

CHARGE syndrome: an update

CHARGE syndrome is a rare, usually sporadic autosomal dominant disorder due in 2/3 of cases to mutations within the *CHD7* gene. The clinical definition has evolved with time. The 3C triad (Coloboma-Choanal atresia-abnormal semicircular Canals), arhinencephaly and rhombencephalic dysfunctions are now considered the most important and constant clues to the diagnosis. We will discuss here recent aspects of the phenotypic delineation of CHARGE syndrome and highlight the role of *CHD7* in its pathogeny. We review available data on its molecular pathology as well as cytogenetic and molecular evidences for genetic heterogeneity within CHARGE syndrome.

In brief

- CHARGE syndrome is an autosomal dominant disorder with a prevalence of one in 10 000. Most cases are sporadic, but, in rare instances, transmission from a mildly affected parent has been reported.
- The acronym CHARGE is based on the cardinal features identified when the syndrome was delineated: Coloboma, Heart malformation, choanal Atresia, Retardation of growth and / or development, Genital anomalies, and Ear anomalies. Since that time, new frequent clinical finding have been added to the clinical spectrum of CHARGE syndrome, such as dysmorphic features, rhombencephalic dysfunction, hypoplasia of the semicircular canals and arhinencephaly.
- Diagnostic criteria for CHARGE syndrome were updated by Blake and later Verloes, who stressed the importance of the 3C triad (Coloboma-Choanal atresia-abnormal semicircular Canals).
- CHARGE syndrome results from a dysblastogenetic and dysneurulative process and could be related by a common pathogenetic mechanism resulting in disturbed neural crest development.
- Using a CGH microarray approach, the gene *CHD7* (in 8q12) was identified as causative for CHARGE syndrome in approximately 2/3 of patients with a clinical diagnosis of CHARGE syndrome.
- *CHD7* belongs to a large family of evolutionarily conserved proteins thought to play a role in chromatin organization. *CHD7* is a regulatory element that potentially affects a large number of developmental pathways, explaining the pleiotropic nature of its phenotypic spectrum
- *CHD7* (chromodomain helicase DNA-binding protein) mutations were in majority truncating mutations including nonsense mutations and frameshift mutations. Rarely, 8q12 microdeletion was present. No phenotype / genotype correlation could be proposed.

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Introduction

'CHARGE association' (or Hall-Hittner syndrome¹) was originally delineated by Bryan Hall in 17 children with multiple congenital anomalies (MCA) with choanal atresia² and, independently, by HM Hittner in 10 MCA patients with coloboma.³ In a further review of 21 patients with choanal atresia and/or coloboma, R Pagon coined the term 'CHARGE', an acronym summarizing six cardinal clinical features: ocular Coloboma, Heart defects of any type, Atresia of the choanae, Retardation (of growth and/or of development), Genital anomalies and Ear anomalies (abnormal pinnae or hearing loss).⁴ Patients with CHARGE syndrome were reported before its formal delineation (ie Edward's case,⁵ or patient 3 in Angelman's report⁶) As CHARGE is a multiple anomaly pattern proven to be pathogenetically related, 'CHARGE syndrome' should be used instead of 'CHARGE association'.⁷

In a national study of CHARGE syndrome conducted through the Canadian Pediatric Surveillance Programme, its incidence was evaluated at 1/8500, in accordance with a previous reports of 1/12 500 in North America.^{8,9} By contrast, extrapolation from the incidence of choanal atresia lead to far lesser apparent incidence in Europe: 1/110 000 births.¹⁰ Almost all cases are sporadic, and mean paternal age is increased (34 years¹¹). Recurrence rate is close to 1%. Recurrences in a sibship^{4,12}, parent-to-child transmission^{3,4,13,14} and concordant MZ twins have been reported.^{15–18}

Clinical manifestations

More than 400 patients have been reported in the world literature, including several large series and reviews^{4,9,11,19–21} of which frequencies of the major signs are extrapolated. In the present review, we will stress the more recently delineated anomalies. Figure 1 presents recommended diagnostic algorithm.

Dysmorphia

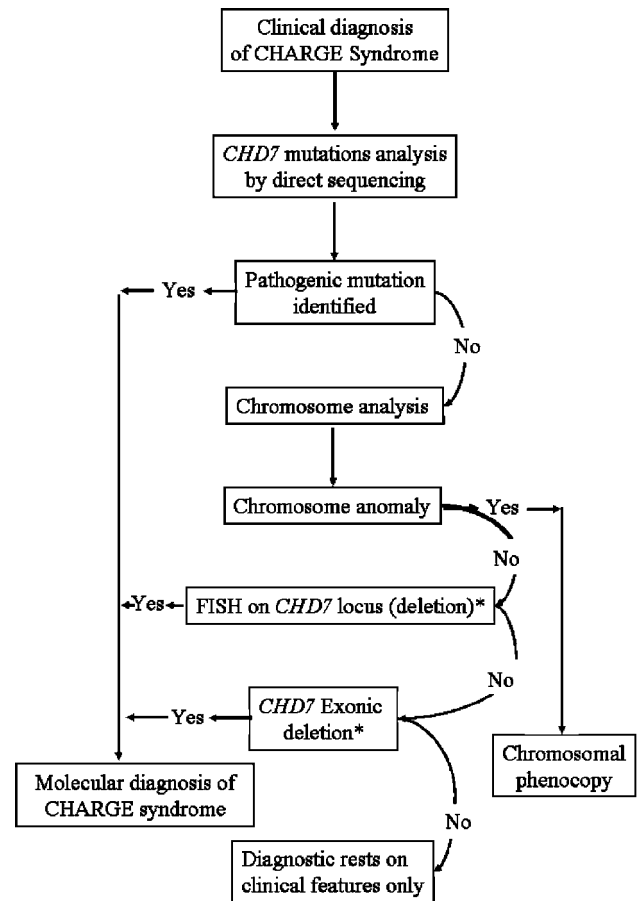
The face is typically square-shaped, with narrow bifrontal diameter, broad nasal bridge, small mouth and inverted V-shaped upper lip. Facial palsy and dysplasia of the pinnae often lead to significant facial asymmetry. Cleft lip and palate are present in 15–20%. Several dental anomalies are reported such as oligodontia and delayed eruption of teeth.²²

Ocular anomalies

Chorioretinal coloboma, with (40%) or without microphthalmia, is present in 75–90%. Coloboma is often of small size, and must be actively searched by funduscopy. In a minority of case, they may extend to the iris and/or the optic disk. Bilateral involvement is present in 70–80%. Among patients with uveal coloboma, CHARGE could represent almost 20%.²³ Other anomalies include hypoplasia of optic nerve, anophthalmia, nystagmus, squint and refractive errors. Palpebral defects have been reported such as ankyloblepharon, coloboma of the lid and atresia of the lachrymal ducts.^{11,18,20,24} Most patients with CHARGE syndrome have preserved vision.

Auditory and vestibular anomalies

The three segments of the ear are affected. In 95–100%, the pinnae are asymmetrically misshaped, low set, anteverted, cup-shaped, wide, but with reduced vertical height (Figure 2). Preauricular tags, hypoplastic auditory canal and microtia can be associated. Middle ear is sometimes affected:^{25,26} absence of the stapedius muscle, absence of the oval window, and hypoplastic incus and stapes, with ossicular chain fixation.^{25,27} Chronic serous otitis is often present, and may contribute to hypoacusia.



* : extremely rare

Figure 1 Diagnostic algorithm for CHARGE syndrome. This algorithm would lead to definite molecular confirmation in 60% of clinically validated CHARGE patients.

Initially described in 1976,²⁸ but definitively settled by Guyot²⁹ who described the ‘CHARGE type of Mondini dysplasia’, malformation of the inner ear has become increasingly important in the diagnostic workup, with the development of inner ear imaging. The prevalence of inner ear anomalies could be higher than 90%.^{26,30} These consist of a specific form of labyrinthine dysplasia that includes complete absence of the pars superior (utricle and semi-circular canals) with or without Mondini dysplasia of the pars inferior (cochlea and saccule). Although Mondini dysplasia is relatively nonspecific, and can be observed in many syndromal deafness, aplasia of the semicircular canals and hypoplastic uncus are probably the most specific anomaly of CHARGE syndrome.^{31,32} In a recent series, nine out of 15 patients ascertained by this anomaly had CHARGE.³³ In the newborn, agenesis of the semicircular canals can be visualized on plain profile X-ray of the skull (Figure 3a, b). In older patients, computerized tomography (Figure 3c, d) or MRI is necessary.

Malformation of the inner ear results in complete absence of nystagmic response to bithermal caloric stimuli. Vestibulo-ocular responses to earth-vertical axis rotation are abolished, but otolith vestibulo-ocular responses to the off-vertical axis rotation test are preserved.³⁴

Deafness affects 60–90% of cases. The commonest hearing defects are severe conductive or mixed loss, and usually affects more the high frequencies than the low ones.³⁵



Figure 2 Typical cup-shaped aspect of 'CHARGE ear'.

Choanal atresia and other ENT anomalies

This sign is present in 35²¹ to 65% of cases. Atresia is most often osseous and bilateral. In a series of patients with choanal atresia, CHARGE syndrome was found in 38/130 cases.³⁶ Laryngeal cleft, subglottic stenosis, tracheomalacia, vocal cord palsy have been reported.²⁶ Gastro-esophageal reflux affects at least half of the cases. Velar incompetence seems common.^{15,18} Choanal atresia is usually absent when palate is cleft.

Central nervous system malformations

Depending on ascertainment and mode of investigation, 55–85% of patients have definite CNS anomalies.^{37,38} Arhinencephaly or hypoplastic olfactory bulbs, with or without other defects, is the most common defect.³⁹ It was observed by MRI scan in 18/18 patients.³⁸ Anosmia has recently be found to be a common anomaly,⁴⁰ in relation with abnormal olfactory bulbs. Holoprosencephaly sequence is the next most common anomaly. Many other defects have been reported, in the forebrain (eg dysgenesis of the frontal lobe, agenesis of the septum pellucidum, nodular heterotopias, lissencephaly) and the hindbrain (Dandy–Walker anomaly, vermis hypoplasia, stenosis of Sylvius aqueduct). CNS malformations are strongly associated with choanal atresia.

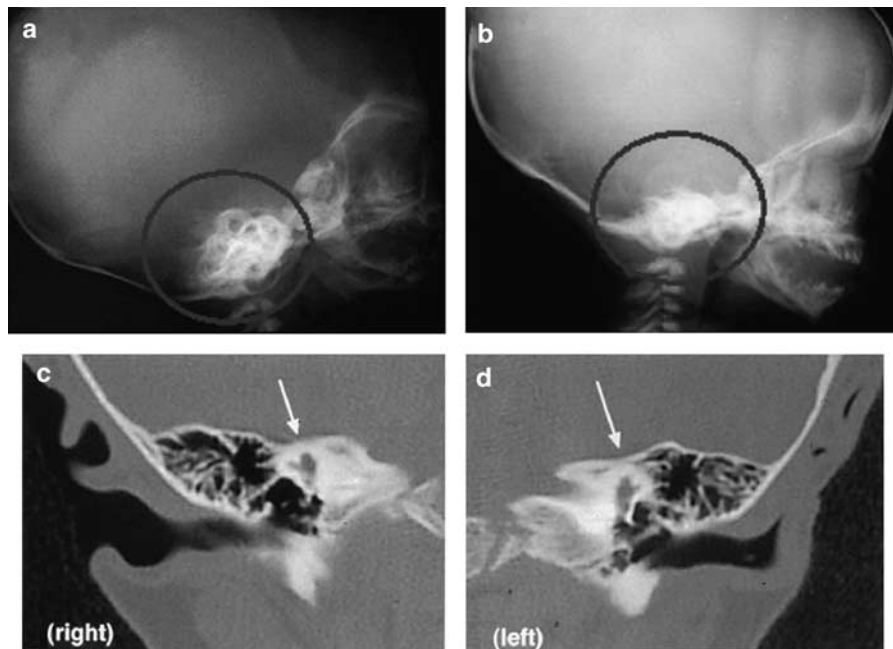


Figure 3 (a) Profile view of normal newborn skull. The semicircular canals are densely mineralized, bulging over the petrous bone (circle). (b) Profile of the skull in an affected newborn. Note absence of the canals. (c–d) Computerized tomography scan of the inner ear of an affected child, revealing absence of the canals.

Rhombencephalic dysfunction (excluding sensorineural deafness)

Besides the acoustic nerve VIII, all other cranial nerves can be affected in CHARGE syndrome, usually in association.⁴¹ Of children with the CHARGE syndrome, 50–90% have congenital facial paralysis and 80% have feeding and swallowing difficulties.⁴² In Byerly's review of 150 cases, the affected nerves were nerve VII (43%), IX and/or X (31%), III and VI (2% each). Facial palsy is usually asymmetric or unilateral. Multiple involvements may result in Moebius sequence. Tellier *et al.*¹¹ stressed the complex dysregulation of brainstem functions in neonate and infants with CHARGE syndrome. Pharyngeal incoordination is associated with a high risk of aspiration (30%⁴²). Many children require G-tube feeding or gastrostomy for several months or years. Thermal dysregulation and irregularities of cardiac rhythm are other signs of rhombencephalic dysfunction.

Hypothalamo-hypophyseal dysfunction and Kallmann syndrome

Hypothalamo-hypophyseal dysfunction is the cause of growth retardation, genital hypoplasia and delayed puberty. Seven out of nine boys tested before the age of 5 months during the neonatal peak period had extremely low testosterone levels; LH response to GnRH stimulation was variable during the first year of life and not correlated with existing clinical abnormalities.³⁸ By contrast, no endocrine dysfunction were demonstrable in four children with short stature and micropenis,⁴³ indicating that the hypothalamo-hypopituitary control of gonadotrophin may be variably affected during life. Delayed or absent puberty affected 2/3 of 21 cases, in relation with hypogonadotropic hypogonadism.⁴⁴ In Jongmans series (with *CHD7* mutation), gonadotrophin deficiency was present in 7/10 girls and 6/7 boys age over 14–15 years.⁴⁵ Hormone substitution is recommended.

Growth retardation

Despite its presence in the original acronym, growth retardation is seldom a primary problem in CHARGE syndrome. Most patients have low normal birth weight and length (around 10th centile).⁴⁶ Postnatal growth retardation should be in relation to feeding difficulties and/or surgical problems.⁴⁷ Growth tends to catch up after infancy, but insufficient and inadapted intakes may have adverse outcome on growth and bone mineralization. Osteoporosis seems frequent in elder patients with CHARGE, because of 'relative' malnutrition and insufficient secretion of steroids. True GH deficiency is exceptional.

Genito-urinary anomalies

Genital anomalies are observed in 50–70% of cases (males: 80–90%, females: 15–25%). Thus, the use of this sign in

diagnostic score biases diagnosis to boys. Hypoplastic external genitalia is the most common anomaly: 80% of boys have micropenis and/or cryptorchidism, and 1/3 of the girls have hypoplastic labiae majorae. Mullerian tract agenesis has been reported twice.⁴⁸ Sex steroid therapy has been tried for penile growth and descent of testis in males with CHARGE syndrome. The main use for testosterone is for delayed male puberty in adolescence. Females often require hormone replacement at puberty.⁴⁹ Anomalies of the urinary tract may be present in 10–40%, such as renal ectopia, horseshoe kidneys, ureteral anomalies, but are nonspecific and may be asymptomatic, or diagnosed beyond infancy because of infections.

Other visceral malformations

Congenital heart defects (CHD) are observed in 50–85% of cases. Conotruncal defects as Fallot tetralogy (1/3 of cases), atrioventricular canal, VSD, ASD, aortic coarctation and PDA represent 75% of the defects. Aberrant subclavian arteries are often present. CHD are statistically associated with tracheo-esophageal fistula, renal anomalies and cleft lip/palate.⁵⁰

Immune deficiency

Immune deficiencies is uncommon and variable in CHARGE: (impaired T-cell proliferation, T-cell lymphopenia, immunoglobulin G2 subclass deficiency^{20,51,52}). Rarely patients may exhibit true DiGeorge syndrome.⁵³

Orthopedic problems

Limb anomalies, usually minor, are reported in 37%.⁵⁴ Spine may be malformed: vertebral fusions, hemivertebrae, abnormal cervico-cranial junction, abnormal number of ribs. Scoliosis is a frequent problem in older children with CHARGE (60% of patients beyond infancy⁵⁵). It is promoted by hypotonia, but may be worsened in case of vertebral defects.

Mental retardation and behavioral disturbances

Motor milestones are commonly delayed, at least partially because of vestibular and visual impairments. Sitting is possible after 1 year and walking occurs between 24 and 36 months. Mental retardation (MR), that is, IQ < 70, is present in more than 70% of cases. IQ range from normal (115 in Money and Norman⁵⁶) to severe retardation. In Jongmans series of patients with *CHD7* mutations, 8/32 patients had IQ above 70. There is strong mixture of primary developmental factors and acquired postnatal damages in the etiology of MR. In a recent series,¹¹ almost 50% of patients had MRI evidences of anoxo-ischemic brain lesions. In series of 21 children,⁵⁷ extensive bilateral coloboma with poor vision, microcephaly and brain malformation are the only three parameters that were predictive of poor intellectual outcome; the main psychomotor milestones (normally reached before age 4 years)

Table 1 Frequencies of major anomalies in patients with CHD7 from two series compared with the CHD7-negative patients from some of them. No significant differences can be shown

Sign	Jongmans	Lalani CHD7 mutated	Combined	Lalani CHD7 (not mutated)	P (χ^2)
CHD	31/47	54/59	85/106 (80%)	30/42	0.68
Coloboma	33/47	55/62	88/109 (81%)	30/43	0.60
Facial asymmetry	10/47	36/56	46/103 (45%)	13/39	0.42
Choanal atresia	17/47	34/57	51/104 (49%)	23/39	0.56
Hearing loss	37/41	54/59	91/100 (91%)	36/38	0.88
Inner ear anomaly	21/21	21/22	43 (42?)/?/44 (98%)	9/10	0.87
External ear anomaly	47/47	59/62	106/109 (97%)	39/42	0.86
Urogenital anomalies	17/22 (males)	29/53	46/75 (61%)	26/39	0.79
Cleft lip/palate	17/47	18/60	35/107 (33%)	9/41	0.37
TE fistula	8/47	10/55	18/102 (18%)	3/40	

were severely delayed, although intellectual outcome (at primary-school age) was satisfactory for half the children. There has been a recent interest on behavioral aspects of CHARGE syndrome.^{58,59} Children with CHARGE syndrome frequently have relatively low adaptive behavior skills, motor impairments being particularly significant. Adaptive skills are negatively correlated with age at walking, degree of hearing and visual impairment and medical involvement, and tend to decrease with aging. Nevertheless, individual adaptive behavior scores revealed a broader and higher reaching range of ability.⁶⁰ Patients with CHARGE syndrome often are diagnosed with obsessive-compulsive disorder, attention deficit disorder, Tourette syndrome and autism. The diagnosis of autism remains challenging in individuals with sensory impairments, although autistic features appear more common in CHARGE than in other syndromes sharing a similar level of mental handicap. Whether that symptomatology is really autism or only the consequence of sensory deprivation remains disputed.

Clinical particularities of CHARGE syndrome owing to CHD7 mutation

The frequency of signs in the subgroup of patients carrying a CHD7 mutation has recently been reported in 69/107 patients⁴⁵ and 59/110 patients⁶¹ (Table 1) In a recent small series, Aramaki *et al.*⁶² found CHD7 mutations in 17/24 (74%). Comparison between patients with or without mutation of CHD7 is only available in Lalani's series. They showed a relative excess of coloboma, PDA and facial palsy, whereas the two groups were not different for the other types of CHD, choanal atresia, deafness, external and internal ear anomaly, urogenital and tracheoesophageal anomalies and cleft.⁶¹ These differences disappear when the two large series are combined (see Table 1). Postnatal growth also appeared more severely impaired in the mutated group (mean stature: -2.53 vs -1.76SD). Vestibulo-cochlear defects appears to be the most important feature of CHD7-related CHARGE syndrome; altogether, 57/58 mutation-positive patients for which appropriate

studies were performed, had anomalies of the semicircular canal.

It must be stressed that the three series are made of clinically typical cases and are thus self-limiting. As for other developmental genes, the spectrum of clinical manifestations linked to CHD7 mutations is expected to become wider and more variable than the strict clinical phenotype that can be driven from these early series.

Diagnostic criteria

Originally, to make a diagnosis of CHARGE complex, four to six cardinal signs were needed to be present, one being either choanal atresia or a coloboma.^{4,63} These criteria were updated by Blake *et al*⁸, who proposed new diagnostic criteria, expanding the original picture to encompass brainstem anomalies and visceral malformations. Verloes proposed renewed clinical criteria, focusing on the coloboma-choanal atresia-abnormal semicircular canals (3C) triad, and giving a formal definition for partial and atypical CHARGE syndromes.³² Table 2 presents the three diagnostic systems with their inclusion criteria. At this point of knowledge, they remain important in preselecting patients for CHD7 mutational screen (although this may change when the full clinical spectrum of CHD7 mutants will be known). A combination of coloboma, choanal atresia and abnormal semicircular canals (3C) is highly predictive of the presence of a CHD7 mutation.⁶¹ On the other hand, only 1/47 screen-positive patients did not fulfill these criteria. Nevertheless, the clinical spectrum of CHD7 mutations is not yet fully defined and further series are still necessary to refine the diagnostic criteria to search a CHD7 mutation.

Prenatal diagnosis and fetal pathology

Prenatal diagnosis of CHARGE syndrome has been reported at least twice.^{64,65} Many developmental anomalies of CHARGE syndrome can be detected by fetal ultrasonography. Hydramnios is present in 50% of cases with choanal atresia²⁰ and with tracheo-esophageal fistula. Absence of semicircular canal is detectable by ultrasound,

Table 2 Clinical criteria

	<i>Major criteria</i>	<i>Minor criteria</i>	<i>Inclusion rule</i>
Pagon	1. Choanal atresia 2. Ocular coloboma	1. Heart defects of any type 2. Retardation (of growth and/or of development), 3. Genital anomalies 4. Ear anomalies (abnormal pinnae or hearing loss)	Four criteria out of six, and at least one major
Blake	1. Coloboma – of iris, retina, choroid, disc; microphthalmia 2. Choanal atresia – unilateral/bilateral, membranous/bony, stenosis/atresia 3. Characteristic ear abnormalities – external ear (lop or cup-shaped), middle ear (ossicular malformations, chronic serous otitis), mixed deafness, cochlear defects 4. Cranial nerve dysfunction – facial palsy (unilateral or bilateral), sensorineural deafness and/or swallowing problems	1. Genital hypoplasia – males: micropenis, cryptorchidism; females: hypoplastic labia; both males and females: delayed, incomplete pubertal development 2. Developmental delay – delayed motor milestones, language delay, mental retardation 3. Cardiovascular malformations – all types, especially conotruncal defects (eg, tetralogy of Fallot), AV canal defects, and aortic arch anomalies 4. Growth deficiencies – short stature, growth hormone deficiency 5. Orofacial cleft – cleft lip and/or palate 6. Tracheoesophageal-fistula – tracheoesophageal defects of all types 7. Characteristic face – sloping forehead, flattened tip of nose	Four majors OR three majors +three minors
Verloes	1. Ocular coloboma 2. Choanal atresia 3. Hypoplasia of semicircular canals	1. Rhombencephalic dysfunction (brainstem and cranial nerve III to XII anomalies, including sensorineural deafness) 2. Hypothalamo-hypophyseal dysfunction (including GH and gonadotrophin defects) 3. Malformation of the ear (internal or external) 4. Malformation of mediastinal organs (heart, esophagus,) 5. Mental retardation	Typical CHARGE: three majors OR two majors + two minors Partial CHARGE : two majors +one minor Atypical CHARGE: two majors but no minors OR one major + two minors

and should be searched for, in fetuses with conotruncal heart defects. In a series of 10 fetuses with *CHD7* mutations, arhinencephaly and semicircular canal agenesis were two constant features.³⁹ *CHD7* mutational screening on CVS is an option for recurrence screening, when a parent is affected, or to exclude gonadal mosaicism.

Etiologies

CHARGE syndrome is etiologically heterogeneous. Chromosome anomalies and single gene defects are responsible for CHARGE syndrome. Exposure to teratogenic agents may result in a phenocopy. Recently, the *CHD7* gene has been shown to be mutated in roughly 58% of cases in an American series⁶¹ and 64% of cases in the Dutch series.^{45,66} Both series used Blake's and Verloes' criteria and similar analytical strategy (complete sequencing). Several explanations could be proposed to explain the patient without mutation: genetic heterogeneity, intronic mutations in the *CHD7* gene, exonic deletion (missed by sequencing) and 3' or 5' UTR mutations.

CHD7 A truncated version of *CHD7*, initially labeled *KIAA1416*, was cloned in 2000.⁶⁷ *Visser et al*⁶⁸ reported two CHARGE patients with *de novo* overlapping microdeletions on 8q12, including a previously reported patient with an apparently balanced chromosome 8 translocation.⁶⁹ They sequenced the nine known genes located in the shortest region of deletion overlap and detected mutations in the chromodomain helicase DNA-binding protein 7 (*CHD7*) gene in 10 of these 17 patients. Almost simultaneously, a patient with an interstitial deletion 8q11.2–q13⁷⁰ and affected twins with apparently balanced translocation involving the 8q12 region⁷¹ were reported. For these twins, the translocation breakpoint occurred between exons 3 and 8 of *CHD7* leading to *CHD7* haploinsufficiency.

CHD7 is located in 8q12.1. It is 188 kb in size, consists of 37 coding exons and one non-coding exon, and codes for a 2997 amino-acid protein. *CHD7* belongs to the MI-2/*CHD* family of chromodomain proteins, which counts at least nine members (*CHD1–CHD9*). The *CHD* family proteins act by modifications of chromatin structure-altering access of the transcriptional apparatus to its chromosomal DNA

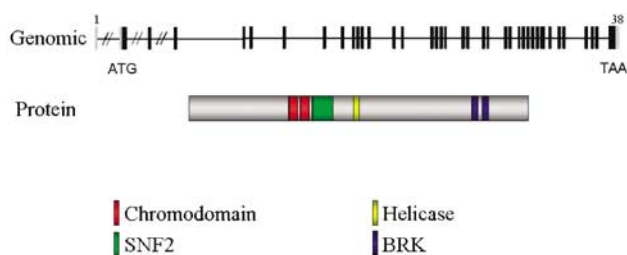


Figure 4 Genomic (up) and protein (bottom) map of *CHD7*.

template.⁷² *CHD7* protein counts several functional domains: two chromo (chromatin organization modifier), one SNF2/SWI, one helicase and two BRK domains (Figure 4). Chromo domains are implicated in the recognition of lysine-methylated histone tails and of DNA (and RNA) targets. They mediate chromatin interactions in a variety of different protein context.⁷³ SWI/SNF2 domains are found in proteins characterized by DNA-dependent ATPase activity, involved with the maintenance, transmission and expression of the eukaryotic genome by regulating the structure of chromatin.⁷⁴ The helicase domain plays a role in DNA strand separation during replication, repair, recombination and transcription. Function of the BRK domain is unknown.

By semi-quantitative reverse transcriptase-polymerase chain reaction assay, *CHD7* is expressed ubiquitously in fetal and adult tissues: including eye, olfactory epithelium, inner ear and vascular system.⁶⁸ By contrast, in 10.5-day mice embryos, *Chd7* is selectively expressed in the outflow tract of the heart, optic vesicle, facio-acoustic preganglion complex, forebrain, hindbrain, olfactory pit and mandibular component of the first branchial arch.⁶¹ Microarray gene-expression analysis of lymphoblastoid cell lines showed a signature pattern of gene-expression differences that distinguished the individuals with CHARGE syndrome with *CHD7* mutation from the controls.

The function of *CHD7* protein is still largely unknown. The closely related *CHD1*, *CHD3* and *CHD4* are known to contribute to the nucleosome remodeling and deacetylating complex, leading to anticipate a similar function for *CHD7*. *CHD7* has recently been shown to be a nuclear protein, physically associated with chromatin. It colocalizes with promoters specifically marked with methylation of lysine 4 on histone H3 (H3K4me), suggesting that *CHD7* targets a specific subset of transcriptionally active genes, including several genes within the *HOXA* cluster, and may act as an activator of transcription.⁷⁵

Published mutations in *CHD7* are scattered throughout the gene, and do not show preferential domain aggregation, nor hot spot (although some identical mutations were found in two or three unrelated individuals.) They include missense, nonsense and splicing mutations. Furthermore, small intragenic deletions have been shown in one case⁷⁶ (Table 3). In Lalani's series 61, 73% of

mutations were predicted to be truncating, clearly indicating haploinsufficiency as the pathogenic mechanism. There is no clear genotype/phenotype relationship. Mutations were shown to be dominantly transmitted in some families, and proven recurrence of a mutation in two sibs confirmed the possibility of germinal mosaicism. Interestingly, a pair of affected MZ twins⁶¹ showed remarkable discrepancies in the cardiac malformation patterns. Somatic mosaicism was detected in the unaffected mother of a sib pair, supporting the existence of germline mosaicism.⁴⁵

With the ENU mutagenesis program, Bosman *et al*⁷⁷ reported the identification of mutations in the *Chd7* gene in nine of these mice mutant, including six nonsense and three splice-site mutations. These *Wheels* heterozygous mutant mice revealed a range of defects with reduced penetrance, such as truncations of the lateral semicircular canal, cleft palate, choanal atresia, septal defects of the heart, hemorrhages, prenatal death, vulva and clitoral defects and keratoconjunctivitis sicca that mimics human CHARGE syndrome. Lalani *et al*⁶¹ studied mouse embryo whole-mount and section *in situ* hybridization and showed the expression of *Chd7* in the outflow tract of the heart, optic vesicle, facio-acoustic preganglion complex, brain, olfactory pit and mandibular component of the first branchial arch. The *in situ* hybridization analysis of the *CHD7* gene during early human development by Sanlaville⁷⁸ showed a good correlation between specific *CHD7* expression pattern and the developmental anomalies observed in CHARGE syndrome. In particular, *CHD7* is expressed in the semicircular canals, the forebrain, pituitary gland, olfactory bulbs and nerves, the nasal epithelia, the neural retina, the optic nerve sheath and the anterior and median lobes of the pituitary gland.

SEMA3E After having mapped a *de novo* balanced t(2;7)(p14;q21.11) translocation, in a CHARGE patient, interrupting the *SEMA3E* gene located in 7q21, Lalani⁷⁹ reported a *de novo* missense mutation S703L of *SEMA3E* in another affected patient with iris coloboma, choanal atresia, ear malformation, profound deafness, tetralogy of Fallot, developmental delay and growth retardation. Array-based expression analysis showed a non-*CHD7* pattern in this patient. Although this amino acid is conserved in human, mouse and rat, the role of *SEMA3E* remains putative and functional relations between *CHD7* and *SEMA3E* unexplained.

Chromosomal anomalies

Several chromosomal anomalies (including trisomy 13 and 18) show elements of CHARGE syndrome. More specifically, 11qter deletion and 13qter deletion are confusing, as coloboma, heart defects and holoprosencephaly/arhinencephaly are commonly reported. Table 4 summarizes the chromosomal anomalies reported with convincing

Table 3 Types and frequency of *CHD7* mutations reported in CHARGE patients

	<i>Jongmans</i>		<i>Lalani</i>		<i>Aramki</i>		<i>French series^a</i>		<i>Total</i>	
Nonsense	31	45%	28	44%	6	35%	27	36%	92	44%
Frame shift	17	25%	19	30%	7	41%	32	43%	75	36%
Splice site	13	19%	10	16%	3	18%	7	9%	33	16%
Missense	8	12%	7	11%	0	0%	8	11%	23	11%
intragenic deletion	0		0		1	6%	0		1	0.5%
	69		64		17		74		207	

^aPersonal communication (Sanlaville *et al*, Assises de Génétique, 2006, Montpellier, France).

Table 4 Cytogenetic anomalies, not involving the 8q12 region, reported with CHARGE syndrome

<i>Ref</i>	<i>Karyotype</i>	<i>C</i>	<i>H</i>	<i>A</i>	<i>R</i>	<i>G</i>	<i>E</i>	<i>Criteria</i>
92	45,XX,-3,-22,+der(3)t(3;22)(p25;q11.1)	—	+	+	+	girl	—	3/6
	46,XY,-18,+der(18)t(2;18)(q37.3;q22.3)	—	+	+	+	+	?	4/6
93	46,XY,-3,+del 3(pterp21.2::p12qter)	+	+	+	+	+	+	6/6
94	46,XX,invdup(14)(q22-q24.3)	+	+	+	+	+	+	6/6
95	47,XX,+18	+	+	+	?	?	+	4/6
96	46,XX,der(21)t(19;21)(q13.1;q22.3)mat	+	+	?	+	?	+	4/6
80	46,XX,del 22q11.2	+	+	+	+	?	+	5/6
97	46,X,der(X)t(X;2)(p22.1;q33)	—	+	+	+	+	+	5/6
98	46,XY,dup(1)(q31-q43)	—	+	—	+	+	+	4/6
99	46,XX,t(5;14)(p15;q13)	+	—	—	+	+	+	4/6
100	Trisomie 8q22qter	—	+	+	?	+	—	4/5

CHARGE phenotype. Although the overlap of CHARGE and DiGeorge syndromes has been repeatedly reported, reports of the deletion in bona fide CHARGE remains exceptional⁸⁰ A single patient has been reported with both del(22)(q11) and *CHD7* mutation.⁴⁵

Phenocopies CHARGE syndrome has been reported in patients exposed *in utero* to thalidomide, retinoic acid, hydantoines, alcohol or maternal diabetes.^{81–85} Those reports are anecdotic and could be fortuitous.

Pathogeny

Abnormal events, in CHARGE occurs at the end of the first month of development (closure of colobomic cleft: days 34–44; rupture of bucopharyngal membrane: day 35–38, colonization of cardiac tube by neural crest cells: day 32–45; cochlea: begins at day 36) to after the 9th week of gestation. CHARGE syndrome results from a dysblastogenetic and dysneurulative process: abnormal differentiation of cephalic mesoderm and ectoderm (otic placode and first branchial cleft), abnormal setting (differentiation, migration, survival) of neural crest cells, abnormal interaction of neural crests (forming e.a. first and second arch) with the cephalic mesoderm and the developing forebrain, and concomitant disorder in the development of the prosencephalon, mesencephalon and rhombencephalon out of

which the neural crest cells have migrated. CHARGE is thus a complex neurocristopathy.^{37,86–88}

Van Meter,⁸⁸ and later Kallen,⁸⁹ suggested that oculo-auriculo-vertebral, VATER and CHARGE could be related by a common pathogenetic mechanism resulting in disturbed neural crest development. In particular, agenesis of semicircular canals was reported in Goldenhar syndrome.⁹⁰ From a nosographic point of view, CHARGE syndrome can also be regarded as a syndromal form of Kallmann syndrome, which is defined by hypogonadotropic hypogonadism combined with a defective sense of smell and abnormal olfactory bulb development. Hill⁹¹ reported Kallmann syndrome with agenesis of the semicircular canals.

Conclusions

CHARGE syndrome is a clinical diagnosis. The clinical scores have shown their robustness, as almost all patients with *CHD7* mutation fit them (Figure 1). Pathogeny of the CHARGE remains puzzling: *CHD7* is a regulatory element that potentially affects a large number of developmental pathways, explaining the pleiotropic nature of its phenotypic spectrum. The next step will be to understand precisely which genes and pathways are under the control of *CHD7*, whether *SEMA3E* is or not another CHARGE gene

and which molecular partner(s) can explain the 40% of non-mutated CHARGE syndrome.

References

- Graham Jr JM: A recognizable syndrome within CHARGE association: Hall-Hittner syndrome. *Am J Med Genet* 2001; **99**: 120–123.
- Hall BD: Choanal atresia and associated multiple anomalies. *J Pediatr* 1979; **95**: 395–398.
- Hittner HM, Hirsch NJ, Kreh GM, Rudolph AJ: Colobomatous microphthalmia, heart disease, hearing loss, and mental retardation—a syndrome. *J Pediatr Ophthalm Strab* 1979; **16**: 122–128.
- Pagon RA, Graham Jr JM, Zonana JYSL: Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: CHARGE association. *J Pediatr* 1981; **99**: 223–227.
- Edwards JA, Young RB, Finlay HLV: Coloboma with multiple congenital anomalies. *BMJ* 1961; **26**: 586–587.
- Angelman H: Syndrome of coloboma with multiple congenital abnormalities in infancy. *BMJ* 1961; **29**: 1212–1214.
- Lubinsky MS: Properties of associations: identity, nature, and clinical criteria, with a commentary on why CHARGE and Goldenhar are not associations. *Am J Med Genet* 1994; **49**: 21–25.
- Blake KD, Davenport SL, Hall BD *et al*: CHARGE association: an update and review for the primary pediatrician. *Clin Pediatr* 1998; **37**: 159–173.
- Issekutz KA, Graham Jr JM, 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**: 309–317.
- Harris J, Robert E, Kallen B: Epidemiology of choanal atresia with special reference to the CHARGE association. *Pediatrics* 1997; **99**: 363–367.
- Tellier AL, Cormier-Daire V, Abadie V *et al*: CHARGE syndrome: report of 47 cases and review. *Am J Med Genet* 1998; **76**: 402–409.
- Finel E, Parent P, Giroux JD, de PL: The CHARGE association. *Arch Pediatr* 1996; **3**: 1020–1025.
- Metlay LA, Smythe PS, Miller ME: Familial CHARGE syndrome: clinical report with autopsy findings. *Am J Med Genet* 1987; **26**: 577–581.
- Mitchell JA, Giangiacomo J, Hefner MA, Thelin JW, Pickens JM: Dominant CHARGE association. *Ophthalmic Paed Genet* 1985; **6**: 271–276.
- Oley CA, Baraitser M, Grant DB: A reappraisal of the CHARGE association. *J Med Genet* 1988; **25**: 147–157.
- Levin DL, Muster AJ, Newfeld EA, Paul MH: Concordant aortic arch anomalies in monozygotic twins. *J Pediatr* 1973; **83**: 459–461.
- Farquhar J, Carachi R, Raine PA: Twins with oesophageal atresia and the CHARGE association. *Eur J Pediatr Surg* 2002; **12**: 56–58.
- Chestler RJ, France TD: Ocular findings in CHARGE syndrome. Six case reports and a review. *Ophthalmology* 1988; **95**: 1613–1619.
- Davenport SL, Hefner MA, Mitchell JA: The spectrum of clinical features in CHARGE syndrome. *Clin Genet* 1986; **29**: 298–310.
- Blake KD, Russell-Eggitt IM, Morgan DW, Ratcliffe JM, Wyse RK: Who's in CHARGE? Multidisciplinary management of patients with CHARGE association. *Arch Dis Childhood* 1990; **65**: 217–223.
- Stromland K, Sjogreen L, Johansson M *et al*: CHARGE association in Sweden: malformations and functional deficits. *Am J Med Genet A* 2005; **133**: 331–339.
- Al ST, Cottrell DA, Hughes CV: Dental findings associated with the malformations of CHARGE. *Pediatr Dent* 2002; **24**: 43–46.
- Ozeki H, Shirai S, Nozaki M, Ikeda K, Ogura Y: Maldevelopment of neural crest cells in patients with typical uveal coloboma. *J Pediatr Ophthalmol Strabismus* 1999; **36**: 337–341.
- Russell-Eggitt IM, Blake KD, Taylor DS, Wyse RK: The eye in the CHARGE association. *Br J Ophthalmol* 1990; **74**: 421–426.
- Wright CG, Brown OE, Meyerhoff WL, Rutledge JC: Auditory and temporal bone abnormalities in CHARGE association. *Ann Otol Rhinol Laryngol* 1986; **95**: 480–486.
- Morgan D, Bailey M, Phelps P, Bellmanj S, Grace A, Wyse R: Ear–nose–throat abnormalities in the CHARGE association. *Arch Otol Head Neck Surg* 1993; **119**: 49–54.
- Lemmerling M, Dhooge I, Mollet P, Mortier G, Van CP, Kunnen M: CT of the temporal bone in the CHARGE association. *Neuroradiology* 1998; **40**: 462–465.
- Sekhar HK, Sachs M: Mondini defect in association with multiple congenital anomalies. *Laryngoscope* 1976; **86**: 117–125.
- Guyot JP, Gacek RR, DiRaddo P: The temporal bone anomaly in CHARGE association. *Arch Otolaryngol Head Neck Surg* 1987; **113**: 321–324.
- Abadie V, Wiener-Vacher S, Morisseau-Durand MP *et al*: Vestibular anomalies in CHARGE syndrome: investigations on and consequences for postural development. *Eur J Pediatr* 2000; **159**: 569–574.
- Amiel J, Attié-Bitach T, Marianowski R *et al*: Temporal bone anomaly proposed as a major criteria for diagnosis of CHARGE syndrome. *Am J Med Genet* 2001; **99**: 124–127.
- Verloes A: Updated diagnostic criteria for CHARGE syndrome: a proposal. *Am J Med Genet A* 2005; **133**: 306–308.
- Satar B, Mukherji SK, Telian SA: Congenital aplasia of semicircular canals. *Otol Neurotol* 2003; **24**: 437–446.
- Wiener-Vacher SR, Amanou L, Denise P, Nancy P, Manach Y: Vestibular function in children with the CHARGE association. *Arch Otolaryngol Head Neck Surg* 1999; **125**: 342–347.
- Thelin JW, Mitchell JA, Hefner MA, Davenport SL: CHARGE syndrome Part II. Hearing loss. *Int J Pediatr Otorhinolaryngol* 1986; **12**: 145–163.
- Leclerc JE, Fearon B: Choanal atresia and associated anomalies. *Int J Pediatr Otorhinolaryngol* 1987; **13**: 265–272.
- Lin AE, Siebert JR, Graham Jr JM: Central nervous system malformations in the CHARGE association. *Am J Med Genet* 1990; **37**: 304–310.
- Pinto G, Abadie V, Mesnage R *et al*: CHARGE syndrome includes hypogonadotropic hypogonadism and abnormal olfactory bulb development. *J Clin Endocrinol Metab* 2005; **90**: 5621–5626.
- Sanlaville D, Genevieve D, Bernardin C *et al*: Failure to detect an 8p22–8p23.1 duplication in patients with Kabuki (Niikawa-Kuroki) syndrome. *Eur J Hum Genet* 2005; **13**: 690–693.
- Chalouhi C, Faulcon P, Le BC, Hertz-Pannier L, Bonfils P, Abadie V: Olfactory evaluation in children: application to the CHARGE syndrome. *Pediatrics* 2005; **116**: e81–e88.
- Byerly KA, Pauli RM: Cranial nerve abnormalities in CHARGE association. *Am J Med Genet* 1993; **45**: 751–757.
- White DR, Giambra BK, Hopkin RJ, Daines CL, Rutter MJ: Aspiration in children with CHARGE syndrome. *Int J Pediatr Otorhinolaryngol* 2005; **69**: 1205–1209.
- Khadilkar VV, Cameron FJ, Stanhope R: Growth failure and pituitary function in CHARGE and VATER associations. *Arch Dis Childhood* 1999; **80**: 167–170.
- Wheeler PG, Quigley CA, Sadeghi-Nejad A, Weaver DD: Hypogonadism and CHARGE association. *Am J Med Genet* 2000; **94**: 228–231.
- Jongmans M, Admiraal R, van der DK: CHARGE syndrome: the phenotypic spectrum of mutations in the CHD7 gene. *J Med Genet* 2005, eprint.
- Blake K, Kirk JM, Ur E: Growth in CHARGE association. *Arch Dis Childhood* 1993; **68**: 508–509.
- Dobbelsteyn C, Marche DM, Blake K, Rashid M: Early oral sensory experiences and feeding development in children with CHARGE syndrome: a report of five cases. *Dysphagia* 2005; **20**: 89–100.
- Ragan DC, Casale AJ, Rink RC, Cain MP, Weaver DD: Genitourinary anomalies in the CHARGE association. *J Urol* 1999; **161**: 622–625.

- 49 Blake KD, Salem-Hartshorne N, Daoud MA, Gradstein J: Adolescent and adult issues in CHARGE syndrome. *Clin Pediatr (Philadelphia)* 2005; **44**: 151–159.
- 50 Wyse RK, al-Mahdawi S, Burn J, Blake K: Congenital heart disease in CHARGE association. *Pediatr Cardiol* 1993; **14**: 75–81.
- 51 Allouche C, Sarda P, Tronc F, Jalaguier J, Montoya F, Bonnet H: [The CHARGE association]. *Pediatrie* 1989; **44**: 391–395.
- 52 Theodoropoulos DS: Immune deficiency in CHARGE association. *Clin Med Res* 2003; **1**: 43–48.
- 53 De Lonlay-Debeney P, Cormier-Daire V, Amiel J *et al*: Features of DiGeorge syndrome and CHARGE association in five patients. *J Med Genet* 1997; **34**: 986–989.
- 54 Brock KE, Mathiason MA, Rooney BL, Williams MS: Quantitative analysis of limb anomalies in CHARGE syndrome: correlation with diagnosis and characteristic CHARGE anomalies. *Am J Med Genet A* 2003; **123**: 111–121.
- 55 Doyle C, Blake K: Scoliosis in CHARGE: a prospective survey and two case reports. *Am J Med Genet A* 2005; **133**: 340–343.
- 56 Money J, Norman BF: Pedagogical handicap associated with micropenis and other CHARGE syndrome anomalies of embryogenesis: four 46, XY cases reared as girls. *Am J Psychother* 1988; **42**: 354–379.
- 57 Raqbi F, Le BC, Morisseau-Durand MP, Dureau P, Lyonnet S, Abadie V: Early prognostic factors for intellectual outcome in CHARGE syndrome. *Dev Med Child Neurol* 2003; **45**: 483–488.
- 58 Graham Jr JM, Rosner B, Dykens E, Visootsak J: Behavioral features of CHARGE syndrome (Hall-Hittner syndrome) comparison with Down syndrome, Prader-Willi syndrome, and Williams syndrome. *Am J Med Genet A* 2005; **133**: 240–247.
- 59 Smith IM, Nichols SL, Ssekutz K, Blake K: Behavioral profiles and symptoms of autism in CHARGE syndrome: preliminary Canadian epidemiological data. *Am J Med Genet A* 2005; **133**: 248–256.
- 60 Salem-Hartshorne N, Jacob S: Adaptive behavior in children with CHARGE syndrome. *Am J Med Genet A* 2005; **133**: 262–267.
- 61 Lalani SR, Safiullah AM, Fernbach SD *et al*: Spectrum of CHD7 Mutations in 110 Individuals with CHARGE Syndrome and Genotype-Phenotype Correlation. *Am J Hum Genet* 2006; **78**: 303–314.
- 62 Aramaki M, Udaka T, Kosaki R *et al*: Phenotypic spectrum of CHARGE syndrome with CHD7 mutations. *J Pediatr* 2006; **148**: 410–414.
- 63 Mitchell JA, Davenport SL, Hefner MA, Shei MM: Use of an expert model to test diagnostic criteria in CHARGE syndrome. *J Med Syst* 1985; **9**: 425–436.
- 64 Hertzberg BS, Kliewer MA, Lile RL: Antenatal ultrasonographic findings in the CHARGE association. *J Ultrasound Med* 1994; **13**: 238–242.
- 65 Becker R, Stiemer B, Neumann L, Entezami M: Mild ventriculomegaly, mild cerebellar hypoplasia and dysplastic choroid plexus as early prenatal signs of CHARGE association. *Fetal Diagn Ther* 2001; **16**: 280–283.
- 66 Jongmans M, Sistermans EA, Rikken A *et al*: Genotypic and phenotypic characterization of Noonan syndrome: new data and review of the literature. *Am J Med Genet A* 2005; **134**: 165–170.
- 67 Nagase T, Kikuno R, Ishikawa KI, Hirotsawa M, Ohara O: Prediction of the coding sequences of unidentified human genes. XVI. The complete sequences of 150 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res* 2000; **7**: 65–73.
- 68 Vissers LE, van Ravenswaaij CM, Admiraal R *et al*: Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet* 2004; **36**: 955–957.
- 69 Hurst JA, Meinecke P, Baraitser M: Balanced t(6;8)(6p8p;6q8q) and the CHARGE association. *J Med Genet* 1991; **28**: 54–55.
- 70 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 A* 2005; **133**: 326–330.
- 71 Johnson DS, Morrison N, Grant L *et al*: Confirmation of CHD7 as a cause of CHARGE association identified by mapping a balanced chromosome translocation in affected monozygotic twins. *J Med Genet* 2006; **43**: 280–284.
- 72 Woodage T, Basrai MA, Baxevanis AD, Hieter P, Collins FS: Characterization of the CHD family of proteins. *Proc Natl Acad Sci USA* 1997; **94**: 11472–11477.
- 73 Brehm A, Tufteland KR, Aasland R, Becker PB: The many colours of chromodomains. *Bioessays* 2004; **26**: 133–140.
- 74 Dirscherl SS, Krebs JE: Functional diversity of ISWI complexes. *Biochem Cell Biol* 2004; **82**: 482–489.
- 75 Schachen PC, Tie F, Lalani SR, Belmont JW, Collins FS: The CHD7 protein, mutated in CHARGE syndrome, binds to specific sites on chromatin (A19). *Am J Med Genet* 2006; **79** (suppl. 1): 20.
- 76 Kosaki K, Udaka T, Okuyama T: DHPLC in clinical molecular diagnostic services. *Mol Genet Metab* 2005; **86**: 117–123.
- 77 Bosman EA, Penn AC, Ambrose JC, Kettleborough R, Stemple DL, Steel KP: Multiple mutations in mouse Chd7 provide models for CHARGE syndrome. *Hum Mol Genet* 2005; **14**: 3463–3476.
- 78 Sanlaville D, Etchevers HC, Gonzales M *et al*: Phenotypic spectrum of CHARGE syndrome in fetuses with CHD7 truncating mutations correlates with expression during human development. *J Med Genet* 2005.
- 79 Lalani SR, Safiullah AM, Molinari LM, Fernbach SD, Martin DM, Belmont JW: SEMA3E mutation in a patient with CHARGE syndrome. *J Med Genet* 2004; **41**: e94.
- 80 Devriendt K, Swillen A, Fryns JP: Deletion in chromosome region 22q11 in a child with CHARGE association. *Clin Genet* 1998; **53**: 408–410.
- 81 Smithells RW: Defects and disabilities of thalidomide children. *BMJ* 1973; **1**: 269–272.
- 82 James WH: Clomiphene, anencephaly, and spina bifida. *Lancet* 1977; **1**: 603.
- 83 Grix Jr A: Malformations in infants of diabetic mothers. *Am J Med Genet* 1982; **13**: 131–137.
- 84 Sulik KK, Johnston MC, Daft PA, Russell WE, Dehart DB: Fetal alcohol syndrome and DiGeorge anomaly: critical ethanol exposure periods for craniofacial malformations as illustrated in an animal model. *Am J Med Genet Suppl* 1986; **2**: 97–112.
- 85 Stromland K, Miller MT: Thalidomide embryopathy: revisited 27 years later. *Acta Ophthalmol (Copenhagen)* 1993; **71**: 238–245.
- 86 Siebert JR, Graham JMJ, McDonald C: Pathologic features of the CHARGE association: support for involvement of the neural crest. *Teratology* 1985; **31**: 331–336.
- 87 Kirby ML, Waldo KL: Role of neural crest in congenital heart disease. *Circulation* 1990; **82**: 332–340.
- 88 Van Meter TD, Weaver DD: Oculo-auriculo-vertebral spectrum and the CHARGE association: clinical evidence for a common pathogenetic mechanism. *Clin Dysmorphol* 1996; **5**: 187–196.
- 89 Kallen K, Robert E, Castilla EE, Mastroiacovo P, Kallen B: Relation between oculo-auriculo-vertebral (OAV) dysplasia and three other non-random associations of malformations (VATER, CHARGE, and OEIS). *Am J Med Genet A* 2004; **127**: 26–34.
- 90 Lemmerling MM, Vanzieleghem BD, Mortier GR, Dhooge IJ, Kunnen MF: Unilateral semicircular canal aplasia in Goldenhar's syndrome. *AJNR Am J Neuroradiol* 2000; **21**: 1334–1336.
- 91 Hill J, Elliott C, Colquhoun I: Audiological, vestibular and radiological abnormalities in Kallman's syndrome. *J Laryngol Otol* 1992; **106**: 530–534.
- 92 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**: 246–250.
- 93 Wieczorek D, Bolt J, Schweddeheimer K, Gillessen-Kaesbach G: A patient with interstitial deletion of the short arm of chromosome 3 (pter- > p21.2::p12- > qter) and a CHARGE-like phenotype. *Am J Med Genet* 1997; **69**: 413–417.
- 94 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**: 610–614.

- 95 Lee WT, Hou JW, Yau KI, Wang TR: Trisomy 18 in a patient with CHARGE association. *J Formos Med Assoc* 1995; **94**: 60–62.
- 96 De Krijger RR, Mooy CM, Van Hemel JO *et al*: CHARGE association-related ocular pathology in a newborn with partial trisomy 19q and partial monosomy 21q, from a maternal translocation (19;21) (q13.1;q22.3). *Pediatr Dev Pathol* 1999; **2**: 577–581.
- 97 Lev D, Nakar O, Bar-Am I *et al*: CHARGE association in a child with *de Novo* chromosomal aberration 46, X,der(X)t(X;2)(p22.1;q33) detected by spectral karyotyping. *J Med Genet* 2000; **37**: E47.
- 98 Dev VG, Bulter MG, Phillips JA: 1q duplication due to unequal crossover in a patient with CHARGE association and DiGeorge sequence. *Am J Hum Genet* 1985; **37**: A90 (264).
- 99 Abeliovich D, Yagupsky P, Bashan N. 3:1 meiotic disjunction in a mother with a balanced translocation, 46,XX,t(5,14)(p15;q13) resulting in tertiary trisomy and tertiary monosomy offspring. *Am J Med Genet* 1982; **12**: 83–89.
- 100 Townes PL, White MR: Inherited partial trisomy 8q (22 leads to qter). *Am J Dis Children* 1978; **132**: 498–501.