Hennekam syndrome due to a novel homozygous CCBE1 mutation presenting as pediatric-onset CVID

Tessarin G[1], Baronio M[1], Gazzurelli L[1], Rossi S[1], Chiarini M[2], Moratto D[2], Badolato R[1], Lougaris V[1]

[1]Pediatrics Clinic and Institute for Molecular Medicine "A. Nocivelli", Department of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili of Brescia, Brescia, Italy
[2]Flow Cytometry Laboratory, Diagnostic Department, ASST Spedali Civili, Brescia, Italy

Corresponding author:
Vassilios Lougaris, MD
Pediatrics Clinic and Institute of Molecular Medicine "A. Nocivelli",
Department of Clinical and Experimental Sciences, University of Brescia.
Piazzale Spedali Civili 1, 25123, Brescia, Italy
E-mail: vlougarisbs@yahoo.com

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Collagen- and calcium-binding EGF domain-containing protein 1 (*CCBE1*) encodes for an extracellular matrix protein which 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 dysmorphisms, intellectual disability and variable additional features such as congenital alterations, seizures, and thyroid hormones alterations [3–5]. Homozygous loss-of-function mutations in *CCBE1* or *FAT4* have been identified in 25% and 20% of HKLLS affected patients, respectively [1,6]. Recently, biallelic missense mutations in ADAMTS3 were also described as causative of this syndrome in two affected siblings [7]. Patients with HKLLS may also present hypogammaglobulinemia which is commonly thought to be related to intestinal lymphangiectasia and protein-losing related enteropathy; to date, limited data regarding immunological evaluation and long-term follow-up of such patients are available [8,9]. We report on an Italian male patient affected with HKLLS due to a novel *CCBE1* homozygous mutation with an initial diagnosis of pediatric-onset common variable immune deficiency (CVID)-like disorder.

The index patient is currently a 25-year-old male born from consanguineous parents of Italian descent. He was born at 37th gestational week, from hepatitis C virus positive mother. At birth, he presented generalized edema, mitral valve insufficiency of moderate degree and minor dysmorphic features (flat nasal bridge, hypertelorism, round flat face, small mouth). Laboratory evaluation at three 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 pediatric neuropsychiatric evaluation suggested mild neurodevelopmental delay. Human purified

albumin infusions were required, and edema remission was achieved within the 3rd month of life. During the first year of life the patient suffered from recurrent respiratory tract infections. Immunoglobulin serum levels resulted below age-related normal values with defective antibody responses against 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 six years. He presented in good clinical conditions, with minor dysmorphic features and mild neurodevelopmental delay. Immunological work up showed normal white blood cell counts, with normal $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+CD56^+$ cell counts, in slight contrast with previously reported data [9]. T-cell proliferation upon aCD3, aCD3+IL-2, and phytohemagglutinin stimulation resulted normal (Table 1, 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 resulted heterozygous for the *CCBE1* mutation (Supplementary Figure 1A, 1B). The p.G256D variant has not been reported in the gnomAD or 1000 Genomes databases, nor in patients affected by HKLLS. The glycine in position 256 is highly conserved among species and its alteration affects the collagen 1 domain, which has been proved to be crucial for the activation of vascular endothelial growth factor C in vitro and in vivo (Supplementary Figure 1C, 1D) [10]. Predictive software analysis such as PolyPhen-2 (1.00), SIFT (0.006), PROVEAN (-3.45), CADD (1.252) suggested a probably damaging or deleterious effect of the G256D variant. In more recent years detailed lymphocyte subset evaluations were performed always showing an adequate production of newly generated T cells (RTE), confirmed also by normal values of T cell receptor excision circles (TRECs) (data not shown), and a normal T-cell maturation profile as well as T-cell receptor repertoire (Table 1, second column and data not shown). Immunophenotype analysis of B cell subsets displayed a progressive accumulation of $CD19^+IgD^+IgM^+CD27^+$ IgM-memory cells and of $CD19^{hi}CD21^{lo}$ cells, most of which presenting a $CD27^+$ memory phenotype, which in the last evaluation presented values exceeding normal range (Table 1, second column). Conversely, percentages of $CD19^+CD20^-CD38^{hi}CD27^+$ terminally differentiated cells were constantly low and often close to the detection limit of the test, a phenotype resembling that of CVID.

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Of note, during the 25-year follow-up, the index patient did not suffer from invasive or recurrent infectious episodes and did not present edema or endocrine alterations. His differential blood counts remained within normal range and stable over time without development of lymphopenia; at last evaluation at the age of 25 years, serum total protein level and serum albumin levels were 6.9 and 4.3 g/dL, respectively (normal value 6.0 – 8.0 and 3.1 – 5.2 g/dL, respectively).

Immunological alterations in HKLLS are not always present and their evolution over-time has not been defined yet. In the small number of affected patients that underwent immunological evaluation, lymphocyte subset evaluation ranged from increased peripheral B and NK cell numbers to normal B, T and NK cell counts [8,9]. The index patient carrying the novel CCBE1 mutation presented B cell maturational disturbances with normal T and NK cell compartment, a phenotype that has not been associated with this syndrome before. 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 affects the collagen 1 domain, differently form previously reported patients that harbour mutations affecting the calcium-binding EGF domain. Homozygous missense mutations in CCBE1 in proximity of the calcium-binding EGF domain have also been reported in two siblings with Lymphedema-Cholestasis Syndrome (LCS; Aagenaes syndrome) [11], possibly suggesting that the clinical presentation of mutations affecting this region may result in more severe clinical phenotypes.

In conclusion, we report on a novel pathogenic loss-of-function homozygous 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 allow to identify HKLLS in patients with a clinical history of neonatal edema, typical dysmorphic features and hypogammaglobulinemia. Additional studies are warranted in order to unveil the specific role of *CCBE1* in human lymphocyte maturation and function.

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Disclosure of conflicts of interest

The authors declare no conflict of interest.

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Table 1. Laboratory and immunological data from the patient harboring the novel p.G256D mutation, at first (6 years) and more recent (25 years) evaluation. White text in black background represents values higher than normal ranges; black text with grey background represents values lower than normal ranges.

	Index patient 6 years (normal values)	Index patient 25 years (normal values)
Red Blood Cells ($x10^6$/uL)	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 ($x10^3$/uL)	232 (150 - 300)	192 (130 - 400)
White Blood Cells ($x10^3$/uL)	6.66 (5.00 - 14.50)	4.99 (4.00 - 10.80)
Lymphocytes ($x10^3$/uL)	2.40 (1.50 - 7.00)	1.19 (0.90 - 4.00)
Neutrophils ($x10^3$/uL)	3.30 (1.50 - 8.00)	3.36 (1.50 - 8.00)
IgG (mg/dL)	650* (633 - 1916)	935* (690 - 1500)
IgA (mg/dL)	25 (41 - 315)	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/uL)	1590 (1200 - 2600)	1036 (721 - 2562)
$CD3^+CD4^+$ (%)	38.4 (37.0 - 55.0)	36.3 (28.5 - 65.6)
$CD3^+CD4^+$ (cells/uL)	921 (650 - 1500)	596 (273 - 1882)
Naive ($CD45RA^+CCR7^+$) (%)	n.e	50.7 (20.4 - 63.6)
RTE ($CD45RA^+CCR7^+CD31^+$) (%)	n.e	29.1 (11.4 - 48.1)
Central memory ($CD45RA^-CCR7^+$) (%)	n.e	30.6 (18.7 - 46.2)
Effector memory ($CD45RA^-CCR7^-$) (%)	n.e	15.0 (7.1 - 38.0)
Terminally differentiated ($CD45RA^+CCR7^-$) (%)	n.e	3.7 (0.3 - 9.1)
$CD3^+CD8^+$ (%)	23.2 (16.0 - 37.0)	21.4 (10.5 - 37.7)
$CD3^+CD8^+$ (cells/uL)	556 (370 - 1100)	351 (177 - 783)
Naive ($CD45RA^+CCR7^+$) (%)	n.e	32.3 (13.1 - 66.5)
Central memory ($CD45RA^-CCR7^+$) (%)	n.e	8.8 (2.6 - 24.5)
Effector memory ($CD45RA^-CCR7^-$) (%)	n.e	32.5 (10.1 - 47.4)
Terminally differentiated (CD45RA+CCR7-) (%)	n.e	26.4 (5.2 - 63.5)
$CD4^+/CD8^+$	1.7	1.7
$CD4^-CD8^-$ TCR γ/δ^+ (%)	n.e	5.8 (0.9 - 11.2)
B cells ($CD19^+$) (%)	23.8 (2.0 - 17.0)	18.9 (5.8 - 22.1)
B cells ($CD19^+$) (cells/uL)	571 (270 - 860)	310 (86 - 648)
RBE ($CD38^{hi}CD10^+$) (%)	n.e	4.6 2.1 - 26.1)
Naive ($IgD^+CD21^{hi}CD10^-CD27^-$) (%)	n.e	37.5 (33.7 - 74.0)
$CD19^{hi}CD21^{lo}$ (%)	n.e	14.7 (1.4 - 13.6)
Switched memory ($IgD^-IgM^-CD27^+$) (%)	n.e	8.0 (2.8 - 23.4)
IgM memory ($IgD^+IgM^+CD27^+$) (%)	n.e	35.0 (5.1 - 25.5)
Terminally differentiated ($CD38^{hi}CD27^+CD20^-$) (%)	n.e	0.1 (0.2 - 8.1)

NK cells ($CD3^-CD16^+CD56^+$) (%)	7.8 (2.0 - 30.0)	17.4 (3.4 - 28.4)
NK cells ($CD3^-CD16^+CD56^+$) (cells/uL)	187 (100 - 480)	289 (40 -741)
T-cells proliferation (aCD3, aCD3+IL-2, phytohemagglutinin)	normal	normal

*Under immunoglobulin replacement treatment.