Exploration of potential immunodeficiency unveils Hennekam Lymphangiectasia-Lymphedema Syndrome

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To the Editor,

Hennekam lymphangiectasia-lymphedema syndrome (HKLLS) is a rare autosomal recessive disorder characterized by lymphangiectasia, lymphedema, facial anomalies and mental retardation (1). The lymphangiectasia primarily affects the intestines, but also the pleura, pericardium, kidney and thyroid gland. Common dysmorphic features are a flat face and broad nasal bridge, hypertelorism, epicanthal folds, a small mouth, and dental anomalies such as irregular placement, supernumerary molars, and agenesis of maxillary incisors. Mental retardation is common but varies in severity. One third of patients have seizures (1, 2). In some cases, fetal abnormalities such as hydrops fetalis can be diagnosed on prenatal ultrasound scans (3). HKLLS is a genetically heterogeneous disorder caused by biallelic loss-of-function mutations in *CCBE1* (18q21) or *FAT4* (4q28) in 25% and 20% of the cases, respectively (4, 5). Recently, loss-of-function mutations in *ADAMTS3* (4q13), were also associated with HKLLS (6, 7). More than twenty other genes, including *FLT4*, *FOXC2*, *GATA2* and *SOX18*, are involved in lymph vessel formation and their mutations cause primary lymphedema (4, 6). Here we present a patient, referred for a suspected inborn error of immunity, in whom we diagnosed HKLLS.

The patient was the first child of non-consanguineous parents of Belgian descent, born at term without any prenatal abnormalities. He presented with neonatal generalized edema within the first week (Figure 1). Primary investigations showed hypoalbuminemia and hypogammaglobulinemia (IgG 1.4 g/L, IgA 0.085 g/L) without proteinuria. Congenital nephrotic syndrome was excluded. Gastroscopy was performed and revealed intestinal lymphangiectasia. He was started on a diet with high percentage of medium-chain fatty acid and low percentage of long-chain triglycerides (Lipistart). At the age of one month, the patient was admitted to the pediatric intensive care unit for febrile presentation complicated by pleural and pericardial effusion, not requiring mechanical ventilation or circulation support. Monotherapy with Lipistart did not sufficiently increase his serum protein levels and the patient received a central catheter for administration of albumin and immunoglobulins (IVIG). Albumin administration was then gradually decreased and could be stopped at the age of fifteen months. Over the years, the patient's diet was gradually expanded to low-fat foods, prepared in coconut oil. In childhood he suffered from recurrent otitis and lower airway infections, which improved after a switch to subcutaneous immunoglobulin therapy at the age of 4 years, and at the age of seven years he developed refractory molluscum contagiosum with secondary abscesses. Furthermore, he has atopic eczema and dust mite allergy.

At the age of eight years old, the boy was referred to our center for a second opinion. He showed facial dysmorphism with flat nasal bridge, hypertelorism, long philtrum, double tooth row and a bifid uvula. He had pitting edema in the lower limbs, hypermobile joints and hoarseness, and diffuse molluscum

contagiosum. Molluscum improved with zinc supplementation, administered because of low serum zinc levels, and prophylactic antibiotics helped in prevention of secondary skin infection. His blood work up, including white blood cells, immunoglobulins levels, complement, lymphocyte subsets and thyroid function, was completely normal. Natural Killer cell function as measured by CD107a surface expression upon stimulation with K562 cells was normal (Figure S1). A targeted gene panel analysis for immunodeficiency, including *GATA2* copy number analysis and targeted *NEMO* sequencing, was normal. The complex clinical presentation of the patient prompted us to perform whole-exome sequencing, which revealed compound heterozygous variants (c.683_684insT, p.Leu229Profs*8 – inherited from the mother, and c.829G>A, p.Gly277Arg – a private variant inherited from the father) in *CCBE1*, suggestive for HKLLS1 (Fig S2).

The main driver of lymphangiogenesis in embryonic development is vascular endothelial growth factor (VEGF)-C, which acts via VEGF receptor 3 (VEGFR3). Mutations in VEGF-C and VEGFR3 are associated with hereditary lymphedema. Collagen- and calcium-binding EGF domain-containing protein 1 (CCBE1) plays an important role in the lymphangiogenesis, as it regulates the activation of VEGF-C. It depends on the A disintegrin and metalloprotease with thrombospondin motifs-3 (ADAMTS3) metalloprotease for the proteolytic cleavage of pro-VEGF-C into active VEGF-C (6, 8). CCBE1 contains a calcium-binding epidermal growth factor domain (N-terminal) and a collagen domain (C-terminal). Almost all known mutations in *CCBE1* affect the calcium-binding EGF domain (8). There is intra-familial variability in the phenotype (2). In the present patient, p.Leu229Profs*8 introduces a frameshift and a premature stop codon and leads to a loss-of-function. This mutation was published before in patients with Hennekam syndrome (4). The other variant, p.Gly277Arg, is a private variant predicted to be pathogenic by in silico analysis (combined annotation-dependent depletion (CADD) score 33 with a mutation significance cutoff (MSC) of 24.9, Sorting Intolerant From Tolerant (SIFT) damaging, Polyphen probably damaging) (Fig S2).

We here report a patient with the typical clinical features of Hennekam syndrome, namely lymphedema, lymphangiectasia and dysmorphism, who also experienced severe molluscum, not responding to conventional treatment. Hypogammaglobulinemia due to protein loss is not typically associated with viral skin infections. Cutaneous involvement such as haemangiomas is rare in Hennekam syndrome. Association with viral skin infections and formation of verrucae has been reported previously (9), while susceptibility for molluscum has not been described before.

Other syndromes are characterized by the triad lymphangiectasia, lymphedema and dysmorphism, such as Noonan, Njolstad and Turner syndrome (10, 11). Combined with the cytopenia, immune deficiency and dental anomalies displayed by our patient, osteopetrosis-lymphedema-ectodermal

dysplasia anhidrotic with immunodeficiency (OLEDAID) or NEMO deficiency also entered in the differential diagnosis, as well as GATA2 deficiency and CD55 deficiency with hyper-activation of complement, angiopathic thrombosis, and severe protein-losing enteropathy (CHAPLE syndrome) (9-11).

In our case, there was a diagnostic delay of nine years because of the initial search of specific gene mutations, which was inconclusive. In undiagnosed diseases, exome sequencing could therefore be the key to make a prompt diagnosis and initiate a more targeted treatment.

In conclusion, we present a case report with a novel, probably pathogenic mutation in the CCBE1 gene, resulting in Hennekam lymphangiectasia-lymphedema syndrome. It is an eye opener to not forget about phenocopies of rare PIDs.

Conflict of interest statement

IM receives the CSL-Behring Chair of Primary Immunodeficiencies in Children, paid to Institution.

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Authorship

SK and IM were the principal investigators and drafted the manuscript; SK, GB, and IM were involved in the clinical care. All authors revised and corrected the manuscript.

Availability of data and material (data transparency): not applicable

Code availability: not applicable

Ethics approval

This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments, and was approved by the Ethics Committee for Research of Leuven University Hospitals.

Consent to participate: the subject has given consent to participate.

Consent for publication: the subject has given consent for findings based on his samples and history to be published.

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Figure 1: A) Schematic representation of the major mediators of lymphangiogenesis. Mutations in the proteins highlighted in red have been reported in patients with Hennekam lymphangiectasia-lymphedema syndrome. B) Edema of the lower limb during the patient's first week of life.

ADAMTS3: A disintegrin and metalloprotease with thrombospondin motifs-3; CCBE1: collagen- and calcium-binding EGF domain-containing protein 1; DCHS1: dachshous cadherin-related 1; ERK and MEK: extracellular signal-regulated kinases, also known as MAPK: mitogen-activated protein kinase; FAT4: FAT atypical cadherin 4; PROX1: Prospero homeobox protein 1; SOX18: SRY-related HMG-box 18; VEGFC: vascular endothelial growth factor C; VEGFR3: vascular endothelial growth factor receptor 3.

Figure S1: NK cell degranulation, monitored by CD107a surface expression on CD56+ cells, upon stimulation with K562 cells in patient compared to healthy control (HC ($n = 1$)). Percentage of CD107a⁺CD56⁺ cells is depicted and noted in the blots. **Figure S2.** Pedigree of the index patient.