

# More Clinical Overlap between 22q11.2 Deletion Syndrome and CHARGE Syndrome than Often Anticipated

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## Key Words

CHARGE syndrome · *CHD7* · 22q11.2 deletion syndrome · *TBX1*

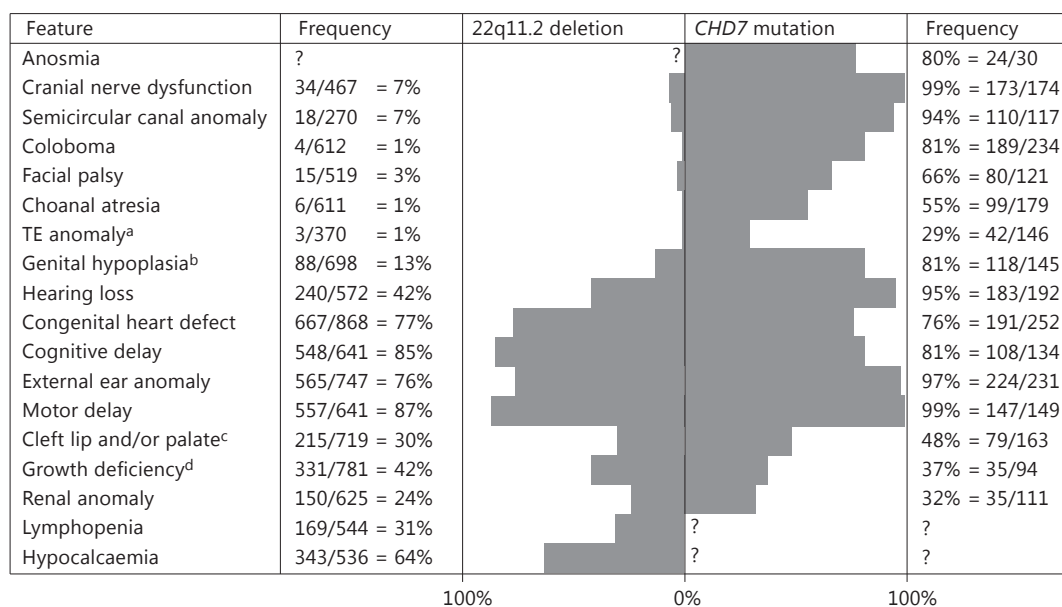
## Abstract

CHARGE (coloboma, heart defects, atresia of choanae, retardation of growth and development, genital hypoplasia, and ear abnormalities) and 22q11.2 deletion syndromes are variable, congenital malformation syndromes that show considerable phenotypic overlap. We further explored this clinical overlap and proposed recommendations for the genetic diagnosis of both syndromes. We described 2 patients clinically diagnosed with CHARGE syndrome, who were found to carry a 22q11.2 deletion, and searched the literature for more cases. In addition, we screened our cohort of *CHD7* mutation carriers (n = 802) for typical 22q11.2 deletion features and studied *CHD7* in 20 patients with phenotypically 22q11.2 deletion syndrome but without haploinsufficiency of *TBX1*. In total, we identified 5 patients with a clinical diagnosis of CHARGE syndrome and a proven 22q11.2 deletion. Typical 22q11.2 deletion features were found in 30 patients (30/802, 3.7%) of our *CHD7* mutation-positive cohort. We found

truncating *CHD7* mutations in 5/20 patients with phenotypically 22q11.2 deletion syndrome. Differentiating between CHARGE and 22q11.2 deletion syndromes can be challenging. *CHD7* and *TBX1* probably share a molecular pathway or have common target genes in affected organs. We strongly recommend performing *CHD7* analysis in patients with a 22q11.2 deletion phenotype without *TBX1* haploinsufficiency and conversely, performing a genome-wide array in CHARGE syndrome patients without a *CHD7* mutation.

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CHARGE syndrome (OMIM 214800, coloboma, heart defects, atresia of choanae, retardation of growth and development, genital hypoplasia, and ear abnormalities) is a highly variable, multiple congenital malformation syndrome that shows considerable clinical overlap with other syndromes like Kallmann syndrome [Kim et al., 2008; Jongmans et al., 2009], VACTERL association (vertebral anomalies, anal atresia, cardiac defects, tracheo-oesophageal fistula, oesophageal atresia, renal anomalies, and limb defects) [Källén et al., 2004; Solomon, 2011], Goldenhar syndrome (oculo-auriculo-vertebral spectrum)



**Fig. 1.** Frequency of the most common clinical features seen in patients with a 22q11.2 deletion and patients with a *CHD7* mutation. Frequency is depicted as number of patients with feature/number of patients investigated; data is based on 943 patients from Children's Hospital of Philadelphia Database for 22q11.2 deletion group and on 280 patients indentified at the Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands) with a

*CHD7* mutation as described in Bergman et al. [2011b]. TE anomaly = Tracheo-oesophageal anomaly. <sup>a</sup> In 22q11.2 deletion group only tracheo-oesophageal fistula. <sup>b</sup> Including 28 patients with hypospadias in 22q11.2 deletion group. <sup>c</sup> 148 patients in 22q11.2 deletion group had a submucosal cleft. <sup>d</sup> Height <2.5 SD in *CHD7* cohort or below 5th percentile in 22q11.2 deletion cohort.

[Van Meter and Weaver, 1996; Källén et al., 2004], and SOX2 anophthalmia syndrome [Engelen et al., 2011]. The most striking similarity of clinical features, however, is seen with 22q11.2 deletion syndrome as illustrated by Jyonouchi et al. [2009]. Here, we further explored the clinical similarities between CHARGE syndrome and 22q11.2 deletion syndrome.

CHARGE syndrome has an estimated birth incidence of 5.8–6.7 per 100,000 live births [Janssen et al., 2012]. A patient is currently diagnosed with CHARGE syndrome if the clinical diagnostic criteria of Blake et al. [1998] or of Verloes [2005] are fulfilled. The major clinical features include choanal atresia, coloboma of the eye, hypoplastic semicircular canals, external ear anomalies, and cranial nerve dysfunction (as summarised in fig. 1) [Bergman et al., 2011b]. CHARGE syndrome is inherited in an autosomal dominant fashion, but most cases are sporadic due to de novo mutations in the *CHD7* gene [Zentner et al., 2010b; Bergman et al., 2011b]. *CHD7* codes for a chromodomain helicase DNA-binding protein that has a cell type-specific and embryonic stage-dependent function in regulating the expression of other developmental genes [Vissers et al., 2004; Schnetz et al., 2009; Zentner et al.,

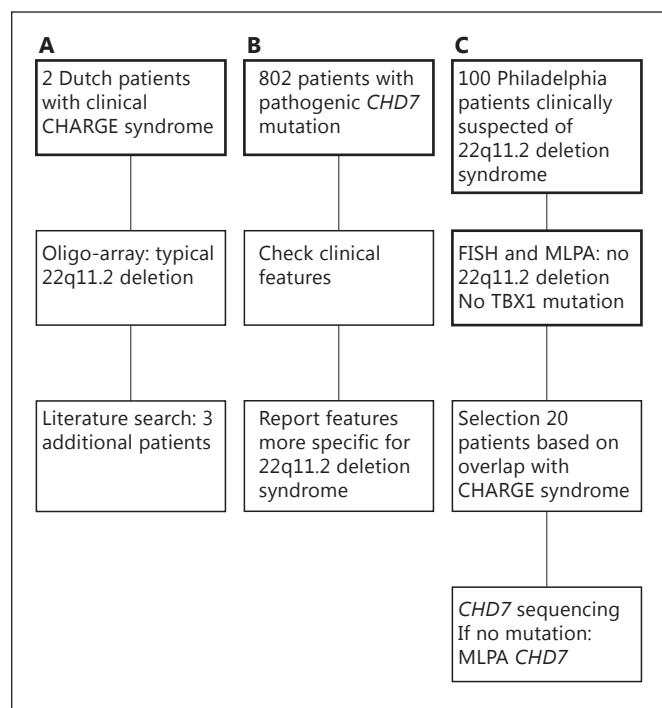
2010a]. Heterozygous *CHD7* mutations are found in more than 90% of the patients, who fulfil the clinical criteria of CHARGE syndrome, but can also be detected in patients with an atypical phenotype [Jongmans et al., 2006; Bergman et al., 2011b].

Chromosome 22q11.2 microdeletions have an estimated birth incidence between 10 and 26 per 100,000 live births and cause a highly variable clinical phenotype including velocardiofacial syndrome (OMIM 192430) which is the combination of velopharyngeal incompetence and other palate abnormalities, congenital heart defects, and dysmorphic facial features. DiGeorge syndrome (OMIM 188400) is another associated phenotype that includes features of congenital heart defects of the outflow tract, hypocalcaemia and immunodeficiency [McDonald-McGinn et al., 1993; Schwinger et al., 2010; McDonald-McGinn and Sullivan, 2011]. Since velocardiofacial syndrome and DiGeorge syndrome describe variable clinical expressions of the same entity, the term 22q11.2 deletion syndrome is now more commonly used (features summarised in fig. 1) [Jyonouchi et al., 2009; McDonald-McGinn and Sullivan, 2011]. This syndrome is inherited in an autosomal dominant manner from a

parent in 10% of new cases but mostly occurs de novo [McDonald-McGinn and Sullivan, 2011]. The majority of patients with velocardiofacial syndrome and DiGeorge syndrome have a 3.0- (90%) or 1.5-Mb (8%) hemizygous deletion of chromosome 22q11.2 that can be identified using fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) or genome-wide array analysis [Schwinger et al., 2010; Gennery, 2012]. Mutations in the *TBX1* gene located in the commonly deleted region cause a similar phenotype, and thus *TBX1* haploinsufficiency appears to significantly contribute to the features of 22q11.2 deletion syndrome [Merscher et al., 2001].

The overlap between CHARGE syndrome and 22q11.2 deletion syndrome has long been recognised [Emanuel et al., 1992; de Lonlay-Debeney et al., 1997; Digilio et al., 1997; Devriendt et al., 1998; Herman and Siegel, 1998]. The overlapping clinical features include congenital conotruncal heart defects, cleft palate, ear abnormalities, hearing loss, growth deficiency, developmental delay, renal abnormalities, hypocalcaemia, and immune deficiency [Jyonouchi et al., 2009; Bergman et al., 2011b]. Clinical features like coloboma, choanal atresia, facial nerve palsy, tracheo-oesophageal fistula, hypoplastic semicircular canals, micropenis, or hypogonadotropic hypogonadism generally occur more often in patients with CHARGE syndrome than in those with 22q11.2 deletion syndrome, although not exclusively (see fig. 1). Because of the clinical resemblance between the 2 syndromes, in our recent review, we recommended that *CHD7* is a good candidate gene to analyse in patients with clinical features of 22q11.2 deletion syndrome, but who do not have a deletion or mutation of *TBX1*. We further recommended that a whole-genome array should be performed in patients suspected of CHARGE syndrome but without a *CHD7* mutation or deletion [Bergman et al., 2011b].

The overlap between CHARGE and 22q11.2 deletion syndrome and the variable expression of both syndromes can hamper clinical diagnosis but also provides interesting clues to the aetiology and pathogenesis of both syndromes. We provided further details of the overlap between the 2 syndromes by describing case reports of patients diagnosed with CHARGE syndrome but carrying a 22q11.2 deletion, by reporting the typical 22q11.2 deletion features present in a *CHD7*-positive cohort and by describing the results of *CHD7* sequencing in a cohort of patients with features of 22q11.2 deletion syndrome but without a deletion or mutation of *TBX1*. The molecular pathways underlying this clinical resemblance and the implications for genetic diagnostic work were discussed.



**Fig. 2.** Overview of our study design. This flow diagram illustrates the 3 different parts of our study. The data in thickened boxes was available at the start of the study. **A** Clinical CHARGE patients with 22q11.2 deletions; **B** patients with a *CHD7* mutation and features of 22q11.2 deletion syndrome; **C** *CHD7* analysis in patients clinically presenting as 22q11.2 deletion syndrome.

## Methods

A schematic overview of our study design is shown in figure 2.

### Patients

We described the clinical findings of 2 Dutch patients, who were diagnosed with CHARGE syndrome according to the clinical criteria of Blake et al. [1998] and/or Verloes [2005], but who appeared to have a 22q11.2 deletion. We further summarised the available clinical data of 3 patients described in the literature with clinically typical CHARGE syndrome and a 22q11.2 deletion.

We screened our database of 802 patients with a pathogenic *CHD7* mutation for clinical features more specific for 22q11.2 deletion syndrome, like hypocalcaemia, thymus anomalies and immunological problems, or who were reported to have a DiGeorge or 22q11.2 deletion phenotype.

In addition, we analysed *CHD7* in a cohort of 20 patients from the Department of Paediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pa., USA. These patients were selected from a cohort of 100 cases, who were clinically suspected of 22q11.2 deletion syndrome, but in whom FISH did not detect a deletion of chromosome 22q11.2 and MLPA did not find any atypical deletions of the 22q11.22 region. In the patients selected for *CHD7* analysis, point mutations in the *TBX1* gene had also been excluded. The 20 patients were selected, because their

**Table 1.** Clinical features in patients with clinical CHARGE syndrome and a 22q11.2 deletion

Features	Patients				
	Emanuel et al., 1992	Digilio et al., 1997	Devriendt et al., 1998	our patient 1	our patient 2
Coloboma	u	+	+	+	-
Heart defect	u	+	+	+	+
Choanal atresia*	u	+	+	+	+
Growth retardation	u	u	+	u	u
Developmental delay	u	u	+	+	+
Genital hypoplasia	u	+	-	-	-
Ear anomaly	u	+	+	-	+
SCC hypoplasia	u	u	u	+	-
Cleft lip and/or palate	u	-	-	-	-
Hearing loss	u	u	+	+	-
Feeding difficulties**	u	u	u	+	+
Facial palsy	u	u	u	-	-
TE anomaly	u	-	u	-	-
Hypocalcaemia	u	+	-	-	-
Immunological abn.	u	+	u	-	-
Thymus abn.	u	-	-	u	-
Other	CHARGE association, no further information	unilateral absent radius, hypoplastic ulna	preauricular tags, pit in left cheek	broncho-tracheomalacia, GERD	pharyngomalacia

Features more commonly seen in CHARGE syndrome than in 22q11.2 deletion syndrome are highlighted in grey. abn. = Abnormalities; GERD = gastro-oesophageal reflux disease; SCC = semicircular canal; TE = tracheo-oesophageal anomaly; u = unexamined/unknown; + = present; - = not present.

\* Atresia or stenosis of choanae. \*\* Only if necessitating tube feeding.

clinical features overlapped with features seen in CHARGE syndrome, including the presence of congenital heart defects, coloboma, immune defects, ear anomalies, renal malformations, and hearing loss.

#### CHD7 Analysis

Blood samples from all patients were drawn after informed consent. DNA was isolated according to standard procedures. The 37 coding exons of *CHD7* (exons 2–38, RefSeq NM\_017780.02) and their flanking intronic sequences were amplified by PCR and sequenced as described earlier [Jongmans et al., 2006]. If no mutations were identified, *CHD7* was screened for whole-exon deletions and duplications by MLPA using a commercially available set of probes: the SALSA P201 kit (MRC-Holland, Amsterdam, The Netherlands; <http://www.mrc-holland.com>) [Bergman et al., 2008].

#### Analysis of 22q11.2 Deletions and *TBX1* Sequencing

Patients gave informed consent, and blood samples were taken. 22q11.2 deletions were detected or excluded in the Dutch patients by array comparative genomic hybridisation (CGH) using an Agilent 180 K oligonucleotide array (custom design no. 023363, Agilent Technologies Incorporation, Santa Clara, Calif., USA) and/or FISH using probe RP11-481H20 and RP11-590C5. The array CGH procedures were carried out according to the manufacturer's protocols. Normal male or female reference DNA was used as a control, and DNA analytics version 4.0.81 (Agilent Technologies Incorporation) was used to analyse the results.

The 20 Philadelphia cases were studied by FISH using the commercially available probe N25 and then analysed by an MLPA assay specific for the chromosome 22q11.22 region (MRC Holland; SALSA P250 kit) to identify deletions, whose proximal endpoints are distal to the standard FISH probes and would not have been identified in the original cohort [Jalali et al., 2008]. Point mutations in the *TBX1* gene were excluded by capillary sequencing following PCR amplification as previously reported [Gong et al., 2001].

## Results

### Clinical CHARGE Patients with 22q11.2 Deletions

Table 1 lists all the patients with a CHARGE syndrome phenotype known to carry a 22q11.2 deletion including the 2 described below.

*Patient 1.* This girl was briefly described by Bergman et al. [2011b]. She was born by caesarean section because of foetal distress at 33 + 3 weeks of gestation. The pregnancy was complicated by gestational diabetes mellitus and polyhydramnios. Her birth weight was 2,830 g (97.7th percentile), and her Apgar scores were 9 and 9 after 1 and 5 min, respectively. During the first 4 weeks of life, she had feeding problems necessitating nasogastric tube feed-

ing. She was diagnosed with bronchotracheomalacia and gastro-oesophageal reflux disease. Several congenital anomalies were noticed: coloboma of the left iris and both retinas, unilateral choanal stenosis, mild pulmonic stenosis, and bilateral mixed hearing loss. A CT scan of the mastoid showed abnormal semicircular canals as well as an abnormal vestibulum and an abnormal basal convolution of the cochlea. The girl had a developmental motor delay; she started walking at the age of 2 years and 3 months. At her last examination, at the age of 3 years, she had a normal language comprehension (quotient score of 91, comprehension scales of the Dutch Reynell Developmental Language Scales). Physical examination showed simple ears, an anteriorly placed anus and a hockey-stick line crease on both palms. The diagnosis CHARGE syndrome was made based on the clinical diagnostic criteria of both Blake et al. [1998] and Verloes [2005]. Analysis of *CHD7* did not show a mutation or deletion. Subsequently, array CGH showed a de novo 2.6-Mb loss of 22q11.2 (proximal breakpoint 17,210,818–17,270,293; distal breakpoint 19,891,492–19,870,318). The deletion was confirmed by FISH analysis.

*Patient 2.* This female infant was born prematurely at 33 + 4 weeks with a birth weight of 1,565 g (10th–20th percentile). The pregnancy was complicated by polyhydramnios. Postnatally, she experienced respiratory distress and was suspected of having a partial choanal atresia. She had a congenital heart defect consisting of arterial septal defect, ventricular septal defect and patent ductus arteriosus. Morphologic evaluation at the age of 2 days revealed microcephaly (28 cm; –5.6 SD below mean), short palpebral fissures, dysmorphic ears with broad superior crus of the antihelices, overfolded helices, and a bulbous nasal tip. CHARGE syndrome or a chromosomal abnormality was suspected, and array CGH, fundoscopy, renal ultrasound, and brain imaging with special attention for the semicircular canals were suggested. Array CGH showed a 2.9–3.0 Mb deletion of 22q11.21 (proximal breakpoint 17,210,818–17,270,293; distal breakpoint 20,142,009–20,247,225) that was confirmed by FISH analysis. The other investigations had normal results, except for subtle abnormalities of the brain MRI scan with slightly delayed myelination and mildly enlarged ventricles.

At the age of 2 months, the ventricular septal defect was surgically corrected. From age 4–22 months, she required a tracheostomy because of the combination of very narrow choanae and pharyngomalacia. At the age of 10 months, a gastrostoma with feeding tube were placed. At her last examination at the age of 2 years and 4 months,

her height was 86 cm (–1 SD). Her head circumference was not measured, but she was normocephalic before (at the age of 22 months, her head circumference was 47.7 cm (–0.7 SD)). She was still being fed through her feeding tube and had just started to use some spoken words in addition to sign language after the removal of her tracheotomy.

#### *CHD7 Mutations in Phenotypic 22q11.2 Deletion Syndrome*

*Patients with a CHD7 Mutation and Features of 22q11.2 Deletion Syndrome.* Table 2 summarises the 30 patients out of our international database of 802 patients, in which typical features of 22q11.2 deletion syndrome were described [Janssen et al., 2012]. We also included 3 additional patients from the literature, in whom the precise *CHD7* mutation was not mentioned [Chopra et al., 2009]. All 33 patients had features that are more commonly seen in deletion 22q11.2 syndrome than in CHARGE syndrome. A 22q11.2 deletion was excluded in 25 of the 33 patients (76%). In one patient, a paternally inherited 2.5-Mb 22q11.23 deletion located distal to the 22q11.2 deletion syndrome region was identified in addition to the *CHD7* mutation [Kaliakatsos et al., 2010]. At least, 16 of the 33 patients died in infancy.

*CHD7 Analysis in Patients Clinically Presenting as 22q11.2 Deletion Syndrome.* We identified 5 pathogenic *CHD7* mutations in our group of 20 patients that were clinically suspected of 22q11.2 deletion syndrome.

Table 3 summarises the mutations and known clinical features of the 5 patients clinically presenting as deletion 22q11.2 syndrome, but in whom a *CHD7* mutation was identified. Remarkably, all 5 patients carried a truncating mutation in the *CHD7* gene. In addition, we identified 2 silent *CHD7* mutations (c.4014C>T, p.Gly1338Gly and c.6216C>G, p.Pro2072Pro) in 2 other patients. Both silent mutations had been identified in the 1,000 Genomes project (DbSNP rs199828744, rs188679907).

## Discussion

We highlighted the clinical overlap between 22q11.2 deletion and CHARGE syndrome. Although some of the clinical features are present far more often in one of the syndromes (fig. 1), none are seen exclusively in either 22q11.2 deletion or CHARGE syndrome. For example, the presence of choanal atresia or semicircular canal hypoplasia does not exclude a 22q11.2 deletion, and severe T-cell dysfunction also occurs in CHARGE syndrome.

**Table 2.** Patients with a *CHD7* mutation, who show clinical features of 22q11.2 deletion syndrome

ID	Type of mutation	Features															immunological abn.	thyrmus abn.	other
		coloboma	heart defect	choanal atresia*	growth retardation	developm. delay	genital hypoplasia	ear anomaly	SCC hypoplasia	cleft lip and/or palate	hearing loss	feeding difficulties**	facial palsy	TE anomaly	hypocalcaemia				
0 <sup>d</sup>	del	+	+	-	-	u	+	+	-	-	u	+	u	+	+	+	+	+	22q11 phenotype, long slender fingers
0 <sup>a</sup>	del wg	-	+	-	u	+	u	+	u	-	u	u	u	u	u	u	u	u	
P99	fs	+	+	-	u	+	u	+	u	u	+	+	u	+	u	+	+	+	
P129 <sup>d</sup>	fs	+	+	+	+	+	+	+	+	-	+	u	+	u	+	+	+	u	PTH def
P137	fs	+	+	-	+	+	u	+	+	u	+	+	u	-	u	u	+	+	bronchomalacia, torticollis, GERD, absent thumbs
P147	fs	+	+	-	+	u	+	+	u	u	+	u	u	-	u	u	+	+	fissure upper lobe right long, hypothyroidism
P197 <sup>e</sup>	fs	+	+	-	u	u	+	+	u	u	u	u	+	u	+	+	+	+	laryngomalacia, erythroderma, total alopecia
P238	fs	u	u	u	u	u	u	u	u	u	u	u	u	u	u	+	+	u	
P245 <sup>a</sup>	fs	+	+	u	u	+	u	+	u	u	u	u	u	u	+	+	+	+	
P293 <sup>m</sup>	fs	+	+	u	u	u	u	u	u	u	+	u	u	+	+	+	+	+	tracheomalacia, PTH def, ectopia of one kidney
P304	fs	+	u	+	u	u	+	+	-	u	+	u	u	u	+	+	u	u	
P767	fs	-	+	u	-	+	+	+	+	-	+	+	+	-	+	u	u	u	PTH def, OSAS
P800 <sup>i</sup>	fs	+	+	-	-	+	+	+	+	-	+	+	+	+	u	-	+	+	obstructive apneus
P866	fs	u	u	u	u	u	+	+	u	u	u	u	u	u	+	u	u	u	
P875	fs	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	laryngeomalacia, hypothyroidism, vermiform dysplasia
P987 <sup>h</sup>	fs	+	+	u	u	u	+	+	u	u	+	u	u	u	+	u	u	u	PTH def, limb abnormality
P29 <sup>a</sup>	non	+	+	u	u	u	+	+	u	+	u	u	u	u	u	u	u	+	abnormal limbs
P40	non	+	+	+	+	u	u	+	u	u	u	u	u	u	u	+	u	+	
P37 <sup>b</sup>	non	-	+	+	u	u	+	+	u	+	u	-	-	-	+	u	+	+	PTH def
P44 <sup>c</sup>	non	+	+	+	u	u	-	+	u	u	u	u	+	u	+	+	+	+	PTH def, laryngomalacia, abnormal limbs
P122 <sup>a</sup>	non	+	+	+	u	u	+	u	u	u	u	u	u	+	+	+	u	u	
P189 <sup>b</sup>	non	u	+	-	u	u	+	+	u	u	-	u	u	+	+	+	u	u	hypothyroidism, partial agenesis corpus callosum, horseshoe kidney
P256 <sup>f,g</sup>	non	u	+	u	u	u	+	u	u	+	u	u	u	u	u	u	u	+	terminated pregnancy, left isomerism, hypoplastic optic nerves
P279 <sup>i,k,l</sup>	non	-	+	+	+	+	+	+	+	-	+	+	u	-	u	-	+	+	cortical brain atrophy
P780 <sup>i,k</sup>	non	-	+	+	+	+	+	+	+	-	+	-	-	+	+	-	-	-	NEC, hydrocephalus, GERD
P834 <sup>i</sup>	non	+	+	u	+	+	+	+	+	+	+	u	+	u	u	u	u	+	laryngomalacia, PTH def, corpus callosum hypoplasia, horseshoe kidney
P898	non	+	+	u	u	u	+	+	u	+	+	u	u	u	u	+	+	u	hydrocephaly
P1003 <sup>j</sup>	non	-	+	+	u	u	-	+	+	u	u	u	u	u	u	+	+	+	glottic web, laryngomalacia, vertical talus
0 <sup>a</sup>	non	u	+	u	u	u	+	+	u	u	u	u	u	u	+	u	u	+	preauricular tags
P616	spl	+	+	-	+	u	+	+	+	u	u	u	+	-	+	u	+	+	scalp cutis aplasia, PTH def
0 <sup>a</sup>	U	+	+	u	u	u	+	u	u	-	+	u	u	+	u	+	+	+	seizures
0 <sup>a</sup>	U	+	+	+	+	u	u	u	u	-	+	u	+	u	u	+	+	u	
0 <sup>a</sup>	U	+	+	u	u	u	u	u	u	-	+	u	-	u	u	+	+	u	bulbar palsy

Features more commonly seen in 22q11.2 deletion syndrome than in CHARGE syndrome are highlighted in grey.

u = Unexamined/unknown; + = present; - = not present; abn. = abnormalities; del = deletion exon 4; fs = frameshift; developm. = developmental; GERD = gastro-oesophageal reflux disease; ID = patient ID in online database of *CHD7* mutations ([www.chd7.org](http://www.chd7.org)); non = nonsense; NEC = necrotising enterocolitis; OSAS = obstructive sleep apnea syndrome; PTH def = low parathyroid hormone or hypoparathyroidism; SCC = semicircular canal; spl = splice site; TE = tracheo-oesophageal; U = mutations unknown (all patients described in Chopra et al. [2008]); wg = whole gene; 0 = not mentioned in the online database.

<sup>a</sup> Gennery et al., 2008. <sup>b</sup> Writzl et al., 2007. <sup>c</sup> Inoue et al., 2010. <sup>d</sup> Wincent et al., 2008. <sup>e</sup> Kaliaatsos et al., 2010. <sup>f</sup> Vuorela et al., 2007. <sup>g</sup> Vuorela et al., 2008. <sup>h</sup> Van de Laar et al., 2007. <sup>i</sup> Jongmans et al., 2006. <sup>j</sup> Hoover-Fong et al., 2009. <sup>k</sup> Vissers et al., 2004. <sup>l</sup> Bergman et al., 2011a. <sup>m</sup> Sanka et al., 2007. <sup>n</sup> Randall et al., 2009. <sup>o</sup> Chopra et al., 2008.

\* Atresia or stenosis of choanae. \*\* Only if necessitating tube feeding.

**Table 3.** Features of 5 patients with clinical 22q11.2 deletion syndrome but without *TBX1* haploinsufficiency, in whom *CHD7* mutations were found

Patient ID	<i>CHD7</i>	CHD7	Clinical features*
CH95-172	c.493_496delinsGG	p.Pro165fs	suspected of having 22q11.2 deletion syndrome, no further information available
CH95-218	c.2440C>T	p.Gln814X	ADHD, speech therapy, <b>sensorineural/conductive hearing loss</b> , scoliosis, myopia, <b>retinal coloboma</b> , dysmorphic features
CH94-143	c.3024T>G	p.Tyr1008X	thymic hypoplasia, CLP, hypocalcemia, normal cardiac ultrasound, <b>auricular dysplasia</b>
CH96-184	c.4357C>T	p.Gln1453X	low PTH, low Ca, low T cells, small thymus, dysmorphia, micrognathia, <b>low-set malformed ears</b> , <b>choanal atresia</b> , velopharyngeal incompetence, laryngotracheomalacia, ASD
CH99-214F	c.4424del	p.Glu1475fs	suspected of having 22q11.2 deletion syndrome, no further information available

\* Typical CHARGE features are indicated in bold.

Remarkably, almost exclusively, we found truncating *CHD7* mutations in both groups of patients suspected of having *TBX1* haploinsufficiency (tables 2, 3). This is in line with our previous observation that *CHD7* missense mutations result in a milder phenotype. Features also seen in 22q11.2 syndrome, like congenital heart defects and cleft palate, occur more often in patients with a truncating mutation than in those with a missense mutation [Bergman et al., 2012b]. Thus, the clinical overlap between these syndromes is predominantly seen in the more severely affected patients with a 22q11.2 deletion or *CHD7* mutation.

How can we explain the remarkable clinical overlap between these 2 syndromes? It is possible that both syndromes could be present simultaneously, but this must be extremely rare, as no cases have been reported yet. However, it cannot be excluded, since not all mutations in *CHD7* and *TBX1* are detectable by current techniques. It is well known that no *CHD7* mutation can be detected in 5–10% of the patients with a clinical diagnosis of CHARGE, suggesting either undetectable *CHD7* mutations (e.g. in the promoter region) or the existence of a second gene that can cause CHARGE syndrome when mutated. Nonetheless, both syndromes should be included in a common differential diagnosis as discussed in our recent reviews [McDonald-McGinn et al., 1993; Bergman et al., 2011b; McDonald-McGinn and Sullivan, 2011].

The most likely explanation for the phenotypic overlap between both syndromes is that the causative genes, *CHD7* and *TBX1*, function in the same embryonic path-

way or in pathways with a common target. The *CHD7* gene is expressed ubiquitously during human embryonic development with a high expression in the foetal inner ear, eye, central nervous system, and in the neural crest of the pharyngeal arches [Sanlaville et al., 2006]. The *CHD7* protein belongs to the chromodomain helicase DNA-binding (CHD) family [Woodage et al., 1997] and is thought to regulate gene transcription by ATP-dependent chromatin modification during embryogenesis [Schnetz et al., 2009]. *CHD7* cooperates with, amongst others, PBAF (polybromo- and BRG1-associated factor containing complex) in controlling neural crest gene expression and cell migration [Bajpai et al., 2010].

The *TBX1* gene codes for the T-box transcription factor *TBX1* that regulates the expression of downstream growth and transcription factors that are involved in the development of the heart, thymus, parathyroid, and palate. *TBX1* physically interferes with *SMAD1*, influencing its binding to *SMAD4* and thus signal transduction [Fulcoli et al., 2009]. Interestingly, *CHD7* was found to colocalise with *SMAD1* (OMIM 601595) and other transcription factors at enhancer elements near genes that are repressed [Schnetz et al., 2010]. Thus, both *TBX1* and *CHD7* regulate gene transcription and might well regulate the transcription of the same genes.

Mice with heterozygous *Chd7* mutations show semi-circular canal defects, septal heart defects, cleft palate, choanal atresia, hyposmia, olfactory bulb anomalies, testes hypoplasia, hearing loss, and low body weight [Bosman et al., 2005; Adams et al., 2007; Hurd et al., 2007,

Gene	Disease	Choanal atresia	Cranial nerve dysfunction	Semicircular canal anomalies	Congenital heart defect	Renal anomalies	Intellectual disability	Growth deficiency	Genital hypoplasia	Pituitary problems	Microphthalmia/coloboma	External ear anomalies	Hearing loss	Cleft lip and/or palate	Oesophageal atresia	Hypocalcaemia	Immunodeficiency
<i>CHD7</i>	CHARGE syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>TBX1</i>	22q11.2 deletion syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>JAG1</i>	Alagille syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>GLI3</i>	Pallister Hall syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>FGFR1</i>	Kallmann syndrome Pfeiffer syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>FGF8</i>	Kallmann syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>SOX2</i>	Syndromic microphthalmia type 3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>OTX2</i>	Syndromic microphthalmia type 5	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>MYCN</i>	Feingold syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>TCOF1</i>	Treacher Collins syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>FGF10</i>	Lacrimo-auriculo-dento- digital syndrome	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

**Fig. 3.** Multiple congenital anomaly syndromes having clinical overlap with CHARGE syndrome and 22q11.2 microdeletion syndrome. In figure 3, we show the overlapping clinical features of CHARGE and 22q11.2 deletion syndrome with known genetic syndromes. All the genes mentioned in this figure or their proteins have been associated with either *CHD7* or *TBX1*. The expression of *FGFR1*, *OTX2* and *TBX1* depends on *CHD7* in some tissues; *TBX1* and *FGF8* are in epistasis in ectodermal development; bind-

ing of the protein treacle which is encoded by *TCOF1* partly depends on the presence of *CHD7*, and *SOX2* and *CHD7* are co-factors that regulate the expression of *JAG1*, *MYCN* and *GLI3*, amongst others [Randall et al., 2009; Hurd et al., 2010; Zentner et al., 2010a; Engelen et al., 2011; Layman et al., 2011]. Figure 3 shows that the linked molecular pathways are reflected by the shared clinical features of the syndromes.

2011; Layman et al., 2009; Bergman et al., 2010 ]. Other CHARGE features, e.g. coloboma, external ear anomalies and tracheo-oesophageal defects, have not been reported in *Chd7*-deficient mice. This discrepancy in phenotype between man and mice might be caused by species-specific differences in *CHD7* requirements or differences in genetic background [Zentner et al., 2010b].

Mice with haploinsufficiency for *Tbx1* show the full range of malformations that can be present in the 22q11.2 deletion syndrome [Merscher et al., 2001]. Tissue-specific conditional mutagenesis of *Tbx1* has shown its role in the secondary heart field [Xu et al., 2004], pharyngeal mesoderm [Zhang et al., 2006], pharyngeal endoderm [Arnold et al., 2006], pharyngeal epithelia [Zhang et al., 2005], and otic epithelium [Xu et al., 2007].

Surprisingly, mice with a double heterozygous mutation of *Chd7* and *Tbx1* show a severe cardiovascular phenotype and severely reduced postnatal viability compared to mice with a heterozygous mutation of *Chd7* or *Tbx1* [Randall et al., 2009]. The synergistic haploinsufficiency of both genes resulted in an enhanced effect on the fourth pharyngeal arch morphogenesis, abnormal thymus development and malformations of the semicircular canals. These observations in mice together with our observations in the patients presented in this paper suggest that both genes act in the same developmental pathway. Randall et al. [2009] hypothesised that *Chd7* might modulate *Tbx1* expression but were unable to prove that the expression of either gene changed in mouse embryos mutated at the other locus. Hurd et al. [2011] showed that *Tbx1* expression was expanded more ventrally in the develop-



ing inner ear of a *Chd7* null mouse conditional mutant (*Chd7<sup>Gt/Gt</sup>*) compared to the wild-type mouse embryo. This effect was not seen in heterozygously mutated mice (*Chd7<sup>Gt/+</sup>*), suggesting that there is a dose-dependent inhibiting effect of *Chd7* on *Tbx1* in the inner ear which might be essential for inner ear neurogenesis [Hurd et al., 2011].

As an alternative theory, a shared convergent pathway via fibroblast growth factor 8 (FGF8, OMIM 600483) has been suggested, but has not been proven [Randall et al., 2009]. It was shown that reduced *Chd7* dosage in the olfactory placode, pituitary and hypothalamus in mice reduced the expression of the FGF8 receptor *Fgfr1* (OMIM 136350) [Layman et al., 2011]. FGF8 and its receptor FGFR1 are interesting linking factors, since both are also involved in the pathogenesis of other organs frequently affected in CHARGE syndrome, like the combination of hypogonadotropic hypogonadism and anosmia [Pallais et al., 1993; Bergman et al., 2011a, 2012a]. The fact that this combination is seldom seen in patients with 22q11.2 deletion could be explained by the more ubiquitous expression of *CHD7* compared to *TBX1*.

The tight relationship between 22q11.2 deletion and CHARGE syndrome is not an isolated observation. Both syndromes share common features with other syndromes that may reveal further clues for interaction of their causative genes and underlying embryonic pathways. For instance, *SOX2* mutations (OMIM 184429) result in a phenotype characterised by anophthalmia, tracheo-oesophageal abnormalities, pituitary defects, and genital abnormalities. Like *CHD7*, *SOX2* is assumed to play a role in neural stem cells, and Engelen et al. [2011] showed that *CHD7* is a *SOX2* transcriptional cofactor with their com-

mon target genes being *JAG1*, *GLI3* and *MYCN* (Notch and Shh signalling pathways). Shh signalling regulates the expression of *TBX1* in the pharyngeal arch probably through transcription factors of the FOX family [Yamagishi et al., 2003]. *TBX1* has also been described as an upstream regulator of the Notch-signalling effector *HES1* in the pharyngeal arch and a downstream target of *JAG2* in tooth morphogenesis [Mitsiadis et al., 2010; van Bueren et al., 2010]. *CHD7* and *TBX1* have been described to interact with proteins known from other overlapping syndromes (fig. 3). Thus, *CHD7* and *TBX1* may also interact indirectly through different pathways, like the Notch and Shh signalling pathways.

In conclusion, the clinical diagnosis of 2 highly variable syndromes, CHARGE and 22q11.2 deletion syndrome, can prove challenging. The syndromes should therefore be included in a common differential diagnosis, and we strongly recommend performing *CHD7* analysis in any patients with a 22q11.2 deletion phenotype but without *TBX1* haploinsufficiency and performing a genome-wide array for 22q11.2 deletions in clinical CHARGE patients without a *CHD7* mutation. We have shown that there is strong clinical evidence that both molecular pathways are linked, although the precise nature of this link needs further exploration.

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