT	p.Tyr2056fs
4	F	26		-	-		-	+	-	+	+	+	-	NM	-	+	+	-	+	-		Atypical	c.5405-7G>A	
5	F	16	+	+	+	+	-	+		+	+	+		+	+	+	+	+	+	-	-	Typical	Deletion of the locus	
6	F	16	+		-	+	+	+	-	+			-	+	+	+	+	+	+	-	-	Typical	c.219dupT	p.Gln74fs
7	F	21		+	+	+	-		-	+	+	+		+	+	+	+		+			Typical	c.934C>T	p.Arg312Ter
8	M	12		+	-	-	-	-	+	+	+	+	+	+	+	+	+	+		-	+	Typical	c.3490C>T	p.Gln1164Ter
9	F	8		+		-	+	+	-	+	+			+	-	+	+	+		+	-	Typical	c.2915A>G	p.Glu972Arg
10	M	35	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	Typical	c.[908_909insG;910dupA]	p.Ile304Ter
11	M	24				+	+	-	+	+	+	+	+	+	+	+	+	-		-	-	Typical	c.5405-17G>A	
12	F	7	+	+	+	+	-	+	-	-			-	+	+	+	+	-	+	-	-	Typical	c.4645-1G>C	
13	F	3	+	+	+	-	+	-	-	+			-	+	+	+	+	-	+	-	-	Typical	c.6268dupT	p.Trp2090fs
14	F	3	+	+	+	-	-	-	-	+			-	-	+	+	+	-	-	+	-	Typical	c.4145T>C	p.Ile1382Ser
15	F	18	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	Typical	c.5405-17G>A	
16	M	13m	+	+		-	-	-	+	-	+	+	+	-	+	+	+	-	+	-	-	Typical	c.5210+3A>G	
17	M	8	+	+	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	-	-	Typical	c.5405-17G>A	
18	F	31m		+	+	-	-	-	+	+				-	+	+	+	+	-	+	+	Typical	c.5355G>A	p.Trp1785Ter
19	F	31m	+	+	+	+	-	-	-	+			-	+	+	+	+	-	+	+	-	Typical	c.2918-2927del	p.Leu973Ter
20	F	4	+	+		+	-	+	+	-	+		-	+	+	+	+	-	+	+	-	Typical	c.718C>T	P Gln240Ter
21	F	5		+	+	-	-	+	-	+			-	+	+	+	+	-	+	-	-	Typical	c.7891C>T	p.Arg2631Ter
22	M	9	+	+	+	-	-	-	-	-			+	-	+	+	+	-	-	-	-	Typical	c.8491delT	p.Ser2831fs
23	M	19		+	-	-	-	+	-	+	+	+	+	-	+	+	+	-	-	-	+	Typical	c.3847C>T	p.Gln1283Ter
24	M	12	+	+		-	-	-	-	+	+	+	+	+	+	+	+	-	+	-	-	Typical	c.284_285delGG	p.Gly95fs
25	F	6	+	+	-	-	-	+	-	+			+	-	+	+	+	-	-	-	-	Typical	c.6393delT	p.Phe2131fs
26	F	24	+	+	+	+	-	+	+	-	+	+		-	+	+		+	+	+		Typical	c.2504_2508delATCTT	p.Tyr835fs
27	M	6	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	-	+	-	-	Typical	c.7384delT	p.Ser2462fs
28	F	15	+	+	+	-	+	+	-	+	+	+		-	+	+	+	+	+	-	-	Typical	c.6103+5G>A	
29	F	8	+	+		+	-	+	+	-			-	+	+	+	+	-	-	-	-	Typical	c.2145delC	p.Asn715fs
30	F	24		+	-	-	-	+	+		+	+		-	+	+	+			-		Typical	c.1480C>T	p.Arg494Ter
31	M	19	-	+	+	-	+	+		+	-	-		-	+	+		-	+	+		Typical	c.1665+1G>C	
32	M	13	+	+	+	+	-	+	+	+				+	+	+	+	-	-	-	-	Typical	c.4667dupC	p.Arg1557fs
33	M	5	+	+		-	-	+	-	+	+	+	+	+	+	+	+	-	+	+	-	Typical	c.4138dupA	p.Thr1380fs
34	M	20		+	+	-	+	+	+		-	-	-	-	+	+	+	-			-	Typical	c.1167_1168dup	p.Tyr390fs
35	M	23m	-	+		+	-	+	-	+			+	+	+	+	+	+	-	+	+	Typical	c.7344_7345delAA	p.Glu2450fs
36	M	17	+	+		+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	-	Typical	c.5991delT	p.Phe1997fs
37	F	33		+	+	+	-	+	-	-	+	+	+	-	-	+	+	-	-	-	-	Typical	c.8055G>A	p.Trp2685Ter
38	F	36	+	+		-	-	-	-	-		-	-	+		+	-	-	+			Typical	c.7165-15G>A	
39	M	8	+	+	-	-	-	+	-			-	+	-	-	+	+	-	-	-	+	Typical	c.2219A>G	p.Asp740Gly
40	M	10	+	+		+	-	+	-	+	+	+	+	+	-	+	+	+	+	-	+	Typical	c.7314_7332del19ins42	p.Glu2439fs
41	F	6	+	+		+	-	+	-	+			-	+	+	+	+	-	-	-	+	Typical	c.7456>T	p.Gln2487Ter
42	M	13		+			+	+	-	+	+	+	+	-		+	+	+	-	-	-	Atypical	c.3905T>C	p.Leu1302Pro
43	F	31	+	+	-	-	+	-	-		+	+	-	+	-	+	+		-	+	-	Typical	c.7988_7989insATA	p.Pro2664Ter
44	F	16		+		+	-	-	-	-			-	-	+	+	+		-	-	-	Typical	c.4353+1G>A	
45	M	13		+		-	-	+	-	+	+	+	+	-	-	+	+	-	+	-	-	Atypical	c.5050G>A	p.Gly1684Ser
46	M	19m	-	+	-	-	-	-	-	-			+	-	+	-	+		-	-	-	Typical	c.4215G>C	p.Gln1405His
47	F	21		+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	+	-	+	Typical	c.3106C>T	p.Arg1036Ter
48	M	10	+	+	-	+	-	+	-	-	-	-	+	+	+	+	+	-	-	-	+	Typical	Deletion of exons 30 and 31	
49	F	5		+	+	+	-	+	-	-	-	-	-	+	+	-	+	-		-	+	Typical	Deletion of exons 30 and 31	
50	F	5	+	+		-	-	+	-	+			-	+	+	+	+	-	-	+	-	Typical	c.7727A>G	p.Asp2576Gly
51	M	31m		+		+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	Typical	c.2499-2A>C	
52	F	7		+		-	-	+	-	-			+	-	+	+	+	-	+	-	-	Atypical	c.5405-18C>A	
53	M	31m	+	+	+	-	+	+	-	-			+	-	+	+	+	-	+	-	-	Typical	c.5405-7G>A	p.Gly1802fs
54	M	8			-	-	-	+	-	-	+	+	+	-	-	+	+			-	-	Atypical	c.5297C>G	p.Ser1766Ter
55	M	10	+	+	-	+	-	+	-	-			-	-	-	+	+	-	-	-	+	Typical	c.7282C>T	p.Arg2428Ter
56	F	5		+	+	+	-	+	-	+			-	-	+	+		+	+	-	-	Typical	c.8700_8704del	p.Gly2901fs
57	M	4		+	-	-	+	+	-	-			+	+	+	+	+	-		-	-	Typical	c.2238+2T>A	
58	M	6		+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	+	-	-	Typical	c.5300+1delG	
59	F	13	-	+	+	-	-	-	-	+	+	+	-	+	-	+	+	-	+	-	-	Typical	c.8458_8459dupCT	p.Ser2821fs
60	F	32	+	+	+	-	-	-	+	-	+	+		+	+	+	+	-	-	-	-	Typical	c.3091T>C	p.Trp1031Arg
61	M	29m	-	+		+	+	-	-	+	+	-	+	+	-	+	+	-	+	-	+	Typical	c.7879C>T	p.Arg2627Ter
62	F	8m		+		+	-	+	-				-	-	+	+	+	-	-	-	-	Typical	c.669_670insG	p.Asn224fs
63	M	5	+	+	+	-	-	+					+	-	-	+	+	+	-	-	+	Typical	c.2886T>G	p.Tyr962Ter
64	F	8	+	+		+	-	+		+			+	-	+	+	-	-	-	-	+	Typical	c.5210+3A>G	
65	M	4	+	+	-	+	-	+	+	+	+		+	+	+	+	+	+	+	-	+	Typical	c.2561delA	p.Ile856fs
66	F	22			+	+	-	+	-	+	+		+	+	+	+	+			-	-	Typical	c.4480C>T	p.Arg1494Ter
67	F	4m		+	-		-	+					-	-			+	-	-	-	-	Typical	c.6712G>T	p.Glu2238Ter
68	F	32m		+		-	-	+	-	+			-	-	+	+	+	-	-	-	+	Atypical	c.2906delG	p.Glu970fs

Patient no.	Sex	Age	Anosmia or olfactory-bulb agenesis	SCC hypoplasia	Coloboma	Choanal atresia	Cleft lip and/or palate	Heart defect	Esophageal atresia	Intellectual disability	Pituitary defect	Hypogonadism	Genital anomalies	Growth retardation	Cranial nerve defects	Deafness	Ear anomalies	Kidney anomalies	CNS anomalies	IUGR	Microcephaly	Classification	Mutation	
69	M	13m	+	+	-	+	-	+	-		+	+	+	-	+	+	+	-	+	-	+	Typical	c.3655C>T	p.Arg1219Ter
70	F	9m		+	+	-	-	-	-	-				+	+	+	+		-	+	-	Typical	c.2835+2T>C	
71	F	31		+	+	-	-	-	-	-				-	+	+	+		-	-	-	Typical	c.2835+2T>C	
72	F	1m	-	+	+	+		-	-					NM	-	+	+		+	+		Typical	c.3655C>T	p.Arg1219Ter
73	F	1m	-	+	+	-	-	+	-				-	NM			+	+	+	-	-	Typical	c.1294delC	p.His432fs
74	F	3	-	+		-	-	-	-	-			-	+	+	+	+	-	+	+	-	Atypical	c.5607+17A>G	
75	M	14		+	-	-	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	Typical	c.8077-2A>G	
76	M	34m		+		+	-	+	-				+	+	+	+	+	+	+	+	+	Typical	c.4851-1G>T	
77	F	9	+	+	+	-	-	-	+	-				-	+	+	+	-	-	-	-	Typical	c.7879C>T	p.Arg2627Ter
78	F	34m		+	-	-	-	+	+				-	-	+	+	+	-	-	-	-	Typical	c.755C>A	p.Ser252Ter
79	M	13	+	+	+	-	-	-	-	+			+	-	+	+	+	-		-	-	Typical	c.1135_1136delCA	p.Gln379fs
80	M	26m	+	+	+	+	+	-	-				+	-	+	+	+	+	-	-	-	Typical	c.5604_5607del	p.Ile1868fs
81	M	15		+	-	-	-	-	+	+		-	+	-	-	+	+	-	-	+	-	Typical	c.5405-17G>A	
82	F	27		+	-	+	+	+		+			-	+	-	+	+	-		-		Typical	c.2377-2A>G	
83	F	1m	+	+		-	-	+						NM	+		+		+	-	-	Typical	c.5157_5185dup	p.Lys1729fs
84	M	0m		+	-	+		+	-				-	NM			+	+		-	-	Typical	c.7614delT	p.Asp2538fs
85	F	3	+	+	+		-	+		-	+		-	+	+	+	+	-	-	-	-	Typical	c.4675delA	p.Arg1559fs
86	M	18		+		-	+	+	-	+	+	+	+	+	+	+	+	-		-	-	Typical	c.3999T>G	p.Tyr1333Ter
87	F	17	-	+	+	-	-	+	-	+	+	+	-	+	+	+	+		+	-	-	Typical	c.5929C>T	p.Arg1677Ter
88	F	17	-	+	+	+	-	+	+	+	+	-		+	-	+	+		+	+	+	Typical	c.6525insTdelGA	p.Glu2176fs
89	F	14		+	+	+	-	-	-	-			+	+	-	+		-	-	+	-	Typical	c.2504_2508delATCTT	p.Tyr835fs
90	M	14	-	+		+	-	+	-	+	+		+	+	+	+	+	-	-	-		Typical	c.2443-2A>G	
91	F	32m	+	+	+	-	+	+	-	-	+	-	-	+	+	+	+	-	+	+	-	Typical	c.6070C>T	p.Arg2024Ter
92	M	25		+	+	-	-	-		+	+	+	+	-		+	+	-	+	-	+	Typical	c.7282C>T	p.Arg2428Ter
93	F	15			+	-	+	-	-	+	-	-	-	-	-	+		-	+	-	-	Typical	c.6103+1delG	

CNS, central nervous system; F, Female; IUGR, intra-uterine growth retardation; M, Male; m, months; NM, not mentioned; SCC, semicircular canal.

3.2.3 | Central nervous system and cranial nerve anomalies

Arhinencephaly/anosmia: arhinencephaly was found in 77% (41/53) of patients; 76% (32/42) of *CHD7*-positive patients. Hyposmia or anosmia, when tested, was present in 82% (23/28) of patients; 83% (19/23) of *CHD7*-positive patients. 79% (56/71) of patients had one or both of these features.

TABLE 3 Frequency of clinical features of the patients with CHARGE syndrome

Clinical feature	No. of patients with the diagnostic criterion (%)
Major criteria	
Coloboma	85/117 (73)
Choanal atresia and/or cleft lip and/or palate	65/51 (56)
SCC hypoplasia or agenesis	107/113 (95)
Arhinencephaly and/or anosmia	56/70 (80)
Minor criteria	
Cranial nerve defects	112/114 (98)
Hypothalamo-hypophyseal dysfunction	55/62 (89)
External-ear anomalies	100/115 (87)
Heart defects or esophageal anomalies	86/115 (75)
Intellectual disability	63/96 (66)

SCC, semicircular canal.
The given percentages are the number of patients with a particular feature divided by the number of patients with information about the feature.

Cranial nerve defects were found in 73% (84/115) of all patients, 74% (63/87) of *CHD7*-positive patients. They were velopharyngeal dysfunction or swallowing problems in 65/77 (84%) or facial palsy in 54/77 (70%). For seven patients, we did not have details on which cranial nerve was affected. Central nervous system (CNS) anomalies (olfactory-bulb defect excluded) were found in 49% (50/102) of patients; 51% (40/79) of *CHD7*-positive patients. Hindbrain anomalies were not rare: 18/70 (26%) patients had cerebellar defect, 13/67 (19%) brainstem anomalies, and 2 Dandy Walker malformation. The other CNS anomalies included ventricular enlargement or hydrocephaly in 15/72 patients (21%), white matter defect in 12/66 (18%), hypoplastic corpus callosum in 11/73 (15%), and holoprosencephaly in 2/73 (3%). In addition, microcephaly was found in 31/108 patients (29%) and macrocephaly in one case. Features of hemorrhagic events were found in four patients and morphological anomalies of the hypothalamo-hypophyseal pituitary system in five.

3.2.4 | Heart/oesophageal defects

Classifying the diagnostic criteria, Sanlaville (2007) proposed to combine these two different defects into one minor diagnostic criterion as a malformation of mediastinal organs (heart, esophagus).

A congenital heart defect was found in 65% (76/117) of patients; 63% (58/92) of *CHD7*-positive patients, including various malformations, atrial (35/74) or ventricular (18/72) septal defects, that can be part of atrioventricular septal defects (8/72), tetralogy of Fallot (9/72), transposition of great vessels (4/72), double-outlet right ventricle (2/71), or hypoplastic left heart syndrome (1/71). Vascular anomalies included patent ductus arteriosus (30/72 cases), aberrant subclavian artery (6/69 cases), and coarctation of aorta (5/72). Three patients

TABLE 4 Frequency of clinical features in CHARGE syndrome cases with or without a pathogenic *CHD7* variant

Clinical features	Patients with *CHD7* variant (%)	Patients without identified *CHD7* variant (%)
SCC hypoplasia or agenesis	87/88 (99)	17/21 (81)
Deafness	86/89 (97)	17/20 (85)
External-ear anomalies	78/91 (86)	18/20 (90)
Anosmia	19/23 (83)	4/5 (80)
Olfactory-bulb agenesis	32/42 (76)	9/11 (82)
Cranial nerve defects	64/87 (74)	15/20 (75)
Hypogonadism	34/46 (74)	8/12 (67)
Coloboma	67/92 (73)	15/22 (68)
Inner-ear anomalies (SCC defects excluded)	50/77 (65)	11/18 (61)
Heart defects	58/92 (63)	16/21 (76)
Intellectual disability	48/77 (62)	11/15 (73)
Genital anomalies	43/77 (56)	10/20 (50)
CNS defect	40/79 (51)	8/20 (40)
Choanal atresia	38/89 (43)	9/21 (43)
Kidney anomalies	25/80 (31)	5/19 (26)
Esophageal anomalies	20/84 (24)	1/20 (5)
IUGR	21/89 (24)	6/20 (30)
Cleft lip and/or palate	18/91 (20)	2/22 (9)

SCC, semicircular canals; IUGR, intra uterine growth retardation; CNS, central nervous system.
The given percentages are the number of patients with a particular feature divided by the number of patients with information about the feature.

showed an anomalous pulmonary venous drainage, one aortic arch anomalies, two a right-sided aortic arch and one dextrocardia.

Esophageal defects occurred in 20% (21/107) of patients; 24% (20/84) of *CHD7*-positive patients, mostly esophageal atresia (50%). In all, 72% (75/104) had gastroesophageal reflux disease.

Finally, 75% (86/115) of patients had heart disease or esophageal defects.

3.2.5 | Ocular defects

Coloboma was present in 72% (82/114) of patients; 73% (67/92) of *CHD7*-positive patients and affected the iris in 15% and the retina in 80%. It was associated with microphthalmia in 38 patients. Nystagmus was present in 21% (25/119) of patients.

3.2.6 | Choanal atresia

Choanal atresia was found in 43% (49/114) of patients; 43% (38/89) of *CHD7*-positive patients. It was bilateral in 59% of cases and associated with a cleft lip or palate in five cases. Among the 65 remaining patients, 14 had an isolated cleft lip and/or palate. Overall, 18% (21/117) of patients had a cleft lip and/or palate and 56% had one or both of these features.

Choanal atresia was found in 43% (49/114) of patients; 43% (38/89) of CHD7-positive patients. It was bilateral in 59% of cases and associated with a cleft lip or palate in five cases.

3.2.7 | Endocrinological anomalies

Genitalia were abnormal in 47/54 males (87%) and 7/47 females (15%). Overall, 89% (55/62) of patients had pituitary deficiency, 72% (42/58) had hypogonadism. The item "hypogonadism" was assessed variously according to whether patients had had hormonal analysis or not. For those who did not have hormonal analysis, hypogonadism was diagnosed with under-development of the external genitalia in males or delayed puberty. Growth hormone deficiency, analyzed by GH stimulation tests, was found in 23/67 patients (34%) and hypothyroidism in 6/76 (8%). We did not have the information about the cause, peripheral or central, of the hypothyroidism.

3.2.8 | Intellectual disabilities

Intellectual disabilities were found in 66% (63/96) of patients; 62% (48/77) of *CHD7*-positive patients. This item was estimated by the clinicians who included patients, without details. Assessing the disabilities in these patients who have two sensorial defects, deafness and visual impairment, is difficult. Another study is evaluating this item. When the condition was known, all patients had delayed motor milestones.

3.2.9 | Other clinical features

Mean height, weight and head circumference were −1.7 SD (±1.6), −0.9 SD (±1.8), and −1.2 SD (±1.7), respectively, of patients' age at the time of the study. Vertebral malformations of varying severity were observed in 49% (50/103) of patients. A large spectrum of limb anomalies were present in 29% (31/108) of patients. The urinary



FIGURE 1 The typical shape of ears: asymmetric square ears with a triangular concha and without an earlobe

system was affected in 29% (30/103) of patients; 31% (25/80) of *CHD7*-positive patients, with variable severity, including pyelo-ureteral duct dilatation (5 cases), horseshoe kidney (1 case), unilateral (7 cases), or bilateral (1 case) renal hypoplasia, or unilateral renal agenesis (4 cases).

3.3 | Phenotype of patients with or without an identified pathogenic CHD7 variant

In all, 79% (93/118) of patients had a pathogenic *CHD7* variant; three had another molecular defect [pathogenic variant c.2245dup, p.(thr749asn fs*5) in the *EFTUD2* gene in one case, 8q21.3 deletion in one case and a chromosomal anomaly (arr 3p26.3p24[73,914–18,784,667] × 3,14q32.31q32.33[103,123,635–107,278,770] × 1 in one case), and 18% (22/118) had no identified anomaly. The frequency of each clinical feature and prognostic factor in the two groups are summarized in Table 4. No item significantly discriminated the groups. Hearing loss and SCC anomalies were present in almost all patients with a pathogenic *CHD7* variant (97–99%, respectively) versus 81–85% in patients without a *CHD7* variant.

3.4 | Molecular analysis

Overall, 98/119 patients had a karyotype, which was normal in 88 cases. One patient with a pathogenic *CHD7* variant had a chromosomal inversion, inv(7)(p15.2q33) (and a normal CGH array), and the karyotype result was not mentioned for 9 patients. The 22q11.2 deletion was excluded in 62 patients. In total, 35/100 patients without an identified

pathogenic *CHD7* variant underwent CGH array. Four other patients had 46,XX/47,XXY mosaic, an 8q12 deletion including *CHD7*, a de novo 8q21.3 locus deletion and an unbalanced translocation t(3;14)(arr3p26.3p24[73,914–18,784,667] × 3,14q32.31q32.33[103,123,635–107,278,770] × 1) derivative from a maternal translocation, respectively.

For 79% (93/118) of patients, the *CHD7* gene (pathogenic variant or deletion) was involved in the syndrome; 29% (27) patients had nonsense pathogenic variants, 34% (31) a frameshift mutation predicting a premature stop codon, 28% (26) a splice pathogenic variant, and 5% (5) a missense variant. Two familial cases showed deletion of exons 30 and 31, and one a complete deletion of the gene. In 71 cases, both parental DNA profiles were studied, and all pathogenic variants had occurred de novo except for one familial case (affected mother with typical CS). The intragenic deletion found in two brothers, with suspected germinal mosaicism in one parent, was not found in blood samples of parents. Seven pathogenic variants were found more than once in exons 8 (c.2504_2508del), 15 (c.3655C>T), 34 (c.7282C>T), and 36 (c.7879C>T) and introns 23 (c.5210 + 3A>G) and 25 (c.5405-17G>A and c.5405-7G>A). Among the 79 different pathogenic variants carried by our patients, 8 were previously described in other patients and 72 (90%) were novel. Pathogenic variants were located in the functional protein domain in 28/92 cases, particularly for the five missense pathogenic variants.

Pathogenic variants are summarized in Figure 2 and Table 2.

3.5 | Pathogenic variant classification in terms of the expected impact on the CHD7 protein structure

To study the relation between patients' phenotype and type of pathogenic variant they carried, we classified pathogenic variants as truncating or not. Nonsense and frameshift pathogenic variants represented 59 truncating pathogenic variants. Minigene assay of pathogenic variants c .5405-18C>A and c.5405-7 (data not shown) allowed us to consider that these pathogenic variants were truncating, whereas study of the pathogenic variant c.5405-17G>A concluded that it was non-truncating. Deletion of exon 30 and 31 was considered truncating. Because truncating pathogenic variants are predicted to not produce protein (due to a nonsense-mediated mRNA decay mechanism), we classified the complete deletion of the gene with truncating pathogenic variants. We excluded from the comparison the pathogenic variants with unknown truncating status: missense pathogenic variants with a possible effect on splicing mechanism and splice pathogenic variants not studied by minigene assay or for which a wild-type transcript was present in ex vivo study were not considered, thus excluding 20 patients. In silico and in vitro predictions concluded that five missense pathogenic variants were pathogenic and did not affect splicing (data not shown) (Table 5).

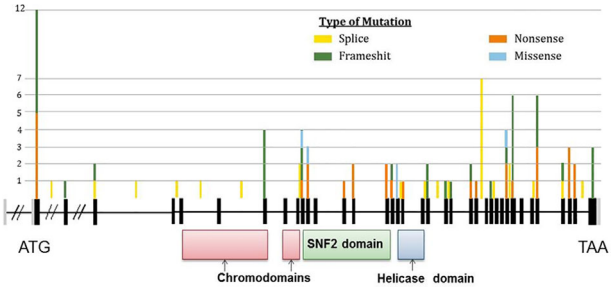


FIGURE 2 Pathogenic *CHD7* variants in CHARGE syndrome

3.6 | Molecular study by the phenotypic form of CS (typical vs. atypical)

Among the 119 patients, 90% (107) had typical CS according to our diagnostic criteria, and 10% (12) had atypical CS. We found a pathogenic *CHD7* variant in 83% (89/107) of typical CS cases and 58% (7/12) of atypical cases. One patient with atypical CS carried a frameshift pathogenic variant, one a nonsense pathogenic variant, three a splice pathogenic variant, and two a pathogenic missense mutation. No pathogenic variant was identified for 5/12 patients. When the variant status was known, pathogenic variants were truncating.

In all, 30 patients with typical CS carried a frameshift pathogenic variant, 25 a splice pathogenic variant, 24 a nonsense pathogenic variant, 5 a missense pathogenic variant, 1 a deletion of the locus, 2 familial cases an intragenic deletion, and 1 a pathogenic variant in *EFTUD2*. For 16 patients, no pathogenic variant was identified. When the status was known, pathogenic variants were truncating in 87% (60/69) of cases (Figure 3).

4 | DISCUSSION

For half of our series of CS, anomalies were detected during pregnancy, mainly heart defects and cleft lip or palate, which are not specific features. This finding agrees with the study of Busa et al. (2016) who, in a series of 12 children with a diagnosis of CS in the first 3 months of life and a pathogenic *CHD7* variant, found 58% of pregnancies complicated by the identification of isolated or multiple congenital anomalies. Such circumstances should lead to propose a systematic careful prenatal US examination to identify typical external ears and/or SCC anomalies which is possible around the 20–22th weeks of gestation. When the diagnosis is highly suspected, fetal brain MRI, feasible from the 28th week of gestation, and molecular analysis of *CHD7* can be proposed to confirm the diagnosis.

4.1 | Diagnostic criteria

As our study took place before the publication of Hale et al. in 2016, which introduced pathogenic *CHD7* variants as a diagnosis criteria, we did not take this criteria into account for inclusion of our patients. We chose to include patients on the basis of clinical data according to Verloes (2005) classification, updated by Blake et al. (2006) and Sanlaville et al. (2006). In this cohort of 119 patients, we did not find any partial form of CS (i.e., 2 major and 1 minor criteria). 90% had a typical

TABLE 5 Frequency of known or presumed prognostic factors in patients carrying a truncating or non-truncating mutation in *CHD7*

Clinical feature	Truncating mutation (%)	Non-truncating mutation (%)
Heart defect	44/63 (70)	2/9 (22)
CNS anomaly	31/46 (67)	1/6 (17)
Bilateral choanal atresia	19/58 (33)	0/8 (0)
Intellectual disability	34/51 (67)	4/8 (50)
SCC hypoplasia	60/61 (98)	8/8 (100)
Coloboma	48/63 (76)	7/9 (78)
Impaired vision	44/55 (80)	7/8 (87.5)
Impaired hearing	59/60 (98)	7/9 (78)
Cranial nerve defect	47/60 (78)	5/9 (56)
Atresia of esophagus	14/58 (24)	3/9(33)
Typical CHARGE syndrome	60/64 (94)	9/9 (100)

CNS, central nervous system; SCC, semicircular canals. The given percentages are the number of patients with a particular feature divided by the number of patients with information about the feature.

form and 10% an atypical one. Because of the small number of atypical cases (12), we could not statistically compare the two groups of typical CS form (3 major, or 2 major and 2 minor criteria) and atypical form (2 major, or 1 major and 3 minor criteria). Three patients were excluded because they lacked clinical criteria, but because the clinical practitioners suspected the diagnosis mainly on dysmorphic features, these patients were found, after inclusion, to have a pathogenic *CHD7* variant. Recently, Hale et al. (2016) reported a series of 28 patients including one with atypical presentation and a pathogenic *CHD7* variant and proposed to broaden the diagnostic criteria, adding a pathogenic *CHD7* variant as a major criterion. If we had adopted this classification, these three patients would not have been excluded from our cohort.

As our study took place before the publication of Hale et al. in 2016, which introduced pathogenic CHD7 variants as a diagnosis criteria, we did not take this criteria into account for inclusion of our patients. We chose to include patients on the basis of clinical data according to Verloes (2005) classification, updated by Blake et al. (2006) and Sanlaville et al. (2006)

Regarding major and minor criteria, our results confirm that SCC hypoplasia/agenesis and arhinencephaly/anosmia, present in 95% and 80% of patients, respectively, are major features of the syndrome. In

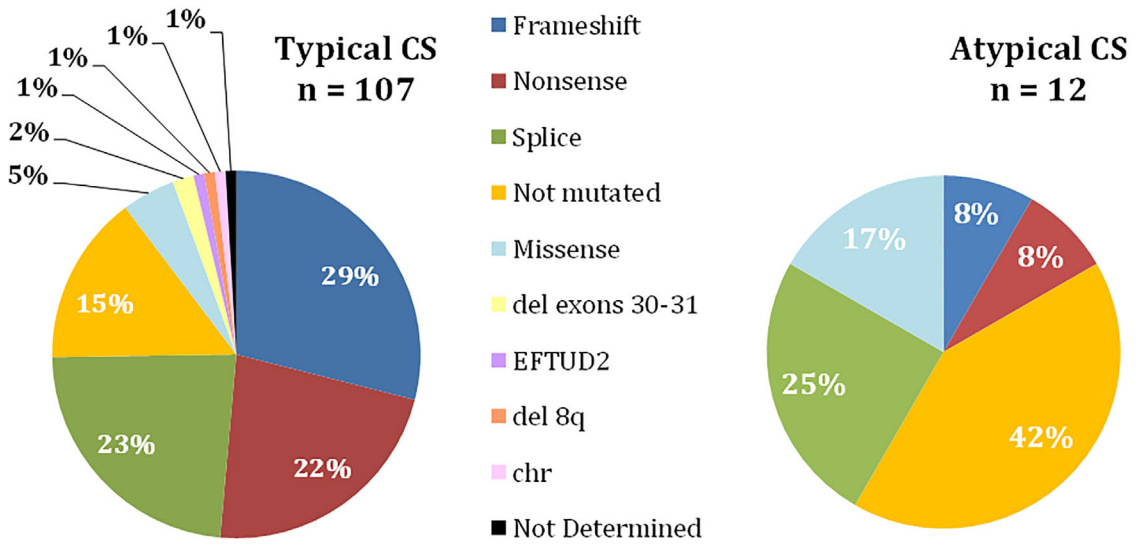


FIGURE 3 Type of mutations in CHARGE syndrome. Chr, chromosomal anomaly; CS, CHARGE syndrome

our series, we had adults with a diagnosis of CS for a long time, who did not undergo cerebral MRI but had anosmia. Because arhinencephaly or absent olfactory bulbs or tracts are more objective criteria than is anosmia, these features must be checked with a cerebral MRI in young patients as soon as a CS diagnosis is suspected. This item, absent of the Hale's classification, should be kept as a criteria of diagnosis, even if less specific than SCC hypoplasia/agenesis. Coloboma was present in 73% of our patients, thereby corroborating it as a major sign of the syndrome. Bilateral and asymmetric external-ear anomalies were present in 87% of our patients and the middle ear was abnormal in only 38%. In the Verloes' classification, external-ear anomalies were considered a minor sign, but we agree with Hale that they should be considered a major sign, especially in atypical forms, for which these are often the first feature that suggests the diagnosis of CS. Choanal atresia was found in only 43% of patients, and so cannot be considered alone as a major sign. In 2006, Blake and Prasad proposed to associate isolated cleft palate (i.e., not associated with choanal atresia in a given patient) with choanal atresia and consider the item "choanal atresia and/or cleft palate" as a major sign (Blake & Prasad, 2006). In our cohort, 56% of patients had one or both of these features.

Finally, 72% of our patients had hypogonadotrophic hypogonadism (HH). All patients did not have a biological analysis to ascertain HH, and this feature is often under-diagnosed in girls, because underdevelopment of external genitalia cannot be seen. For patients with a biological analysis, nearly 90% had HH. Recently, HH and agenesis of the uterus and ovaries was reported in a girl with CS due to a deletion of the *CHD7* gene (Reynaert et al., 2016). HH should be searched in patients in the first months of life if CS is suspected, because it can be treated at expected pubertal age, but biological diagnosis is not possible between age 6 months and puberty. Regarding this result of 90% of HH in CS patients, we suggest this item could be considered as a major criteria of the syndrome, even if not specific.

Dysmorphic traits of the face should be better considered for diagnosis of CS. A square-shaped face was found in 81% of our patients.

4.2 | Molecular analysis

In total, 79% (93/118) of patients had a pathogenic *CHD7* variant, 83% with a typical phenotype and 58% an atypical one. In 2010, Zentner et al., reviewing 379 cases of CS, found that 67% were *CHD7* mutation-positive, whereas 33% were mutation-negative. Then, identification of intragenic deletion by MLPA identified other *CHD7* mutation positive cases. Jongmans et al. (2006) found that mutation detection rate rises above 90% if only those CHARGE patients who met the clinical diagnostic criteria of Blake et al. (1998) and/or Verloes (2005) are taken into account. We have no explanation why the detection rate is lower in our study, while Verloes' criteria were taken into account. In our cohort, all patients underwent *CHD7* sequencing and MLPA. The advent of Whole Exome Sequencing (WES) should enable to identify some more mutations in the *CHD7* gene. Study by WES of our negative patients is going on, but data are not yet available. Moreover, we can presume that some negative patients could have a variant in non-coding parts of the gene, which could be identified either by sequencing the whole gene (i.e., exons and introns) either by Whole Genome Sequencing.

In total, 79% (93/118) of patients had a pathogenic CHD7 variant, 83% with a typical phenotype and 58% an atypical one.

Among the 79 different pathogenic variants carried by our patients, 8 were previously described in other patients and 72 (90%) were novel. These results confirm that most of mutations of *CDH7* gene are private ones.

Regarding the atypical phenotype, about the half (7/12) had a pathogenic truncating *CHD7* variant. We would have expected less severe mutations. Therefore, we could not conclude to a phenotype–genotype correlation.

Two of our patients had a holoprosencephaly. It is of interest that one of them had a mutation in *CHD7* (c.2504_2508delATCTT). This malformation had never been reported before in CS patients with a mutation in *CHD7*. In the other patient, no mutation was identified.

Unlike splice pathogenic variants (30% in our series vs. 11% in the literature), nonsense (29%) and missense (5%) pathogenic variants were less frequent in our cohort than previously described (44% and 8%; Janssen et al. 2012). Improvement in the interpretation of intronic or exonic variants, even when recurrent, probably improves the detection rate of pathogenic variants. In most cases, CS is due to *CHD7* haploinsufficiency. Pathogenic variants lead to a transcript carrying a premature stop codon that will be eliminated by nonsense-mediated mRNA decay. These pathogenic variants are truncating. Some patients have missense pathogenic variants or splice pathogenic variants involving sensitive interpretation. Sequence defects lead to a functional or structural alteration of the protein. These pathogenic variants are usually predicted to be responsible for a less severe phenotype.

Predicting the impact of a splice pathogenic variant is difficult. Most are probably responsible for *CHD7* haploinsufficiency and some may create an in-frame transcript. Depending on the pathogenic variant, the transcript is not generated by the cell or is an alternative transcript of the wild type. An active protein rate is thus variable, as was described by Lee et al. (2016) regarding the c.2443-2A>G pathogenic variant.

In our series, one patient had a locus deletion and typical CS. Among seven patients reported with a deletion involving the *CHD7* gene, two did not have CS in terms of Verloes' diagnostic criteria. This is a rare cause of CS that should be systematically searched when direct sequencing results for *CHD7* are negative. With the current technology of next-generation sequencing, these deletions should not be missed.

4.3 | Comparison of features of patients with and without a pathogenic *CHD7* variant

As was previously reported by (Zentner et al., 2010) and (Lalani et al., 2006), the frequency of hearing loss and SCC defects was greater with than without a pathogenic *CHD7* variant ($p = 0.03$ and $p = 0.004$, respectively). However, we did not find a significant difference between the groups in choanal atresia, heart defect, coloboma, growth retardation, milestone delay or kidney defects, which was reported by Hale et al., 2016; Lalani et al. (2006); and Zentner et al. (2010). Arhinencephaly, intellectual disability, heart defects and IUGR were more frequent without than with a pathogenic variant. Heart defects could be a selection bias in our cohort because they are frequent sign indicating consultation with a geneticist.

Pathogenic variants were more frequent with typical than atypical CS (83% vs. 58%), but the number of patients (10%, 12) with atypical CS was not sufficient to conclude. Clinical classification was established to predict the pathogenic *CHD7* variant but remains imperfect perhaps due to missed deep intronic pathogenic variants or regulatory region defects or misinterpreting *CHD7* variants. For example, deletion of a region upstream of the *CHD7* START codon has been described (Pisaneschi et al., 2015). Some of the patients in the non-mutating group were probably misclassified. *EFTUD2* was found involved in patients with a CS diagnosis and a specific feature, microcephaly (Lehalle et al., 2014).

4.4 | Comparison of patients with truncating or non-truncating pathogenic variants

We studied all clinical features and known or presumed prognostic factors and found no statistically significant difference regarding term at birth or Apgar score. The mean Apgar score at 10 min was 7.7 for patients with a truncating pathogenic variant and 10 with a non-truncating pathogenic variant ($p = 0.036$) probably due to the absence of bilateral choanal atresia in patients with a non-truncating pathogenic variant (33% of patients with a truncating pathogenic variant had choanal atresia). Heart defects, CNS anomalies, and cranial nerve defects were more frequent with truncating than non-truncating pathogenic variants. This finding, expected to impact the prognosis of the syndrome, is in agreement with Bergman et al. (2012) who showed that *CHD7* missense mutations are, in general, associated with a milder phenotype than truncating mutations.

4.5 | Limitations of the study

As our study took place before the publication of Hale et al. in 2016, which introduced pathogenic *CHD7* variants as a diagnosis criteria, we did not take this criteria into account for inclusion of our patients. If we had adopted this classification, three patients would not have been excluded from our cohort. Although our patients met Verloes' criteria for inclusion, our detection rate of mutations in the *CHD7* gene in typical cases (83%) is lower than the one of 90% reported by Jongmans et al. (2006). At the moment, WES is going on for our *CHD7*- negative patients, which should raise this rate.

5 | CONCLUSION

In conclusion, we had an opportunity to analyze a large cohort of 119 patients with CS. Considering the Verloes' classification, we aimed to identify a phenotype–genotype correlation regarding the different groups of typical, partial and atypical forms. As we found no partial form and only 10% of atypical ones, we could not demonstrate any correlation. When mutations were identified in atypical cases, they were truncating ones. It does not seem appropriate to keep this classification in three groups anymore. To avoid to exclude very light phenotypes, we agree with Hale et al. (2016) that presence of a pathogenic *CHD7* variant should be a major criterion of the CS diagnosis. On the other hand, we think that arhinencephaly, present in 80% of our patients, should be considered as a criteria. Nevertheless, as no mutation can be found in about 10% of clinical typical CS patients, this syndrome remains a clinical diagnosis.

From a molecular viewpoint, we found no pathogenic *CHD7* variant in 21% of all patients and 17% of typical cases. At least some of these patients probably have an intronic pathogenic *CHD7* variant, which, until now, could not be searched for. New technologies of next-generation sequencing should allow us to analyze the whole gene to solve this question. If genes other than *CHD7* (and *EFTUD2* in very rare cases) were involved in CS, they should be identified in the very near future by whole-exome and even genome sequencing.

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CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare. All other authors are clinical geneticists in different university hospitals in France, involved in certified centers for development anomalies. Most are professors of genetics. They included their patients with CHARGE syndrome in this study.

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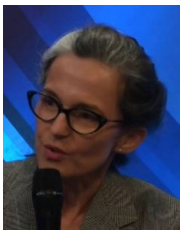
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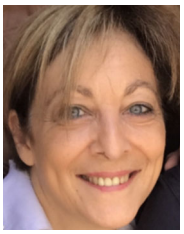
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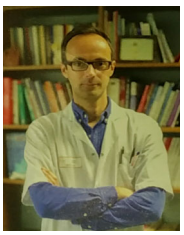
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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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