

REVIEW

CHARGE syndrome: a review of the immunological aspects

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CHARGE syndrome is caused by a dominant variant in the *CHD7* gene. Multiple organ systems can be affected because of haploinsufficiency of *CHD7* during embryonic development. CHARGE syndrome shares many clinical features with the 22q11.2 deletion syndrome. Immunological abnormalities have been described, but are generally given little attention in studies on CHARGE syndrome. However, structured information on immunological abnormalities in CHARGE patients is necessary to develop optimal guidelines for diagnosis, treatment and follow-up in these patients. Here, we provide an overview of the current literature on immunological abnormalities in CHARGE syndrome. We also explore immunological abnormalities in comparable multiple congenital anomaly syndromes to identify common immunological phenotypes and genetic pathways that might regulate the immune system. Finally, we aim to identify gaps in our knowledge on the immunological aspects in CHARGE syndrome that need further study.

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INTRODUCTION

Patients with a combination of coloboma and other multiple congenital abnormalities were first described in 1961.^{1,2} However, the association between coloboma, congenital heart defects and choanal atresia was first recognized by both Hall³ and Hittner *et al*⁴ in 1979. Then in 1981, Pagon *et al*⁵ proposed the acronym CHARGE, which stands for the following symptoms: Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genital abnormalities, and Ear abnormalities (external, middle and inner ear including deafness). The incidence of CHARGE syndrome (MIM# 214800) is estimated at 1 in 15 000–17 000 newborns⁶ and its clinical diagnosis is based on the criteria proposed by Blake *et al*⁷ or by Verloes.⁸ Since 2004, when the major genetic cause of CHARGE syndrome was identified as a dominant variant in the *CHD7* gene (MIM# 608892), which usually occurs *de novo*, CHARGE syndrome can also be diagnosed by molecular diagnostics. A variant in *CHD7* can be found in over 90% of all children who fulfil the clinical diagnostic criteria.^{6,9–11} The *CHD7* gene encodes a member of the chromodomain helicase DNA-binding protein family that regulates the transcription of genes during embryonic development. Haploinsufficiency of *CHD7* affects multiple organ systems, including the heart, the inner ear and the eye. There is no clear genotype–phenotype correlation, but variants leading to a premature stop codon are, in general, associated with a more severe phenotype than variants with a non-truncating effect, that is, missense variants.¹²

Since the genetic cause of CHARGE syndrome was identified, its phenotype has been further explored. In addition to the above symptoms, other common clinical features of CHARGE syndrome are: absent or hypoplastic semicircular canals, cranial nerve dysfunction (including facial nerve palsy), cleft lip and/or palate, anosmia, feeding difficulties and skeletal abnormalities.^{8,9,13} Furthermore,

deficits in the immune system have been described in CHARGE patients, which might lead to morbidity and even mortality.¹⁴

Here, we provide an overview of the current literature on immunological dysfunction in CHARGE syndrome. We also explore what can be learned from the clinical overlap between CHARGE syndrome and other multiple congenital anomaly (MCA) syndromes. Do these syndromes share immunological phenotypes? Last but not least, we try to identify gaps in our knowledge that need further study.

METHODS

A systematic search via the online database PubMed of publications on immunological aspects of CHARGE syndrome was performed (Supplementary 1). All patients with a proven variant in *CHD7*, which affects the normal function of the protein, were included in our review. We also decided to include articles prior to the identification of *CHD7* as the causative gene, in order to have a complete overview. Before *CHD7* was discovered, some patients with CHARGE syndrome were described as having DiGeorge syndrome (#MIM 188400) or as having DiGeorge syndrome with CHARGE association. For our review, we only selected patients from these papers who fulfilled the diagnostic criteria of Blake *et al*⁷ and/or Verloes,⁸ and in whom a deletion of 22q11.2 (the cause of DiGeorge syndrome) had been excluded or was unknown. The titles and abstracts of English language articles were reviewed for relevance. Relevant articles were studied in detail and their reference lists were scanned for additional publications (Supplementary 2). Information on the thymus and the results of the following immunological tests were used: lymphocyte subset phenotyping (if available, absolute numbers of T-, B- and NK-cells), quantitative immunoglobulin analysis, and T-cell function by response on mitogens (phytohaemagglutinin). Owing to frequently missing absolute values and the differences in reporting laboratory results, especially whether age-related reference values were used or not, we decided to follow the interpretations of the authors instead of using our own cutoff values. For papers that only mentioned laboratory results without interpretation, we used the age-related reference values according to Comans-Bitter *et al*¹⁵ for interpretation.

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RESULTS

The collected cohort

All literature on immunology or immune dysfunction in CHARGE syndrome consists of case reports or retrospective studies. We identified 26 publications, comprising 59 patients in total ('our collected cohort'), which fulfilled our inclusion criteria (Table 1). Thirty-six of 59 (61%) patients had a proven variant in *CHD7* with functional effect (Table 1A). Detailed information on the variant in *CHD7* has been reported for 23 of these patients and all variants were known to lead to a premature stop in *CHD7* (data not shown). The remaining 23 of 59 (39%) patients fulfilled the clinical criteria of CHARGE syndrome, but the results of *CHD7* analysis (Table 1B) or the results of both *CHD7* analysis and 22q11.2 deletion testing (Table 1C) were unknown. The median age at which immunological tests ($n=64$) were performed in our collected cohort was 15 weeks (range, 1 day to 8 years). In some patients, immunological tests were performed at different ages.

Immunological abnormalities reported in CHARGE syndrome

Clinical presentation related to immune abnormalities included recurrent otitis media in 4 patients (6.8%), sinusitis in 2 patients (3.4%), conjunctivitis in 2 patients (3.4%), dermatitis in 4 patients (6.8%), infections of the respiratory tract in 6 patients (10%), including pneumonia in 3 (5.1%), and sepsis in 5 (8.5%) of 59 patients.^{14,16–22} Three patients had features resembling Omenn syndrome, a form of severe combined immune deficiency, characterized by autoimmunity-like features and macular skin rash due to the formation of abnormal, autoreactive T cells.^{18,23} Other severe presentations included a patient with recurrent oral candidiasis, recurrent severe infections, and septic shock due to colonization with multi-resistant species of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinobacter* (Janda *et al*,²² Table 1B); another patient with severe general dermatitis and ulcers of the colon (Boudny *et al*,²⁴ Table 1B); and a patient with CHARGE association and T-cell deficiency with a chronic viral infection of the gut (Wood *et al*,²⁵ Table 1C).

Information on T-cell numbers were reported in 44 of 59 patients and in 24 of 36 patients with a proven variant in *CHD7*. As shown in Table 1, 35 of the 44 (80%) patients with available information on T-cell numbers had low or absent T-cell numbers. Of the 35 patients, 1 patient had T-cell lymphopenia and a transient B-cell lymphopenia, which normalized with ageing.^{26–28} Thymic aplasia was reported in 27 of 59 patients and in 16 of 36 patients with a proven variant in *CHD7*. T-cell lymphopenia in our collected cohort was associated with thymic aplasia or hypoplasia in 21 of 22 (95%) patients.^{18–36} T-cell function by response on mitogens was available for 28 of 59 patients and of these 28 patients 24 (86%) had a low or absent response on mitogen. For 23 of 26 (88%) patients, T-cell lymphopenia was concomitant with T-cell dysfunction.^{14,17,18,20,22–31,33,35–37}

In our collected cohort, B- and/or NK-cell numbers were reported in only 29 of 59 patients, and of these 29 patients, 1 had low B-cell numbers, 1 had high B-cell numbers, 1 had low NK-cells numbers and 3 had high NK-cell numbers. The reported B- and NK-cells numbers of other patients were normal.^{14,23,26–28,34–36}

Immunoglobulin levels were reported in 33 of 59 patients and in 20 of 36 patients with a proven variant in *CHD7*. Hypogammaglobulinaemia was reported in 20 of 33 (61%) patients and measured along with T-cell lymphopenia in 14 of 18 (78%) patients.^{14,17,22–29,35,36} Thirteen of 33 (39%) patients with known immunoglobulin levels had IgG deficiency and 2 of these patients had received immunoglobulin replacement therapy.^{14,16–18,22–29,38} Of these 13 patients with IgG

deficiency, 6 had recurrent or chronic infections and 1 had absent specific antibody responses.^{14,16–18,22,24,25} Isolated immunoglobulin deficiencies were rarely reported in CHARGE syndrome. One patient with an isolated IgG2 subclass deficiency¹⁷ and two patients with an isolated IgA deficiency¹⁴ were reported. One patient developed low IgG and elevated IgE while the T-cells normalized.¹⁸

Jyonouchi *et al*¹⁴ reported on the largest cohort of patients with CHARGE syndrome ($n=25$) proven by a variant in *CHD7*, and retrospectively collected their immunological data. However, lymphocyte subset phenotyping and quantitative immunoglobulin analysis were performed in only nine and eight patients, respectively. They concluded that a greater proportion of CHARGE patients had immunological abnormalities than previously thought (lymphopenia in 60% and humoral defects in 16% of patients, respectively). They confirmed the overrepresentation of T-cell dysfunction and also reported two patients with a severe combined immune deficiency phenotype.

In conclusion, T-cell lymphopenia is common (80%) in CHARGE patients and is associated with a reduced T-cell function and hypogammaglobulinaemia, but normal B-cell and NK-cells numbers. Thymic aplasia or hypoplasia might be the underlying cause of T-cell lymphopenia, leading to increased frequencies of infection and other clinical presentations.

According to Jyonouchi *et al*,¹⁴ immune dysfunction in CHARGE might contribute to the mortality of the syndrome. Two patients died from infectious complications and two patients with a confirmed severe combined immune deficiency phenotype died from respiratory failure. Assing *et al*³⁴ reported a patient with CHARGE syndrome who had severely reduced thymic function and a severe lymphopenia, but showed a functional and diverse T-cell receptor repertoire, and a good response to vaccines. An uneventful infection history was reported up to the age of 34 months, but thereafter the clinical status is unclear.

Immunological abnormalities reported in overlapping MCA syndromes

CHARGE syndrome overlaps clinically with other MCA syndromes (see Figure 1).³⁹ The most remarkable phenotypic overlap is with 22q11.2 deletion syndrome (#MIM 192430, *TBX1*), including the DiGeorge phenotype, where immunodeficiency is an important symptom. Congenital heart defects, cognitive and motor delay, hearing loss, external ear anomaly, cleft lip and palate, growth deficiency, and renal anomaly are seen in both syndromes.^{14,39} The total absence of the thymus, and therefore complete T-cell lymphopenia (DiGeorge phenotype), is rare and is seen in less than 1.5% of patients with 22q11.2 deletion syndrome.⁴⁰ Patients with the typical 1.5 or 3.0 Mb deletion of 22q11.2 rarely show the full presentation of the syndrome and only a minority develop opportunistic infections. Much more common is a phenotype with T-cell lymphopenia (67%)⁴¹ combined with mild to moderate functional immunological impairment which improves with age. This T-cell lymphopenia mostly present as frequent viral infections during childhood, with or without secondary bacterial infections, but can also remain clinically unrecognized.^{40,41} Owing to their abnormal palatal anatomy, which may compromise drainage, most patients are susceptible to upper airway infections, as is also seen in patients with CHARGE syndrome. There are more publications on immunoglobulin abnormalities in 22q11.2 deletion syndrome compared with CHARGE syndrome.^{42,43} Recent studies indicate that the antibody deficiency in 22q11.2 deletion syndrome is likely to be underestimated.^{44,45} Patel *et al*⁴⁴ have reported immunoglobulin deficiency in the largest cohort ($n=855$) to date and concluded that 6% of the patients over the age of three had

Table 1 Clinical and immunological findings in patients with CHARGE syndrome, as reported in the literature

Reference	n	Sex	Age	R			G	E	HL	SSC	Other and immunological symptoms	Mitogen			Immunoglobulins	
				C	H	A						Growth	Cognition	Dysmorph		Thymus
<i>A. CHARGE syndrome with proven variant in CHD7 with functional effect</i>																
Santaville <i>et al</i> ⁶⁰	10 ^a	M	21 wg	+	+	+	U	-	+	+					U	
		M	21 wg	+	+	+	U	+	+	+					U	
		M	23 wg	+	+	+	U	-	+	+					U	
		M	23 wg	+	+	+	U	+	+	+					U	
		M	28 wg	-	+	+	U	+	+	+					U	
		M	29 wg	-	+	+	U	+	+	+					U	
		F	29 wg	+	+	+	U	+	+	+					U	
		F	32 wg	+	+	+	U	+	+	+		TE			U	
		F	36 wg	-	+	+	U	-	+	+		TE			U	
		F	1 d	-	+	+	U	+	+	+		TE			U	
Witzl <i>et al</i> ⁹	2	M	2 d	-	+	+	U	+	+	+		HP			Low IgG, high IgM and IgA	
		F	2 mo	-	+	+	U	+	+	+		HP			U	
Sanka <i>et al</i> ⁶⁰ Gennery <i>et al</i> ⁶³	4	F	7 wk	+	+	+	U	+	+	+					N	
		M	4 mo	+	+	+	U	+	+	+						Low IgA
		M	2.5 mo	+	+	+	U	+	+	+						Low IgG and IgA
		F	at birth	-	+	+	U	+	+	+						Low IgA, high IgE
Hoover-Fong <i>et al</i> ⁶¹ Wincent <i>et al</i> ⁷⁵ Chopra <i>et al</i> ⁶² Jyonouchi <i>et al</i> ¹⁴	10	M	3.5 mo	-	+	+	U	+	+	+					Low IgA	
		M	5 mo	-	+	+	U	-	+	+						N
		M	3 wk	-	+	+	U	-	+	+						Low IgA
		M	1 y	+	+	+	U	+	+	+						U
		M	1 y	+	+	+	U	+	+	+						U
		M	16 d	+	+	+	U	+	+	+						U
		F	12 mo	+	+	+	U	+	+	+						N
		F	4 mo	+	+	+	U	+	+	+						N
		F	2 y	+	+	+	U	+	+	+						N (3 y)
		U	2 y	+	+	+	U	+	+	+						N
Kaliakatsos <i>et al</i> ¹⁸	10	U	3 mo	+	+	+	U	+	+	+						Low IgG, IgM and IgA
		U	5 wk	+	+	+	U	+	+	+						Low IgA (3 mo)
		U	5 mo	+	+	+	U	+	+	+						Low IgG, IgM and IgA
		U	4 y	+	+	+	U	+	+	+						N
		U	2 mo	+	+	+	U	+	+	+						U
		U	1 wk	+	+	+	U	+	+	+						U
		U	4 mo	+	+	+	U	+	+	+						N
		U	7 y	+	+	+	U	+	+	+						Low IgM, absent IgA (8 y)
		U	6 y	+	+	+	U	+	+	+						Absent IgA
		M	1 mo	+	+	+	U	+	+	+						N
Inoue <i>et al</i> ⁶³ Assing <i>et al</i> ⁶⁴	1	M	7 mo	+	+	+	U	+	+	+						Low IgG, High IgE
		F	neonate	+	+	+	U	-	+	+						N
		F	1 mo	+	+	+	U	-	+	+						N
		F	2 mo	+	+	+	U	+	+	+						N (3 mo)
		F	6 mo	+	+	+	U	+	+	+						N (4 mo)
		F	14 mo	+	+	+	U	+	+	+						N (13 mo)

Table 1 (Continued)

Reference	n	Sex	Age	C	H	A	R	Growth	Cognition	G	E	HL	SSC	Other and immunological symptoms	Thymus	T-cells	Mitogen response	B-cells	NK-cells	Immunoglobulins
<i>B. Clinically diagnosed CHARGE syndrome, CHD7 status unknown, but deletion 22q11.2 excluded</i>																				
North <i>et al</i> ¹⁶	1	F	4.5 y	+	+	+	+	+	+	+	+	+	+	Recurrent otitis media and sinusitis	U	N	U	U	U	Hypogammaglobulinaemia with low IgG2
De Lonlay-Debeney <i>et al</i> ¹⁹	5	M	17 d	+	+	+	-	+	+	+	+	+	+	CN	HP	N	U	U	U	U
		M	19 mo	+	+	+	+	+	+	+	+	+	+	CN	HP	U	U	U	U	U
		F	4 mo	+	-	+	+	+	+	+	+	+	+	CN	-	U	U	U	U	U
		M	6 mo	+	+	+	+	+	+	+	+	+	+	CN	-	U	U	U	U	U
		F	13 d	+	+	+	-	+	+	+	+	+	+	Sepsis, hepatic abscess	N	Low	U	U	U	U
		M	23 d	+	+	+	+	+	+	+	+	+	+	CN, TE	-	-	-	N	Low	Low IgA (41 d)
Markert <i>et al</i> ^{26,27,35,36}	1	M	3 mo	+	+	+	+	+	+	+	+	+	+	CN, TE	U	Low	Low	U	U	U
Rice <i>et al</i> ²⁸	1	M	14 d	+	+	+	+	+	+	+	+	+	+	CN, TE	U	-	Low	N	N	U
Squires <i>et al</i> ²⁷	1	U	3 mo	+	+	+	+	+	+	+	+	+	+	CN, TE	U	Low	Low	U	U	U
Markert <i>et al</i> ²⁶	1	M	3 d	+	+	+	-	+	+	+	+	+	+	CN, TE	-	Low	U	Low	High	High IgA
Markert <i>et al</i> ^{26,27}	1	M	31 d	+	+	+	+	+	+	+	+	+	+	CN, dermatitis, ulcers colon, recurrent respiratory infections	-	Low	-	N	High	Not reliable because of treatment with immunoglobulins
Rice <i>et al</i> ²⁸	1	F	44 d	+	+	+	+	+	+	+	+	+	+	recurrent respiratory infections, bronchiolitis, pneumonia, otitis media, urticaria, allergy	U	Low	Low	N	N	Low IgG, IgM and IgA
Boudny <i>et al</i> ²⁴	2	F	59 d	+	+	+	+	+	+	+	+	+	+	recurrent respiratory infections, bronchiolitis, pneumonia, otitis media, conjunctivitis, otitis media	U	Low	U	N	N	Low IgG2
Theodoropoulos ¹⁷	2	F	30 mo	+	+	+	+	+	+	+	+	+	+	CN, dermatitis, sepsis, PCP	U	N	N	N	N	Low IgE
Markert <i>et al</i> ²⁷	2	U	43 d	+	+	+	+	+	+	+	+	+	+	CN	U	Low	-	U	U	U
Rice <i>et al</i> ²⁸	2	U	41 d	+	+	+	+	+	+	+	+	+	+	CN, dermatitis, infections	U	-	-	U	U	U
Markert <i>et al</i> ^{20,21}	2	M	255 d	+	+	+	+	+	+	+	+	+	+	CN, dermatitis, infections	-	N	Low	U	U	U
		M	316 d	+	+	+	+	+	+	+	+	+	+	CN, dermatitis, sepsis, PCP	-	N	Low	U	U	U
		M	158 d	+	+	+	+	+	+	+	+	+	+	CN, dermatitis	-	Low	Low (318 d)	U	U	High IgE
Markert <i>et al</i> ²¹	1	U	2 mo	+	+	+	+	+	+	+	+	+	+	TE, recurrent severe infections and septic shocks	-	-	-	U	U	Hypogammaglobulinaemia
Janda <i>et al</i> ²²	1	M	42 d	+	+	+	+	+	+	+	+	+	+	TE	-	U	U	U	U	Low
Lee <i>et al</i> ²⁸	1	F	6 d	+	+	+	+	+	+	+	+	+	+	otitis media, conjunctivitis	-	-	U	U	N	N
Chopra <i>et al</i> ²²	1	F	3 y	+	+	+	+	+	+	+	+	+	+	CN, chronic enteric virus infection	HP	Low	Low	U	U	Treatment with immunoglobulins
Theodoropoulos ¹⁷	1	M	3 y	+	+	+	+	+	+	+	+	+	+	CN, pneumonia, otitis media, conjunctivitis	U	Low	-	N	N	Low IgG1
<i>C. Clinically diagnosed CHARGE syndrome, CHD7 and 22q11.2 deletion status unknown</i>																				
Wood <i>et al</i> ⁵	1	M	5 mo	-	+	+	+	+	+	+	+	+	+	CN, coloboma or microphthalmia; CN, cranial nerve dysfunction; d, day(s); E, ear anomalies; F, female; G, genital anomalies; H, heart anomalies; HL, hearing loss; HP, hypoplasia; M, male; mo, month(s); N, normal; n, number of patients; PCP, <i>Pneumocystis carini</i> pneumonia; R, retardation in development and/or growth; SCID, severe combined immune deficiency; SSC, semicircular canals anomalies; TE, tracheoesophageal defects; U, unknown; wg, weeks of gestation; wk, week(s); Y, year(s); +, present; -, absent (including thymic aplasia).	U	Low	Low	U	U	Treatment with immunoglobulins

Abbreviations: A, choanal atresia or stenosis, including cleft palate since these anomalies rarely occur together; C, coloboma or microphthalmia; CN, cranial nerve dysfunction; d, day(s); E, ear anomalies; F, female; G, genital anomalies; H, heart anomalies; HL, hearing loss; HP, hypoplasia; M, male; mo, month(s); N, normal; n, number of patients; PCP, *Pneumocystis carini* pneumonia; R, retardation in development and/or growth; SCID, severe combined immune deficiency; SSC, semicircular canals anomalies; TE, tracheoesophageal defects; U, unknown; wg, weeks of gestation; wk, week(s); Y, year(s); +, present; -, absent (including thymic aplasia).
[#]Foetuses.

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/palate	Oesophageal atresia	Hypocalcaemia	Immunodeficiency
<i>CHD7</i>	CHARGE syndrome																
<i>TBX1</i>	22q11.2 deletion syndrome																
<i>JAG1</i>	Alagille syndrome																
<i>SOX2</i>	Syndromic microphthalmia type 3																
<i>FGFR1</i>	Pfeiffer syndrome/ Kallmann syndrome																
<i>FGF8</i>	Kallmann syndrome																
<i>KMT2D</i>	Kabuki syndrome																

Figure 1 Clinical overlap of CHARGE syndrome with other MCA syndromes. The overlapping clinical features of CHARGE syndrome with other MCA syndromes are shown. All the genes mentioned, or their proteins, have been associated with CHD7. Adapted from Corsten-Janssen *et al.*¹⁴

hypogammaglobulinaemia, 19% of the total cohort had a low level of IgG and 2–3% of the patients were receiving immunoglobulin replacement therapy. Björk *et al.*⁴⁵ found that 6 of 26 adult patients (23%) had low immunoglobulin levels. Autoimmune diseases, like rheumatoid diseases and idiopathic thrombocytopenia purpura, are seen in approximately 10% of patients with 22q11.2 deletion syndrome,⁴⁶ whereas no autoimmune diseases were seen in the CHARGE syndrome patients, although 3 of 59 (5%) patients with CHARGE syndrome had an Omenn-like syndrome, which has autoimmune-like features. An explanation of the autoimmune predisposition in 22q11.2 deletion syndrome might be a reduced level of natural regulatory T-cells (nTreg).⁴⁷ These nTregs are important in the maintenance of self-tolerance, that is, suppression of the immune response against self-antigens. In our collected cohort of CHARGE patients, nTreg levels were mentioned in only two patients: one patient had few nTregs (no value was mentioned)²³ and the other patient had higher proportions of nTregs compared with two adult controls.³⁴ So CHARGE syndrome shares a large clinical overlap with 22q11.2 deletion syndrome and this seems to be also true for the prevalence of T-cell lymphopenia, leading to susceptibility for viral (and secondary bacterial) infections.

Immunological functions in other overlapping MCA syndromes are less well described. Alagille syndrome (#MIM 118450), caused by variants in *JAG1*, shares abnormalities in the semicircular canals, heart defects, renal anomalies and intellectual disabilities with CHARGE syndrome.³⁹ Recurrent ear and respiratory tract infections are seen in about a quarter of patients with Alagille syndrome.⁴⁸ The underlying immunological dysfunction was described in one paper as a diminished T-helper 1 response.⁴⁹

Patients with Pfeiffer syndrome (#MIM 101600, *FGFR1*) have overlapping features with CHARGE syndrome regarding cleft-lip and/or palate and hearing loss.³⁹ The immunological function in 12 patients with craniofacial malformation syndromes, including Pfeiffer syndrome, was studied by Scheuerle *et al.*⁵⁰ Unfortunately, they did not specify the immunological dysfunction per syndrome. However, seven patients were tested for T-cell numbers and T-cell lymphopenia was reported in one patient, decreased T-helper cells in three patients and decreased T-killer cells in three patients. Three patients had additional abnormalities in immunoglobulin counts and in

lymphocyte stimulus and response function. Two were brothers, who both had Pfeiffer syndrome.

Kabuki syndrome (#MIM 147920, *KMT2D*) has the following clinical overlap with CHARGE syndrome: cleft palate, mental retardation, short stature, genital hypoplasia, congenital heart defects, abnormalities of the eye and ear, renal abnormalities, scoliosis, and recurrent otitis media in infancy.^{51,52} Hoffman *et al.*⁵³ evaluated the immune condition of 19 patients with Kabuki syndrome and concluded that hypogammaglobulinaemia is a frequent finding (84%). However, they tested lymphocyte subsets in only three patients, who showed no abnormalities in T- and B-cell numbers. Diminished T-cell function by response on phytohaemagglutinin and reduced naive T-helper cells were reported in one patient with Kabuki syndrome.⁵⁴ Autoimmune disorders, including idiopathic thrombocytopenia purpura, are common in children with Kabuki syndrome.^{53,55}

Thus, CHARGE syndrome and the clinically overlapping 22q11.2 deletion syndrome share an increased prevalence of T-cell dysfunction. Although T-cell dysfunction is only mentioned sporadically in Alagille syndrome, Pfeiffer syndrome and Kabuki syndrome, the clinical overlap of these syndromes with CHARGE syndrome indicates that their underlying genetic defects may result in shared embryonic pathways leading to T-cell abnormalities.

DISCUSSION

Does CHD7 share T-cell-related pathways with *TBX1*, *JAGGED1*, *FGFR1* and *MLL2*?

The 22q11.2 region contains the *TBX1* gene (#MIM 602054), which has been identified as a candidate gene for most of the phenotypic features seen in the 22q11.2 deletion syndrome.⁴⁰ The existence of a shared embryonic pathway or pathways of CHD7 and *TBX1* has been studied for heart development⁵⁶ and inner ear development⁵⁷ in animal models. Both genes are expressed in the pharyngeal arches, of which the third and fourth arches contain the precursors of the thymic stromal cells.^{58–60} This might explain the shared underlying pathogenesis of abnormal thymic development and the high proportion of thymic aplasia (44%) in our collected cohort with a proven *CHD7* variant, which can lead to impaired T-cell development. However, it

should be noted that the high proportion of thymic aplasia might be due to reporting bias.

It is also known that impaired T-cell development affects further maturation of the thymic stromal cells resulting in thymic epithelial cells with lack of Aire expression, a transcription factor that regulates the expression of self-antigens. These self-antigens are important for the deletion of autoreactive T-cells.⁶¹ McLean-Tooke *et al*⁴⁷ suggested that the reduced number of nTregs seen in 22q11.2 deletion syndrome is related to thymic function and structure. So the autoimmune predisposition in 22q11.2 deletion syndrome might be explained by both impaired thymic and T-cell development. In CHARGE syndrome, only three patients were reported to have Omenn-like syndrome, which has autoimmune-like features.^{18,23} Well-defined autoimmune diseases have not yet been described in CHARGE syndrome, suggesting that the nTregs physiology might be different in CHARGE syndrome compared with 22q11.2 deletion syndrome. It would therefore be interesting to further study nTregs in CHARGE syndrome.

JAG1 (#MIM 601920) is the underlying causative gene of Alagille syndrome and its protein JAGGED1 is a ligand of the Notch receptor. The Notch signalling pathway regulates cell-fate decisions during ontogeny, including the development of lymphoid cells, and there is

accumulating evidence to suggest that Notch signalling is also involved in the maturation of peripheral T-cells.^{62,63} In animal models, Jagged1 seems to play a role in the generation of regulatory T-cells.^{64,65} *CHD7* and *JAG1* might be linked indirectly via *SOX2* (#MIM 184429), because *Chd7* has been identified as a *Sox2* transcription cofactor in the regulation of common target genes including *Jag1*.⁶⁶ Functional affective variants in *SOX2* result in syndromic microphthalmia type 1, a phenotype that shares several features with CHARGE syndrome: microphthalmia, motor disability, neurocognitive delays, sensorineural hearing loss, oesophageal atresia, pituitary defects and gonadotropin deficiency.⁶⁷ Immune dysfunction has not been described in syndromic microphthalmia type 1 thus far.

The underlying causative gene for Pfeiffer syndrome is *FGFR1* (#MIM 136350), encoding the fibroblast growth factor receptor 1 (*FGFR1*). *FGFR1* and *FGF8* (#MIM 600483) are also involved in Kallmann syndrome, which shares features such as anosmia and hypogonadotropic hypogonadism with CHARGE syndrome.⁶⁸ A common pathway for *CHD7* and fibroblast growth factor 8 (*FGF8*)/*FGFR1* has therefore been suggested.^{56,69,70} *FGFR1* is expressed in a subset of T-cells and is believed to interact with the T-cell receptor to enhance the activation of T-cells.⁷¹

Table 2 Phenotypic comparison of our collected cohort with a proven variant in *CHD7* compared with cohorts with a proven variant in *CHD7* from the literature

Feature	Our collected <i>CHD7</i> -positive cohort (n = 36)	<i>CHD7</i> -positive cohort from Bergman <i>et al</i> ¹³ (n = 280)	<i>CHD7</i> -positive cohort from Zentner <i>et al</i> ⁷² (n = 254) ^a
Coloboma	19/26 ^b 73% (53–81%) ^c	189/234 81% (68–84%)	190/253 75%
Heart defect	25/26 96% (69–97%)	191/252 76% (68–78%)	193/250 77%
Choanal atresia	17/20 ^d 85% (47–92%)	99/179 55%	95/247 38%
Cleft lip and/or palate	No isolated cleft lip	79/163 48% (28–70%)	79/242 33%
Growth retardation	3/3 100% (8–100%)	35/94 37% (13–79%)	101/141 72%
Developmental delay	Developmental delay 4/5 80% (11–97%)	Delayed motor milestones 147/149 99% (53–99%) Intellectual disability 108/134 74% (39–91%)	Developmental delay 107/141 76%
Genital hypoplasia	13/20 65% (36–81%)	118/145 81% (42–90%)	116/187 62%
External ear anomaly	21/21 100% (58–100%)	224/231 97% (80–98%)	214/235 91%
Semicircular canal anomaly	14/14 100% (39–100%)	110/117 94% (39–98%)	94/96 98%
Cranial nerve dysfunction	12/12 100% (33–100%)	173/174 99% (62–100%)	Unknown
Facial palsy	6/6 100% (17–100%)	80/121 66% (29–85%)	72/187 39%
Tracheoesophageal anomaly	9/17 53% (25–78%)	42/146 29% (15–63%)	35/185 19%

Adapted from Bergman *et al*¹³ and Zentner *et al*.⁷²

^aThis cohort partially overlaps with the cohort of Bergman *et al*¹³ because the phenotypes of 64 of the patients in that study had been published previously.

^bFrequencies are represented as the number of patients with a particular feature/the total number of patients that were tested for that particular feature.

^cThe range of percentages presented between brackets was calculated as: (positive/total) × 100% - (positive + unknown/total) × 100%.

^dCleft palate is included in choanal atresia since these anomalies rarely occur together.

Kabuki syndrome is caused by variants in *KMT2D* (#MIM 602113), affecting the function of a methyltransferase named MLL2 that is involved in transcriptional regulation. Recently, it has been shown that CHD7 interacts with the same transcriptional proteins as MLL2.⁵² It is unknown whether MLL2 plays a role in the immunological dysfunction in Kabuki syndrome, but it is possible that both MLL2 and CHD7 regulate expression of genes which are involved in the immune system.

CHD7 regulates the expression of genes and shares pathways with other regulatory proteins in embryonic development.³⁹ The interaction of CHD7 with TBX1 and their role in thymic development has been well established in the literature.^{58–60} Evidence for the interaction of CHD7 with JAG1, FGFR1 and MLL2 during embryonic development, especially the development of the immune system, is less strong, but reports are emerging describing possible links in the genetic pathways. Although we only have detailed information on the variant in *CHD7* for 23 patients, the fact that all reported variants lead to a premature stop in *CHD7* is interesting, because truncating *CHD7* variants are in general associated with a more severe phenotype.¹²

Are certain phenotypic features more common in our collected cohort?

In Table 2, we compare the clinical features of 36 patients in our collected cohort for whom a variant in *CHD7* with functional effect had been proven (Table 1A) with two other cohorts described by Bergman *et al.*¹³ These two cohorts consist of 280 patients with a proven variant in *CHD7* from Bergman *et al.*¹³ and 254 patients from Zentner *et al.*⁷² Most features show similar frequencies in all three cohorts. We see a higher frequency in our collected cohort for heart defects (96% *versus* 76% and 77%), choanal atresia (85% *versus* 55% and 38%), and tracheoesophageal anomaly (53% *versus* 29% and 19%). Facial palsy (100%) and growth retardation (100%) also seem to occur more frequently in our collected cohort, but we note that we only have clinical information on six and three patients, respectively. It should also be noted that two cases²⁹ were included in all three cohorts. As the differences found might be due to the predominance of truncating variants in our cohort, Table 3 shows the phenotypic comparison of patients carrying variants leading to a premature stop in *CHD7* in our collected cohort ($n=23$) and in that of Bergman *et al.*¹² ($n=315$). The frequencies between the two cohorts are more comparable than the frequencies shown in Table 2. However, Table 3 shows that our collected cohort still had a higher frequency for heart

defects (95.7 *versus* 82.5%), choanal anomaly (84.2 *versus* 60.4%), and tracheoesophageal anomaly (50.0 *versus* 33.6%). This is interesting because heart defects, choanal atresia and tracheoesophageal anomalies are all midline defects, including abnormal thymus development.

Suggested future studies

As shown in Table 1, the publications on immunology in CHARGE syndrome are scarce and most studies describe only one or a few patients, with data collected retrospectively. The differences in reporting the laboratory results, for example, absolute *versus* relative values, and the use of different reference values, whether age-related or not, make it difficult to draw conclusions. A limitation of our approach is that reporting bias might play a role, because most publications are case reports. Furthermore, the median age is quite young in our collected cohort. As the T-cell number may well improve with age, as has been shown in 22q11.2 deletion syndrome,⁷³ our collected cohort might represent the more severe phenotypes.

We found lymphopenia in 80% of our collected cohort, which is in contrast to the 60% found in the study of Jyonouchi *et al.*¹⁴ Our higher percentage might reflect reporting bias. Another contrast is the reported prevalence of immunoglobulin abnormalities in 22q11.2 deletion syndrome of 6% in recent studies^{44,45} and 40% in older reports,^{42,43} with the prevalence of 61% in our collected cohort. However, we only had information on immunoglobulin levels from 33 of 59 (56%) patients. Nonetheless, the percentages that we found indicate that immunological dysfunction in CHARGE syndrome might play a greater role in the phenotype than previously thought. There is a need to broaden our knowledge on the frequency and exact nature of immune abnormalities in CHARGE syndrome, including information on B- and NK-cells. As the pathophysiology of the immunological dysfunction in 22q11.2 deletion syndrome has been extensively studied, it is worthwhile to explore whether the same pathophysiology also applies to CHARGE syndrome.

Anatomical deviations, such as palatal defects, contribute to the susceptibility to infections and their duration by impeding drainage or clearance of infectious debris. It is important to know whether immunological issues complicate the severity of infections in order to optimize care management in patients. This is especially true for children with CHARGE syndrome, who have other co-morbidities (such as heart defects and tracheoesophageal defects) that require operative procedures, because these also constitute risk factors for infections.

Because immunological problems might contribute to the morbidity and even the mortality of patients with CHARGE syndrome, timely diagnosis of an immune dysfunction is relevant. Information on the type of immune abnormality will provide clues for future studies that could develop guidelines to protect these children from excess morbidity (and maybe mortality) due to infections. Such guidelines could include clinical follow-up strategies, prescribing prophylactic antibiotics, or even a procedure to restore T-cell lymphopenia by replacing thymic tissue with a thymic transplant.^{40,74}

To better understand the frequency and nature of immune dysfunction in CHARGE syndrome, a prospective study is needed to systematically collect immunological data on more patients with CHARGE syndrome, including those without overt symptoms of immunological defects. Correlating immunological laboratory results with clinical data will yield more insight into the immune dysfunction. In addition, new genome-wide sequencing techniques can be applied to identify patients with atypical CHARGE syndrome and further expand the phenotypic spectrum of CHARGE syndrome, including its immunological features.

Table 3 Phenotypic comparison of patients carrying a variant in *CHD7* leading to a premature stop in *CHD7*

Feature	Cohort of	
	Our collected cohort ($n=23$) ^a	Bergman <i>et al.</i> ¹² ($n=315$) ^b
Coloboma	16/23 (69.6%)	199/229 ^c (86.9%)
Heart defect	22/23 (95.7%)	212/257 (82.5%)
Choanal atresia	16/19 ^d (84.2%)	110/182 (60.4%)
Cleft lip and/or palate	No isolated cleft lip	80/144 (55.6%)
External ear anomaly	20/20 (100%)	217/221 (98.2%)
Semicircular canal anomaly	14/14 (100%)	121/121 (100%)
Cranial nerve dysfunction	5/5 (100%)	119/131 (90.8%)
Tracheoesophageal anomaly	8/16 (50.0%)	43/128 (33.6%)

The patients come from our collected cohort and the cohort of Bergman *et al.*¹²

^a23 truncating mutations: 2 deletions, 8 frameshift mutations, 13 nonsense mutations.

^b315 truncating mutations: 5 deletions, 139 frameshift mutations; 171 nonsense mutations.

^cOwing to lacking clinical data, the number of patients is lower than the total number of patients.

^dCleft palate is included in choanal atresia as these anomalies rarely occur together.

CONCLUSION

Immunological dysfunction, which predominantly affects T-cell function, has occasionally been described in patients with CHARGE syndrome. A prospective study, with systematically collected immunological and clinical data, is needed to explore the frequency and nature of their immune dysfunction. It would help identify clinical management issues in this infection-prone group of patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest. NutsOhra Foundation had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

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