European Journal of Medical Genetics 53 (2010) 280–285

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Original article

Novel *CHD7* mutations contributing to the mutation spectrum in patients with CHARGE syndrome

Kathrin Wessels [a], Bettina Bohnhorst [b], Ingrid Luhmer [c], Susanne Morlot [d], Axel Bohring [e], Jon Jonasson [f], Jörg T. Epplen [g], Dorothea Gadzicki [h], Stefanie Glaser [h], Gudrun Göhring [h], Madeleine Mälzer [a], Anke Hein [a], Mine Arslan-Kirchner [a], Manfred Stuhrmann [a], Jörg Schmidtke [a], Brigitte Pabst [a,*]

[a] *Institute of Human Genetics, Carl-Neuberg-Str. 1, 30625, Hannover Medical School, Hannover, Germany*
[b] *Clinic for Paediatric Pneumology and Neonatology, Hannover Medical School, Hannover, Germany*
[c] *Clinic for Paediatric Cardiology and Internal Medicine, Hannover Medical School, Hannover, Germany*
[d] *MVZ wagnerstibbe, Human Genetics, Hannover, Germany*
[e] *Institute of Human Genetics, Westphalian Wilhelms-University, Münster, Germany*
[f] *Clinical genetic, University Linköping, Linköping, Sweden*
[g] *Human Genetic Department, Ruhr University Bochum, Bochum, Germany*
[h] *Institute of cell and molecular pathology, Hannover Medical School, Hannover, Germany*

ARTICLE INFO

Article history:
Received 11 February 2010
Accepted 4 July 2010
Available online 30 July 2010

Keywords:
CHARGE syndrome
CHD7
Mutation
MLPA
Detection rate
Clinical variability

ABSTRACT

CHARGE syndrome is an autosomal dominant inherited multiple malformation disorder typically characterized by coloboma, choanal atresia, hypoplastic semicircular canal, cranial nerve defects, cardiovascular malformations and ear abnormalities. Mutations in the chromodomain helicase DNA-binding protein 7 (*CHD7*) gene are the major cause of CHARGE syndrome. Mutation analysis was performed in 18 patients with firm or tentative clinical diagnosis of CHARGE syndrome. In this study eight mutations distributed across the gene were found. Five novel mutations – one missense (c.2936T > C), one nonsense (c.8093C > A) and three frameshift mutations (c.804_805insAT, c.1757_1770del14, c.1793delA) – were identified. As far as familial data were available these mutations were found to have arisen *de novo*. Comparison of the clinical features of patients with the same mutation demonstrates that expression of the phenotype is highly variable. The mutation detection rate in this study was 44.4% in patients with a clinically established or suspected diagnosis of CHARGE syndrome.

1. Introduction

Hall [9] and Hittner et al. [10] were the first to describe independently a nonrandom cluster of malformations which was later referred to as CHARGE association by Pagon et al. [20]. The acronym summarizes the main clinical features of the syndrome, which are **c**oloboma, **h**eart defect, choanal **a**tresia, **r**etardation in development and growth, **g**enital hypoplasia and **e**ar anomalies [20]. Other common clinical findings include hypoplasia of the semicircular canals, rhombencephalic dysfunction, facial nerve palsy, cleft lip/palate, tracheoesophageal fistula, arhinencephaly and distinctive facial features. Renal anomalies, thymic/parathyroid hypoplasia, hand and spine anomalies are occasional findings that are less specific [12,14,22,26]. Mental development varies from near normal to profound retardation [5]. Low adaptive behavior skills and symptoms of autistic spectrum disorder have been described [6]. Expression of the clinical features varies, leading to a great phenotypic diversity. Diagnostic criteria were set up to help to identify individuals with the clinical diagnosis of CHARGE syndrome [5,18], and these have been recently redefined by Verloes [26]. According to Blake et al. [5] the diagnosis can be confirmed by the presence of four major criteria, namely coloboma, choanal atresia, external and internal inner ear malformations and cranial nerve dysfunction. The combination of three out of four major and three out of seven minor criteria including cardiovascular malformations, tracheoesophageal fistula, growth deficiency, genital hypoplasia, developmental delay, orofacial cleft and characteristic facial features describes the typical CHARGE syndrome as well. Verloes [26] focused on the embryological defects and defined semicircular canals, coloboma and choanal atresia as the three major criteria. The combination of two major and two minor signs also reflects typical CHARGE syndrome. Rhombencephalic anomalies, hypothalamo-hypophyseal dysfunction, external/middle ear malformations, malformations of

* Corresponding author. Tel.: +49 0511 5328550; fax: +49 0511 5325865.
E-mail address: pabst.b@mh-hannover.de (B. Pabst).

1769-7212/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmg.2010.07.002

mediastinal viscera and mental retardation are the five possible minor criteria stated by Verloes [26].

CHARGE syndrome (OMIM 214800) follows an autosomal dominant mode of inheritance. Its incidence of 1:8500 was evaluated in a Canadian study [11] and is suggested to range between 0.1and1.2/10,000 live births [23]. Mutations in the chromodomain helicase DNA-binding protein 7 (*CHD7*) gene in 8q12.1 were identified as causative for CHARGE syndrome [27]. The gene has 37 coding exons and encodes a 2997-amino acid protein that belongs to the family of chromodomain helicase DNA-binding (CHD) proteins. In humans at least nine CHD genes are known. Functional domains such as chromo (chromatin organization modifier), SNF2-related helicase/ATPase and BRK were identified in the CHD7 protein [1,17,31]. The majority of *CHD7* gene mutations is unique and occurs *de novo*. Familial transmission and germline mosaicism have rarely been described [7,12–14,21,28,29]. Since the identification of the molecular etiology, the term CHARGE syndrome has been used instead of CHARGE association, as had already been proposed by Lubinsky [16].

In the present study 18 patients either with typical features of CHARGE syndrome or suspected to have CHARGE syndrome were screened for mutations in the *CHD7* gene in order to confirm the clinical diagnosis of CHARGE syndrome. Altogether eight mutations were identified, five of which were new and contribute to the mutation spectrum found in CHARGE patients. These analyses confirm and emphasize the clinical variability of the phenotype.

2. Material and methods

2.1. Subjects

Blood or DNA samples from 18 patients, six females and 12 males, were referred to our institute for mutation analysis in the *CHD7* gene. DNA samples from 50 anonymous healthy individuals served as controls. Institutional review board (IRB) approval for this study was obtained.

2.2. Mutational analysis

Genomic DNA was extracted from peripheral blood lymphocytes using *QIAGEN* Blood mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The coding regions of exons 2–38 and flanking intronic sequences were amplified by polymerase chain reaction (PCR). *CHD7* exons were amplified using mainly newly designed primers, except for some exons which were previously described [12]. For PCR conditions and primer sequences see Supplementary material. PCR products were cleaned from unbound nucleotides either by polyethylene glycol precipitation and ethanol washing or by enzymatic digestion (ExoSAP-IT, USB Cooperation, Cleveland, OH, USA). Sequencing analysis was performed using the ABI Big Dye Terminator cycle sequencing kit 3.1 (Applied Biosystems, Foster City, CA, USA). The products were purified with DyeEx. 2.0 Spin Kit (QIAGEN GmbH, Hilden, Germany) and analyzed on an ABI genetic analyzer 3130xl. Multiplex ligation probe dependent amplification (MLPA) analysis was performed to screen for exon duplication and deletion in the *CHD7* gene using the SALSA MLPA Kit P201-B1 CHARGE (MRC Holland, Amsterdam, The Netherlands). The kit contains probes for 33 of the 38 *CHD7* exons.

The novel sequence variations c.-66C > T and c.2936T > C were confirmed by restriction enzyme digests suitable to detect the sequence changes and analyzed in 50 control individuals. Parental samples, as far as available, were analyzed for inheritance.

3. Results

In a cohort of 18 unrelated patients with either firm or tentative clinical diagnosis of CHARGE syndrome a mutation screening of the 37 coding exons of the *CHD7* gene and the adjacent intron/exon boundaries was performed by direct sequencing. About one third of our patients were under the age of one year. In this study eight presumably disease-causing mutations, five novel and three recurrent, were identified in the *CHD7* gene (Table 1). The mutation spectrum comprises four nonsense, three frameshift and one missense mutation. The mutations were scattered throughout the gene. The clinical features of individuals with and without a mutation in the *CHD7* gene are summarized in Tables 2 and 3, respectively. The missense mutation c.2936T > C in exon 11 leads to an exchange of the amino acid leucine to proline and has not been described before. This mutation is located within the SNF2 domain of the CHD7 protein. The aforementioned amino acid leucine is conserved within the CHD protein family and in CHD orthologues between higher eukaryotes (Table 3). The missense mutation were proven to be de novo and was not identified in 50 healthy individuals.

The three nonsense mutations c.469C > T, c.6070C > T and c.7879C > T have been described previously in other patients [3,7,12,27]. Furthermore, a sequence variation (c.-66C > T) was identified within the noncoding 5′ region of the *CHD7* gene. The nucleotide exchange was not found in 50 healthy individuals. The parents were not available for analysis. In silico analysis performed with the program mutation taster (www.mutationtaster.org) interpret the nucleotide exchange as nonpathogenic.

In ten patients no causal *CHD7* mutation was identified by sequencing or MLPA analysis.

4. Discussion

Mutations in the *CHD7* gene on chromosome 8 (8q12.1) have been found to be a major cause for the clinical phenotype of CHARGE syndrome [27]. In this study eight pathogenic mutations – five novel and three recurrent mutations – were identified in the *CHD7* gene in 44.4% of the patients with either firm or tentative clinical diagnosis of CHARGE syndrome. The mutations are distributed throughout the gene with no mutational hotspot. The frequency of the different mutation types, 57% nonsense, 29% frameshift and 14% missense, summarized in the HGMD database [24] correlates with the reported *CHD7* mutational spectrum in this study. The main mutation type recognized is truncation mutation of the *CHD7* gene causing a premature termination codon (PTC) which in the majority of cases can be predicted to cause nonsense-mediated decay. Nonsense mutations in the last two exons of the *CHD7* gene which are supposed to escape NMD are described only rarely in the HGMD database [24] and once in our study (case 8). Therefore it is suggested that haploinsufficiency might be the major disease-causing mechanism in CHARGE syndrome [14].

The *CHD7* gene has been described to encompass several important domains, e.g. the chromodomain, the SNF2 helicase/ATPase domain and the BRK domain. These domains have been reported to be responsible for regulating chromatin structure and transcriptional activity [31]. The occurrence of missense mutations resulting in a less severe and less specific phenotype with milder mental retardation was recently suggested by Jongmans et al. [12]. The novel missense mutation (c.2936T > C) identified in case 6 is considered to be pathogenic, because the affected amino acid is evolutionarily conserved (see Table 4) and is located in the conserved SNF domain which plays an important role in regulation of transcription and chromatin unwinding as well as in DNA repair

Table 1
CHD7 mutations identified in this study.

Individual	Exon	Mutation type	Nucleotide change	Protein change	Segregation	References
13	2	nonsense	c.469C > T	p.Arg157X	n.a.	[27]
15	2	frameshift	c.804_805insAT	p.Val269MetfsX37	de novo	this study
18	3	frameshift	c.1757_1770del14	p.Tyr586X	n.a.	this study
4	3	frameshift	c.1793delA	p.Lys598ArgfsX10	de novo	this study
6	11	missense	c.2936T > C	p.Leu979Pro	de novo	this study
14	30	nonsense	c.6070C > T	p.R2024X	de novo	[12,27]
2	36	nonsense	c.7879C > T	p.Arg2627X	de novo	[3,7,12]
8	38	nonsense	c.8093C > A	p.Ser2698X	n.a.	this study

n.a. not analyzed.

and DNA recombination [8,17]. The patient fulfills all four major and four minor criteria of Blake's scoring scheme [5].

Three previously described nonsense mutations (c.469C > T case 13, c.7879C > T case 2, c.6070C > T case 14) were identified in the patient cohort. The clinical features of these patients were compared with those in previous studies.

The nonsense mutation (c.469C > T) in exon 2 (case 13) was already described in two sporadic [12,27] and in a familial case with a mildly affected father and two severely affected sons [7]. The affected father has a cup-shaped external ear on the right side as the only sign of CHARGE syndrome. Characteristic features of CHARGE syndrome like coloboma, cardiovascular malformations and external and inner ear anomalies were described in the two affected sons, in the girl reported by Vissers et al. [27] and in the case presented here. Interestingly, none of the patients had atresia of the choanae, but cleft lip with cleft palate was present in our patient who already fulfills Blake's criteria for CHARGE syndrome and in the one reported by Vissers et al. [27]. Cleft lip with cleft palate is rarely found in combination with choanal atresia and it is suggested that it might replace choanal atresia in some cases [5].

Cleft lip and/or cleft palate without choanal atresia is described in a further case (18) with a novel frameshift mutation. Following the interpretation of Blake [5] this patient could be categorized as typical CHARGE syndrome with three major and three minor criteria instead of two major and three minor criteria.

The nonsense mutation c.6070C > T in exon 30 was already described by Vissers et al. [27] and Jongmans et al. [12]. The patient (case 14) presented here has no coloboma as reported in the two other patients, but has bilateral choanal atresia which is not described in the published cases.

The nonsense mutation (c.7879C > T) in exon 36 was independently described by Jongmans et al. [12] and Aramaki et al. [3]. No clinical information about the affected patient is given in the report by Aramaki et al. [3]. Jongmans et al. [12] described a female infant with bilateral coloboma which is also present in case no. 2. This girl shows no facial palsy like the one reported earlier, but has atresia of both choanae.

Table 2
Clinical features in eight patients with mutation in the *CHD7* gene.

patient	2	4	6	8	13	14	15	18	n (%) features in this study	% features published in Zentner et al. [32]
Gender	f	m	m	f	m	m	m	m		
Age at examination	2 m	12 d	11 y	2 m	11 y	5 d	7 m	5 w		
Coloboma[MB,MV]	+	+	+	+	+	−	−	−	5/8 (63%)	75%
Choanal atresia/stenosis[MB,MV]	+	+	+	+	−	+	−	−	5/8 (63%)	38%
Ear anomalies, mixed deafness, cochlear defects[MB,mV]	+	+	+	+	+	+	+	+	8/8 (100%)	89%[a], 98%[b], 91%[c]
Cranial nerve dysfunction − facial palsy, sensorineural deafness, swallowing problems[MB,mV]	+	+	+	−	+	+	+	+	7/8 (88%)	39%[d]
Hypoplastic semicircular canals[MV]	n.i.	n.i.	n.i.	n.i.	+	n.i.	n.i.	n.i.	1/1	
Cleft lip and/or palate[mB]	−	+	−	−	+	−	−	+	3/8 (38%)	33%
Tracheoesophageal fistula[mB]	−	+	−	−	−	−	+	−	2/8 (25%)	19%
Cardiovascular malformations[mB,mVa]	+	+	+	−	+	+	+	+	7/8 (88%)	77%
Genital hypoplasia, delayed, incomplete pubertal development[mB]	n.i.	+	+	−	+	+	+	+	6/7 (86%)	62%
Developmental delay, mental retardation[mBmV]	+	+/n.i.	+	n.i.	+	n.i.	n.i.	n.i.	4/4 (100%)	76%
Growth defiency[mB]	−	n.i.	+	n.i.	+	−	n.i.	n.i.	2/4 (50%)	72%
Distinctive CHARGE facies[mB]	n.i.	+	n.i.	−	+	n.i.	n.i.	n.i.	2/3 (67%)	
Hypothalamo-hypophyseal dysfunction[mV]	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	−	
Esophagus atresia[mVa]	−	+	n.i.	n.i.	−	−	+	n.i.	2/5 (40%)	
Additional findings										
Renal anomalies	−	+	n.i.	−	+	+	n.i.	+	4/6 (67%)	
Hand anomalies − clinodactyly, camptodactyly, cutanoeus syndactyly	−	−	n.i..	−	+	−	+	n.i.	2/6 (34%)	
Skeletal anomalies	+	+	n.i.	n.i.	−	+	n.i.	n.i.	3/4 (75%)	
Brain anomalies	n.i.	+	n.i.	−	+	+	n.i.	n.i.	3/4 (75%)	
Thymus hypoplasia	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	+	1/1	
Blake's criteria Major/minor	4M 2m	4M 6m	4M 4m	3M 0m*	3M 6m	3M 2m*	2M 3m*	2M 3m*		
Verloes' criteria Major/minor	2M 3m	2M 3m	2M 4m	2M 1m*	2M 4m	1M 3m*	0M 3m*	0M 3m*		

[+]present, [−]absent, n.i. no information, *incomplete data may underestimate the score and therefore the clinical category. [a]ear abnormalities incl. deafness, [b]temperal bone anomalies, [c]external ear malformation, [d]only facial nerve palsy, [MB MV]Major Blake Major Verloes criteria, [mB mV]minor Blake minor Verloes criteria, [mVa]one minor Verloes criteria.

Table 3
Clinical features in ten patients without mutation in the *CHD7* gene.

patient	1	3	5	7	9	10	11	16	17	12	n (%) features in this study	% features published in Zentner et al. [32]
Gender	m	f	m	m	f	m	m	m	f	f		
Age at examination	4 m	9 y	10 y	13 y	13 y	10 y	13 y	2 y	19 y	4 y		
Coloboma[MB,MV]	n.i.	+	−	−	+	−	+	−	−	n.i.	3/8 (38%)	65%
Choanal atresia/stenosis[MB,MV]	n.i.	−	+	+	−	−	−	−	+	+	4/9 (57%)	47%
Ear anomalies, mixed deafness, cochlear defects[MB,mV]	n.i.	+	−	+	+	+	+	−	+	n.i.	6/8 (75%)	86%[a], 75%[b] 90%[c]
Cranial nerve dysfunction − facial palsy, sensorineural deafness, swallowing problems[MB,mV]	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	+	+	n.i.	2/2 (100%)	19%[d]
Hypoplastic semicircular canals[MV]	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	−	n.i.	n.i.	0/1	
Cleft lip and/or palate[mB]	n.i.	−	−	+	−	−	−	−	n.i.	+	2/8 (25%)	29%
Tracheoesophageal fistula[mB]	n.i.	−	+	−	−	−	−	−	n.i.	−	1/8 (13%)	18%
Cardiovascular malformations[mB,mVa]	n.i.	−	+	−	−	−	+	−	−	+	3/9 (33%)	72%
Genital hypoplasia, delayed, incomplete pubertal development[mB]	n.i.	−	+	+	−	+	−	+	n.i.	n.i.	4/7 (57%)	70%
Developmental delay, mental retardation[mBmV]	+/n.i.	+	+	+	+	+	+	+	+	n.i.	9/9 (100%)	94%
Growth defiency[mB]	n.i.	+	n.i.	n.i.	+	n.i.	−	+	n.i.	n.i.	3/4 (75%)	
Distinctive CHARGE facies[mB]	n.i.	+	−	+	−	−	−	−	n.i.	n.i.	2/7 (29%)	
Hypothalamo-hypophyseal dysfunction[mV]	n.i.	n.i.	n.i.	n.i.	n.i.	−	n.i.	n.i.	n.i.	n.i.	0/1	
Esophagus atresia[mVa]	n.i.	−	+	−	−	−	−	−	−	−	1/9 (11%)	
Renal anomalies	+	−	−	−	−	−	+	+	−	n.i.	3/9 (33%)	
Hand anomalies − clinodactyly, camptodactyly, cutanoeus syndactyly	n.i.	−	−	−	−	−	+	−	+	n.i.	2/8 (25%)	
Skeletal anomalies	n.i.	−	−	−	−	−	+	n.i.	n.i.	n.i.	1/6 (17%)	
Brain anomalies	+	+	+	+	−	+	−	+	+	+	7/8 (88%)	
Thymus hypoplasia	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	−	
Blake's criteria Major/minor	0H1N*	2H3N*	1H4N*	2H3N*	2H2N*	1H2N*	2H2N*	1H2N*	3H1N*	1H2N*		
Verloes' criteria Major/minor	0H0N*	1H2N*	1H2N*	1H1N*	1H2N*	0H2N*	1H3N*	0H3N*	1H3N*	1H1N*		

[+]present, [−]absent, n.i. no information, *incomplete data may underestimate the score and therefore the clinical category, [a]ear abnormalities incl. deafness, [b]temperal bone anomalies, [c]external ear anomalies, [d]only facial nerve palsy, [MB MV]Major Blake Major Verloes criteria, [mB mV]minor Blake minor Verloes criteria, [mVa]one minor Verloes criteria.

The clinical features of these patients show considerable variability and support the observation that identical mutations are associated with variable phenotypes. This suggests that additional, not yet identified modifier genes contribute to the phenotype of patients with CHARGE syndrome.

Copy number variation of the *CHD7* gene due to duplication or deletion of several exons or the whole gene have been described in individual cases [2,4,15,19,25,29,30]. None of the patients who were tested negative in sequencing analysis had a duplication and or deletion of one or several exons of the *CHD7* gene. It might be possible that some of our patients carry mutations outside the *CHD7* gene region not covered in our study.

Diagnostic criteria for CHARGE syndrome as suggested by Blake et al. [5] and later by Verloes [26] were applied to all patients (Tables 2 and 3). Direct sequencing and MLPA analysis failed to identify mutations in ten out of 18 patients. In the mutation

Table 4

Comparison of alignment of amino acid sequences

4a) alignment of the amino acid sequences of CHD7 in human and orthologues

```
NP_060250.2|Homo sapiens                REPETERVERPPADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMR
XP_519780.2|Pan troglodytes             REPETERVERPPADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMR
XP_544097.2|Canis familiaris            REPETERVERPPADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMR
NP_001074886.1|Mus musculus             REPETERVERPPADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNMR
NP_001071054.1|Gallus gallus            REPEMERVERPPADDWKKSESSREYKNNNKLREYQLEGVNWLLFNWYNTR
NP_523441.1|Drosophila melanogaster     QRSEWKSKKRPHPELWKKLEKTPVYKGGNSLRPYQLEGLNWLKFSWYNTH
                                        :..* :  :** .: *** *.:  **..*.** *****:*** *.*** :
```

4b) alignment of the amino acid sequences of CHD protein family

```
NP_001261.2|CHD1        ---KQPSYIGGHEGLELRDYQLNGLNWLAHSWCKGNSCILADEMGLGKTI
NP_001262.3|CHD2        ---KQPAYLGG-ENLELRDYQLEGLNWLAHSWCKNNSVILADEMGLGKTI
NP_065971.2|CHD8        ---KLELSHEYKNRNQLREYQLEGVNWLLFNWYNRQNCILADEMGLGKTI
NP_079410.4|CHD9        ---KIDQSRDYKNGNQLREYQLEGLNWLLFNWYNRRNCILADEMGLGKTI
NP_060250.2|CHD7        ---KSESSREYKNNNKLREYQLEGVNWLLFNWYNMRNCILADEMGLGKTI
NP_115597.3|CHD6        ---KLEKSREYKNSNQLREYQLEGMNWLLFNWYNRKNCILADEMGLGKTI
NP_001264.2|CHD4        VKYERQPEYLDATGGTLHPYQMEGLNWLRFSWAQGTDTILADEMGLGKTV
NP_056372.1|CHD5        VKFDKQPWYIDSTGGTLHPYQLEGLNWLRFSWAQGTDTILADEMGLGKTV
NP_001005273.1|CHD3     VKYETQPRFITATGGTLHMYQLEGLNWLRFSWAQGTDTILADEMGLGKTI
                           .            *: **::*:*** ..* :  . ***********:
```

The affected amino acid leucine mutated in case 6 is highlighted.

negative group case 11 and 17 belong to the category of atypical CHARGE syndrome according to Verloes scoring scheme. Case 16 does not fulfill the criteria of Blake or Verloes even if additional clinical information would be available. The other cases do not fulfill either Blake's or Verloes criteria but could relate to typical CHARGE syndrome in case further relevant clinical features e.g. hypoplasia of the semicircular canals would be present in the patient.

All patients who fulfilled the diagnostic criteria according to Blake's et al. and/or Verloes' scoring schemes were heterozygous carriers of a disease-causing *CHD7* mutation. The analysis included seven children under the age of one year. In six children mutations were identified, although according to the scoring scheme of Blake only two of them (case 2, case 4) fulfilled the criteria with four major features each. This underlines the recommendation by Blake to consider a CHARGE syndrome in infants already when one or two major and several minor criteria are met [5].

Applying the scoring scheme of Verloes, case 2 and 4 can be assigned typical CHARGE syndrome while case 8 relates to partial and case 14 to atypical CHARGE syndrome. In two of the six infants with an identified *CHD7* mutation (case 15 and 18) neither Coloboma nor Atresia of Choanae were reported. Additional clinical information e.g. about the semicircular canals were missing which could otherwise result in the definition typical CHARGE or partial CHARGE.

Thus it is advisable to consider a CHARGE syndrome in infants even if they do not fulfill the diagnostic score.

Zentner et al. [32] recently summarized the clinical features and molecular data from 379 patients with CHARGE syndrome. They concluded that inner ear malformations including semicircular canal aplasia/dysplasia, facial nerve palsy and ocular colobomas are more frequent in patients with a *CHD7* gene mutation. In Tables 2 and 3 are the frequencies of the clinical features found in our study listed adjacent to the data summarized by Zentner et al. [32]. The frequencies of most of the features are in a similar range as far as the data are comparable because of different types of listing. In our group the frequency of choanal atresia is higher than the frequency reported by Zentner. In our mutation negative group the frequencies of coloboma and cardiovascular malformation are lower compared to the frequencies reported [32].

The mutation detection rate in previous studies ranged between 58 and 70% in patients who fulfilled the diagnostic criteria of CHARGE syndrome [2,12,14,27]. The mutation detection rate of about 44% in this study is in good accordance with the detection rate of 40.5% reported in a previously published study with a more unselected patient cohort [28].

The analysis supports the observation that besides recurrent mutations a high proportion of private mutations are reported. The variability of clinical features found in recurrent mutations confirms and emphasizes the wide variability of the clinical phenotype of patients with CHARGE syndrome.

Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejmg.2010.07.002.

References

[1] M.D. Allen, T.L. Religa, S.M. Freund, M. Bycroft, Solution structure of the BRK domains from CHD7, J. Mol. Biol. 371 (2007) 1135–1140.
[2] M. Aramaki, T. Udaka, R. Kosaki, et al., Phenotypic spectrum of CHARGE syndrome with CHD7 mutations, J. Pediatr. 148 (2006) 410–414.
[3] M. Aramaki, T. Udaka, C. Torii, H. Samejima, R. Kasaki, T. Takahashi, K. Kosaki, Screening for CHARGE syndrome mutations in the CHD7 gene using denaturing high- performance liquid chromatography, Genet. Test. 10 (2006) 244–251.
[4] J.E. Bergman, I. de Wijs, M.C. Jongmans, R.J. Admiraal, L.H. Hoefsloot, C.M. van Ravenswaaij-Arts, Exon copy number alterations of the CHD7 gene are not a major cause of CHARGE and CHARGE-like syndrome, Eur. J. Med. Genet. 51 (2008) 417–425.
[5] K.D. Blake, S.L. Davenport, B.D. Hall, M.A. Hefner, R.A. Pagon, M.S. Williams, A.E. Lin, J.M. Graham Jr., CHARGE association: an update and review for the primary paediatrician, Clin. Pediatr. 37 (1998) 159–173.
[6] K.D. Blake, C. Prasad, CHARGE syndrome, Orphanet J. Rare. Dis. 1 (2006) 34.
[7] A. Delahaye, Y. Sznajer, S. Lyonnet, M. Elmaleh-Bergès, I. Delpierre, S. Audollent, S. Wiener-Vacher, A.L. Mansbach, J. Amiel, C. Baumann, D. Bremond-Gignac, T. Attié-Bitach, A. Verloes, D. Sanlaville, Familial CHARGE syndrome because of CHD7 mutation: clinical intra- and interfamilial variability, Clin. Genet. 72 (2007) 112–121.
[8] A. Flaus, D.M. Martin, G.J. Barton, T. Owen-Hughes, Identification of multiple distinct Snf2 subfamilies with conserved structural motifs, Nucleic Acids Res. 34 (2006) 2887–2905.
[9] B.D. Hall, Choanal atresia and associated multiple anomalies, J. Pediatr. 95 (1979) 395–398.
[10] H.M. Hittner, N.J. Hirsch, G.M. Kreh, A.J. Rudolph, Colobomatous microphthalmia, heart disease, hearing loss, and mental retardation—a syndrome, J. Pediatr. Ophthalmol. Strabismus 16 (1979) 122–128.
[11] K.A. Issekutz, J.M. Graham Jr., C. Prasad, et al., An epidemiological analysis of CHARGE syndrome: preliminary results from a Canadian study, Am. J. Med. Genet. A 133 (2005) 309–317.
[12] M.C.J. Jongmans, R.J. Admiraal, K.P. van der Donk, L.E. Vissers, A.F. Baas, L. Kapusta, J.M. van Hagen, D. Donnai, T.J. de Ravel, J.A. Veltman, A. Geurts van Kessel, B.B. de Vries, H.G. Brunner, L.H. Hoefslot, C.M. van Ravenswaaij, CHARGE syndrome: the phenotypic spectrum of mutations in the CHD7 gene, J. Med. Genet. 43 (2006) 306–314.
[13] M.C. Jongmans, L.H. Hoefsloot, K.P. van der Donk, R.J. Admiraal, A. Magee, I. van de Laar, Y. Hendriks, J.B. Verheij, I. Walpole, H.G. Brunner, C.M. van Ravenswaaij, Familial CHARGE syndrome and the CHD7 gene: a recurrent missense mutation, intrafamilial recurrence and variability, Am. J. Med. Genet. A 146(A) (2008) 43–50.
[14] S.R. Lalani, A.M. Safiullah, S.D. Fernbach, K.G. Harutyunyan, C. Thaller, L.E. Peterson, J.D. McPherson, R.A. Gibbs, L.D. White, M. Hefner, S.L. Davenport, J.M. Graham, C.A. Bacino, N.L. Glass, J.A. Towbin, W.J. Craigen, S.R. Neish, A.E. Lin, J.W. Belmont, Spectrum of CHD7 mutations in 110 individuals with CHARGE syndrome and genotype-phenotype correlation, Am. J. Hum. Genet. 78 (2006) 303–314.
[15] A.M. Lehman, J.M. Friedman, D. Chai, F.R. Zahir, M.A. Marra, L. Prisman, E. Tsang, P. Eydoux, L. Armstrong, A characteristic syndrome associated with microduplication of 8q12, inclusive of CHD7, Eur. J. Med. Genet. 52 (2009) 436–439.
[16] M.S. Lubinsky, Properties of associations: identity, nature, and clinical criteria, with a commentary on why CHARGE and Goldenhar are not associations, Am. J. Med. Genet. 49 (1994) 21–25.
[17] C.G. Marfella, A.N. Imbalzano, The Chd family of chromatin remodelers, Mutat. Res. 618 (2007) 30–40.
[18] J.A. Mitchell, S.L.H. Davenport, M.A. Hefner, M.M. Shei, Use of an expert system to test diagnostic criteria in CHARGE syndrome, J. Medical. Syst. 9 (1985) 425–436.
[19] S. Monfort, M. Roselló, C. Orellana, S. Oltra, D. Blesa, K. Kok, I. Ferrer, J.C. Cigudosa, F. Martínez, Detection of known and novel genomic rearrangements by array based comparative genomic hybridisation: deletion of ZNF533 and duplication of CHARGE syndrome genes, J. Med. Genet. 45 (2008) 432–437.
[20] R.A. Pagon, J.M. Graham Jr., J. Zonana, S.L. Yong, Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: CHARGE association, J. Pediatr. 99 (1981) 223–227.
[21] S. Pauli, L. Pieper, J. Häberle, P. Grzmil, P. Burfeind, M. Steckel, U. Lenz, H.W. Michelmann, Proven germline mosaicism in a father of two children with CHARGE syndrome, Clin. Genet. 75 (2009) 473–479.
[22] D. Sanlaville, H.C. Etchevers, M. Gonzales, J. Martinovic, M. Clément-Ziza, A.-L. Delezoide, M.-C. Aubry, A. Pelet, S. Chemouny, C. Cruaud, S. Audollent, C. Esculpavit, G. Goudefroye, C. Ozilou, C. Fredouille, N. Joye, N. Morichon-Delvallez, Y. Dumez, J. Weissenbach, A. Munnich, J. Amiel, F. Encha-Razavi, S. Lyonnet, M. Vekemans, T. Attié-Bitach, Phenotypic spectrum of CHARGE syndrome in fetuses with CHD7 Truncating mutations correlates with expression during human development, J. Med. Genet. 43 (2006) 211–217.
[23] D. Sanlaville, A. Verloes, CHARGE syndrome: an update, Eur. J. Hum. Genet. 15 (2007) 389–399.
[24] P.D. Stenson, M. Mort, E.V. Ball, K. Howells, A.D. Phillips, N.S. Thomas, D.N. Cooper, The human gene mutation database: 2008 update, Genome Med. 1 (2009) 13.
[25] T. Udaka, N. Okamoto, M. Aramaki, C. Torii, R. Kosaki, N. Hosokai, T. Hayakawa, N. Takahata, T. Takahashi, K. Kosaki, An Alu retrotransposition-mediated deletion of CHD7 in a patient with CHARGE syndrome, Am. J. Med. Genet. A 143 (2007) 721–726.
[26] A. Verloes, Updated diagnostic criteria for CHARGE syndrome: a proposal, Am. J. Med. Genet. A 133 (2005) 306–308.
[27] L.E. Vissers, C.M. van Ravenswaajj, R. Admiraal, J.A. Hurst, B.B.A. de Vries, I.M. Janssen, W.A. van der Vliet, E.H. Huys, P.J. de Jong, B.C. Hamel, E.F. Schoenmakers, H.G. Brunner, J.A. Veltman, A.G. van Kessel, Mutations in a new member of the chromodomain gene family cause CHARGE syndrome, Nat. Genet. 36 (2004) 955–957.

[28] P. Vuorela, S. Ala-Mello, C. Saloranta, M. Penttinen, M. Pöyhönen, K. Huoponen, W. Borozdin, B. Bausch, E.M. Botzenhart, C. Wilhelm, H. Kääriäinen, J. Kohlhase, Molecular analysis of the CHD7 gene in CHARGE syndrome: identification of 22 novel mutations and evidence for a low contribution of large CHD7 deletions, Genet. Med. 9 (2007) 690–694.
[29] J. Wincent, E. Holmberg, K. Strömland, M. Soller, L. Mirzaei, T. Djureinovic, K. Robinson, B. Anderlid, J. Schoumans, CHD7 mutation spectrum in 28 Swedish patients diagnosed with CHARGE syndrome, Clin. Genet. 74 (2008) 31–38.
[30] J. Wincent, A. Schulze, J. Schoumans, Detection of CHD7 deletions by MLPA in CHARGE syndrome patients with a less typical phenotype, Eur. J. Med. Genet. 52 (2009) 271–272.
[31] T. Woodage, M.A. Basrai, A.D. Baxevanis, P. Hieter, F.S. Collins, Characterization of the CHD family of proteins, Proc. Natl. Acad. Sci. U S A 94 (1997) 11472–11477.
[32] G.E. Zentner, W.S. Layman, D.M. Martin, P.C. Scacheri, Molecular and phenotypic aspects of CHD7 mutation in CHARGE syndrome, Am. J. Med. Genet. A 152A (2010) 674–686.