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CHARGE Syndrome and Chromosome 22q11.2 Deletion Syndrome: A Comparison of Immunologic and Non-Immunologic Phenotypic Features

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

[a]Division of Allergy and Immunology, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

[b]Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia

[c]Clinical Director, Gene Dx

[d]Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia

[e]Division of Allergy and Immunology, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Abstract

Objectives—CHARGE 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, hearing loss, and cleft palate. Immunodeficiency has been well documented in 22q11.2 deletion, but there is 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 immune deficiency found in CHARGE patients.

Methods—This study includes 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 22q11.2 deletion syndrome patients 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 males. A high incidence of marked hypocalcemia was observed in our study group (72%). We found a spectrum of cell-mediated immune deficiency in our study group, which ranged from lymphopenia (60%) to severe-combined immune deficiency (8%). Defects in humoral immunity were documented in 4

Corresponding Author: Soma Jyonouchi, MD., Division of Allergy and Immunology, The Children's Hospital of Philadelphia, 34th St and Civic Ctr Blvd, Philadelphia, PA 19104, (215) 590 2549 (215) 590 4529 (fax), Jyonouchi@email.chop.edu.

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patients and included severe hypogammaglobulinemia with decreased T-cell numbers, transient hypogammaglobulinemia during infancy, and IgA deficiency.

Conclusion—The presence of coloboma, choanal atresia, facial nerve palsy, tracheoesophageal fistula, or genital hypoplasia in males 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 CHARGE syndrome patients than in 22q11.2 deletion syndrome patients. Early inclusion of immunologists to the multi-disciplinary care team (as with 22q11.2 deletion) may be of great benefit to affected patients.

Keywords

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

INTRODUCTION

CHARGE syndrome (OMIM 214800) is an autosomal dominant condition with occurrence between 1 per 10,000 and 1 per 8,500 live births [1,2]. The clinical picture of CHARGE syndrome was originally described in 1979 by Hall *et al.* and Hittner *et al.*[3,4]. In 1981, Pagon *et al.* developed the popular acronym of CHARGE (**C**oloboma, **H**eart defect, **A**tresia choanae, **R**etarded growth and development, **G**enital hypoplasia, **E**ar anomalies/deafness) [5]. 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 non-random 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 out of 17 patients with CHARGE syndrome [7]. With improved detection techniques, *CHD7* mutations were later identified in 16 out of 17 patients [8]. In a large cohort of 110 patients with CHARGE, Lalani *et al*. demonstrated the presence of *CHD7* mutation in 58% of patients [9]. Similarly, Jongmans *et al*. reported that 69 out of 107 (65%) patients, clinically diagnosed with CHARGE syndrome, in their cohort carried a *CHD7* mutation [8]. 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, immune deficiency) 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 more than 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 two 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 two conditions. We also focused on analyzing the immunologic phenotype present in our population of patients with CHARGE 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 – 2008 in the Clinical Genetics Program at the Children's Hospital of Philadelphia. All patients were evaluated for the presence of phenotypic criteria for diagnosis of CHARGE syndrome proposed by Blake *et al*. 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 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 using phytohemagglutinin, concanavalin A, and pokeweed mitogens. For 8 patients, serum concentrations of 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, while for the 3 remaining patients no information was available regarding the type of pneumococcal vaccine administered. In addition, no information was available regarding the exact time lapse following 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 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, since 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% (8/25) 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, two of whom had a confirmed phenotype of severe combined immune deficiency (SCID).

We found a surprisingly high incidence of hypocalcemia in our CHARGE syndrome population (72%), compared to 26% in the 22q11.2 deletion cohort (Tables 1 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.*, our patients had lymphopenia at high frequency (60%) (Table 1) [22]. Marked lymphopenia of less than 2000 cells/mm^3 was present in 7 out of 8 patients who had died during infancy. Lymphocyte immunophenotyping was available for 9/25 patients, including 2 deceased patients. Table 3 summarizes the findings.

Moderate decreases in CD4+ T-helper and CD8+ cytotoxic T cells were documented in 2/9 patients. Severe T cell deficiency was documented in 2/9 patients as evidenced by an almost complete absence of T-cells and markedly low proliferative responses to T cell mitogens (Con A and PHA). The remaining 5/9 patients had normal T-cell numbers appropriate for their age. The CD56+ natural killer cell population and CD19+ B-cell populations were normal except for 1/9 patient who had elevated CD19+ cells.

Quantitative immunoglobulin studies and specific antibody levels for vaccine antigens were available for 8/25 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 out of 8 patients had IgA deficiency based on undetectable levels of IgA at the age of 6 years and 8 years. Low antibody titers to tetanus and diphtheria were documented in 2/8 and 1/8 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. The differential diagnosis for suspected CHARGE syndrome includes 22q11.2 deletion syndrome due to 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 males 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 to 0.5% (3/564) 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*. have previously reported choanal atresia to be present in only 1% of their 22q11.2 deletion patients (n = 558) [23]. Cranial nerve dysfunction is one of Blake's major criteria for CHARGE syndrome. Facial nerve (CN V) palsy was noted to be present in 20% of patients with CHARGE syndrome. While 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, due to dysfunction of cranial nerve 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 two syndromes. Tracheoesophageal fistula was found in 20% of patients with CHARGE syndrome compared to 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 patients with CHARGE, 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 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. Therefore, we propose that hypocalcemia can be a common feature of CHARGE syndrome as well as the 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 transplant or thymic transplant.

While it is common practice to screen for immune deficiency in patients with the 22q11.2 deletion syndrome, the same cannot be said for CHARGE syndrome. Immune deficiency 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*. described 4 patients with *CHD7* mutations and clinical features of CHARGE syndrome. Two of these patients had a T-B+NK+ form of SCID while 2 other patients had features of Omenn syndrome (a SCID variant with eosinophilia and elevated IgE) [32]. Writzl *et al*. described 2 *CHD7* mutation positive patients with CHARGE syndrome with significant T-cell lymphopenia, but normal B-cell and NK cell numbers [37]. Markert and colleagues have additionally described 8 patients diagnosed with DGS, who had overlapping clinical features of CHARGE. 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 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. In our 22q11.2 deletion cohort, 30% (39/131) of patients had an absolute lymphocyte count less than 2800 cells/mm^3 during the first year of life and 0.7% (1/131) had an absolute lymphocyte count less than 1000 cells/mm^3. 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 immune deficiency 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: 14/18 patients with hypocalcemia revealed lymphopenia while only 1/7 patients without hypocalcemia revealed lymphopenia. In 22q11.2 deletion, impaired embryogenesis of the 3rd and 4th 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% (8/25) died during infancy. In contrast, the overall mortality rate among our patients with a 22q11.2 deletion is only 5% [21]. Of particular concern is the finding that 7 out of 8 patients with CHARGE syndrome who had died during infancy had profoundly low absolute lymphocyte counts below 2000 cells/mm^3. Flow cytometric analysis of lymphocyte populations was available for two 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 immune deficiency that may have contributed to their death.

Abnormalities in humoral immunity were also documented, with 2 patients having low total IgG (one 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 immune deficiency. Patients with significantly reduced T-cell numbers and function are susceptible to opportunistic infections and are at risk for developing life-threatening GVHD or severe CMV infections if given non-irradiated 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 is relatively small compared to the 22q11.2 deletion cohort. There is a potential skewing towards 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 immune deficiency) 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

In summary, 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 males to occur with greater frequency in CHARGE syndrome than in the 22q11.2 deletion. 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 the 22q11.2 deletion syndrome when evaluating patients with these features. A spectrum of cell-mediated immune deficiency 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 the 22q11.2 deletion syndrome.

Abbreviations

DGS	DiGeorge syndrome
CHD7	Chromodomain helicase DNA-binding protein-7
SCID	Severe combined immune deficiency

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Table 1

Phenotypic features in 25 patients with a *CHD7* mutation

	n	%
Heart Defect†	23/25	92%
Ear Anomaly†	21/25	84%
Hearing Loss†	20/25	80%
Genital Hypoplasia	**11/14***	**78%**
Hypocalcemia†	18/25	72%
Lymphopenia†	15/25	60%
Coloboma	**14/25**	**56%**
Cranial Nerve Dysfunction	14/25	56%
- Swallowing (IX, X, XI)	11/25	44%
- Facial Nerve Palsy (V)	**5/25**	**20%**
Developmental Delay†	13/25	52%
Choanal Atresia	**9/25**	**36%**
Growth Deficiency†	11/25**	44%
Renal anomalies†	8/25	32%
Cleft Palate†	6/25	24%
TE Fistula	**5/25**	**20%**

*14 male patients with *CHD7* mutation

**14 patients were infants (growth deficiency is usually seen in older CHARGE patients)

†Features common to both CHARGE and DiGeorge syndrome

Table 2

Phenotypic features in 22q11.2 deletion (Children's Hospital of Philadelphia database)

	n	%
Heart Defect	397/547	72%
Ear Anomaly	493/554	89%
Hearing Loss	79/193	41%
Hypocalcemia	93/357	26%
Genital Hypoplasia	6/334	2%
Lymphopenia*	39/131	30%
Coloboma	3/547	0.5%
Cranial Nerve Dysfunction	n/a	n/a
Choanal Atresia	n/a	n/a
Growth Deficiency (ht < 5%)	190/534	35%
Renal Abnormalities	62/226	27%
Developmental Delay		
- Motor	37/40	92%
- Mental	31/40	77%
- Language	33/40	82%
Cleft Palate	30/456	6%
Submucosal Cleft Palate	25/456	5%
TE Fistula	1/102	1%

*Absolute lymphocyte count of less than 2800 cells/mm^3 during the first year of life

Table 3

Immunophenotyping in 9 patients with CHARGE syndrome

Reference	Age*	CD3 Cells/uL (900–4500)	CD4 Cells/uL (500–2400)	CD8 Cells/uL (300–1600)	CD16/CD56 Cells/uL (100–1000)	CD19 Cells/uL (200–2100)	CD45RA Cells/uL (41–1121)	CD45 RO Cells/uL (153–582)
Patient #1	2yo	324 (L)	203 (L)	82 (L)	214	456	105	94
Patient #2	3mo	149 (L)	98 (L)	45 (L)	602	120	50	202
Patient #3	5wk	3 (L)	1 (L)	2 (L)	827	340	12 (L)	1 (L)
Patient #4	5mo	34 (L)	5 (L)	5 (L)	443	1370		
Patient #5	4yo	960	530	312	340	4503 (H)	379	134
Patient #6	2mo	2647	1818	912	232	447	1625	175
Patient #7	1wk	2359	1681	631	155	669	1449	140
Patient #8	4mo	5815	4221	1125				
Patient #9	7yo	1011	300	563	338	418	85	316

*Age of patient at the time of testing

Table 4

Humoral Immunity in 8 patients with CHARGE syndrome

Reference	Age*	IgG mg/dl (477–1334)	IgM mg/dl (51–194)	IgA mg/dl (40–251)	Tetanus Ab IU/ml >0.60 IU/ml	Diptheria Ab IU/ml >0.10 IU/ml	Pneumococcal Ab ug/ml
Patient #1	3yo	493	63	78	4.16	>5.00	14/14 protective
Patient #2	3mo	171 (L)	38 (L)	13 (L)	n/a	n/a	n/a
Patient #3	3mo	249	50	7.1 (L)	n/a	n/a	n/a
Patient #4	5mo	56 (L)	38 (L)	<6 (L)	0.05 (L)	<0.1	0/14 protective
Patient #5	4yo	976	89	187	0.45 (L)	0.17	
Patient #8	4mo	399	65	26	1.71	< 0.1 (L)	2/14 protective
Patient #9	8yo	1160	44 (L)	<6 (L)	0.37 (L)	0.42	4/14 protective
Patient #10	6yo	950	52	<6 (L)	1.38		8/14 protective

*Age at the time of testing