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CASE REPORT

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Novel mutation in *CCBE 1* as a cause of recurrent hydrops fetalis from Hennekam lymphangiectasia-lymphedema syndrome-1

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Key Clinical Message

Whole exome sequencing (WES) was used to determine the etiology of recurrent hydrops fetalis in this case of Hennekam lymphangiectasia-lymphedema syndrome-1. WES is a useful approach for diagnosing rare single-gene conditions with nonspecific phenotypes and should be considered early in the diagnostic process of investigating fetal abnormalities.

KEYWORDS

congenital abnormalities, exome sequencing, genetic testing, Hennekam syndrome, hydrops fetalis, prenatal diagnosis

1 | INTRODUCTION

Hennekam lymphangiectasia-lymphedema syndrome-1 (HKLLS1) is a disorder of lymphedema, lymphangiectasis, and development delay. The edema is usually congenital and often progressive, generally including the limbs, genitalia, and face. Maldevelopment of the lymphatic system preferentially affects the intestines and limbs, but can also affect other systems, such as the pleura, pericardium, and kidneys.[1] This syndrome was first reported by Dr. Hennekam in 1989 as an autosomal recessive condition causing intestinal lymphangiectasia, diffuse lymphedema, facial dysmorphisms (depressed nasal bridge, hypertelorism, narrow mouth, and deformities of the teeth and ears), and various degrees of growth impairment, cognitive disorders, and seizures.[2] HKLLS1 is often caused by homozygous or compound heterozygous pathogenic variants in the collagen and calcium-binding EGF domain 1 (*CCBE1*) gene, an extracellular matrix protein essential to lymphatic system development.[3] However, *CCBE1* variants are only found in 25% of suspected cases.[4] Isolated or syndromic primary lymphedema has been associated with variants in 27 additional genes, including *EPHB4, FLT4, FOXC2, GATA2, GJA1, GJC2, HGF, HRAS, IKBKG, ITGA9, KIF11, KRAS, PTPN11, PTPN14, RAF1, RASA1, SOS1, SOX18, VEGFC, CELSR1, EPHB4, FAT4, PIEZO1, RELN, RIT1, TSC1, TSC2*, and *ADAMTS3*.[5-7]

Hydrops fetalis is defined as excessive fluid collections within fetal cavities and extravascular compartments: skin edema, ascites, pleural or pericardial effusions, thickened placenta, and polyhydramnios. It represents the end stage of a variety of disorders.[8] Hydrops can be immune (isoimmunization) and nonimmune, with up to 90% of current cases attributed to nonimmune causes,[8,9] of which lymphatic dysplasias make up 5%-15%. Nonimmune hydrops carries a poor

prognosis, and fetal mortality ranges from 50%-98%.[8,10,11] Unfortunately, despite recent advances in diagnostic capabilities, 16%-19% of cases remain idiopathic.[9,11]

Here, we report a case in which whole exome sequencing was used to diagnose the etiology of recurrent hydrops fetalis as HKLLS1 and to reveal a new pathologic variant in the *CCBE1* gene.

2 | CLINICAL CASE

The patient was a 39-year-old G4P1211 female of Northern European origin who presented to our institution with recurrent fetal hydrops. In her first pregnancy (managed at an outside institution), hydrops was diagnosed at 18 weeks. Anatomical survey was significant for unilateral clubfoot, without other abnormalities. Cardiac rhythm and middle cerebral artery (MCA) Doppler velocimetry were normal, without evidence of fetal anemia. The patient's blood type was Rh-positive and antibody-negative. Amniocentesis revealed normal karyotype. Infectious workup was negative for parvovirus, cytomegalovirus, toxoplasmosis, herpes simplex virus, adenovirus, and coxsackie virus. Diabetes and thyroid studies were unremarkable. The patient elected termination, and a dilation and evacuation was performed at 22 weeks. No placental pathology was available. The final diagnosis was idiopathic hydrops.

In her second pregnancy, hydrops was again diagnosed at 18 weeks. Anatomical survey was normal, other than echogenic bowel. A full workup was performed, as described above for her first pregnancy, which was unremarkable. Maternal TSH was low (0.33 uIU/mL), but FT4 and FT3 were normal. TPO antibody was elevated at 152 IU/mL. Additional normal testing included a Kleihauer-Betke acid elution for fetomaternal hemorrhage, testing for adenovirus, hemoglobin electrophoresis, and G6PD. She was referred to our institution for consultation, and cordocentesis was performed which confirmed normal fetal hematocrit. A lysosomal storage disease panel was negative for GM1 gangliosidosis, mucopolysaccharidosis I and VII, Niemann-Pick disease types A and B, Gaucher disease, and sialidosis. The patient underwent induction termination at 22 weeks of gestation. An autopsy described a female fetus with a cystic hygroma, serous (nonchylous) pleural and pericardial effusions, congested liver, hypoplastic lungs, and a "markedly enlarged, hyperplastic thyroid gland". Cardiac anatomy, placental pathology, and karyotype were normal. Placental cultures were positive for common vaginal flora (*Lactobacillus*, *Streptococcus viridans*, and diphtheroids) and negative for fungi and anaerobes. Maternal postpartum testing was negative for inherited thrombophilia and antiphospholipid antibodies. Antinuclear antibodies and TPO antibodies were positive.

In her third pregnancy, prenatal laboratories were unremarkable. Thyroid function was normal (TSH, FT4, T3, TSH, TRAB) other than elevated anti-TPO antibody (201 IU/mL). Given a concern that fetal thyroid stimulation may be driving pathogenesis of hydrops, she was treated with propylthiouracil for fetal thyroid suppression from 12 to 31 weeks with add-back maternal levothyroxine. The pregnancy progressed to term, and the patient had spontaneous rupture of membranes at 38 weeks. Labor was augmented, and she delivered a morphologically normal male infant via normal spontaneous vaginal delivery, weighing 3625 g, with Apgars of 8 and 9. The infant was healthy and without thyroid or other metabolic disease. Neonatal thyroid studies were normal. Incidentally, following delivery, the patient was diagnosed with papillary thyroid cancer. This was not believed to be connected to the presence of anti-TPO antibodies. She underwent total thyroidectomy. Pathology revealed a stage 1 multifocal papillary thyroid carcinoma arising in the background of lymphocytic thyroiditis with negative margins. She was started on levothyroxine and liothyronine sodium for postsurgical hypothyroidism. Radioiodine ablation was deferred due to desired future pregnancy.

In her fourth pregnancy, fetal hydrops was first noted at 15 weeks (Figure 1). Prior to this diagnosis, she had normal first trimester screening and noninvasive prenatal testing. Amniocentesis at 18 weeks returned 46, XY, inv(2)(q14.2q23) male karyotype with suspected paracentric inversion of 2q, which appeared balanced at the level of standard cytogenetic analysis. Fetal microarray was normal suggesting no detectable deletions or duplications at the breakpoints of this inversion. Maternal peripheral blood chromosome analysis showed the same inversion. An expanded carrier screen for 218 hereditary genetic disorders via Progenity's Global Panel was negative. Paternal karyotype was normal. Fetal blood sampling was unremarkable, including normal fetal thyroid studies.

Given the history of recurrent hydrops, without any identifiable diagnosis, WES trio (with full exome sequencing of the parents as controls) was performed on fetal amniocytes. The next generation sequencing results showed compound heterozygous variants in the collagen and calcium-binding EGF domains 1 (*CCBE1,* NM_133459.3) gene, leading to the diagnosis of HKLLS1. Targeted Sanger sequencing was performed on parental and fetal samples confirming the identified variants (Figure 2).

Following fetal demise, dilation and evacuation was performed. Autopsy was significant for mild-to-moderate lymphocyte depletion of the thymus, consistent with intrauterine hypoxic stress, and an excessively long, hyper-twisted umbilical cord.

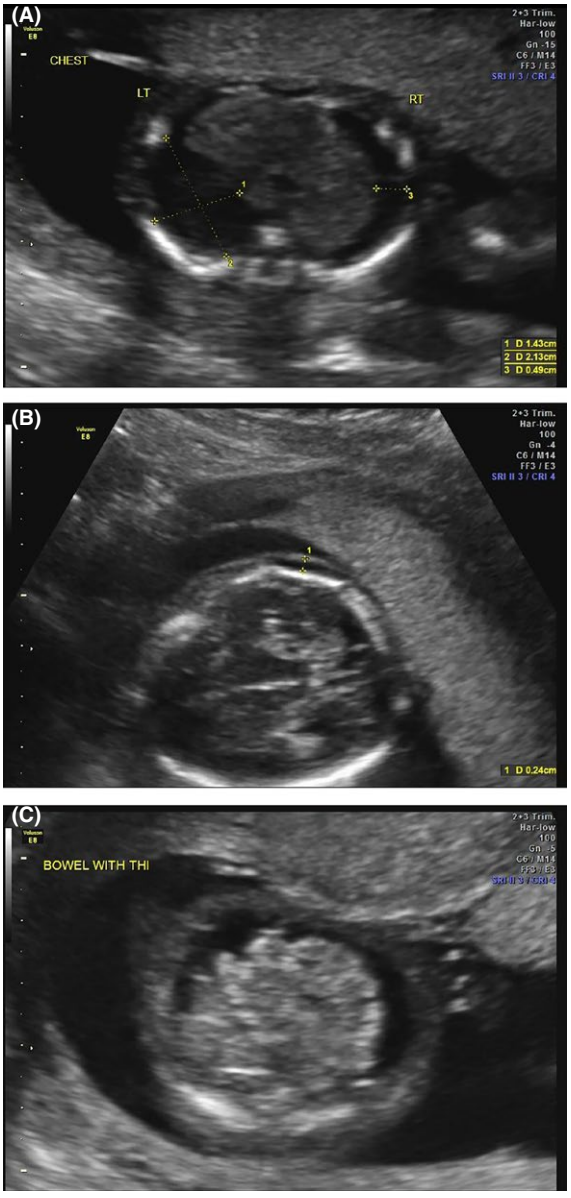

FIGURE 1 Ultrasound images of hydropic fetus from G4 pregnancy. A, Fetal chest demonstrating pleural effusions; B, fetal head demonstrating scalp edema; C, fetal abdomen demonstrating ascites

The maternally inherited p.Leu229fs (c.683_684insT) pathogenic, loss-of-function variant in the *CCBE1* gene is listed in the Genome Aggregation Database (gnomAD) Browser in 14 out of 277 032 chromosomes (rs563023244). This frameshift variant has been reported in a 20-year-old male patient who also carried another *CCBE1* variant, p.Arg158Cys[3] and in a 52-year-old patient who also carried p.Asp104Asn *CCBE1* variant on the other allele.[12]

The paternally inherited p.Thr112Ile (c.335C>T) likely pathogenic variant is rare, has not been published in the literature, and is listed in one out of 246 160 chromosomes in the gnomAD Browser. Threonine 112 is highly conserved, and the pathogenicity of this variant is supported by computational prediction programs (SIFT, MutationTaster, and PolyPhen-2). Thus, given this rare variant is located in the EGF-like calcium-binding domain, on the opposite chromosome of the loss-of-function variant, it is likely pathogenic, supporting the diagnosis of HKLLS1. Formalin-fixed fetal tissue from the second affected pregnancy was submitted for targeted analysis of the identified *CCBE1* variants but good quality DNA could not be extracted on multiple attempts. DNA from the first affected pregnancy was not available. Therefore, confirmation of the variants was not possible in the other affected pregnancies. The unaffected sibling has not yet been tested.

3 | DISCUSSION

Currently, first-line testing for fetal abnormalities identified by ultrasound usually consists of chromosome analysis and/or genomic microarray testing. Chromosome analysis determines the etiology of abnormalities in 9%-19% of cases while genomic microarray provides additional clinically relevant information in 6% of such cases.[13,14] Therefore, in most cases, a cause for the fetal ultrasound abnormalities is not determined.

Next generation sequencing, including whole genome sequencing (WGS) and WES, was first popularized in the research realm to aid in the discovery of new, previously uncharacterized diseases. More recently, reverse phenotyping has employed the same technology to help make rare clinical diagnoses that are suspected to be of Mendelian (single-gene) genetic origin.[15,16] Previously, targeted analysis of candidate genes using specific predetermined probes, for example, florescent in situ hybridization, was needed. This, however, requires at least a suspicion of a particular disease or candidate gene, either by family history or clinical phenotype.[17] Unfortunately, many phenotypes discovered via fetal ultrasound, especially nonimmune hydrops, are nonspecific.

Whole genome sequencing detects genetic variation in a patient's entire genetic code, whereas WES investigates only the protein-coding regions and intron-exon borders. While exons make up only 1%-2% of the human genome, 85% of variants implicated in genetic disorders are located in these coding regions.[18] Exome sequencing is favored over genome sequencing in order to improve efficiency and decrease cost, thereby making it more accessible as a clinically available diagnostic tool.[19]

There are many case reports that describe using WES to aid in clinical diagnosis. More recent series have reported diagnostic yields of fetus and parents trios up to 24% in cases where standard genetic testing is normal.[17] Unfortunately, WES remains limited by cost, lengthy turnaround time, diagnostic gaps, and the need for sophisticated genetic counseling.[20] Furthermore, WES is not reliable in detecting large

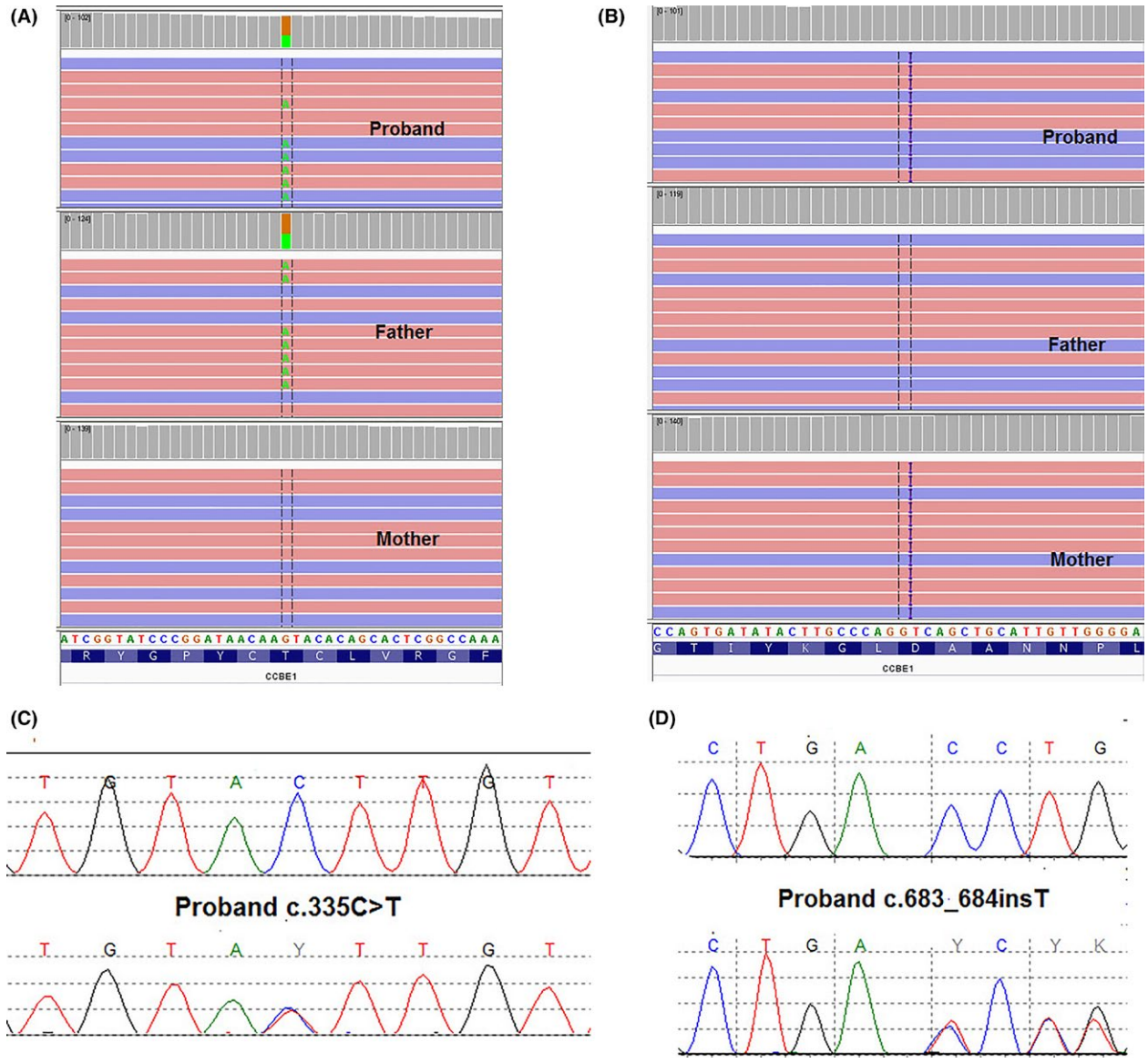

FIGURE 2 A compound heterozygous pathogenic mutations in *CCBE1* gene were detected. (A and C) A paternally inherited variant, p.Thr112Ile (c.335C>T) variant was detected and confirmed by Sanger sequencing. (B and D) A maternally inherited variant, p.Leu229fs (c.683_684insT), was detected and confirmed by Sanger sequencing

deletions/duplications/insertions, structural rearrangements, triple repeat expansions, mitochondrial DNA variants, or mosaic variants. Causative variants may be located in non-coding regions analyzed by WGS, but this test is currently more expensive.[21]

In the above case, WES was used to identify a cause of recurrent fetal hydrops, not otherwise detected on extensive workup of multiple pregnancies, which included fetal karyotype and microarray. If WES had been ordered on the patient's second pregnancy with fetal hydrops, perhaps after the normal microarray analysis, earlier diagnosis could have been established. WES identified compound heterozygosity in the *CCBE1* gene consisting of a known pathogenic variant of maternal origin and a likely pathogenic variant of paternal origin. Unfortunately, complex segregation analysis could not be performed due to inability to test the prior affected pregnancies.

CCBE1, located on chromosome 18q21, codes for a secreted protein that is required for lymphangiogenesis. During embryogenesis, development of the lymphatic system occurs through a process of budding, migration, and proliferation of lymphocytic progenitor cells beginning during the 6th week of gestation. The *CCBE1* gene encodes a signaling protein that is essential to the budding of lymph vessels from venous endothelium.[10,22] *CCBE1* knockout mice models have demonstrated an absence of lymphatic vessels, and affected mice die prenatally with severe hydrops.[23] Homozygosity mapping has identified *CCBE1* gene variants to be a cause of HKLLS1.[3] So far, at least

12 different *CCBE1* variants have been reported in at least seventeen probands/families.[3,4,12,24-29]

In conclusion, WES was able to identify compound heterozygous pathogenic variants in the *CCBE1* gene as a cause of HKLLS1 and recurrent fetal hydrops. The p.Thr112Ile variant has never been described, but alters a highly conserved sequence and is predicted to be pathogenic. As in our case, WES may provide a definitive diagnosis for cases of idiopathic nonimmune hydrops, allowing recurrence risk counseling, facilitating preconception counseling, and leading to informed future reproductive choices, such as preimplantation genetic diagnosis and earlier prenatal diagnosis with chorionic villus sampling.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

DJM, TSA, and LML-S: involved in drafting the manuscript. RM, TT, CEM, TRM, and DAW: revised the manuscript.

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