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CHARGE (Coloboma, Heart Defect, Atresia Choanae, Retarded Growth and Development, Genital Hypoplasia, Ear Anomalies/Deafness) Syndrome and Chromosome 22q11.2 Deletion Syndrome: A Comparison of Immunologic and Nonimmunologic Phenotypic Features

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Pediatrics 2009;123;e871-e877
DOI: 10.1542/peds.2008-3400

ARTICLE

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Soma Jyonouchi, MD[a], Donna M. McDonald-McGinn, MS, CGC[b], Sherri Bale, PhD[c], Elaine H. Zackai, MD[b], Kathleen E. Sullivan, MD, PhD[a]

Divisions of [a]Allergy and Immunology and [b]Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; [c]Gene Dx, Gaithersburg, Maryland

Financial Disclosure: Dr Bale is the clinical director of Gene Dx, a company that offers genetic testing for the *CHD7* mutation; the other authors have no financial relationships relevant to this article to disclose.

What's Known on This Subject

CHARGE syndrome and 22q11.2 deletion syndrome have clinical overlap, but a direct comparison of features from 2 cohorts has not been evaluated. Immunodeficiency in CHARGE consists of case reports only. Immune function is not routinely evaluated in this syndrome.

What This Study Adds

This study identifies clinical features useful for differentiating between CHARGE syndrome and 22q11.2 deletion syndrome. Marked hypocalcemia and immunodeficiency can be seen in CHARGE syndrome. The latter indicates a need for immunologists to be involved in the care team.

ABSTRACT

OBJECTIVES. CHARGE (coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, ear anomalies/deafness) syndrome and chromosome 22q11.2 deletion syndrome are known to have significant clinical overlap including cardiac anomalies, ear abnormalities, hearing loss, developmental delay, renal abnormalities, and cleft palate. Immunodeficiency has been well documented in 22q11.2 deletion, but there has been limited recognition of this potentially serious complication in CHARGE syndrome. The goals of our study were to identify clinical features unique to CHARGE syndrome or 22q11.2 deletion and to describe the spectrum of immunodeficiency found in patients with CHARGE syndrome.

METHODS. This study included 25 children diagnosed with CHARGE syndrome with positive *CHD7* mutations through the Children's Hospital of Philadelphia genetics program. Clinical features and laboratory findings were reviewed retrospectively. We compared our findings to data available for a large cohort of patients with 22q11.2 deletion syndrome followed in our clinical genetics program.

RESULTS. Features found more commonly in CHARGE syndrome included coloboma, choanal atresia, facial nerve palsy, tracheoesophageal fistula, and genital hypoplasia in boys. A high incidence of marked hypocalcemia was observed in our study group (72%). We found a spectrum of cell-mediated immunodeficiency in our study group, which ranged from lymphopenia (60%) to severe combined immunodeficiency (8%). Defects in humoral immunity were documented in 4 patients and included severe hypogammaglobulinemia with decreased T-cell numbers, transient hypogammaglobulinemia during infancy, and immunoglobulin A deficiency.

CONCLUSIONS. The presence of coloboma, choanal atresia, facial nerve palsy, tracheoesophageal fistula, or genital hypoplasia in boys should alert the clinician to the possibility of CHARGE syndrome rather than the 22q11.2 deletion. Molecular testing for *CHD7* mutations may help to confirm the diagnosis. In this study, significant hypocalcemia and lymphopenia occurred more frequently in patients with CHARGE syndrome than in those with 22q11.2 deletion syndrome. Early inclusion of immunologists to the multidisciplinary care team (as with 22q11.2 deletion) may be of great benefit to affected patients. *Pediatrics* 2009;123:e871–e877

www.pediatrics.org/cgi/doi/10.1542/peds.2008-3400

doi:10.1542/peds.2008-3400

Key Words
CHARGE syndrome, *CHD7*, DiGeorge syndrome, chromosome 22q11.2 deletion, velocardiofacial syndrome, TBX-1, thymus, SCID, T cell, immunodeficiency, hypocalcemia

Abbreviations
CHARGE— coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, ear anomalies/deafness
DGS—DiGeorge syndrome
CHD7— chromodomain helicase DNA-binding protein 7
SCID—severe combined immunodeficiency
NK—natural killer
Ig—immunoglobulin

Accepted for publication Jan 21, 2009

Address correspondence to Soma Jyonouchi, MD, Children's Hospital of Philadelphia, Division of Allergy and Immunology, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104. E-mail: jyonouchi@email.chop.edu

CHARGE SYNDROME (OMIM 214800) is an autosomal dominant condition with occurrence between 1 per 10 000 and 1 per 8500 live births.[1,2] The clinical picture of CHARGE syndrome was originally described in 1979 by Hall[3] and Hittner et al.[4] In 1981, Pagon et al[5] developed the popular acronym of CHARGE (coloboma, heart defect, atresia

choanae, retarded growth and development, genital hypoplasia, ear anomalies/deafness). Additional features of this syndrome include cleft lip and palate, hearing loss, tracheoesophageal fistula, and cranial nerve dysfunction such as facial nerve palsy.[6] This syndrome has considerable phenotypic variability, with no single feature being present consistently.

Originally, CHARGE was considered to be a nonrandom association of anomalies rather than a syndrome. It was not until 2004 that Vissers and colleagues reported the presence of mutations in the chromodomain helicase DNA-binding protein-7 (*CHD7*) gene in 10 of 17 patients with CHARGE syndrome.[7] With improved detection techniques, *CHD7* mutations were later identified in 16 of 17 patients.[8] In a large cohort of 110 patients with CHARGE, Lalani et al[9] demonstrated the presence of *CHD7* mutation in 58% of patients. Similarly, Jongmans et al[8] reported that 69 of 107 (65%) patients, clinically diagnosed with CHARGE syndrome, in their cohort carried a *CHD7* mutation. The exact function of the *CHD7* gene has not been elucidated. However, chromo domain family proteins are known to regulate gene transcription.[10] In situ hybridization analysis of *CHD7* during human development has demonstrated expression of this gene in the central nervous system, semicircular canals, and the neural crest of the pharyngeal arches. That is, *CHD7* expression occurs in the organs affected in CHARGE syndrome.[11]

Chromosome 22q11.2 microdeletions result in a variable spectrum of clinical phenotypes including DiGeorge syndrome (DGS) and velocardiofacial syndrome. The incidence of 22q11.2 deletion is estimated to be between 1 in 3900 to 1 in 9700 live births.[12,13] Ninety percent of patients diagnosed with DGS (cardiac anomalies, hypocalcemia, immunodeficiency) and velocardiofacial syndrome (cardiac anomalies, pharyngeal dysfunction, dysmorphic facies) have a hemizygous 22q11.2 deletion.[14] The most common deletion, a 3 Mb region on chromosome 22, encompasses >35 genes. *TBX-1* has emerged as a leading gene responsible for the phenotypic features seen in this syndrome. Namely, *TBX-1* regulates the expression of downstream growth factors and transcription factors that are involved in development of the heart, thymus, parathyroid, and palate.[15] Homozygous *TBX-1* knockout mice have been shown to develop heart defects, thymic hypoplasia, cleft palate, and abnormal facial features similar to some patients with 22q11.2 deletion.[16]

It has long been recognized that CHARGE syndrome and chromosome 22q11.2 deletion syndrome have overlapping phenotypic features. These include cleft palate, cardiac malformations, ear abnormalities, hearing loss, growth deficiency, developmental delay, and renal abnormalities.[17–20] The existence of shared features and wide spectrum of clinical manifestation of these 2 syndromes can make initial diagnosis challenging. The current availability of molecular testing for both conditions provides an opportunity for improved early diagnosis, which can lead to better management. Proper diagnosis can also aid with genetic counseling because *CHD7* mutations usually occur sporadically, whereas 22q11.2 deletions are familial in 10% of cases.[9,21]

This study retrospectively reviewed 25 subjects with CHARGE syndrome and confirmed *CHD7* mutations. We compared the phenotypic features in these patients with features of patients with a 22q11.2 deletion available from a large cohort of patients followed at the Children's Hospital of Philadelphia. Our objective was to identify clinical features that would be most useful for differentiating between the 2 conditions. We also focused on analyzing the immunologic phenotype present in our population of patients with CHARGE syndrome for the purpose of improving clinical management.

METHODS

This study was a retrospective review of 25 patients with CHARGE syndrome and *CHD7* mutations diagnosed over a 10-year period from 1998 through 2008 in the Clinical Genetics Program at the Children's Hospital of Philadelphia. All patients were evaluated for the presence of phenotypic criteria for the diagnosis of CHARGE syndrome proposed by Blake et al[1] in 1998. The major criteria include coloboma, choanal atresia, ear anomalies/hearing loss, and cranial nerve dysfunction. The minor criteria include genital hypoplasia, developmental delay, cardiovascular malformations, growth deficiency, cleft palate, tracheoesophageal fistula, and abnormal facies. Developmental delay was defined as the presence of motor delay, hypotonia, or mental retardation.[6] We compared the clinical features of patients with CHARGE syndrome to clinical features of patients with a 22q11.2 deletion syndrome followed at the Children's Hospital of Philadelphia. Analysis of patient information was performed with the permission of the Children's Hospital of Philadelphia Internal Review Board.

Flow cytometric enumeration of lymphocyte subsets was available for 9 patients. For 4 patients, lymphocyte proliferative responses were evaluated by using phytohemagglutinin, concanavalin A, and pokeweed mitogens. For 8 patients, serum concentrations of Immunoglobulin G (IgG), IgA, and IgM were measured by nephelometry. Tetanus, diphtheria, and pneumococcal antibody titers were measured in 6, 5, and 5 patients, respectively. Two infants were vaccinated with pneumococcal protein conjugated vaccine, whereas the 3 remaining patients had no information available regarding the type of pneumococcal vaccine administered. In addition, no information was available regarding the exact time lapse after vaccinations.

RESULTS

The clinical features of patients with CHARGE syndrome were retrospectively analyzed in comparison to patients with a 22q11.2 deletion. The phenotypic features and significant laboratory findings of patients with CHARGE syndrome are summarized in Table 1. Table 2 summarizes the features of 22q11.2 deletion syndrome from a database of patients followed at the Children's Hospital of Philadelphia. It is of note that only 6 patients in our study group with confirmed *CHD7* mutations fulfilled

TABLE 1 Phenotypic Features in 25 Patients With a *CHD7* Mutation

Heart defect, *n/N* (%)[a]	23/25 (92)
Ear anomaly, *n/N* (%)[a]	21/25 (84)
Hearing loss, *n/N* (%)[a]	20/25 (80)
Genital hypoplasia, *n/N* (%)	11/14 (78)[b]
Hypocalcemia, *n/N* (%)[a]	18/25 (72)
Lymphopenia, *n/N* (%)[a]	15/25 (60)
Coloboma, *n/N* (%)	14/25 (56)
Cranial nerve dysfunction, *n/N* (%)	14/25 (56)
Swallowing (IX, X, XI)	11/25 (44)
Facial nerve palsy (V)	5/25 (20)
Developmental delay, *n/N* (%)[a]	13/25 (52)
Choanal atresia, *n/N* (%)	9/25 (36)
Growth deficiency, *n/N* (%)[a]	11/25 (44)[c]
Renal anomalies, *n/N* (%)[a]	8/25 (32)
Cleft palate, *n/N* (%)[a]	6/25 (24)
Tracheoesophageal fistula, *n/N* (%)	5/25 (20)

[a] Features common to both CHARGE and DiGeorge syndromes.
[b] Fourteen male patients with *CHD7* mutation.
[c] Fourteen patients were infants (growth deficiency is usually seen in older patients with CHARGE syndrome).

TABLE 2 Phenotypic Features in 22q11.2 Deletion (Children's Hospital of Philadelphia Database)

Heart defect, *n/N* (%)	397/547 (72)
Ear anomaly, *n/N* (%)	493/554 (89)
Hearing loss, *n/N* (%)	79/193 (41)
Hypocalcemia, *n/N* (%)	93/357 (26)
Genital hypoplasia, *n/N* (%)	6/334 (2)
Lymphopenia, *n/N* (%)[a]	39/131 (30)
Coloboma, *n/N* (%)	3/547 (0.5)
Cranial nerve dysfunction, *n* (%)	—
Choanal atresia, *n* (%)	—
Growth deficiency (height < 5%), *n/N* (%)	190/534 (35)
Renal abnormalities, *n/N* (%)	62/226 (27)
Developmental delay, *n/N* (%)	
Motor	37/40 (92)
Mental	31/40 (77)
Language	33/40 (82)
Cleft palate, *n/N* (%)	30/456 (6)
Submucosal cleft palate, *n/N n* (%)	25/456 (5)
Tracheoesophageal fistula, *n/N* (%)	1/102 (1)

[a] Absolute lymphocyte count of <2800 cells per mm^3 during the first year of life.

Blake's clinical criteria for CHARGE syndrome. The remaining 19 patients lacked the appropriate number of major or minor phenotypic criteria. This may be secondary to the age of patients in our study group, because more than half of the patients studied ($n = 14$) were infants at the time of evaluation. Some CHARGE features, such as growth deficiency and developmental delay may be difficult to detect early in life.[6]

Of the major Blake's criteria, 84% had ear anomalies and 56% of patients had coloboma. Hearing loss was present in 80% of patients. Of the remaining major criteria, 56% of patients had evidence of cranial nerve dysfunction (such as swallowing difficulty or facial nerve palsy) and 36% of patients had choanal atresia. Patients were also evaluated for the presence of Blake's minor criteria for CHARGE syndrome. Congenital heart disease was present in 92% of patients. Seventy-eight percent of male patients with CHARGE syndrome had evidence of genital hypoplasia. Developmental delay was found in 52% of patients and growth deficiency was noted in 44% of patients. Cleft palate was present in 24% of patients and tracheoesophageal fistula was found in 20% of patients.

Of note, 32% (8 of 25) of patients in this study died during infancy. In 3 patients, death was attributed to complications from severe cardiovascular disease. Two patients died from infectious complications (rhinovirus pneumonia and overwhelming sepsis). Three patients died from respiratory failure, 2 of whom had a confirmed phenotype of severe combined immunodeficiency (SCID).

We found a surprisingly high incidence of hypocalcemia in our CHARGE syndrome population (72%), compared with 26% in the 22q11.2 deletion cohort (Tables1 and 2). The hypocalcemia was typically present during the neonatal period and was often significant. In a number of our study patients, DGS was initially considered to be a likely diagnosis, given the presence of hypocalcemia coupled with congenital heart disease.

One of the most notable findings in our study group is that in comparison with age-matched reference ranges published by Comans-Bitter et al,[22] our patients had lymphopenia at high frequency (60%) (Table 1). Marked lymphopenia of <2000 cells per μL was present in 7 of 8 patients who had died during infancy. Lymphocyte immunophenotyping was available for 9 of the 25 patients, including 2 deceased patients. Table 3 summarizes the findings. Moderate decreases in $CD4^+$ T-helper and $CD8^+$ cytotoxic T cells were documented in 2 of 9 patients. Severe T-cell deficiency was documented in 2 of 9 patients as evidenced by an almost complete absence of T cells and markedly low proliferative responses to T-cell mitogens (Concanavalin A and Phytohemagglutinin). The remaining 5 of 9 patients had normal T-cell numbers appropriate for their age. The $CD56^+$ natural killer (NK) cell population and $CD19^+$ B-cell populations were normal except for 1 of 9 patients who had elevated $CD19^+$ cells.

Quantitative immunoglobulin studies and specific antibody levels for vaccine antigens were available for 8 of the 25 patients (Table 4). One patient had a low total IgG of 171 mg/dL during infancy with frequent sinopulmonary infections. Another patient had severe hypogammaglobulinemia (IgG: 56 mg/dL) and absent antibody titers at 5 months of age, despite vaccination, along with almost complete absence of T cells. Two of 8 patients had IgA deficiency based on undetectable levels of IgA at the ages of 6 and 8 years. Low antibody titers to tetanus and diphtheria were documented in 2 of 8 and 1 of 8 patients, respectively.

DISCUSSION

CHARGE syndrome is a disorder associated with multiple congenital malformations. *CHD7* has been identified as a major causative gene for this condition. Here, we report on the clinical features of 25 patients with CHARGE syndrome with confirmed *CHD7* mutations.

TABLE 3 Immunophenotyping in 9 Patients With CHARGE Syndrome

Patient No.	Age[a]	CD3, Cells per μL (Ref: 900–4500)	CD4, Cells per μL (Ref: 500–2400)	CD8, Cells per μL (Ref: 300–1600)	CD16/CD56, Cells per μL (Ref: 100–1000)	CD19, Cells per μL (Ref: 200–2100)	CD45RA, Cells per μL (Ref: 41–1121)	CD45RO, Cells per μL (Ref: 153–582)
1	2 y	324 (L)	203 (L)	82 (L)	214	456	105	94
2	3 mo	149 (L)	98 (L)	45 (L)	602	120	50	202
3	5 wk	3 (L)	1 (L)	2 (L)	827	340	12 (L)	1 (L)
4	5 mo	34 (L)	5 (L)	5 (L)	443	1370		
5	4 y	960	530	312	340	4503 (H)	379	134
6	2 mo	2647	1818	912	232	447	1625	175
7	1 wk	2359	1681	631	155	669	1449	140
8	4 mo	5815	4221	1125				
9	7 y	1011	300	563	338	418	85	316

Ref indicates reference. (L) indicates low numbers based on reference ranges and (H) refers to high numbers based on reference ranges.
[a] Age of the patient at the time of testing.

TABLE 4 Humoral Immunity in 8 Patients With CHARGE Syndrome

Patient No.	Age[a]	IgG, mg/dL (Ref: 477–1334)	IgM, mg/dL (Ref: 51–194)	IgA, mg/dL (Ref: 40–251)	Tetanus Antibody, IU/mL (Ref: >0.60 IU/mL)	Diphtheria Antibody, IU/mL (Ref: >0.10 IU/mL)	Pneumococcal Antibody, μL/mL
1	3 y	493	63	78	4.16	>5.00	14/14 protective
2	3 mo	171 (L)	38 (L)	13 (L)	NA	—	—
3	3 mo	249	50	7.1 (L)	NA	—	—
4	5 mo	56 (L)	38 (L)	<6 (L)	0.05 (L)	<0.1	0/14 protective
5	4 y	976	89	187	0.45 (L)	0.17	
8	4 mo	399	65	26	1.71	<0.1 (L)	2/14 protective
9	8 y	1160	44 (L)	<6 (L)	0.37 (L)	0.42	4/14 protective
10	6 y	950	52	<6 (L)	1.38		8/14 protective

NA indicates not applicable.
[a] At the time of testing.

The differential diagnosis for suspected CHARGE syndrome includes 22q11.2 deletion syndrome because of a number of overlapping clinical features. These include congenital heart defects, cleft palate, ear abnormalities, hearing loss, renal abnormalities, and developmental delay.

In our study, we found coloboma, choanal atresia, facial nerve palsy, tracheoesophageal fitula, and genital hypoplasia in boys to be features that are more often associated with CHARGE syndrome than 22q11.2 deletion syndrome. Coloboma was found in 56% of patients with CHARGE syndrome compared with 0.5% (3 of 564) in patients with a 22q11.2 deletion. Choanal atresia was present in 36% of patients with CHARGE syndrome. Although we do not have data for choanal atresia from our 22q11.2 deletion cohort, Rauch et al[23] have previously reported choanal atresia to be present in only 1% of their 22q11.2 deletion patients (n = 558). Cranial nerve dysfunction is one of Blake's major criteria for CHARGE syndrome. Facial nerve (cranial nerve V) palsy was noted to be present in 20% of patients with CHARGE syndrome. Although this feature is not seen in the 22q11.2 deletion syndrome, it should be noted that asymmetric crying facies has been described in this diagnosis. Swallowing difficulty in CHARGE syndrome, because of dysfunction of cranial nerves IX, X, and XI, was observed in 44% of patients, but this feature can have significant overlap with dysphagia and velopharyngeal insufficiency seen in the 22q11.2 deletion.[24] Thus, feeding or swallowing difficulty is not clinically useful for differentiating the 2 syndromes. Tracheoesophageal fistula was found in 20% of patients with CHARGE syndrome compared with only 1% of patients with the 22q11.2 deletion syndrome.[25] Other esophageal and airway abnormalities have previously been described in the 22q11.2 deletion including esophageal atresia, glottic and subglottic narrowing, tracheomalacia, and laryngeal cleft.[25,26]

Hearing loss was present in 80% of the patients with CHARGE syndrome, but was also present in a significant number of patients with a 22q11.2 deletion (41%). Developmental delay was present in 52% of patients with CHARGE syndrome and up to 92% of patients with a 22q11.2 deletion.[20] Hypocalcemia is a finding that is typically associated with the 22q11.2 deletion. Significant hypocalcemia, however, was documented in 72% of our patients with CHARGE syndrome. Therefore, we propose that hypocalcemia can be a common feature of CHARGE syndrome as well as 22q11.2 deletion syndrome.

Patients with a 22q11.2 deletion can have a small, hypoplastic thymus, which leads to low numbers of T cells. In some patients, thymic tissue may reside in extrathymic locations such as the neck or the retropharyngeal space enabling patients to generate T cells.[27] Patients can have decreased $CD3^+$ and $CD4^+$ T-cell counts, which are most severe during the first

year of life, but then often improve.[28,29] T-cell function in response to mitogen stimulation is typically normal. Total immunoglobulins and specific antibody response to vaccines are also normal in most patients. A minority of patients can have IgA deficiency or specific antibody deficiency.[30] Less than 1% of patients with a 22q11.2 deletion have complete absence of thymic tissue, which results in profound lymphopenia and impaired T-cell function.[31] These patients have a life-threatening form of SCID and are candidates for bone marrow or thymic transplant.

Although it is common practice to screen for immunodeficiency in patients with 22q11.2 deletion syndrome, the same cannot be said for CHARGE syndrome. Immunodeficiency in CHARGE syndrome has been described but is rarely included in the description of this disorder. This likely reflects the limited characterization of this clinical feature in publication. Immune defects reported in CHARGE syndrome include T-cell lymphopenia, impaired T-cell function, and low immunoglobulin levels. Even severe T-cell deficiency resembling SCID has been described.[32,33] The prevalence of immunologic abnormalities in CHARGE syndrome is difficult to estimate, because the current literature mainly consists of case reports, many of which lack genetic confirmation with *CHD7* mutation analysis.[33–36]

The data available for patients with proven *CHD7* mutations is limited. Gennery et al[32] described 4 patients with *CHD7* mutations and clinical features of CHARGE syndrome. Two of these patients had a T-B$^+$NK$^+$ form of SCID, whereas 2 other patients had features of Omenn syndrome (a SCID variant with eosinophilia and elevated IgE).[32] Writzl et al[37] described 2 *CHD7* mutation–positive patients with CHARGE syndrome with significant T-cell lymphopenia, but normal B-cell and NK-cell numbers. Markert and colleagues[38] have additionally described 8 patients diagnosed with DGS who had overlapping clinical features of CHARGE syndrome. These patients had severely depressed T-cell numbers, but the *CHD7* mutation analysis for these patients has not been reported.[33,38,39] We suspect that these patients may actually have CHARGE syndrome, given that they were all negative for the 22q11.2 deletion.

In our study, we found low absolute lymphocyte counts to be present in 60% of patients with CHARGE syndrome. In our 22q11.2 deletion cohort, 30% (39 of 131) of patients had an absolute lymphocyte count of $<$2800 cells per μL during the first year of life and 0.7% (1 of 131) had an absolute lymphocyte count of $<$1000 cells per μL. Two patients with CHARGE syndrome had almost complete absence of T cells with a phenotype of T-B$^+$NK$^+$ SCID. These results indicate that a wide spectrum of T-cell immunodeficiency can be seen in patients with CHARGE syndrome, a finding that is similar to the 22q11.2 deletion syndrome.

The exact etiology of lymphopenia found in CHARGE syndrome is unknown. However, our findings indicate a close association between lymphopenia and hypocalcemia: 14 of 18 patients with hypocalcemia revealed lymphopenia, whereas only 1 of 7 patients without hypocalcemia revealed lymphopenia. In 22q11.2 deletion, impaired embryogenesis of the third and fourth pharyngeal pouches that give rise to the thymus and parathyroid gland is implicated as the cause of T-cell lymphopenia and hypocalcemia. On the basis of our finding, it may be postulated that in CHARGE syndrome, a similar impairment of embryogenesis may result in lymphopenia and hypocalcemia.

Among our patients with CHARGE syndrome, 32% (8 of 25) died during infancy. In contrast, the overall mortality rate among our patients with a 22q11.2 deletion was only 5%.[21] Of particular concern is the finding that 7 of 8 patients with CHARGE syndrome who had died during infancy had profoundly low absolute lymphocyte counts of $<$2000 cells per μL. Flow cytometric analysis of lymphocyte populations was available for 2 of these patients and confirmed a phenotype of SCID. For the remaining 5 patients, lymphopenia was not further evaluated with immunophenotyping. This raises the possibility that these 5 patients had a form of severe combined immunodeficiency that may have contributed to their death.

Abnormalities in humoral immunity were also documented, with 2 patients having low total IgG (1 patient had normal specific antibody responses and the other patient had absent antibody responses along with low T-cell numbers), 2 patients having IgA deficiency, and 3 patients demonstrating low specific antibody titers to protein antigens.

We propose that all patients with CHARGE syndrome should be evaluated for defects in cell-mediated immunity as well as defects in humoral immunity. There was no correlation between the severity of *CHD7* mutations and the degree of immunodeficiency. Patients with significantly reduced T-cell numbers and function are susceptible to opportunistic infections and are at risk for developing life-threatening graft-versus-host disease or severe cytomegalovirus infections if given nonirradiated blood products. Live vaccines need to be avoided in this population to prevent severe, disseminated vaccine strain infections. Patients with SCID will typically die from infectious complications within the first few years of life without bone marrow transplantation. Patients demonstrating defects in humoral immunity may need replacement of circulating antibodies with intravenous immune globulin.

We must acknowledge a number of limitations in our study. Our cohort of patients with CHARGE syndrome was relatively small compared with the 22q11.2 deletion cohort. There is a potential skewing toward more severely affected patients who tend to be referred to tertiary care centers. For example, our finding of apparent increased incidence of DGS (hypocalcemia, cardiac disease, and immunodeficiency) may be a reflection of the limitations described above. Future studies with larger cohorts of patients with CHARGE syndrome would help to address these issues.

CONCLUSIONS

We report 25 subjects with CHARGE syndrome in whom *CHD7* mutations were identified. We found the clinical

features of coloboma, choanal atresia, facial nerve palsy, tracheoesophageal fistula, and genital hypoplasia in boys to occur with greater frequency in CHARGE syndrome than in 22q11.2 deletion syndrome. The finding of significant hypocalcemia, heart disease, and lymphopenia in our study group highlights the need to consider a diagnosis of CHARGE syndrome in addition to 22q11.2 deletion syndrome when evaluating patients with these features. A spectrum of cell-mediated immunodeficiency including SCID (necessitating bone marrow or thymic transplantation) can be present in CHARGE syndrome. This indicates a need for early involvement of immunologists into the multi-disciplinary care team, similar to the approach that is currently used in the care of patients with 22q11.2 deletion syndrome.

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Soma Jyonouchi, Donna M. McDonald-McGinn, Sherri Bale, Elaine H. Zackai and Kathleen E. Sullivan

Pediatrics 2009;123;e871-e877
DOI: 10.1542/peds.2008-3400