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Congenital T Cell Deficiency in a Patient with CHARGE Syndrome

Julie Hoover-Fong, MD, PhD, **William J. Savage, MD**, **Emily Lisi, MS**, **Jerry Winkelstein, MD**, **George H. Thomas, PhD**, **Lies H. Hoefsloot, PhD**, and **David M. Loeb, MD, PhD**
McKusick-Nathans Institute of Genetic Medicine, Department of Pediatrics, Johns Hopkins University, Baltimore, MD (J.H-F., E.L.); Division of Pediatric Hematology, Johns Hopkins University, Baltimore, MD (W.S.); Division of Allergy and Immunology, Department of Pediatrics, Johns Hopkins University, Baltimore, MD (J.W.); Department of Genetics, Kennedy Krieger Institute, Baltimore, MD (G.T.); Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands (L.H.); and Division of Pediatric Oncology, Department of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD (D.L.).

Abstract

CHARGE syndrome is an autosomal dominant condition caused by mutations in chromodomain helicase DNA-binding 7. We report a patient with molecularly confirmed CHARGE syndrome, which included a congenital T cell deficiency, who was treated with peripheral blood mononuclear cell transplantation.

The acronym CHARGE refers to the multiple congenital anomaly syndrome of Coloboma, Heart defects, Atresia of the choana(e), Retardation of growth and development and/or central nervous system anomalies, and Genital and Ear anomalies and/or deafness.[1,2] The incidence and prevalence are estimated at 1:8500 and 1:10 000, respectively.[1,3] Additional features subsequently associated with this diagnosis include cochlear and vestibular anomalies, hypothalamic-pituitary dysfunction, and orofacial clefts.[1,2,4-7] Revised diagnostic criteria include ocular coloboma, choanal atresia, and semicircular canal hypoplasia (as high as 90%, 65%, and 95%, respectively) as the 3 features with the highest concurrent incidence and most predictive of an associated CHD7 mutation in a suspected patient with CHARGE.[2,6,8] Immune dysfunction is rarely described with the CHARGE phenotype.[8-11]

CHARGE syndrome shares several features with DiGeorge syndrome, also known as 22q11 deletion or velo-cardio-facial syndrome.[12,13] Features of DiGeorge syndrome include congenital heart defects, velopharyngeal clefting, hypoparathyroidism, and T-cell immunodeficiency. Characteristic CHARGE features of coloboma, choanal atresia, and inner ear anomalies are not typically found in patients with 22q11 deletion syndrome,

Reprint requests: Julie Hoover-Fong, 600 N Wolfe St, Blalock 1008, Johns Hopkins Hospital, Baltimore, MD 21287. jhoover2@jhmi.edu..

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although haploinsufficiency of Tbx1 at this locus in the murine model is implicated in inner ear malformations.[14] Markert et al reported thymic transplantation outcome for 44 patients with DiGeorge anomaly (defined by the authors as a developmental defect in the thymus and parathyroid glands as a result of malformations of the third and fourth pharyngeal pouches), which included patients with DiGeorge syndrome (22q11 deletion) and others with features of CHARGE syndrome.[15] Bowers et al and Bensoussan et al have reported the successful transplantation of peripheral blood mononuclear cells (PBMC) from an HLA-matched sibling in individual patients with DiGeorge.[16,17] No patients with CHARGE syndrome and immune dysfunction have been treated with PBMC transplantation.

CASE REPORT

A 3-week-old male was born at 41 weeks to a healthy 28-year-old primiparous mother and 29-year-old father with birth length, weight, and head circumference between the 25th to 50th percentiles. Family history was noncontributory, and there was no known consanguinity. The child had unilateral choanal atresia, grade 5 vesicoureteral reflux, glottic web, an aberrant left subclavian artery, perinatal hypocalcemia, and a cupped auricle: features that overlap the CHARGE phenotype and 22q11 deletion syndrome. Bilateral aplasia of the semicircular canals and cochlear hypoplasia were confirmed with computed tomography, features supportive of the CHARGE diagnosis.[2,4,6] There was no coloboma, and genitalia were normal. Other abnormalities included ankyloglossia, micro-gastria, vertical talus, scoliosis, 2 to 3 toe syndactyly, and laryngomalacia that required tracheostomy.

DIAGNOSTIC EVALUATION AND CLINICAL COURSE

Initial absolute lymphocyte count was low (980 cells/ μL; 60% B cells, 40% NK cells, and 0.4% T cells). Additionally, the lymphocytes had poor mitogenic response to phytohemagglutinin stimulation, which is used routinely to promote lymphocyte proliferation in preparation for karyo-type analysis (46, XY, normal). In the next 6 weeks, the percentage of T cells increased spontaneously to 6.6%, although the absolute number of T cells remained low (84/ μL). Fluorescence *in situ* hybridization for 22q11 deletion on interphase nuclei was normal in peripheral blood and fibro-blasts. Mononuclear cells lacked response to mitogenic stimuli. Human immunodeficiency virus polymerase chain reaction was negative. There was no thymus on chest radiograph or computed tomography. Thymic transplant and allogeneic PBMC infusion were proposed as the 2 best treatment options, and the family opted for the latter.

At 3 months of age, the patient received 4 days of 1 mg/kg/day fludarabine, followed by a peripheral blood mono-nuclear cell transplant consisting of 4×10^7 nucleated cells/kg and 5×10^6 T cells/kg. The donor was an unrelated 10/10 HLA-matched, cytomegalovirus-negative, 38-year-old male. DNA microsatellite quantification was used to follow donor engraftment and was also used to exclude maternal engraftment. Ten days after infusion, grade 3 acute graft versus host disease (GVHD) was diagnosed, manifested in the skin, which was treated with daclizumab and cyclosporine. Two months after transplant, functional engraftment was demonstrated when the patient successfully cleared a culture-

proven pertussis infection. After transplant, the patient had increased peripheral T cell counts with detectable donor PBMCs (Figure 1). Mitogen-responsive host cells were also detected. This was surprising because the patient had no demonstrable response to mitogen stimulation before PBMC infusion. However, PBMC T cell receptor excision circles, a marker of naive T cell output from the thymus, assayed 14 months after transplant failed to demonstrate host thymic function.

Concurrent with his PBMC transplant, Vissers et al demonstrated that mutations in the CHD7 gene at chromo-some 8q12 cause the CHARGE phenotype.[18] CHD7 is a member of the chromodomain helicase DNA-binding (CHD) gene superfamily, believed to play an important role in early embryogenesis and regulation of chromatin structure and gene expression.[19] Detection of a heterozygous pathogenic mutation at nucleotides g. 6155_6156CT>AG in exon 31 of CHD7 confirmed the clinical diagnosis (Figure 2). The CT>AG is a nonsense mutation leading to premature termination (S2052X) and subsequent haploinsufficiency. Parental lymphocytes had a wild type sequence; therefore, the nucleotide changes in the proband were most likely *de novo*. A heterozygous sequence variation was detected immediately adjacent (g.6157C>A), but is not predicted to change the wild-type arginine residue.

Ten months after transplant, chronic pleural effusions, pulmonary edema, bowel edema, anasarca, Evans syndrome, and left ventricular dysfunction developed in the patient at the same time he became HHV6 positive. After several courses of ganciclovir, his persistent HHV6 viremia cleared. Pulmonary nodules developed on computed tomography scan, and the patient was found to have pulmonary veno-occlusive disease by means of open biopsy at 14 months after transplant. The patient died suddenly of acute renal failure and intractable hyperkalemia, for which no infectious, transfusion, or pharmacologic cause could be found.

DISCUSSION

Despite the unfortunate clinical outcome for this patient, he maintained low level engraftment throughout his entire course after PBMC transplant. He also demonstrated a host response to mitogen stimulation after transplant. This was not expected on the basis of absent pre-transplant mitogen stimulation response. There is no clear explanation for this apparent spontaneous recovery of host immune function. Perhaps this is evidence of extra-thymic T cell production.[20,21] Considering the role CHD7 is thought to play in gene expression, another possibility for host immune recovery may involve mutation-associated loss of CHD7 function resulting in fortuitous alteration of immune-related gene expression.

Although CHD7 has been clearly implicated as a causative gene for CHARGE syndrome, genetic heterogeneity must be present because this gene is responsible for only 60% to 65% of all cases.[6,8] Because of the high cost of sequencing this relatively large gene and because 35% to 40% of cases will not have a CHD7 mutation, CHARGE syndrome may be considered a clinical diagnosis. However, with discovery of at least 1 causative gene, broadening of the CHARGE phenotypic spectrum,[4,6] and refinement of the clinical diagnostic criteria,[2,6,8] more patients lacking the traditional acronym features will undoubtedly receive the diagnosis. CHD7 molecular confirmation of this patient adds

validity to the recent cohort review of 15 patients with CHARGE syndrome with a clinical diagnosis and similar T cell deficiency[11] that immunodeficiency should be included as part of the CHARGE spectrum.

Immune deficiency should be considered as part of the CHARGE syndrome phenotype. This diagnosis should be considered in patients with congenital T cell deficiency of unknown etiology, particularly when at least 1 of the features—coloboma, choanal atresia, or abnormal semicircular canals—is present. Allogeneic PBMC transplant is a viable treatment option for congenital T cell deficiency on the basis of this patient's successful engraftment and the relative accessibility of PBMCs.

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Glossary

CHARGE	Multiple congenital anomaly syndrome of Coloboma, Heart defects, Atresia of the choana(e), Retardation of growth and development and/or central nervous system anomalies, and Genital and Ear anomalies and/or deafness
GVHD	Graft versus host disease
PBMC	Peripheral blood mononuclear cells

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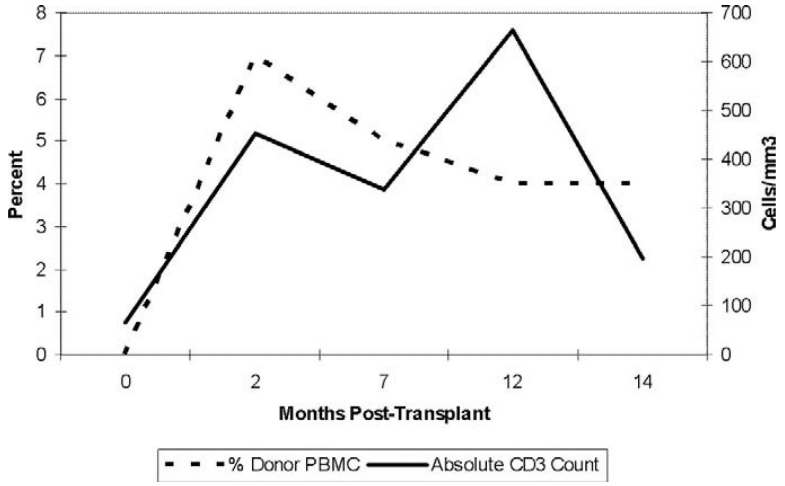

Figure 1.

Recipient peripheral T cell counts and donor chimerism. The percent of the PBMC from the donor is shown simultaneously with the absolute CD3 number from the time of transplantation at time zero. After PBMC transplant, the patient demonstrated increased peripheral T cell counts with detectable donor PBMCs throughout his life.

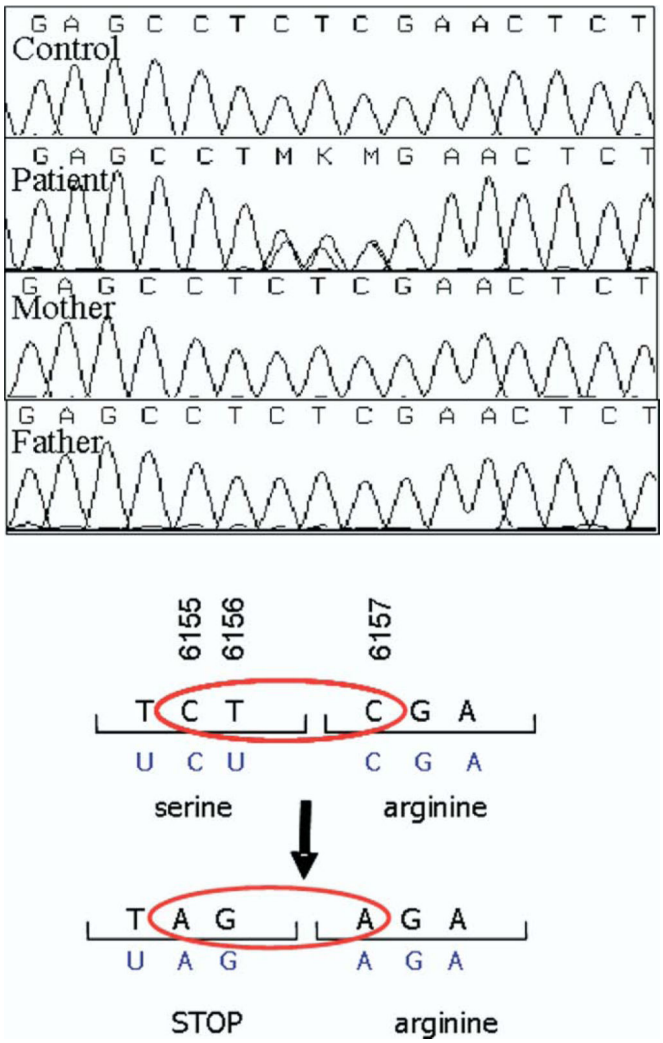

Figure 2.

Sequence analysis. Genomic DNA isolated according to standard procedures from a fibroblast cell line from proband and peripheral blood of the proband's parents. Thirty-seven coding exons of CHD7 (exon 2-38, accession number NM_017780) and their flanking intron sequences amplified by polymerase chain reaction. Sequence analysis performed with 3730 automated sequencer (AB). A heterozygous pathogenic mutation g.6155_6156 CT>AG (S2052X) was detected in exon 31 in CHD7 gene of the proband. This mutation was not detected in either parent. Thus, the nucleotide changes in the proband were *de novo* and most likely on the same allele, causing truncation of the CHD7 protein product. A heterozygous sequence variation was detected immediately adjacent at g.6167C>A, but is not predicted to change the wild-type arginine residue.