

A case of atypical, complete DiGeorge syndrome without 22q11 mutation



A 4-month-old male infant presented in consult to allergy and immunology with eosinophilia (3 separate counts $>3,000/\text{mm}^3$) and an erythrodermic rash. The infant had known unilateral kidney agenesis, tetralogy of Fallot, pulmonary atresia, and ventriculoseptal defect. He had undergone an initial cardiac repair with a Blalock-Taussig shunt at 19 days of life. The patient received 1 transfusion, with irradiated packed red blood cells, at 1 month of age. At 2 months of age, the infant had been admitted to the intensive care unit for hypotension presumed secondary to sepsis from an unclear source. He was treated with vancomycin and cefepime until the presence of respiratory syncytial virus was detected. At 3 months of age, necrotizing enterocolitis was suspected because of hemodynamic instability and blood-flecked stools. He was treated empirically with vancomycin and piperacillin plus tazobactam. One week after completing treatment, he was noted to have increasing transaminases, splenomegaly, eosinophilia, and rash, at which point a drug-related rash was suspected.

The physical examination at 4 months showed an irritable child with normal facies save for low-set posteriorly rotated ears with a slight notch, erythroderma (Fig 1), easily plucked hair (eFigs 1 and 2), hepatomegaly, splenomegaly, and cardiac murmur. Chart review showed absolute lymphopenia, with the absolute lymphocyte count ranging from 500 to $650/\text{mm}^3$ consistently during the first 2 months of life increasing to a normal absolute lymphocyte count of $4,550/\text{mm}^3$ at 4 months (Table 1 lists repeated laboratory values). Absolute eosinophil counts gradually trended from $500/\text{mm}^3$ at 2 months of age to $4,000/\text{mm}^3$ at 4 months. Transaminases were increased, with an aspartate aminotransferase level of 350 U/L and an alanine aminotransferase level of 700 U/L. Review of operative notes and chest x-rays suggested an absent thymus. Fluorescence in situ hybridization for a 22q11.2 deletion was normal, although chromosomal microarray showed several large regions of homozygosity encompassing more than 2.4% of the child's genome, suggesting the parents were related as second cousins. Family history was negative for increased infections or early childhood deaths. The mother had uncontrolled diabetes mellitus during pregnancy and a history of lymph node abscess. The father's history was not available.

Skin biopsy performed at 4 months for Langerhans cell histiocytosis showed acanthosis, spongiotic epidermal changes, and sarcoidal-appearing granulomas without any signs of Langerhans cells. CD3 staining showed that most lymphocytes present in the granulomas were T cells. Bone marrow biopsy to test for myelodysplastic hypereosinophilic syndrome diagnosis was done at 4 months of age, showing 4% B cells, 16% T cells, no increase in blasts by CD33, CD34, and CD45 or on forward scatter or side scatter gating, and normal platelet-derived growth factor gene mutation analysis results. The clinical history was not consistent with drug rash given the length of time from possible offending agents and because the patient's eosinophilia had been present before the initiation of antibiotics. As time went on, the infant's rash and eosinophilia continued despite avoidance of antibiotics that he previously received. Strongyloides titers were negative.

An immunologic workup at 4 months showed normal baseline levels of immunoglobulin M (87 mg/dL, reference 20–90 mg/dL), A (19 mg/dL, reference 8–91 mg/dL), and G (308 mg/dL, reference 110–700 mg/dL), but an increased immunoglobulin E level (1,664 IU/mL, reference 0–30 IU/mL). Tetanus and pneumococcal

immunoglobulin G titers were non-protective; the patient was not yet unvaccinated. Flow cytometry showed an increased total $\text{CD}3^+$ cell count of 3,640 (reference 1,700–3,600) but normal $\text{CD}3^+/\text{CD}4^+$ cell counts of 2,639 (reference 1,000–2,800), $\text{CD}3^+/\text{CD}8^+$ cell counts of 1,001 (reference 800–1,500), and $\text{CD}19^+$ cell counts of 728 (reference 500–1,500). There was a mild decrease in absolute $\text{CD}56^+/\text{CD}16^+$ cell counts at $136/\text{mm}^3$ (reference 200–700/ mm^3), although counts were normal subsequently. Lymphocyte proliferation assays were low to phytohemagglutinin, pokeweed, and concanavalin A. T-cell receptor rearrangement excision circle copies were undetectable despite normal absolute $\text{CD}3^+$, $\text{CD}4^+$, and $\text{CD}8^+$ cell counts. T-cell receptor variable region β spectra typing showed oligoclonal T-cell populations (eFig 3). T-cell phenotyping showed a complete absence of naïve T cells and the T cells that were present were of an activated phenotype (Table 1). Studies of recent thymic emigrants showed absent recent thymic emigrants despite an increased total absolute CD4 cell count of $3,291/\text{mm}^3$ (reference 153–1,745/ mm^3), suggesting no thymic output. Subsequent maternal engraftment studies showed the absence of the maternal XX karyotype in the whole blood sample of the male infant.

The differential diagnosis narrowed to a leaky version of severe combined immunodeficiency disease or Omenn syndrome vs atypical complete DiGeorge syndrome with oligoclonal T cells. Subsequent genetic test results for *RAG1*, *RAG2*, *ARTEMIS*, *CD3D*, *CD3E*, *CD3Z*, *IL2RG*, *IL7R*, *JAK3*, *PNP*, *PTPRC*, *RMRP*, and *ZAP70* gene mutations was negative. The results suggested that the patient had thymic aplasia and an atypical, complete DiGeorge syndrome with autoreactive T cells, although autoreactivity was not confirmed. Subsequent *TBX1* and *CHD7* gene sequence analyses to search for



Figure 1. Patient's rash at 4 months of life. Note alopecia, erythroderma, and desquamation.

Table 1
Trends in Laboratory Testing Over Time

Variable	Reference range (for 4 mo of age) ^a	Birth	2 mo of age	4 mo of age—diagnosis	1 y of age—before thymus transplantation (on cyclosporine)
Complete blood cell count					
Hemoglobin (g/dL)	13.5–22.6	13.4		12.0	14.8
MCV (fL)	93–118	97		94	
RDW (%)	11.1–14.3	15.5		18.4	
Platelet count ($\times 10^3/\mu\text{L}$)	140–300	275		362	306
MPV	10.2–12	9.3		9.9	
WBC count ($\times 10^3/\mu\text{L}$)	4.0–14.6	10 (age-adjusted ref 9.4–31.1)	14.1	21.0	9.6
Absolute lymphocyte count ($\times 10^3/\mu\text{L}$)	2.5–9.8	1.95 (age-adjusted ref: 2.8–9.6)	1.26	7.94	3.17
Absolute neutrophil count ($\times 10^3/\mu\text{L}$)	1.5–7.7	5.95 (age-adjusted ref 5.9–19.9)	11.56	6.85	5.4
Absolute monocyte count ($\times 10^3/\mu\text{L}$)	0.2–1	1.94	0.82	1.47	
Absolute basophil count ($\times 10^3/\mu\text{L}$)	0.01–0.08	0.02	0.02	0.09	
Absolute eosinophil count ($\times 10^3/\mu\text{L}$)	0–0.5	0.08	0.43	4.58	2.00
Flow cytometry^a					
Total CD3 ⁺ cell count	2,500–5,600			3,640	1,137
CD4 ⁺ T-cell count	1,800–4,000			2,639	868
CD8 ⁺ T-cell count	590–1,600			1,001	247
NK-cell count	200–700			136	371 and 1,375
B-cell count	430–3,000			728	1,597
% CD4 ⁺ cells that are CD45RA ⁺ (% CD4 ⁺ T cells)	Ref: comparison with pediatric reference range 2–18 y <50%			0	
% CD4 ⁺ cells that are CD45RO ⁺ (% CD4 ⁺ T cells)	Ref: comparison with pediatric reference range 2–18 y $\geq 15\%$			99	
% CD4 ⁺ /CD31 ⁺ cells that are CD45RA ⁺ (% CD4 ⁺ T cells)	Ref: comparison with pediatric reference range 2–18 y 19.4–60.9%			0	
% CD4 ⁺ /CD62L ⁺ cells that are CD45RA ⁺	Ref: comparison with pediatric reference range 2–18 y 15–71%			0	0.3
% CD8 ⁺ /CD62L ⁺ cells that are CD45RA ⁺	Ref: comparison with pediatric reference range 2–18 y 8–81%			8	0.2
Evaluation for maternal engraftment by karyotyping for XX				Negative	
Lymphocyte proliferation studies					
Phytohemagglutinin 1:20 stimulated (counts/min)	Control 531,285			Patient 86,604 (16% of control)	
Concanavalin A 1:40 stimulated (counts/min)	Control 810,363			Patient 5,134 (0.6% of control)	
Pokeweed mitogen 1:40 stimulated (counts/min)	Control 385,733			Patient 3,575 (0.9% of control)	
Liver–renal function testing					
AST (U/L)	34–518	104 (age-adjusted ref 32–162)		350	8 mo old (on cyclosporine) 380
ALT (U/L)	20–67	21 (age-adjusted ref 5–33)		700	38
Alkaline phosphatase (U/L)	134–518	122 (age-adjusted ref 90–273)		389	380
γ -Glutamyl transferase (U/mL)	8–127	97		238	82
Total bilirubin (mg/dL)	0.2–0.7	8.6 (age-adjusted ref 0.2–12)		0.6	0.4
Blood serum nitrogen (mg/dL)	3–23	8		25	42
Creatinine (mg/dL)	0.42–1.05	0.64		0.39	0.47
CRP (mg/L)	0.1–1.0	0.5		41.5	0.6

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; MCV, mean corpuscular volume; MPV, mean platelet volume; NK, natural killer; RDW, red blood cell distribution width; ref, reference; WBC, white blood cell.

^aFlow cytometric reference range for 4-month-olds from Braun E, *The Harriet Lane Handbook*. 20th ed. Philadelphia, PA: Elsevier Saunders; 2015:334–349. All other laboratory reference ranges are age-adjusted reference ranges at the Vanderbilt University Medical Center.

known DiGeorge or CHARGE syndrome (coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities) variants also returned negative. Data have shown that only half the patients with complete DiGeorge syndrome have 22q11.2 deletion syndrome.¹ Whole exome sequencing was not obtainable because of financial constraints.

The patient was initially treated at 6 months of age with cyclosporine, 2 mg/kg of intravenous (IV) methylprednisolone for 3 days, topical emollients, and immunoglobulin replacement. His rash and organ dysfunction showed significant improvement within days of therapy initiation and at 7 months of age he was able to receive full repair of his cardiac condition. His rash showed further improvement with the addition of daily fluocinolone oil and pimecrolimus creams. Subsequently, he was stable for discharge at 9 months on oral cyclosporine and infusions of IV immunoglobulin. The patient was noted to have rhinovirus and parainfluenza virus 3 infection at routine testing of a nasopharyngeal aspirate at 12 months of age. He developed acute varicella infection at 13 months of life with rash and a varicella zoster virus (VZV) copy number of 11,400 copies/mL. He was treated with IV acyclovir for 3 weeks and then was changed to valacyclovir. At 14 months of age, he underwent laparoscopic esophagogastric fundoplasty. One week later, the VZV copy number increased to 900 and then to 5,500 copies/mL. He developed an oxygen requirement and enlarged skin lesions. He was put back on IV acyclovir and the VZV copy number decreased to 1,000/mL. He received a cultured allogeneic thymus transplant at 14.75 months of age with pretransplantation rabbit antithymocyte globulin and cyclosporine therapy. Unexpectedly, he could not be extubated until 3 days after transplantation. He did fairly well until 17 days after surgery, when he required initiation of high-flow oxygen; he was reintubated 21 days after transplantation. He required the oscillator and high oxygen concentrations, had worsening renal function, and did not respond to diuretic therapy. He succumbed to respiratory failure on day 25 after thymus transplantation, presumably due to parainfluenza virus 3, because 5 days before death his VZV copy number was lower than 278/mL and parainfluenza virus 3 was still present in his secretions.

This patient met the criteria for complete DiGeorge syndrome based on the absence of naïve T cells and congenital heart disease.^{2,3} Importantly, 22q11.2 deletion syndrome is not needed for the diagnosis of DiGeorge anomaly. This patient's mother had diabetes.^{4,5} Infants like this patient represent approximately 15% of infants receiving thymus transplants for complete DiGeorge anomaly.¹ Patients in this group often have single kidneys.^{4,6,7} This patient developed an Omenn syndrome phenotype with oligoclonal T cells and rash at 4 months of age.⁸ These findings are characteristic of atypical, complete DiGeorge syndrome.⁹ This case might be of benefit to those who are faced with the difficult challenge of evaluating persistent eosinophilia and erythroderma in infancy. It is important to realize that not all cases of severe T-cell deficiency present with lymphopenia. Most important for the practicing physician to know from this case is that the final common pathway for the immune dysfunction in DiGeorge syndrome is failure of normal thymus formation and the resulting inability to create normal mature T cells. Potentially, had newborn screening for severe combined immunodeficiency disease been performed for this patient, he would have been identified and treated rapidly, perhaps avoiding the multiple infections that led to his death. Newborn T-cell receptor excision circles screening implementation

is becoming standard practice across state screening programs in the United States and can capture infants who cannot perform T-cell receptor rearrangement successfully.¹⁰ This should result in early diagnosis of patients similar to the present one. As T-cell receptor excision circles screening programs become the standard across the country, it will be crucial to remember that 22q11 mutations are not the only pathway by which atypical, complete DiGeorge syndrome can occur.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.janai.2017.02.025>.

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eFigure 1. Image of patient's leg at age 3 months shows an exfoliative dermatitis with a greasy-appearing crust.



eFigure 2. Image of patient's scalp at age 3 months showing recession of hair line related to dermatitis, exfoliative dermatitis, and easily plucked hair. Compare with image in [Figure 1](#), which was taken at age 4 months, to note the progression of rash and hair loss.

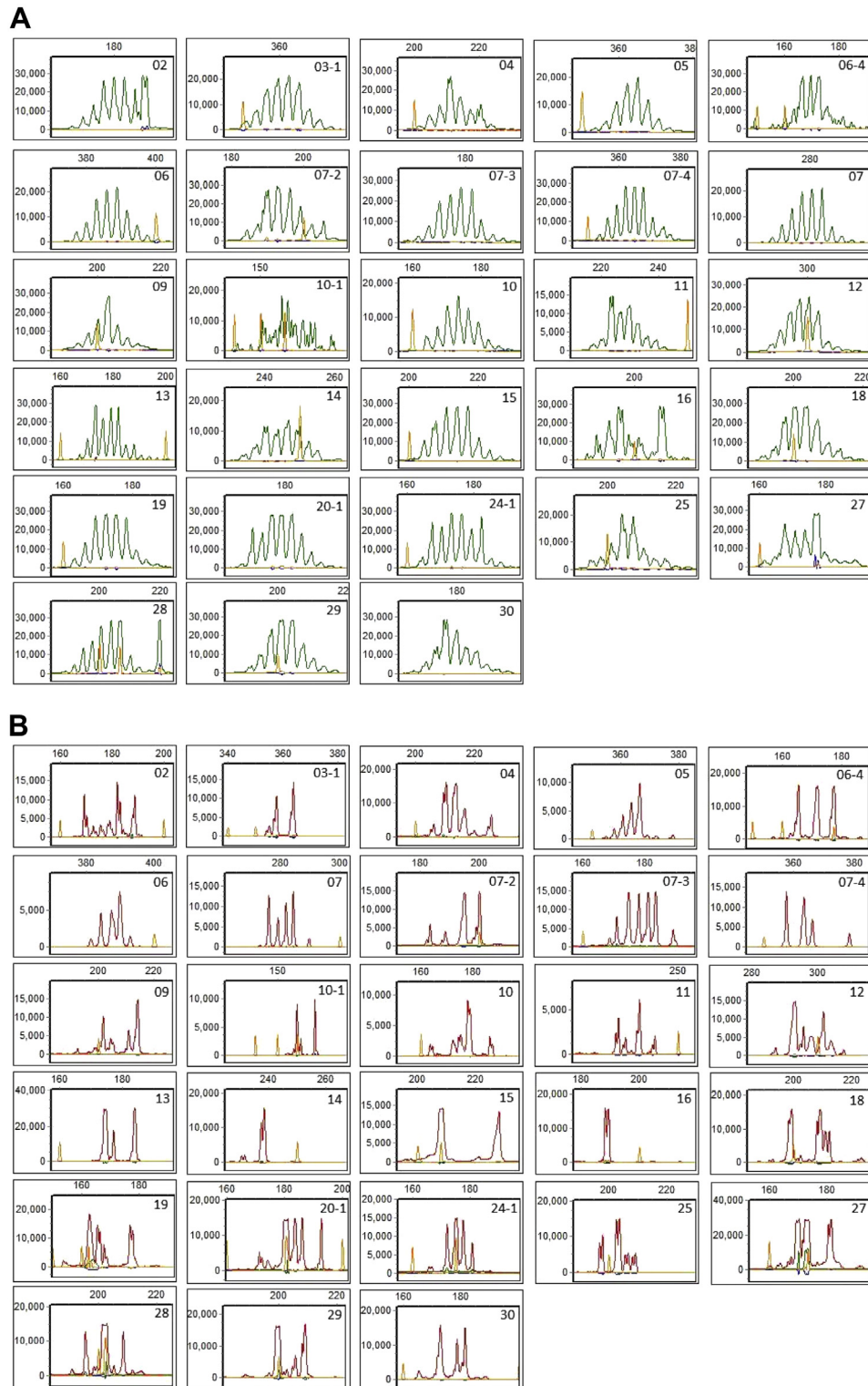


Figure 3. T-cell receptor variable region β spectra type for healthy control (A) and patient (B). T-cell receptor variable region β repertoire diversity analysis is meant to assess the breadth of T-cell diversity, which is an indirect reflection of thymic function and output. T-cell receptor variable region β spectra typing is performed for 24 T-cell receptor variable region β families, including some subfamilies for which consensus primers could not be used. Each graph represents a specific T-cell receptor variable region β family or subfamily. The x-axis (top) provides the size in base pairs and the y-axis provides fluorescence units, which indirectly correlate with the amount of transcript present in each peak, which represents at least 1 CDR3 fragment of the same length. The orange peaks represent size markers used in the assay. The assay is performed by assessing the transcript (RNA) for each family or subfamily in T cells using polymerase chain reaction and subsequent fragment-length analysis for separation of CDR3 fragments of various sizes to reflect diversity within a single family or subfamily. The healthy control spectra type shows a normal polyclonal T-cell receptor variable region β repertoire, with Gaussian distribution of the peaks indicating a normal and diverse T-cell repertoire. The patient sample shows oligo-clonality for most T-cell receptor variable region β families, with non-Gaussian distribution indicating a limited and constrained T-cell repertoire with oligoclonal T cells.