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Short report

De novo heterozygous missense variants in *CELSR1* as cause of fetal pleural effusions and progressive fetal hydrops

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ABSTRACT

Fetal hydrops as detected by prenatal ultrasound usually carries a poor prognosis depending on the underlying aetiology. We describe the prenatal and postnatal clinical course of two unrelated female probands in whom *de novo* heterozygous missense variants in the planar cell polarity gene *CELSR1* were detected using exome sequencing. Using several in vitro assays, we show that the *CELSR1* p.(Cys1318Tyr) variant disrupted the subcellular localisation, affected cell-cell junction, impaired planar cell polarity signalling and lowered proliferation rate. These observations suggest that deleterious rare *CELSR1* variants could be a possible cause of fetal hydrops.

INTRODUCTION

Fetal hydrops is defined as the accumulation of fluid in two or more soft tissues or body cavities including polyhydramnios. The prevalence is estimated to range from 1 in 1700 to 1 in 3000 pregnancies, and perinatal mortality rates are high depending on the primary aetiology.[1] Fetal hydrops is categorised as treatable immune-mediated hydrops, causing fetal anaemia and non-immune hydrops (NIHF). NIHF has many causes and a variety of underlying genetic disorders might be responsible for the cases that have previously been diagnosed as idiopathic.[1] In this report, we describe two unrelated cases of fetal pleural effusions leading to severe fetal hydrops in whom trio exome sequencing (ES) revealed heterozygous *de novo* missense variants in *CELSR1* (OMIM *604523).

Cadherin Epidermal Growth Factor Laminin G seven-pass G-type receptor-1 (*CELSR1*) encodes the planar cell polarity (PCP) protein, CELSR1.[2] PCP is the coordination of cell movement in the plane of a tissue by polarisation signalling pathways. Together with *FZD3* and *VANGL2*, *CELSR1* coordinates the tissue-wide intracellular orientation and displacement of cilia patches.[3] One of the downstream targets of the PCP pathway is the RAS homologue gene family member A (RhoA), which functions as an effector of the PCP pathway activity.[4] Previously, *CELSR1* variants have been associated with neural tube defects (NTDs)[5 6] and lymphatic malformation type 9 (OMIM #619319).[7–9] *CELSR1* is considered as one of the genes in the 22q13.3 microdeletion contributing to Phelan-McDermid syndrome, of which lymphoedema can be part of the clinical features.[10] We are the first to present supportive evidence for pathogenicity of heterozygous *CELSR1* deleterious missense variants as a potential cause for fetal hydrops.

MATERIALS AND METHODS

Medical records were obtained and reviewed. Details on ES procedures and gene panels can be found in the online supplemental materials, as well as details regarding the functional studies.

RESULTS

Clinical report

For details on the prenatal and postnatal clinical course of both probands, see the online supplemental tables. Both cases concerned the second pregnancy of non-consanguineous parents and both were female. In the first case, mild unilateral pleural effusion was detected at the routine second trimester ultrasound. This progressed into severe bilateral hydrothorax, ascites, skin oedema and polyhydramnios (figure 1A), for which intrauterine thoracoamniotic shunting of the left hemithorax and drainage of the contralateral hemithorax at 35 weeks of gestation was successfully performed. Postnatally, she needed constant ventilation and bilateral thoracic drains were placed. Inotropic support was given with medication and a reduction in the drain output was observed by administering octreotide. However, effusions recurred, and thoracentesis had to be performed. Five weeks post partum, MRI thorax lymphangiography[11] (figure 1B) showed no passage of contrast through the thoracic duct and abnormal backflow of contrast to skin. Since this left no options for interventional radiological embolisation, nor was the child's condition good enough for long-term treatment with experimental drugs, clinicians and parents agreed on palliative care. The proband passed away at the age of 8 weeks. Postmortem, immunohistochemistry was performed on a skin biopsy which showed dilated lymphatic vessels in the subcutaneous fat (figure 1C).

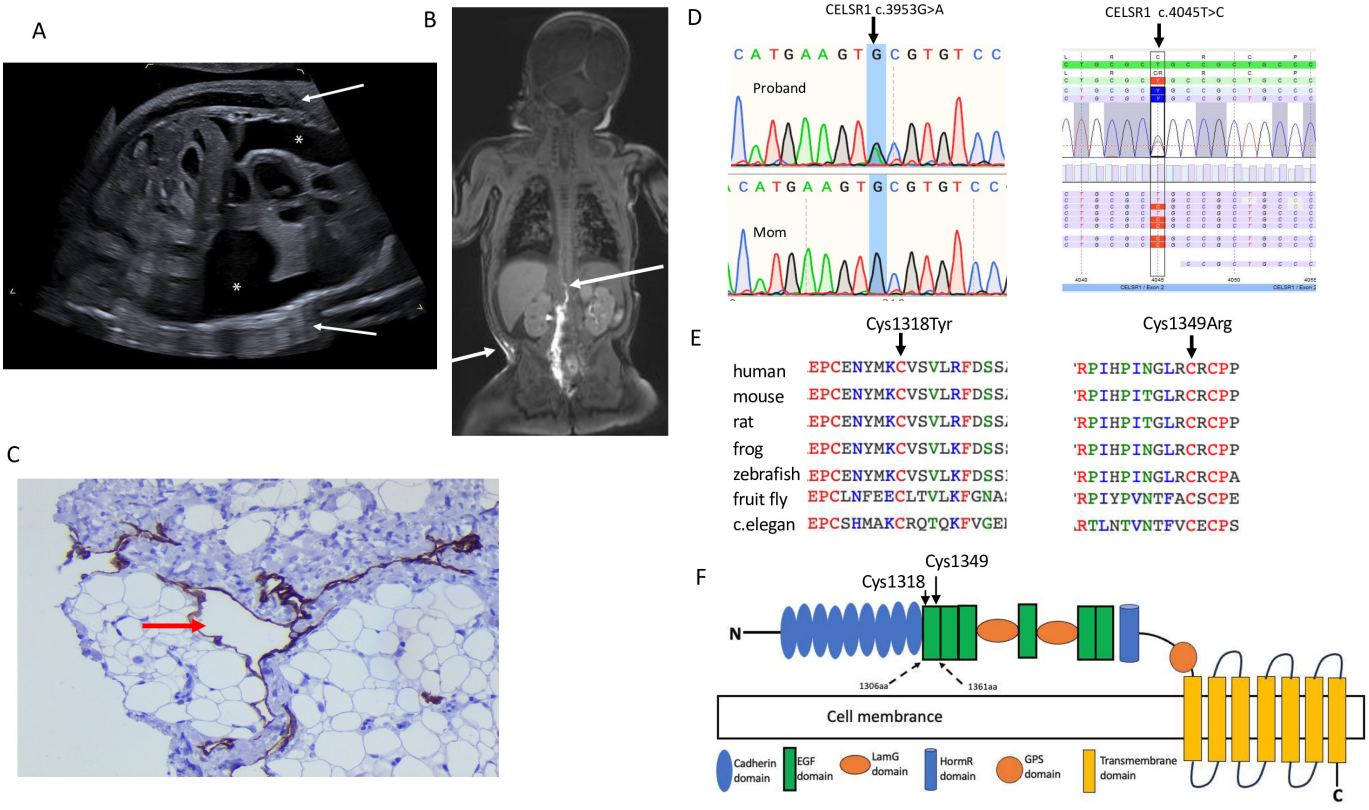

Figure 1 Clinical and genetic analysis of the fetal hydrops cases. (A) Fetal ultrasound of the p.(Cys1318Tyr) proband at 35 weeks 6 days of gestation. Arrows: skin oedema; asterisk: bilateral pleural effusion. (B) MRI lymphangiography of the p.(Cys1318Tyr) proband with contrast injected into the inguinal lymph nodes. Upper arrow: start of the thoracic duct, absence of contrast fluid is clearly visible from the diaphragm and upwards; lower arrow: dermal backflow of contrast fluid. (C) Immunohistochemistry for D2-40 of the p.(Cys1318Tyr) proband skin biopsy. Arrow: dilated lymphatic vessels in subcutaneous fat. (D) Sanger sequencing and exome sequencing analysis of the two cases. (E) Conversation analysis of the *CELSR1* 1318 amino acid cysteine. (Human: NP_001365257.1; mouse: XP_006520442.1; rat: XP_006242229.1; frog: XP_004913058.2; zebrafish: XP_001920772.2; fruit fly: NP_001260871.1; *Caenorhabditis elegans*: NP_506256.3). (F) Schematic diagram of the CELSR1 protein. Both of the CELSR1 mutants targeted the first EGF domain.

The second case was ascertained through GeneMatcher.[12] At routine third trimester ultrasound, fetal hydrops was first detected including severe left hydrothorax and moderate right hydrothorax, ascites, skin oedema and polyhydramnios. Intrauterine thoracoamniotic shunting of the left hemithorax was successfully performed at 30 weeks of gestation. Postnatally, thoracic drains were in place during the first 9 days of life. She received octreotide and similar medication for inotropic support and needed constant ventilation. She was extubated at age 24 days. At the age of 3.5 months, she was put on nasal cannula. Because of feeding intolerance, she received percutaneous endoscopic gastrostomy 1 month after. Currently, she is at home, but still requires low amounts of oxygen and treatment with diuretics. She acquired some respiratory infections but recovered well with slightly higher oxygen demand. Her development, although delayed, progresses continuously.

DNA analysis and functional evaluation

Prenatal genetic analysis was done on amniocytes, and no chromosomal aberration was identified in both cases. Probands and both parents were sequenced in trio postnatally (trio ES) and the following *de novo* variants (figure 1D, online supplemental figure 1) were detected and classified as likely pathogenic according to the American College of Medical Genetics and Genomics guidelines[13]:

Case 1: *CELSR1*: Chr22(GRCh37):g.46859834C>T; NM_001378328:c.3953G>A; p.(Cys1318Tyr).

Case 2: *CELSR1:* Chr22(GRCh37):g.46859742A>G; NM_001378328.1:c.4045T>C; p.(Cys1349Arg).

Both variants have not been previously reported in databases (in-house, national and the Genome Aggregation Database (GnomAD (V.2.1.1)) and affected highly conserved amino acids (figure 1E), which both locate in the first EGF domain of *CELSR1* (figure 1F). Functional prediction scores, including Polyphen-2 and MutationTaster, labelled these variants as damaging and disease-causing, with Combined Annotation Dependent Depletion (CADD) scores of 28.3 and 27.8, respectively.

To verify pathogenicity of the *CELSR1* c.3953G>A; p.(Cys1318Tyr) variant, several in vitro studies were performed. We examined the impact of the p.(Cys1318Tyr) variant on CELSR1 subcellular localisation. The results indicated that the variant affected CELSR1 protein cell membrane localisation compared with wild-type CELSR1 protein in both a plasmid-based overexpression system and the patient fibroblast cells (figure 2A, online supplemental figure 2). Bulk RNA-seq of RNA samples extracted from the proband and the mother's fibroblast cells demonstrated that in the proband mRNA samples, the amount of *CELSR1* mRNA (online supplemental figure 3) was significantly decreased (adjusted p=0.02). Several cytoskeleton-related genes, including *VIM*, *LAMC3*, *COL23A1* and *DNAH5*, were downregulated in the proband compared with the mother (figure 2B). A Gene Ontology (GO) analysis revealed that organelle fission, cell-cell junction, cell-substrate junction and focal adhesion were significantly affected in the proband fibroblast cells (figure 2B).

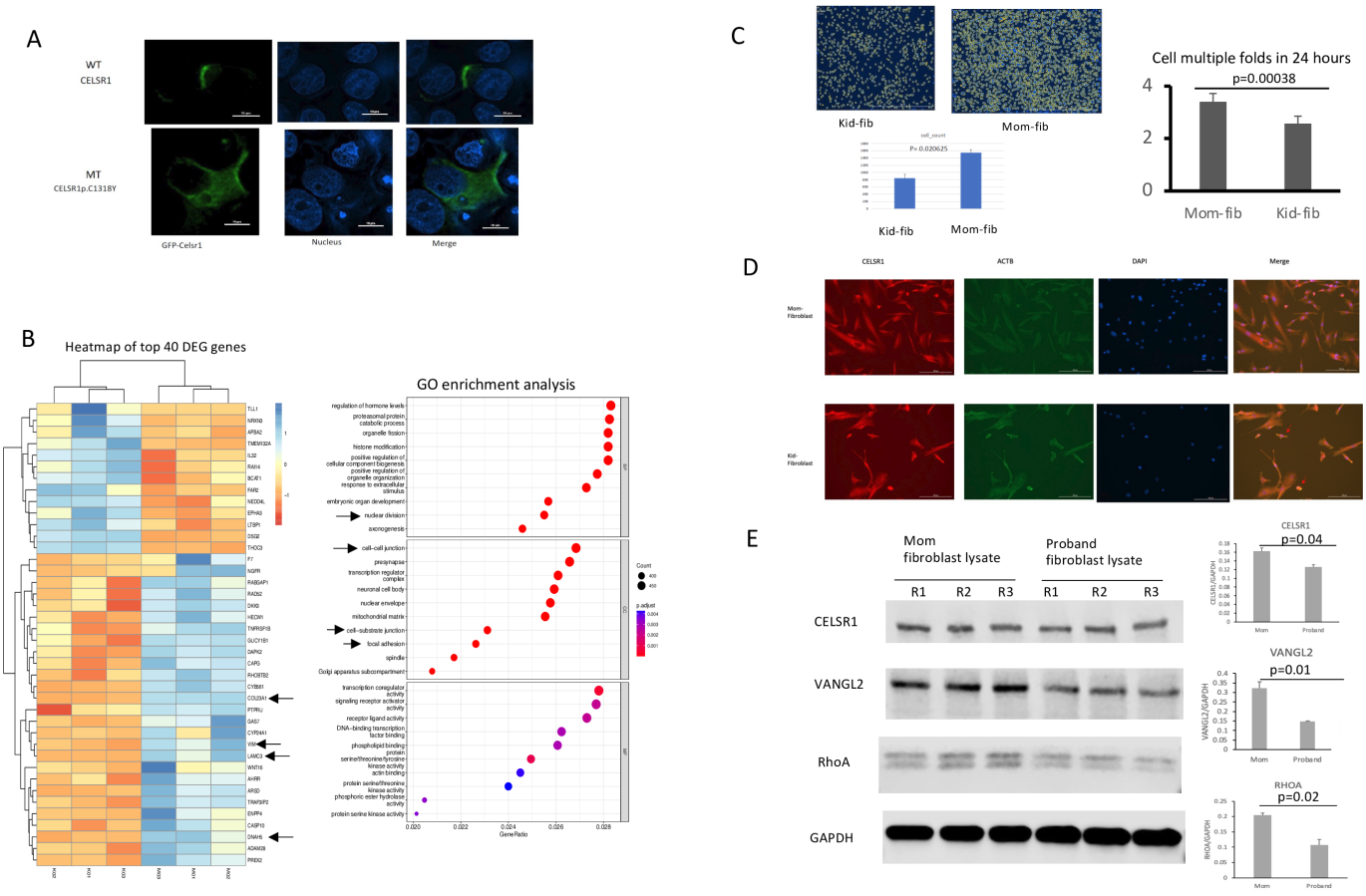

Figure 2 In vitro functional analysis of *CELSR1* p.Cys1318Tyr. (A) Subcellular localisation analysis of GFP-CELSR1 wild-type and mutant protein overexpressed through pGFPN1-CELSR1 plasmids in Hela cells. (B) RNA-seq analysis of RNA samples extracted from fibroblast cells of the mother and the proband. Left: heatmap analysis of the six fibroblast cell RNA samples (three maternal and three proband). Right: GO analysis of RNAseq data. (C) Fibroblast cell proliferation rate analysis. (D) Fibroblast filipodia analysis. Immunofluorescence analysis of CELSR1, β-ACTIN (ACTB) were performed in the maternal and proband fibroblast cells. The arrow indicates the constricted filipodia. (E) PCP pathway signalling analysis by immunoblotting. Left: immunoblotting images of CELSR1, VANGL2, RhoA and GAPDH. Right: semiquantification analysis of CELSR1, VANGL2 and RhoA using GAPDH as reference. BF, biology function; BP, biology process; CC, cellular component; GO, Gene Ontology; PCP, planar cell polarity.

The GO-Biology Process analysis also demonstrated that nuclear division was affected (figure 2B). To assess whether the variant affected cell proliferation, we evaluated cell multiple folds over 24 hours in both the proband and the mother's fibroblast cells. The results indicated that the maternal fibroblast cells increased 3.4 times, while the proband fibroblast cells increased 2.5 times (p=0.00038) (figure 2C). We also demonstrated that cell filopodia were affected in the proband fibroblast cells but not in the maternal fibroblast cells, as indicated by the immunofluorescence of β-actin (figure 2D). As *CELSR1* is a core PCP gene, we examined the PCP pathway signal by immunoblotting analysis of VANGL2 and RhoA. Our results showed that the mutant CELSR1 protein in the proband's fibroblast cells was slightly decreased (figure 2E), which was consistent with the RNA-seq data. Immunoblotting results indicated that VANGL2 and RhoA proteins in the proband's fibroblast cells were significantly reduced compared with the mother's fibroblast cells (figure 2E).

No functional testing was performed on the c.4045T>C; p.(Cys1349Arg) variant, since this case was included after finalising functional testing of the c.3953G>A; p.(Cys1318Tyr) variant. Considering both variants lie within the same domain of the gene (figure 1F), in silico predictions indicate pathogenicity, absence of the variants in GnomAD and similar phenotypes, comparable effects on protein function are expected.

DISCUSSION

To the best of our knowledge, we are the first to describe two heterozygous, *de novo*, missense variants in *CELSR1* possibly associated with fetal hydrops. Previously, loss of function *CELSR1* variants have been reported in probands with distal lymphoedema[7–9] with ages of onset ranging from birth to 77 years. Recently, a heterozygous *CELSR1* missense variant classified as a variant of uncertain significance was detected in a large fetal congenital lymphatic anomalies cohort.[14] When lymphoscintigraphy was performed,[8] signs of lymph rerouting indicating valve deformities were detected. Tatin *et al*[15] showed in mouse embryos that *CELSR1* and *VANGL2* are crucial for the normal development of valve leaflets. Lymphatic dysplasia can lead to fetal hydrops.[1] It is established that *CELSR1* is required for proper cell-to-cell adhesion and lymphatic valve morphogenesis. Our in vitro assays suggest that the *CELSR1* variant p.(Cys1318Tyr) disrupted the subcellular localisation, affected cell-cell junction, impaired PCP signalling and lowered proliferation rate. Since *CELSR1* is expressed in cilia-enriched tissues such as the lymphatic system, it is expected that the decreased cell proliferation rate and damaged cell-cell junctions contribute to the leaking of lymphatic fluid causing the chylothorax. These findings establish the pathogenicity of this variant and substantiate a

potential causal relationship with missense variants in *CELSR1* and the described phenotype. It is unclear if the female sex of our probands may have played a factor in the severity of the phenotypes as suggested by previous publications.[7–9] Treatment of hydrothorax is developing quickly and is currently the subject of further research. Early diagnosis, including knowledge of the aetiology, is imperative to develop effective therapies.

Previous publications linked *CELSR1* variants to a broad spectrum of prenatal and postnatal phenotypes. Truncating and missense variants in *CELSR1* are shown to be associated with NTDs.[5 6] Recent literature identified heterozygous and biallelic *CELSR1* deleterious variants in cohorts suffering from lymphoedema,[8] epilepsy[16] or autism.[17] Elucidating the mechanisms behind the wide variety in expressed phenotypes and the spectrum of *CELSR1* variants (missense variants, truncating variants, whole gene deletions) remains a challenge and is of great importance especially when detecting a *CELSR1* variant prenatally. *CELSR1* can also be part of the 22q13.3 microdeletion including *SHANK3* causing Phelan-McDermid syndrome (OMIM #606232). Smith *et al*[18] recently showed that probands with Phelan-McDermid syndrome having deletions larger than 4 Mb and especially when the deletion includes *CELSR1* are at a greater risk of developing lymphoedema. This substantiates the claim of the importance of *CELSR1* in the development of lymphatic malformations.

The use of prenatal ES diagnostics has significantly increased in the past few years, with diagnostic rates of at least 8%–10% in pregnancies for various ultrasound anomalies.[19] Because of this widespread implementation, variants are increasingly identified in genes that were previously only associated with postnatal phenotypes. Counselling of these novel genetic entities with accompanying uncertain phenotypes remains a challenge. We have previously shown that prenatal ES diagnostics substantially impact parental decision-making regarding termination or continuation of the pregnancy.[20] However, decision-making is complicated when little is known about the (postnatal) prognosis of a case that presents prenatally at the severe end of the phenotypic spectrum. Standardised protocols for reporting prenatal genetic variants are therefore crucial and up-to-date international databases to detect similar cases are essential.

In conclusion, our observations suggest a role of *CELSR1* variants in the development of fetal hydrops and provide evidence for the pathogenicity of the reported variants. Future research needs to be conducted to further validate this finding. Furthermore, we describe the prenatal and perinatal management of probands with severe fetal hydrops.

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Correction notice This article has been corrected since it was published online first. The corresponding author's email address was incorrectly duplicated; this has been corrected.

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