Hennekam Syndrome due to a Novel Homozygous *CCBE1* Mutation Presenting as Pediatric-Onset Common Variable Immune Deficiency

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Collagen- and calcium-binding EGF domain–containing protein 1 (*CCBE1*) encodes an extracellular matrix protein that plays a major role in lymphangiogenesis, in both model organisms and humans [1,2]. Hennekam lymphangiectasia-lymphedema syndrome (HKLLS) is a rare inborn disorder characterized by generalized lymphangiectasia and lymphedema of varying severity, facial dysmorphism, intellectual disability, and variable additional features, such as congenital alterations, seizures, and thyroid hormone alterations [3-5]. Homozygous loss-of-function mutations in *CCBE1* and *FAT4* have been identified in 25% and 20% of HKLLS patients, respectively [1,6]. Recently, biallelic missense mutations in *ADAMTS3* were also reported to cause this syndrome in 2 affected siblings [7]. Patients with HKLLS may also present hypogammaglobulinemia, which is commonly thought to be related to intestinal lymphangiectasia and protein-losing enteropathy. Data regarding immunological evaluation and long-term follow-up of such patients are limited [8,9]. We report the case of an Italian man affected by HKLLS due to a novel homozygous mutation in *CCBE1*. The initial diagnosis was pediatric-onset common variable immune deficiency (CVID)–like disorder.

The patient was a 25-year-old man with consanguineous parents of Italian descent. He was born at gestational week 37 to a hepatitis C virus–positive mother. At birth, he presented generalized edema, moderate mitral valve insufficiency, and minor dysmorphic features (flat nasal bridge, hypertelorism, round flat face, small mouth). Laboratory evaluation at 3 days of life revealed hypoproteinemia (albumin 3.3 g/dL [normal values 4.0-5.0 g/dL]) and hypogammaglobulinemia (IgG 222 mg/dL, IgA <7 mg/dL, IgM 5 mg/dL [normal values, IgG 862-1434 mg/dL, IgA 0 mg/dL, and IgM 5-14 mg/dL, respectively]). Abdominal ultrasound was unremarkable, and the pediatric neuropsychiatric evaluation suggested mild neurodevelopmental delay. Human purified albumin infusions were required, and edema had resolved by the third month of life. During the first year of life, the patient experienced recurrent respiratory tract infections. Immunoglobulin serum levels were below normal values for age, with defective antibody responses to hepatitis B virus and pertussis. Secondary causes of hypogammaglobulinemia were excluded. Hence, the index patient was diagnosed with CVID-like disorder, and immunoglobulin replacement treatment was started. The patient was evaluated at our center at the age of 6 years. His clinical condition was good, with minor dysmorphic features and mild neurodevelopmental delay. The immunological work-up showed normal white blood cell counts, with normal $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD16^+CD56^+$ counts, in slight contrast with previously reported data [9]. T-cell proliferation upon stimulation of aCD3, $aCD3^+$IL-2, and phytohemagglutinin was normal (Table, first column). Targeted next-generation sequencing of the patient's DNA for 264 genes associated with inborn errors of immunity was performed at the age of 21 years and revealed a homozygous mutation in *CCBE1* (c.767G>A, p.Gly256Asp). Each parent and both the proband's siblings were heterozygous for the *CCBE1* mutation (Supplementary Figure 1A, 1B). The p.G256D variant has not been reported in the gnomAD or 1000 Genomes databases or in patients affected by HKLLS. The glycine at position 256 is highly conserved among different species, and its alteration affects the collagen 1 domain, which has proven to be crucial for the activation of vascular endothelial growth factor C in vitro and in vivo (Supplementary Figure 1C, 1D) [10]. Predictive software applications such as PolyPhen-2 (1.00), SIFT (0.006), PROVEAN (-3.45), and CADD (1.252) point to a damaging or deleterious effect of the G256D variant. In more recent years, detailed lymphocyte subset evaluations always showed adequate production of newly generated T cells (recent thymic emigrants), confirmed by normal values of T-cell receptor excision circles (data not shown) and a normal T-cell maturation profile, as well as the T-cell receptor repertoire (Table 1, second column and data not shown). Immunophenotype analysis of B-cell subsets displayed progressive accumulation of $CD19^+IgD^+IgM^+CD27^+$ IgM memory cells and of $CD19^{hi}CD21^{lo}$ cells, most of which present a $CD27^+$ memory phenotype, which in the last evaluation yielded values exceeding the normal range (Table, second column). Conversely, percentages of terminally differentiated $CD19^+CD20^-CD38^{hi}CD27^+$ cells were constantly low and often close to the detection limit of the test. This phenotype resembled that of CVID. Of note, during the 25-year follow-up, the index patient did not experience invasive or recurrent

Table. Laboratory and Immunological Data From the Patient Harboring the Novel p.G256D Mutation at the First Evaluation (6 y) and More Recently (25 y).

	Index patient 6 y (normal values)	Index patient 25 y (normal values)
Red blood cells, $\times 10^6$/μL	4.50 (4.00-5.00)	5.16 (4.50-5.50)
Hemoglobin, (g/dL)	12.5 (11.5-13.5)	15.3 (14.0-18.0)
Platelets, $\times 10^3$/μL	232 (150-300)	192 (130-400)
White blood cells, $\times 10^3$/μL	6.66 (5.00-14.50)	4.99 (4.00-10.80)
Lymphocytes, $\times 10^3$/μL	2.40 (1.50-7.00)	1.19 (0.90-4.00)
Neutrophils, $\times 10^3$/μL	3.30 (1.50-8.00)	3.36 (1.50-8.00)
IgG, mg/dL	650a (633-1916)	935[a] (690-1500)
IgA, mg/dL	25 (41-315)[b]	97 (85-410)
IgM, mg/dL	70 (56-261)	79 (40-240)
T cells ($CD3^+$), %	66.2 (55.0-86.0	63.2 (57.1-87.6)
T cells ($CD3^+$), cells/μL	1590 (1200-2600)	1036 (721-2562)
$CD3^+CD4^+$, %)	38.4 (37.0-55.0)	36.3 (28.5-65.6)
$CD3^+CD4^+$, cells/μL	921 (650-1500)	596 (273-1882)
Naive ($CD45RA^+CCR7^+$), %	NA	50.7 (20.4-63.6)
RTE ($CD45RA^+CCR7^+CD31^+$), %	NA	29.1 (11.4-48.1)
Central memory ($CD45RA^-CCR7^+$), %	NA	30.6 (18.7-46.2)
Effector memory ($CD45RA^-CCR7^-$), %	NA	15.0 (7.1-38.0)
Terminally differentiated ($CD45RA^+$CCR7-), %	NA	3.7 (0.3-9.1)
$CD3^+CD8^+$, %	23.2 (16.0-37.0)	21.4 (10.5-37.7)
$CD3^+CD8^+$, cells/μL	556 (370-1100)	351 (177-783)
Naive ($CD45RA^+CCR7^+$), %	NA	32.3 (13.1-66.5)
Central memory ($CD45RA^-CCR7^+$), %	NA	8.8 (2.6-24.5)
Effector memory ($CD45RA^-CCR7^-$), %	NA	32.5 (10.1-47.4)
Terminally differentiated ($CD45RA^+CCR7^-$), %	NA	26.4 (5.2-63.5)
$CD4^+/CD8^+$	1.7	1.7
$CD4^-CD8^-$ TCR γ/δ^+, %	NA	5.8 (0.9-11.2)
B cells ($CD19^+$), %	23.8 (2.0-17.0)[c]	18.9 (5.8-22.1)
B cells ($CD19^+$), cells/μL	571 (270-860)	310 (86-648)
RBE (CD38hi$CD10^+$), %	NA	4.6 2.1-26.1)
Naïve (IgD^+CD21hi$CD10^-CD27^-$), %	NA	37.5 (33.7-74.0)
$CD19^{hi}CD21^{lo}$, %	NA	14.7 (1.4-13.6)[c]
Switched memory ($IgD^-IgM^-CD27^+$), %	NA	8.0 (2.8-23.4)
IgM memory ($IgD^+IgM^+CD27^+$), %	NA	35.0 (5.1-25.5)[c]
Terminally differentiated (CD38hi$CD27^+CD20^-$), %	NA	0.1 (0.2-8.1)[b]
NK cells ($CD3^-CD16^+CD56^+$), %	7.8 (2.0-30.0)	17.4 (3.4-28.4)
NK cells ($CD3^-CD16^+CD56^+$), cells/μL	187 (100-480)	289 (40-741)
T-cell proliferation (aCD3, $aCD3^+$IL-2, phytohemagglutinin)	Normal	Normal

Abbreviations: NA, not available; RBE, recent bone marrow emigrants; RTE, recent thymic emigrants.
[a]Under immunoglobulin replacement treatment.
[b]Values lower than normal ranges.
[c]Values higher than normal ranges.

infectious episodes and did not present edema or endocrine abnormalities. His differential blood counts remained within the normal range and stable over time, without lymphopenia. At his last evaluation at the age of 25 years, serum total protein and serum albumin levels were 6.9 and 4.3 g/dL, respectively (normal range, 6.0-8.0 and 3.1-5.2 g/dL, respectively).

Immunological alterations are not always present in HKLLS, and their evolution over time has not yet been defined. In the small number of affected patients who underwent immunological evaluation, lymphocyte subsets were found to range from increased peripheral B- and NK-cell counts to normal B-, T-, and NK-cell counts [8,9]. The index patient carrying the novel *CCBE1* mutation presented B-cell maturational disturbances with a normal T- and NK-cell compartment, a phenotype that has not previously been associated with this syndrome. Further experimental data are required to better define the role of *CCBE1* in human lymphocyte homeostasis. In addition, the mild clinical phenotype of the index patient may be related to the homozygous Gly256Asp mutation, which affects the collagen 1 domain differently from previously reported patients who harbor mutations affecting the calcium-binding EGF domain. Homozygous missense mutations in *CCBE1* close to the calcium-binding EGF domain have also been reported in 2 siblings with lymphedema-cholestasis syndrome (Aagenaes syndrome) [11], suggesting that the clinical presentation of mutations affecting this region may result in more severe clinical phenotypes.

In conclusion, we report a novel pathogenic homozygous loss-of-function mutation in *CCBE1* causing HKLLS with a mild clinical phenotype associated with persistent hypogammaglobulinemia requiring regular immunoglobulin replacement treatment. Application of next-generation sequencing techniques may enable us to identify HKLLS in patients with a clinical history of neonatal edema, typical dysmorphic features, and hypogammaglobulinemia. Additional studies are warranted to unveil the specific role of *CCBE1* in human lymphocyte maturation and function.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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