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CHARGE syndrome: the phenotypic spectrum of mutations in the *CHD7* gene

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ABSTRACT

Background: CHARGE syndrome is a non-random clustering of congenital anomalies. The *CHD7* gene on chromosome 8q12.1 was recently discovered as a major gene involved in the aetiology of this syndrome.

Methods: The coding regions of *CHD7* were screened for mutations in 107 index patients with clinical features suggestive of CHARGE syndrome. Clinical data of the mutation positive patients were sampled to study the phenotypic spectrum of mutations in the *CHD7* gene.

Results: Mutations were identified in 69 patients. Here we describe the clinical features of 47 of these patients, including two sib pairs. Most mutations were unique and were scattered throughout the gene. All patients but one fulfilled the current diagnostic criteria for CHARGE syndrome. No genotype-phenotype correlations were apparent in this cohort, which is best demonstrated by the differences in clinical presentation in sib pairs with identical mutations. Somatic mosaicism was detected in the unaffected mother of a sib pair, supporting the existence of germline mosaicism.

Conclusions: *CHD7* mutations account for the majority of the cases with CHARGE syndrome, with a broad clinical variability and without an obvious genotype-phenotype correlation. In one case evidence for germline mosaicism was provided.

INTRODUCTION

CHARGE syndrome (OMIM #214800) is a pleiotropic disorder comprising of coloboma, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, ear anomalies and deafness. A consistent feature in CHARGE syndrome is semicircular canal hypoplasia resulting in vestibular areflexia. [1-3] Other commonly associated congenital anomalies are facial nerve palsy, cleft lip/palate and tracheo-esophageal fistula. Specific behavioural problems, including autistic-like, have been described. [4 5] The combination of abnormalities initially known as CHARGE association was first reported independently by Hall and by Hittner and colleagues in 1979, [6 7] after which Pagon and colleagues proposed the acronym CHARGE in 1981. [8] CHARGE syndrome is an autosomal dominant syndrome with an estimated prevalence at birth between 1 per 10,000 to 1 per 15,000. [9] Recent epidemiological data revealed an occurrence of CHARGE syndrome in 1 in 8,500 live births in the Atlantic Provinces of Canada. [10]

CHARGE syndrome is a phenotypically heterogeneous syndrome and its clinical diagnosis is made using criteria that have been refined several times. Blake et al. suggested diagnostic criteria in 1998. [9] A refinement of these criteria for different age groups was proposed to capture the continuum of the presentation of CHARGE syndrome. [10] Simultaneously, Verloes suggested an update of diagnostic criteria, emphasizing the most specific embryological defects while avoiding non-specific or secondary anomalies. [11] He also suggested the exclusion of sex-dependent criteria. Both sets of diagnostic criteria are depicted in Table I.

CHARGE syndrome was only recently reconsidered to be a syndrome, instead of an association since our group discovered *CHD7* on chromosome 8 (8q12.1) as a major gene involved in this syndrome. [12] *CHD7* encodes a protein of the chromodomain (chromatin organization modifier) family. Members of this family share a unique combination of functional domains consisting of two N-terminal chromodomains, followed by a SWI2/SNF2-like ATPase/helicase domain and a DNA binding domain. [13-14] It is assumed that CHD protein complexes affect chromatin structure and gene expression and, thereby, play an important role in regulating embryonic development.

We report a study of the phenotypic spectrum in 47 patients with a *CHD7* mutation, with special emphasis on differences in presentation in sib pairs that share identical mutations.

Table I. Updated diagnostic Criteria for CHARGE syndrome

Verloes 2005 Typical CHARGE = 3 major criteria or 2 major and 2 minor criteria	**Blake et al. 1998** All four major criteria, or three major and three minor criteria
Major Criteria	**Major criteria**
1) Coloboma (iris or choroid, with or without microphthalmia)	1) Coloboma- iris, retina, choroid, optic disc or microphtalmia
2) Atresia of Choanae	2) Atresia of Choanae
	3) Cranial nerve dysfunction I: Anosmia, VII:Facial palsy, VIII Sensorineural deafness and vestibular problems, IX and/or X: Swallowing problems
3) Hypoplastic semicircular Canals	4) Characteristic external ears (absent or hypoplastic lobes, asymmetry, decreased cartilaginous folds, and triangular concha) and inner ear anomalies (temporal bone findings with cochlear hypoplasia and or absent/ hypoplastic semicircular canals)
Minor criteria	**Minor criteria**
1) Rhombencephalic dysfunction (brainstem dysfunctions, cranial nerve VII to XII palsies and neurosensory deafness)	
	1) Characteristic facial features - broad, sloping forehead, laterally protruding ears, small mouth, and high nasal bridge
2) Malformation of mediastinal organs (heart, esophagus)	2) Congenital cardiovascular malformations of all types
	3) Tracheo-oesophageal fistula
3) Hypothalamo-hypophyseal dysfunction (including GH and gonadotrophin deficiencies)	4) Growth defeciency
	5) Genital hypoplasia - micropenis and/or cryptochordism or hypoplastic labia. Delayed, incomplete pubertal development
4) Abnormal middle or external ear	
	6) Orofacial cleft
5) Mental retardation	7) Developmental delay: delayed motor milestones, hypotonia, MR

METHODS

Patients

The coding regions of the *CHD7* gene were screened for mutations in 107 index patients with clinical features suggestive of CHARGE syndrome. In 69 of these patients a mutation was identified (65 %) and for 47 patients (22 males, 25 females, two sib pairs) sufficient clinical data was available to include them for further studies. The cohort includes fifteen patients reported in our previous study. [12] Parental DNA samples of 22 patients, including one sib pair, were tested for *de novo* occurrence.

Clinical information of the patients was obtained through investigation in our own department or through a written questionnaire when DNA of the patient was referred to the DNA-diagnostics section of our department. Additional information was obtained from clinicians when necessary. The diagnostic criteria by Blake and Verloes (table I) were applied to all cases for which sufficient clinical information was available. [9 11]

All patients or their legal representatives gave informed consent for the DNA studies and the collection of clinical data.

Mutation screening

DNA was isolated according to standard procedures. The 37 coding exons of the *CHD7* gene (exon 2-38, accession number NM_017780) and their flanking intron sequences were amplified by polymerase chain reaction (PCR). Subsequently, sequence analysis was performed using a 3730 automated sequencer (Applied Biosystems).

The primer sets used previously were optimized by using shorter PCR products to exclude allele dropout. [12] Primer information and PCR conditions are given in supplemental tables I and II, available from the *JMG* website.

Whole gene deletions were excluded by Multiplex Ligation-dependent Probe Amplification (MLPA). Specific probe sets were designed for exons 2-11 and exons 33-38. MLPA analysis was performed according to the instructions of the manufacturer (MRC Holland b.v.; www.mlpa.com). Probe information is given in table III of the supplemental data (*JMG* website).

RESULTS

CHD7 mutation analysis

Mutation analysis in our series of 107 index patients revealed 69 mutations in the *CHD7* gene (Fig. 1 and Table II). Two mutations were recurrent, all others were unique. We detected 31 nonsense, 17 frame shift, 13 splice site and 8 missense mutations that were scattered throughout the gene. In the affected sibs identical mutations were identified. Among the *CHD7* positive patients is one girl (no. 22) with a previously identified chromosome 22q11 deletion. Fifteen patients were reported in a previous study. [12] In six of these patients, however, the mutation was not detected initially. After a more thorough investigation with improved primer sets *CHD7* mutations were detected. For 21 index patients the parents were studied. In 20 cases the mutation was proven to be *de novo*. In the sib pair consisting of two boys, mosaicism for the *CHD7* mutation was identified in the mother. In the remaining 38 mutation-negative patients whole gene deletions were excluded by MLPA analysis.

Table II. Overview of *CHD7* mutations

Mutation in *CHD7*	exon	Theoretical effect on RNA(r.) or protein (p.) *	segregation	Pt. in Table III
c.77_78delAA	2	p.Glu26fs	nd	1
c.469C>T	2	p.Arg157X	nd	
c.921_922delAG (e2)	2	p.Gly308fs	nd	
c.1044delC	2	p.Asn349fs	nd	2
c.1078G>T	2	p.Gly360X	one parent excl.	3
c.1388delG	2	p.Gly463fs	de novo	4
c.1465C>T	2	p.Gln489X	nd	5
c.1495C>T	2	p.Gln499X	nd	
c.1714C>T	3	p.Gln572X	de novo	6
c.1973_1974insT	3	p.Glu658fs	nd	7
c.2095A>G	3	r.spl? p.S699G	nd	
c.2194C>G	4	p.Pro732Ala	nd	
c.2238+1G>A	IVS4	r.spl?	de novo	8
c.2442+5G>A	IVS6	r.spl?	nd	9
c.2504_2508delATCTT	8	p.Tyr835fs	nd	
c.2505T>A	8	p.Tyr835X	de novo	10
c.2520G>A	8	p.Trp840X	nd	
c.2572C>T	8	p.Arg858X	de novo	11
c.2958-2A>T	IVS11	r.spl?	de novo	12
c.2959C>T	12	p.Arg987X	nd	13
c.3053_3054insA	12	p.Phe1019fs	nd	14
c.3082A>G	12	p.Ile1028Val	de novo	45
c.3106C>T	12	p.Arg1036X	nd	
c.3302G>A	13	p.Cys1101Tyr	nd	
c.3654C>G	15	p.Tyr1218X	nd	
c.3655C>T	15	p.Arg1219X	nd	15
c.3770T>G	15	p.Leu1257Arg	de novo	46
c.3779-2A>G	IVS15	r.spl?	nd	16
c.4015C>T	17	p.Arg1339X	nd	
c.4157C>G	17	p.Ser1386X	one parent excl.	17
c.4226_4227delTG	18	p.Val1409fs	nd	18
c.4507G>T	19	p.Glu1503X	nd	19

c.4644+1G>A	IVS20	r.spl?			nd	
c.4787A>G	21	p.Asp1596Gly			nd	
c.5050-41_5050-3del39	IVS23	r.spl?			nd	20
c.5402A>C	25	p.His1801Pro			de novo	47
c.5405-17G>A	IVS25	r.spl?			de novo	21
c.5405-7G>A	IVS25	r.spl?			nd	
c.5418C>G	26	p.Asn1807X		22q11del	de novo	22
c.5436C>A	26	p.Asp1812Glu			nd	
c.5534G>A	IVS26	r.spl?			de novo	23
c.5668A>T	29	p.Lys1890X			de novo	24
c.5680_5681delAG	29	p.Ser1894fs			de novo	25
c.5752_5753dupA	29	p.Thr1918fs		sib pair 1	nd	26
c.5752_5753dupA	29	p.Thr1918fs		sib pair 1	nd	27
c.5833C>T	29	p.Arg1945X			de novo	28
c.5893+1G>A	IVS29	r.spl?			nd	
c.5982G>A	30	p.Trp1994X	#		nd	
c.5982G>A	30	p.Trp1994X	#	sib pair 2	mat mosaicism	29
c.5982G>A	30	p.Trp1994X	#	sib pair 2	mat mosaicism	30
c.6051T>A	30	p.Cys2017X			nd	31
c.6070C>T	30	p.Arg2024X			one parent excl.	32
c.6079C>T	30	p.Arg2027X			de novo	33
c.6148C>T	31	p.Arg2050X	$		nd	35
c.6148C>T	31	p.Arg2050X	$		nd	34
c.6155_6157CTC>AGA	31	p.Ser2052X			nd	
c.6157C>T	31	p.Arg2053X			nd	
c.6304delG	31	p.Val2102fs			de novo	36
c.6775+2_6775+3insGT	IVS31	r.spl?			nd	37
c.6955C>T	33	p.Arg2319Cys			nd	
c.7079delA	33	p.Lys2360fs			nd	
c.7165-4A>G	IVS33	r.spl? p.Lys2388_Glu2389insX			nd	
c.7180delC	34	p.Lys2394fs			nd	38
c.7219delA	34	p.Ile2407fs			nd	
c.7252C>T	34	p.Arg2418X			nd	39
c.7400delT	34	p.Leu2467fs			de novo	40
c.7824T>A	35	p.Tyr2608X			de novo	41
c.7879C>T	36	p.Arg2627X			nd	42
c.7884_7885delTA	36	p.His2628fs			de novo	43
c.8016G>A	37	p.Trp2672X			nd	
c.8744_8745dupG	38	p.Leu2916fs			nd	44

* nomenclature according to http://www.genomic.unimelb.edu.au/mdi/mutnomen/ "
nd = not done
#/$ = recurrent mutation

Clinical features

Information obtained through our own investigation and/or through written questionnaires, supplemented with additional information from clinicians, resulted in clinical features of the 47 selected patients as outlined in Table III. Details of these features are provided below. All 47 cases were included in the evaluation unless stated otherwise. The diagnostic criteria by Blake and Verloes (table I) could be applied to 38 cases.[9 11] Only one patient did not fulfil both sets of diagnostic criteria.

Sufficient clinical information could also be obtained for 23 out of the 38 *CHD7* negative patients. Of these patients only two fulfilled the clinical diagnostic criteria by Blake and Verloes. [9 11]
In this section first a summary of all clinical data of the 47 *CHD7* positive patients is given. Subsequently a detailed case report is provided of a girl who did not fulfil the diagnostic criteria by Blake and Verloes (table I) [9 11] and the intra familial variability in sib pairs is delineated.

Neonatal period
The median gestational age of the patients was 38.2 weeks (n=45, range 30-42 weeks). Only one patient was reported to be small for gestational age, while feeding difficulties were reported in 33 (70%) patients. Four patients required a gastrostomy due to severe feeding problems.
Four patients died during the neonatal period, three during the first half year of life and one patient at the age of fourteen years. At the time of investigation four patients were below the age of one year.

Coloboma of the eye
In 33 patients (70%) a coloboma of one (n=4) or both (n=29) eyes was present. In only nine patients the iris was involved, thus in the majority the coloboma was only visible by fundoscopy. In none of the patients the coloboma was restricted to the iris only. Microphthalmia was present in ten patients (21%).

Congenital heart defects
Thirty-one (66%) patients had a congenital heart defect. Fourteen (30%) patients had major heart defects: six tetralogy of Fallot, two double-outlet right ventricle (one combined with hypoplastic left heart and AVSD), three isolated hypoplastic left heart syndrome, one hypoplastic right heart syndrome, one agenesis of the pulmonary valve combined with hypoplastic left heart and one Shone's complex. A right descending aorta was present in three patients and one patient had a vascular ring. The other patients had solitary patent ductus arteriosus beyond infancy (3), patent ductus arteriosus combined with atrium septum defect and/or ventricular septum defect (6) or a solitary septal defect (4).

Retardation of growth and development
A height below the third percentile was reported in 21 out of 32 patients (63%).
Speech development varied from mild speech delay to a severe retardation without speech. Learning disabilities were reported in 24 (75%) out of 32 patients who were above the age of twelve months at last examination. Eight patients (25%) had no cognitive impairment.

Endocrine and urogenital abnormalities
At the moment of *CHD7* testing fifteen patients (8 girls, seven boys) were above fifteen years of age. Gonadotrophin deficiency was present in seven (88%) of these girls, and six (86%) of these boys. Two girls had their menarche at age 14. A hypoplastic uterus was found by ultrasound investigation in three girls. Of all 22 mutation-positive boys four (18%) had cryptorchidism, six (27%) micropenis and seven (32%) had both cryptorchidism and micropenis.
Three patients had a horseshoe kidney and in two patients agenesis of the left kidney was demonstrated. A vesicoureteral reflux was reported in three patients and one patient had renal cysts.

Table III. Anomalies in 47 *CHD7* positive patients

Individual	Sex	Age at clinical evaluation	Gestation (weeks)	Birth weight (grams)	Coloboma		Microphtalmia	Heart defect	Atresia of choanae	Height	Micropenis / Cryptorchidism	Ext. ear anomaly	Hearing loss	Vestibular dysfunction	Facial nerve palsy	Oesophageal fistula	Cleft lip	Cleft palate
					Left	Right												
1	F	17	38	2450	IRCO	IRCO	L/R	-	-	<P3		+	+	?	-	-	-	-
2	F	19	41	3520	RC	RC	R	+	-	?		+	+	?	-	-	-	+
3	F	17	38	2435	-	-	-	+	+	<P3		+	-	+++	-	-	-	-
4	F	6	32	1805	-	-	-	+	-	<P3		+	+	+++	-	+	+	+
5	F	6	36	?	IRCO	IRCO	-	+	-	?		+	?	?	-	-	+	+
6	M	10	38	3025	-	-	-	-	-	P3	+	+	+	+++	+	-	-	+
7	F	11	40	3350	CRO	CRO	L/R	-	-	?		+	+	+++	-	-	-	+
8	M	7	39	2530	CRO	O	L	-	-	<P3	+	+	+	+++	-	-	-	-
9	M	4	39	3200	-	-	-	-	-	>P10	+	+	+	?	-	-	+	+
10	M	<1	31	1650	-	-	-	-	+	?	+	+	+	+++	-	-	-	-
11	F	<1	40	4082	R	R	-	+	-	?		+	+	?	-	-	+	+
12	M	day 6 [A]	38	3060	IRO	RO	-	+	-	?	-	+	?	?	-	-	+	+
13	M	32	37	2900	-	-	-	-	+	<P3	+	+	+	?	-	-	-	-
14	F	35	?	3250	C	-	L	+	+	<P3		+	+	+++	-	-	-	-
15	F	15	39	2910	-	-	-	+	+	<P3		+	+	++	-	-	-	-
16	M	19	42	3700	IRC	IRC	-	-	-	P15	+	+	+	++	-	-	-	-
17	M	19	40	2870	-	-	-	+	+	<P3	+	+	+	+	-	-	-	-
18	M	day 12 [A]	35	2250	IO	O	-	+	+	?	+	+	+	+++	-	-	-	-
19	F	5	40	3840	RO	RO	-	+	-	<P3		+	-	?	-	-	+	+
20	M	month 5 [A]	38	3408	-	-	-	+	+	?	+		?	?	-	-	-	-
21	M	40	40	3250	RO	RO	R	+	-	P10	+	+	+	?	+	+	-	-
22	F	22	36	2000	-	-	-	+	+	<P3		+	+	+++	-	+	-	-
23	M	19	42	2800	IR	R	-	+	-	<P3	+	+	+	+++	-	-	+	+
24	F	month 6 [A]	42	3450	IR	IR	-	+	-	?		+	+	?	+	-	-	-
25	M	1	37	2940	R	R	-	+	-	P50	+	+	+	+++	+	+	-	-
26	F	11	35	1910	RC	IRC	-	+	+	<P3		+	+	+	-	-	-	-
27	F	day 2 [A]	35	1500	-	-	-	+	+	?		+	?	?	-	+	-	-
28	F	5	40	3700	-	-	-	+	-	P3		+	+	+++	-	-	-	-
29	M	7	40	2867	-	-	-	+	-	<P3	+	+	-	?	+	-	+	+
30	M	3	38	3440	RC	-	-	-	-	P3	+	+	+	?	-	+	-	-
31	M	year 14 [A]	?	3500	RC	RC	-	-	-	<P3	-	+	+	+++	-	+	+	+
32	F	12	41	2800	IR	R	L	+	-	<P10		+	+	+++	+	-	-	-
33	M	week 5 [A]	30	1470	RO	RO	U	-	+	?	-	+	+	?	-	+	-	-
34	M	6	37	2700	R	R	-	+	-	?	-	+	+	?	-	-	-	+
35	M	6	35	2835	O	R	R	+	-	<P3	+	+	+	+++	+	-	-	+
36	M	20	40	2820	R	R	-	-	+	<P3	+	+	+	?	-	-	+	-
37	M	<1	39	3114	R	R	-	+	-	?	+	+	?	?	-	-	-	-
38	F	day 21 [A]	35	2393	CRO	CRO	-	+	+	?		+	?	?	-	-	-	-
39	F	3	40	3340	CR	CR	-	+	-	<P3		+	+	+++	-	-	-	-
40	M	20	42	3490	CR	CR	-	+	-	<P3	+	+	+	+	-	-	+	+
41	F	10	37	2650	-	-	-	+	+	<P3		+	+	+++	-	-	-	-
42	F	<1	41	2830	CO	O	-	+	-	?		+	+	?	+	-	-	-
43	F	26	40	2450	R	-	-	+	-	<P3		+	+	+++	+	-	-	-
44	F	20	36	2450	CRO	CRO	-	-	+	?		+	+	+++	+	-	+	+
Missense mutations:																		
45	F	15	38	2980	O	O	L/R	-	-	<P3		+	+	+++	-	-	-	-
46	F	15	41	2500	C	C	-	-	-	P10		+	+	+++	-	-	-	-
47	F	16	40	2100	C	-	-	-	+	P3		+	-	+	-	-	-	-

[A] = deceased
Coloboma: I = iris, R = retina, C = choroidea, O = optic disc
Microphtalmia: R = right, L = Left, U = unilateral, side not known
Vestibular dysfunction: +++ = semicircular canal agenesis on CT scan of inner ear, ++ = vestibular areflexia, + = history of unsteadiness

Individual	Sex	Age at clinical evaluation	Mental Retardation	Neurological abnormalities	Skeletal Abnormalities	Urogenital findings	Gonadadotrophin deficiency	Diagnostic criteria (table I)	
								Blake (1998) [9]	Verloes (2005) [11]
1	F	17	+++				+	+	
2	F	19	?				+		
3	F	17	+				+	+	+
4	F	6	+					+	+
5	F	6	+++						
6	M	10	-					+	+
7	F	11	++		S			+	+
8	M	7	+++			HK		+	+
9	M	4	?						
10	M	<1	?					+	+
11	F	<1	?			C		+	
12	M	day 6 [A]	?	AC + CH	S + HV		+		
13	M	32	+++		HV		+	+	
14	F	35	++	C	S		+	+	+
15	F	15	+					+	+
16	M	19	-		S		+	+	+
17	M	19	++	ACC	S	R	+	+	+
18	M	day 12 [A]	?					+	+
19	F	5	+	H				+	
20	M	month 5 [A]	?	C		A			
21	M	40	+++				+	+	+
22	F	22	++	H + C		R	+	+	+
23	M	19	-					+	+
24	F	month 6 [A]	?						
25	M	1	?					+	+
26	F	11	++					+	+
27	F	day 2 [A]	?						
28	F	5	+					-	-
29	M	7	++					+	
30	M	3	+++					+	
31	M	year 14 [A]	++					+	+
32	F	12	++		K			+	+
33	M	week 5 [A]	?						+
34	M	6	?						
35	M	6	?			HK	+	+	+
36	M	20	+++	C			+	+	+
37	M	<1	?		T	R			
38	F	day 21 [A]	?			HK			+
39	F	3	-			A		+	+
40	M	20	+++		S		+	+	+
41	F	10	+++					+	+
42	F	<1	?						
43	F	26	-				+	+	+
44	F	20	-				+	+	+
Missense mutations									
45	F	15	-					+	+
46	F	15	+					+	+
47	F	16	-	C	HV			+	

[A] = deceased
Mental Retardation: - = normal intelligence, + = mild MR, ++ = moderate MR, +++ = severe MR
Neurological abnormalities: AC = agenesis of corpus callosum, CH = cerebellar hypoplasia, C = convulsions, ACC = atrophy of cerebral cortex, H = hydrocephaly ,
Skeletal abnormalities: S = scoliosis, HV = hypoplastic vertrebrae, K = kyphosis, T = triphalangeal thumb
Urogenital anomalies: HK = horeshoe kidney, C = renal cysts, R = reflux, A = agenesis of one kidney

Ear and vestibular abnormalities
In all patients dysmorphisms of the ears were noted, ranging from "typical CHARGE ears" (small, square, low-set and protruding) to minor structural abnormalities such as absence of an earlobe. One patient had a pre-auricular pit and one patient had narrow external auditory canals. Hearing impairment was demonstrated in 37 out of 41 patients (90%). In 27 patients severe bilateral hearing impairment was observed, whereas five patients showed asymmetric hearing impairment with unilateral normal or mild hearing loss.
In all 21 patients who underwent CT-scanning of the temporal bones, agenesis of the semicircular canals was demonstrated. Vestibular areflexia was demonstrated in two more patients and four patients had a history of balance disturbances. This results in 27 patients (57%) with some evidence of vestibular anomaly. However, from the remaining patients information on this subject was not available, although motor delay (possibly due to vestibular areflexia) was present in all cases on direct questioning.

Nasopharyngeal abnormalities and clefting
Choanal atresia was present in 17 patients (36%) and was unilateral in only three of them. Respiratory insufficiency during the neonatal period was reported in 24 patients (51%). Twenty-two of them had either choanal atresia or a congenital heart defect or both. Tracheomalacia was present in one patient.
Clefting was present in 17 patients (36%). Eleven patients had a cleft lip and palate, five had an isolated cleft palate and one had an isolated cleft lip.

Gastrointestinal abnormalities
Eight patients (17%) had esophageal atresia, which in three was accompanied by a tracheo-esophageal fistula. Two patients had a diaphragmatic hernia and one anal stenosis.

Neurological abnormalities
A minority of the patients (n=4, 9%) had central nervous system abnormalities, including corpus callosum agenesis combined with cerebellar hypoplasia (n=1), hydrocephaly (n=2), and atrophy of the cerebral cortex (n=1). Five patients had convulsions.
Facial nerve palsy was present in ten patients (21%) and mostly (9 out of 10) involved the right-sided facial nerve.

Skeletal abnormalities
Scoliosis was demonstrated in six patients (13%), kyphosis in one, abnormalities of the vertebral bodies in three (6%) and in one patient a triphalangeal thumb was demonstrated.

Aspecific CHARGE syndrome
Patient 28 (born at 40 weeks' gestation; birth weight 3700 g, 70th centile, Fig 2) was a 5 years old girl with developmental delay, slightly dysmorphic ears and severe hearing impairment. CT scan showed bilateral agenesis of the semicircular canals. She required a gastrostomy due to severe feeding problems and she had surgery on a congenital vascular ring. Her height was at the third centile. No choanal atresia, nor cleft palate or coloboma could be detected. In this girl, the only individual in our *CHD7* positive series who did not fulfil the current diagnostic criteria for CHARGE syndrome (table I) [9 11] a *de novo* nonsense mutation was identified, 5833C>T (R1945X) in exon 29 of *CHD7*.

Familial cases
Two sib pairs were included from two families. In both cases, identical *CHD7* mutations were identified in the two sibs. Interestingly, in both cases the affected sib pairs showed distinct clinical features.
Sib pair 1 represented monozygotic twin sisters (born at 35 weeks' gestation, Table III: patients 26+27, Figs. 3a and 3b). Patient 27, who had a birth weight of 1500 g (5th-10th centile), died 29 hours after birth due to the combination of a hypoplastic left heart syndrome

and bilateral choanal atresia. Furthermore, she had a tracheo-esophageal fistula and typical "CHARGE ears". A hearing test was not performed. There were no colobomata of the irides. Patient 26 had a birth weight of 1910 g (25th centile). When examined at the age of 12 years, she had short stature (< 3rd centile) and was functioning four years behind her chronological age. She was born with a large patent ductus arteriosis that required surgery and she needed numerous procedures to correct bilateral choanal atresia. Her first years of life were complicated by feeding problems, for which she had a gastrostomy until the age of six years. She had severe bilateral deafness, abnormal external ears like her twin sister and bilateral chorioretinal colobomata with a right-sided iris coloboma and an unusual inferior pigment pattern in her left iris. Agenesis of the semicircular canals was not tested for by CT-scan, but her gait was unsteady.

Zygosity testing with five unlinked markers was performed and the results were consistent with the twins being monozygotic. In both sisters the spectrum of congenital anomalies was caused by an insertion 5752_5753insA in exon 29 of *CHD7*.

Sib pair 2 consisted of two brothers (Table III: patients 29+30, Figs. 3c and 3d). Patient 29 was 7 years of age (born at 40 weeks' gestation; birth weight 2867 g, 10th centile). He had surgery for cleft lip/palate and a complex heart defect (DORV, AVSD, hypoplastic left heart). He had short stature (3rd centile) and severe developmental delay. His ears showed the typical CHARGE dysmorphisms and he had bilateral hearing loss and unilateral facial nerve palsy. He had no colobomata or choanal atresia.

Patient 30, who was 4 years younger, had bilateral hearing loss and typical "CHARGE ears", a coloboma of the left retina and choroid and he underwent surgery on a tracheo-esophageal fistula. He had short stature (<3rd centile) and was severely mentally retarded. He had vocal cord palsy. This boy had no heart defect and no choanal atresia.

Both brothers have a 5982G>A (W1994X) mutation in exon 30 of CHD7. Sequence analysis of both parents revealed no mutation in the father and a minor aberrant peak in DNA extracted from lymphocytes of the mother. This indicated that a possible mosaicism was present in the mother. This was further investigated and confirmed by an allele-specific PCR, using a primer carrying the 5982G>A mutation at the 3'-end, in combination with the regular exon 30 primer set (Fig. 4). Clinical examination of the mother did not reveal any signs of CHARGE syndrome.

DISCUSSION

At the time of evaluation of our clinical data, *CHD7* sequencing had been performed in 107 index patients referred to our laboratory because of clinical features suggestive of CHARGE syndrome. Pathogenic mutations were identified in 69 patients (65%), including six patients that had previously tested negative. [12] All mutations except two were unique and most mutations had a severe effect on the *CHD7* protein, being either nonsense or frameshift mutations (70%).

From both our previous data and a recent report by Arrington et al. (2005) it is known that microdeletions of the chromosome 8q12.1 region, including the *CHD7* gene, may also result in CHARGE syndrome. [12 15] We excluded the presence of such microdeletions in the patients without *CHD7* mutations by MLPA. From these results we conclude that whole gene deletions of the *CHD7* gene are not a frequent cause of CHARGE syndrome. Currently, we are extending our MLPA analyses in order to assess for the presence of small intragenic deletions.

In 20 out of 21 families a *de novo* occurrence of the *CHD7* mutation could be proven. In the mother of the sibs with the 5982G>A(W1994X) change, this mutation was present as a somatic mosaicism. It is likely that germline mosaicism exists as well. As a consequence prenatal diagnosis should be offered to all parents of children with an apparently *de novo* *CHD7* mutation.

Out of the 69 *CHD7* mutation-positive patients 45 index cases were selected for further clinical study together with two sibs, resulting in a cohort of 47 patients. Due to a short follow-up period, clinical information was limited in eleven patients, especially regarding hearing, growth and development. From the data presented in Table III and the detailed clinical description of our patients, it is clear that within the *CHD7* mutation-positive subset of CHARGE patients an extensive variability in clinical presentation exists, without any obvious genotype-phenotype correlation. This is best demonstrated in the two sib pairs. In the first sib pair, both twin girls had choanal atresia and a heart defect, but they were discordant for the coloboma and tracheo-esophageal fistula. The boys of the other sib pair were discordant for cleft lip/palate, heart defect, tracheo-esophageal fistula, coloboma and hearing loss.

Missense mutations were found in three patients of the clinical study group, one of which was mildly mentally retarded. The other two had normal levels of intelligence. However, normal intelligence was also present in five patients with a nonsense mutation. Overall clinical comparison of these three patients with a missense mutation with the rest of the study group did not reveal any clear differences. However, it is still possible that less severe mutations (i.e. missense mutations) result in a less specific phenotype, not recognized as CHARGE syndrome. Hence, such patients may not be included in this study. On the other hand, patients with a *CHD7* deletion may be more severely affected than patients with a *CHD7* mutation, especially if multiple adjacent genes are deleted. Further studies are needed to explore this.

In Table IV the frequency of the main features of CHARGE syndrome in our group of *CHD7* mutation-positive patients is compared with data from the literature.

Table IV. The frequencies of characteristic CHARGE findings in a population of *CHD7*-positive patients compared to the literature

	This study N=47	(%)	Stromland et al. (2005) N=30 (%)	Issekutz et al. (2005) N=77 (%)	Tellier et al. (1998) N=47 (%)
Coloboma	33/47	70	90	77	79
Choanal atresia	17/47	36	35	64	57
Ear-anomaly/deafness	47/47	100	90	96	100
Cranial nerves	10/47*	21	32*	91	78
Genital hypoplasia (males)	17/22	77	67	65	53

Heart defects	31/47	66	52	84	85
Cleft lip/palate	17/47	36	19	18	17
TE fistula	8/47	17	16	19	15
Growth deficiency	21/32	66	48	58	75
Renal	9/47	19	12	36	19
Spine	9/47	19	27	9	13

* Data on facial nerve only

The distribution of features in the clinically diagnosed CHARGE syndrome patients as reviewed by Stromland et al. (2005), Issekutz et al. (2005) and Tellier et al. (1998) is comparable to the *CHD7* mutation-positive patients. [3 10 16] This indicates that, within the patient group that fulfils the clinical diagnosis of CHARGE syndrome, there is not a specific subgroup that is more likely to have a *CHD7* mutation. None of the clinical features seems to be obligatory for a *CHD7* mutation, with the possible exception of vestibular anomalies. Several reports have stressed the high frequency and the high specificity of anomalies of the semicircular canals. [1 2 17 18] This was also observed in our cohort of patients. All patients that were investigated by CT-scan or vestibular function tests had either abnormal function or an aplasia of the semicircular canals.

The effect of a *CHD7* mutation on a specific organ is variable and does not predict the consequences for other organ systems in which *CHD7* is expressed. For instance, a severe heart defect does not exclude normal intelligence (e.g. individual 43, Table III) and severe mental retardation does not have to be accompanied by severe defects in other organs (e.g. individual 8, Table III). This results in an enormous clinical variability, even within sib pairs. We carefully tested whether both sets of diagnostic criteria could be applied to our patients (see Table III).[9 11] This was not possible in all cases, since for example CT-scanning of the temporal bones is required in order to apply the diagnostic criteria proposed by Verloes. For simplification we decided to use the 1998 Blake criteria as listed in Table I instead of the refined criteria adopted for different age groups. [10] Both Blake and Verloes require that at least a coloboma or choanal atresia is present for the diagnosis CHARGE syndrome. Five patients in our study group (individuals 4-6-9-28-29 in Table III) neither had coloboma nor choanal atresia. Blake et al. (1998) argued that the choanae are usually patent when orofacial clefting is present and palatal clefting can be substituted for choanal atresia in the scoring criteria. [9] As a consequence, only one patient (individual 28 in Table III) failed to fulfil the diagnostic criteria for CHARGE syndrome according to both Blake and Verloes. In 38 patients with features suggestive of CHARGE syndrome no *CHD7* mutation and/or deletion was identified. For 27 of these patients sufficient clinical data were available to apply the clinical diagnostic criteria. Only two of these 27 *CHD7* mutation-negative patients fulfilled the diagnostic criteria. In both patients aplasia of the semicircular canals was demonstrated. As a consequence the positive predictive value of the clinical diagnostic criteria is 37/39 (95%). This is substantiated by the fact that after improvement of the sequencing procedure (table I of supplemental data, *JMG* website) the mutation positive percentage in our first reported cohort reaches 95% (18/19). [12] In the context of the previously suggested genetic heterogeneity, [19-21] this is an interesting observation that needs confirmation.

We would like to stress that CHARGE syndrome remains a clinical diagnosis. Although the high percentage of *CHD7* mutations in clinically diagnosed CHARGE syndrome patients indicates that *CHD7* is the major gene involved, this diagnosis cannot be rejected based on absence of a *CHD7* mutation. On the other hand, based on the clinical criteria alone, one *CHD7* positive patient would have been missed in our series.

In conclusion, we confirm that mostly unique *CHD7* mutations account for the majority of the cases with CHARGE syndrome, with a broad clinical variability and without an obvious genotype-phenotype correlation. In addition we provided evidence for germline mosaicism.

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COMPETING INTERESTS
Non declared

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INFORMED CONSENT
Informed consent was obtained of all patients that are represented by their photograph in this manuscript.

REFERENCES

1 **Morgan D**, Bailey M, Phelps P, Bellman S, Grace A, Wyse R. Ear-nose-throat abnormalities in the CHARGE association. *Arch Otolaryngol Head Neck Surg* 1993;**119**(1):49-54.

2 **Admiraal RJ**, Joosten FB, Huygen PL. Temporal bone CT findings in the CHARGE association. *Int J Pediatr Otorhinolaryngol* 1998;**45**(2):151-62.

3 **Tellier AL**, Cormier-Daire V, Abadie V, Amiel J, Sigaudy S, Bonnet D, de Lonlay-Debeney P, Morrisseau-Durand MP, Hubert P, Michel JL, Jan D, Dollfus H, Baumann C, Labrune P, Lacombe D, Philip N, LeMerrer M, Briard ML, Munnich A, Lyonnet S. CHARGE syndrome: report of 47 cases and review. *Am J Med Genet* 1998;**76**(5):402-9.

4 **Hartshorne TS**, Grialou TL, Parker KR. Autistic-like behavior in CHARGE syndrome. *Am J Med Genet A* 2005;**133**(3):257-61.

5 **Smith IM**, Nichols SL, Issekutz K, Blake K. Behavioral profiles and symptoms of autism in CHARGE syndrome: Preliminary Canadian epidemiological data. *Am J Med Genet A* 2005;**133**(3):248-56.

6 **Hall BD**. Choanal atresia and associated multiple anomalies. *J Pediatr* 1979;**95**(3):395-8.

7 **Hittner HM**, Hirsch NJ, Kreh GM, Rudolph AJ. Colobomatous microphthalmia, heart disease, hearing loss, and mental retardation--a syndrome. *J Pediatr Ophthalmol Strabismus* 1979;**16**(2):122-8.

8 **Pagon RA**, Graham JM Jr, Zonana J, Yong SL. Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: CHARGE association. *J Pediatr* 1981;**99**(2):223-7.

9 **Blake KD**, Davenport SL, Hall BD, Hefner MA, Pagon RA, Williams MS, Lin AE, Graham JM Jr. CHARGE association: an update and review for the primary pediatrician. *Clin Pediatr (Phila)* 1998;**37(**3):159-73.

10 **Issekutz KA**, Graham JM Jr, Prasad C, Smith IM, Blake KD. An epidemiological analysis of CHARGE syndrome: Preliminary results from a Canadian study. *Am J Med Genet A* 2005;**133**(3):309-17.

11 **Verloes A**. Updated diagnostic criteria for CHARGE syndrome: A proposal. *Am J Med Genet A* 2005;**133**(3):306-8.

12 **Vissers LE**, van Ravenswaaij CM, Admiraal R, Hurst JA, de Vries BB, Janssen IM, van der Vliet WA, Huys EH, de Jong PJ, Hamel BC, Schoenmakers EF, Brunner HG, Veltman JA, Geurts van Kessel AG. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet* 2004;**36**(9):955-7.

13 **Delmas V**, Stokes DG, Perry RP. A mammalian DNA-binding protein that contains a chromodomain and an SNF2/SWI2-like helicase domain. *Proc Natl Acad Sci U S A* 1993;**90**(6):2414-8.

14 **Woodage T**, Basrai MA, Baxevanis AD, Hieter P, Collins FS. Characterization of the CHD family of proteins. *Proc Natl Acad Sci USA* 1997;**94**(21):11472-7.

15 **Arrington CB**, Cowley BC, Nightingale DR, Zhou H, Brothman AR, Viskochil DH Interstitial deletion 8q11.2-q13 with congenital anomalies of CHARGE association. *Am J Med Genet* 2005;**133**(3):326-30.

16 **Stromland K**, Sjogreen L, Johansson M, Ekman Joelsson BM, Miller M, Danielsson S, Billstedt E, Gillberg C, Jacobsson C, Norinder JA, Granstrom G. CHARGE association in Sweden: Malformations and functional deficits. *Am J Med Genet A* 2005;**133**(3):331-9.

17 **Amiel J**, Attiee-Bitach T, Marianowski R, Cormier-Daire V, Abadie V, Bonnet D, Gonzales M, Chemouny S, Brunelle F, Munnich A, Manach Y, Lyonnet S. Temporal bone anomaly proposed as a major criteria for diagnosis of CHARGE syndrome. *Am J Med Genet* 2001;**99**(2):124-7.

18 **Satar B**, Mukherji SK, Telian SA. Congenital aplasia of the semicircular canals. *Otol Neurotol* 2003;**24**(3):437-46.

19 **Clementi M**, Tenconi R, Turolla L, Silvan C, Bortotto L, Artifoni L. Apparent

CHARGE association and chromosome anomaly: chance or contiguous gene syndrome. *Am J Med Genet* 1991;**41**(2):246-50.

20 **North KN**, Wu BL, Cao BN, Whiteman DA, Korf BR. CHARGE association in a child with de novo inverted duplication (14)(q22-->q24.3). *Am J Med Genet* 1995;**57**(4):610-4.

21 **Martin DM**, Sheldon S, Gorski JL. CHARGE association with choanal atresia and inner ear hypoplasia in a child with a de novo chromosome translocation t(2;7)(p14;q21.11). *Am J Med Genet* 2001;**99**(2):115-9.

FIGURE LEGENDS

Legend to Figure 1
Distribution of *CHD7* mutations identified in the 69 CHARGE syndrome patients. Coding exons are indicated in black bars, whereas the non-coding sequences are indicated in gray. Colored bars represent chromodomains (red), SNF2 domain (green) and Helicase domain (yellow). Mutations are schematically shown above the exons in which they are located. Nonsense mutations are represented by ● (n=31), missense mutations by ■ (n=8), frameshift mutations (n=17) by ◆ and splice site mutations by ▲ (n=13) respectively.

Legend to Figure 2
Patient 28, who is *CHD7* mutation positive but does not fulfil the diagnostic criteria (see text). (Written consent was obtained for publication of this picture)

Legend to Figure 3
a), twin 1 of sib pair 1 (patient 27 in table III), who died shortly after birth; b) twin 2 of sib pair 1 (patient 26 in table III) at the age of 7 years; c) sib 1 and d) sib 2 of sib pair 2, both at the age of 2 years (patients 29 and 30 in table III). (Written consent was obtained for publication of these pictures)

Legend to Figure 4
Results of allele-specific PCR for 5982G>A mutation in the family with two affected boys (a). DNAs of the indicated family members and an unrelated unaffected control (IV) were subjected to a multiplex PCR using a mutation-specific primer (5982G>A, lower band) and the regular primer set for exon 30 (upper band) (b). The mutation found in the boys (I) was also present in the mother (II). The different relative amounts of the fragments of individual I and II, might reflect the presumed mosaicism in the mother.

Figure 1

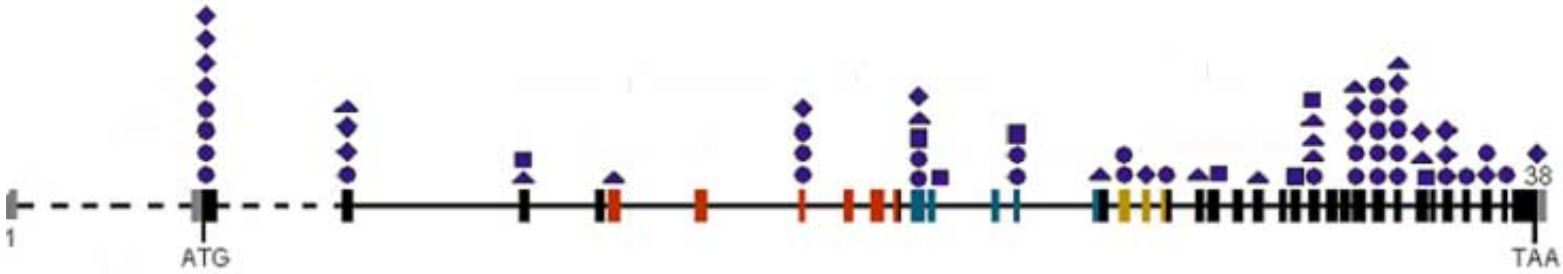


Figure 2

Figure 3a

Figure 3b

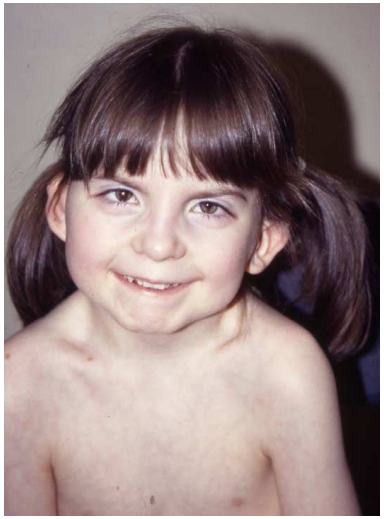


Figure 3c

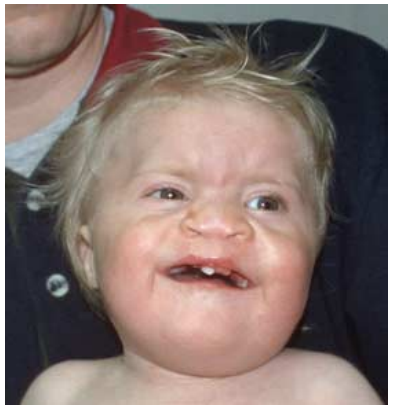


Figure 3d

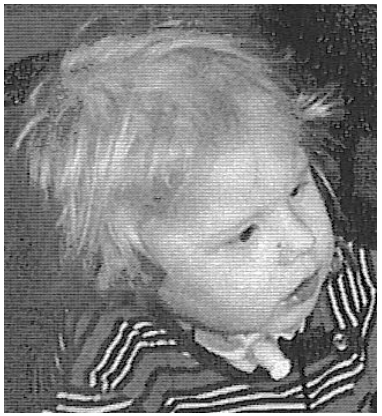
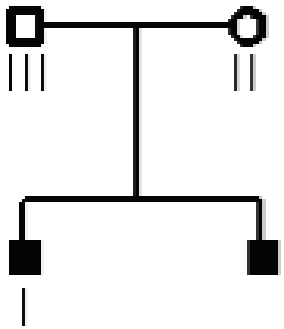


Figure 4

a.



b.