EXCEPTIONAL CASE REPORT

blood advances

Cadherin 17 mutation associated with leaky severe combined immune deficiency is corrected by HSCT

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Key Points

- *CDH17* is expressed in human thymic epithelial cells.
- *CDH17* mutations may be a rare cause of leaky severe combined immune deficiency that can be corrected by HSCT.

Introduction

Cadherin 17 (encoded by the *CDH17* gene) is a member of the cadherin superfamily encoding calcium-dependent membrane-associated glycoproteins. *CDH17* is developmentally regulated and its transcripts are detected in the fetal but not adult liver and intestines. Somatic *CDH17* mutations have been reported in gastrointestinal malignancies and implicated in tumor progression.[1-4] *CDH17* is also expressed in murine precursor and mature B cells,[5] where it contributes to their development and memory B-cell survival.[6,7] *CDH17* expression has also been demonstrated in mouse thymic medullary epithelial cells (mTECs), independent of their expression of the autoimmune regulator (AIRE), a transcriptional facilitator of promiscuous gene expression required for correct T-cell repertoire selection. In contrast, *CDH17* is barely detectable in cortical TECs.[8]

The consequences of *CDH17* mutations for lymphoid development and immune function remain, however, unknown. Here we report on a *CDH17*-mutated patient with leaky severe combined immunodeficiency, who was successfully treated by hematopoietic stem cell transplantation (HSCT), and discuss the possible role of *CDH17* mutations in the pathogenesis of the disease.

Case description

A 5-year-old Saudi Arabian girl presented to us with a long medical history of recurrent sino-pulmonary infections and was noted to have severe T- and B-cell lymphopenia with normal natural killer (NK) cellularity. Thymic output (as quantified by T-cell receptor excision circle [TREC] analysis) and aggregate T-cell function were very low (Table 1). The majority of the peripheral T cells had a memory phenotype, and proliferative response to PHA was low. Tetanus toxoid antibody levels were low. An extensive workup (see Table 1) failed to determine the molecular cause of the observed lymphopenia. During further workup, a stage 4 EBV-positive DLBCL was diagnosed, consistent with reduced immune surveillance. After achieving complete remission following 6 cycles of chemotherapy and surgical resection of tumor tissue in lung and ovaries, the patient underwent myeloablative matched sibling donor HSCT. A radiation containing regimen was chosen given her DLBCL appeared to be only partially responsive to chemotherapy as her ovarian disease was discovered shortly after completing her chemotherapy and her lung lesions still contained active disease after 6 cycles of chemotherapy. Posttransplant, the girl developed grade 2 skin GVHD, CMV viremia/pneumonia, and pulmonary actinomycosis. Remaining in remission from her DLBCL, she had full and stable immune reconstitution by within 2 years following HSCT, and both CD4 and CD8 naïve T-cells, TRECs, T-cell function, and immunoglobulin levels returned to normal values (Table 1; Figure 1). Pre-HSCT peripheral blood WES revealed compound heterozygous mutations in *CDH17*. Each of her parents had one of the noted and distinct mutations in *CDH17* and was healthy with no concern for immune deficiency.

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Table 1. Clinical course and immune function

	Baseline	DLBCL diagnosis	Workup and HSCT	Day 100 post-HSCT	1 Year post-HSCT	2 Years post-HSCT
Clinical course	Multiple lung, sinus, and tonsil infections starting at age 1.5 y requiring frequent hospitalizations and antibiotics SCD with HPFH G6PD	Diagnosis: Chest CT with bilateral, extensive lung infiltrates Lung biopsy revealed a DLBCL, EBV+ 2A level lymph nodes, palatine tonsil, ovary involved CSF and bone marrow were negative	Workup (all negative): HIV PCR FISH for DiGeorge Chromosome breakage studies Genetic workup (all negative): Rag 1/2 AT mutation analysis Nijmegen Artemis XLP XIAP	Grade 2 skin GVHD treated with topical steroids only CMV viremia and possible pneumonia treated with IV immunoglobulin and ganciclovir	Pulmonary actinomyces treated with PCN Bronchiectasis	Clinically well Treatment of actinomyces continues
Immune function						
T cell	Numbers (abs): CD3 = 353, CD4 = 118, CD8 = 163 TREC (nl >3063): 289 copies/10^6 CD3 T cells Proliferation: Low response (<5% of control) to PHA, absent responses to candida and TT TCR diversity: Normal Subsets (%): T_{NAIVE} CD4 33.6 CD8 4.4 T_{CM} 38.7 0.05 T_{EM} 26.5 4.85 T_{EMRA} 1.19 90.7	Treatment: Ritux/pred × 1 cycle Ritux/pred/MTX/Doxo × 2 cycles Ritux/ARAC/MTX × 2 cycles Ritux/Cy/Vinc/pred/MTX/Doxo × 2 cycles Surgical resection of hilar lymph nodes and lung masses Surgical resection of ovary and peritoneal mass	WES Compound heterozygosity for the c.1796+2 T>C mutation and N176S variant in the *CDH17* gene Heterozygous variant in the LIG1 gene Homozygous E7V mutation in the HBB gene Heterozygous S188F mutation in the G6PD gene	Numbers (abs): CD3 = 223, CD4 = 128, CD8 = 92 TREC (nl >3063): 500 copies/10^6 CD3 T cells Proliferation: Normal response to PHA. PWM, candida, and TT not tested. Subsets (%): T_{NAIVE} CD4 — CD8 2.85 T_{CM} — 2.5 T_{EM} — 66 T_{EMRA} — 28.7	Numbers (abs): CD3 = 1350, CD4 = 662, CD8 = 688 TREC (nl >3063): 3800 copies/10^6 CD3 T cells Proliferation: Normal response to PHA and PWM. Absent responses to candida and TT. Subsets (%): T_{NAIVE} CD4 — CD8 31.7 T_{CM} — 3.6 T_{EM} — 19.5 T_{EMRA} — 45.2	Numbers (abs): CD3 = 1089, CD4 = 633, CD8 = 428 TREC (nl >3063): 6010 copies/10^6 CD3 T cells Proliferation: Normal response to PHA and PWM. Candida and TT not tested. Subsets (%): T_{NAIVE} CD4 62.2 CD8 39.4 T_{CM} 17.1 12.9 T_{EM} 18.5 33.7 T_{EMRA} 2.13 13.9
B cell	Numbers (abs): CD19 = 27 Immunoglobulins*: IgG 1270, IgA 199, IgM 524, IgE <2 Vaccine titers: Tetanus Ab 0.06 (minimally protective), diphtheria Ab 0.06 (minimally protective), minimal response to all S.pneumo serotypes	Response: Mixed response to chemotherapy regimens NED at the time of HSCT	HSCT details: MSD HSCT (sibling had normal lymphocyte counts, no SCD or G6PD) Cy/TBI 1320cGy preparative regimen CSA/MTX for GVHD prophylaxis	Numbers (abs): CD19 = 100 Immunoglobulins†: IgG 494, IgA 27, IgM 328, IgE <2	Numbers (abs): CD19 = 1058 Immunoglobulins: IgG 3180, IgA 89, IgM 136, IgE 4	Numbers (abs): CD19 = 756 Immunoglobulins: IgG 1310, IgA 123, IgM 124, IgE 3 Vaccine titers: Tetanus Ab 0.21 (protective), diphtheria Ab 0.21 (protective), protective response to 9 of 14 S.pneumo serotypes.
NK cell	Numbers (abs): CD16/56 = 526 Activity: Normal			Numbers (abs): CD16/56 = 72 Activity: Normal	Numbers (abs): CD16/56 = 185 Activity: Decreased	Numbers (abs): CD16/56 = 120 Activity: Normal
Chimerism				100% Donor chimerism in peripheral blood and bone marrow	100% Donor chimerism in peripheral blood and bone marrow	100% Donor chimerism in peripheral blood and bone marrow

Ab, antibody; abs, absolute; ARAC, cytarabine; AT, ataxia telangiectasia; CMV, cytomegalovirus; CSA, cyclosporine; CSF, cerebrospinal fluid; CT, computed tomography; Cy, cytoxan; DLBCL, diffuse large B-cell lymphoma; Doxo, doxorubicin; EBV, Epstein-Barr virus; FISH, florescence in situ hybridization; G6PD, glucose 6-phosphate deficiency; GVHD, graft-versus-host disease; HBB, β hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; MSD, matched sibling donor; MTX, methotrexate; NED, no evidence of disease; PCN, penicillin; PCR, polymerase chain reaction; PHA, phytohemagglutinin; pred, prednisone; PWM, pokeweed mitogen; Ritux, rituximab; SCD, sickle-cell disease; TBI, total body irradiation; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; T_{EMRA}, effector memory T cells that express CD45RA; T_{NAIVE}, naïve T cells; TT, tetanus toxoid; Vinc, vincristine; WES, whole exome sequencing; XIAP, X-linked inhibitor of apoptosis; XLP, X-linked lymphoproliferative disorder.

*Previous IV immunoglobulin replacement therapy is uncertain.

†Last IV immunoglobulin infusion 1 month prior.

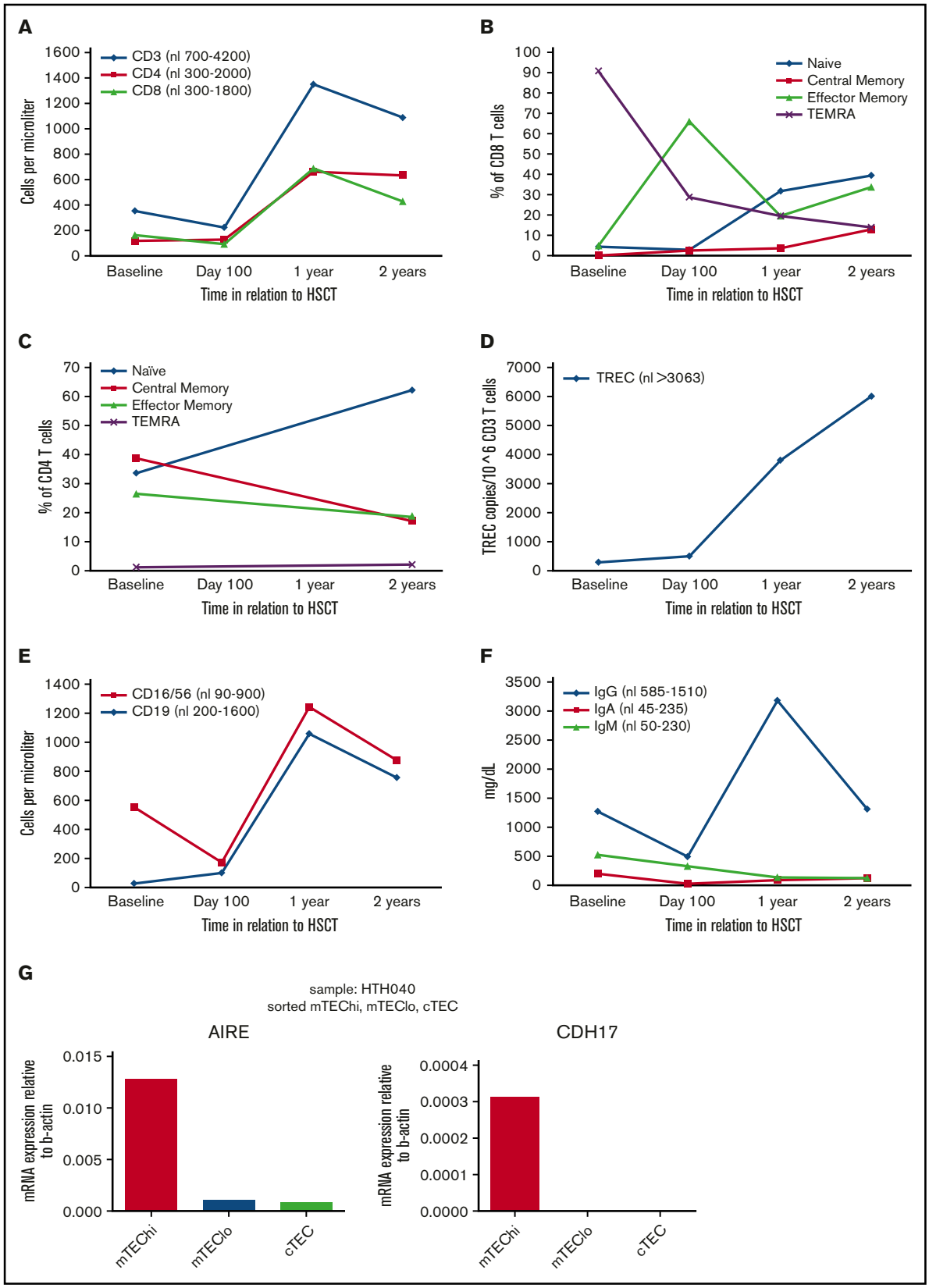

Figure 1. Immune recovery over time and *CDH17* expression in thymic epithelial cells. (A) T-cell numbers. (B) CD8 T-cell subsets. (C) CD4 T-cell subsets. (D) TRECs. (E) B and NK cell numbers. (F) Immunoglobulin levels. (G) *CDH17* expression in the human thymus is restricted to the population of mature medullary epithelial cells (mTEC^{hi}) as shown by quantitative reverse transcription PCR analysis. mTEC^{hi} is a heterogeneous population of thymic epithelial cells that primarily consists of functionally mature, AIRE-expressing cells.

Methods

Patient

The subject was treated on an institutional review board–approved HSCT protocol at the University of Minnesota.

Human thymus samples

Human thymus samples were obtained from children undergoing corrective cardiac surgery at Great Ormond Street Hospital for Children National Health Service trust after parents' informed consent and approval by the Great Ormond Street Hospital for Children National Health Service trust with a Material Transfer Agreement to the chancellor, masters, and scholars of the University of Oxford.

Isolation of thymic epithelial cells

TECs were isolated from the thymic tissue after mechanical and enzymatic tissue disruption followed by magnetic-activated cell sorting and fluorescence-activated cell sorting according to the protocol published by Stoeckle et al.[9]

RNA extraction and quantitative PCR analysis

Total RNA was extracted from sorted TECs using the RNeasy Mini Kit (Qiagen) according to the manufacturers' protocol, including in-column DNase treatment. Complementary DNA was prepared using the SensiFAST kit (Bioline). Complementary DNA was used for quantitative PCR with the relevant forward TTTACATTTTCCCTCGGCAG and reverse CCTCAAACTCTGTGTGCCTG primers (Sigma), using Sensifast SYBR Hi-Rox Kit (Bioline) and StepOnePlus (Applied Biosystems).

Flow cytometry

Standard flow cytometric methods were used for staining of cell-surface proteins. Anti-human monoclonal antibodies to the following molecules, with the appropriate isotype-matched controls were used for staining: CD3 (OKT-3), CD4 (RPA-T4), CD8 (HIT8a), CD19 (HIB19), CD56 (HCD56), CD16 (3G8), CD45RA (HI100), CD45RO (UCHL1), CD31 (390), and CCR7 (4B12), all from BioLegend (San Diego, CA). Data were collected with an LSRFortessa (BD Biosciences, San Jose, CA), cell analyzer and analyzed with FlowJo software (Tree Star Inc., Ashland, OR).

T-cell proliferation

Peripheral blood mononuclear cells were cultured unstimulated or stimulated with 5 μg/mL PHA (Sigma Aldrich; L8754). Proliferation was assayed 4 days later by measuring ^{3}H-thymidine incorporation into genomic DNA added for the last 16 hours of culture.

Results and discussion

Human TECs interrogated by quantitative reverse transcription PCR showed *CDH17* expression only in mature medullary TECs (mTEC^{hi}), a heterogeneous population of TECs that primarily but not exclusively consists of functionally mature cells, as molecularly identified by their expression of *AIRE* (Figure 1G). Pre-HSCT peripheral blood WES revealed compound heterozygous *CDH17* mutations (Table 1). The c.1796+2 T>C mutation (IVS13+2 T>C) in *CDH17* abolishes the splice donor site of intron 13 and is predicted to cause either abnormal transcripts subjected to nonsense-mediated messenger RNA decay or a protein with abnormal sequence. The c.527A>G mutation (N176S) results in a conservative substitution of one neutral, polar amino acid for another. Although the molecular and functional consequence of this mutation is not yet fully established, it is reported at low frequency in the ExAC database (minor allele frequency: 0.0001319), with 16 heterozygous, but no homozygous subjects carrying this variant. Furthermore, given the thymic expression pattern of *CDH17* and the robust donor-derived T-cell recovery post-HSCT, the low thymic output noted at initial presentation appeared to the independent of an intrinsic TEC deficiency.

Overall, predictions would suggest that the *CDH17* mutations identified in this child lead in aggregate to altered protein expression and/or function though as stated previously, the molecular and functional consequences of these mutations are unknown. At baseline, the patient had a markedly reduced number of circulating B cells. Because of the role played by *CDH17* on B-cell development and survival, the *CDH17* mutations may be responsible for B-cell lymphopenia observed in the patient at baseline. Furthermore, because *CDH17* mutations and loss of expression are associated with malignant tumor progression, the propensity for EBV lymphoproliferative disease progression to DLBCL may have been supported by *CDH17* mutations in the malignant B cells. Many primary immune deficiencies are associated with increased risk for malignancies secondary to defective immune surveillance and infection with oncogenic viruses. Non-Hodgkin lymphomas, including DLBCL, are among the most common.[10]

In summary, our patient was a compound heterozygote for 2 *CDH17* mutations without other known immunodeficiency-related genetic mutations, had on presentation severe lymphopenia, and displayed significantly abnormal T-cell function. Our investigations revealed that *CDH17* is also expressed on human mTEC^{hi} cells, but because T-cell lymphopoiesis was restored post-HSCT in our patient, the TEC defect does not appear to be limiting. Based on experimental data that suggests in the mouse that *CDH17* mutations cause B-cell defects,[5,6] we postulate that the patient's compound heterozygosity for *CDH17* mutations affected bipotent lymphoid progenitors and impaired their differentiation. We therefore conclude that the compound heterozygous *CDH17* mutations identified in this patient constitute a rare cause of leaky severe combined immunodeficiency that can be cured with HSCT. Future studies delineating the consequences of the patient's mutations are needed to confirm this conclusion.

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Authorship

Contribution: A.R.S. wrote the manuscript; L.D.N., G.A.H., and B.R.B. designed the experiments; I.A.R., S.M., and M.J.M. performed the experiments and edited the manuscript; and A.R.S., T.C.L., L.D.N., G.A.H., and B.R.B. analyzed and/or interpreted the results and edited the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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