

SYMPOSIUM REVIEW

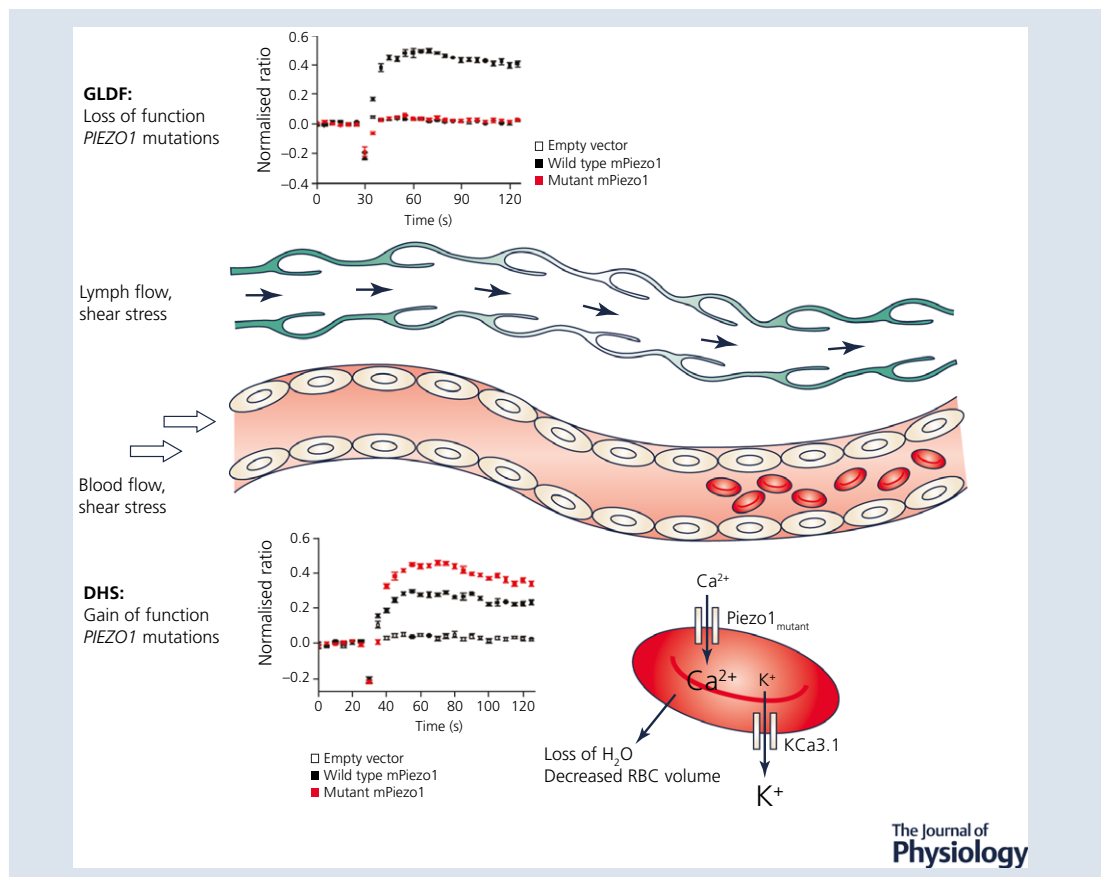
Human phenotypes caused by *PIEZO1* mutations; one gene, two overlapping phenotypes?

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This review was presented at the symposium 'Piezo channel mechanisms in health and disease', which took place at IUPS 38th World Congress, Rio de Janeiro, Brazil, 1–5 August 2017.

Abstract PIEZO1 is a large mechanosensitive ion channel protein. Diseases associated with PIEZO1 include autosomal recessive generalised lymphatic dysplasia of Fotiou (GLDF) and autosomal dominant dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal oedema (DHS). The two disorders show overlapping features, fetal hydrops/perinatal oedema have been reported in both. Electrophysiological studies suggest opposite mechanisms of action: the mutations identified in GLDF patients cause a loss-of-function mechanism of disease and mutations in DHS patients cause gain of function. This raises the question: Is the pathogenic disease mechanism behind the fetal oedema the same in the two phenotypes? In this Symposium Review, we will discuss the two conditions and highlight key questions that remain to be answered. For instance, the perinatal oedema often resolves soon after birth and we are still at a loss to understand why. Are there any mechanisms which could compensate for the faulty PIEZO1 in these patients? Are there physiological changes at birth that are less reliant on the function of PIEZO1? Thus, there is a clear need for further studies into the two disorders, in order to fully understand the role of PIEZO1 in health and disease.

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Abstract figure legend Two human phenotypes, DHS and GLDF, with some overlapping clinical features are caused by mutations in the *PIEZO1* gene. Recombinant PIEZO1 channels with GLDF-associated loss-of-function mutations display a strongly diminished response to stimulus (red squares in upper panel) compared to wild type (black squares). In DHS patients, the slower inactivation of mutant PIEZO1 channels leads to an excess of calcium influx into the cell and an increased potassium efflux from the cell. The ability of the ATP driven Na^+/K^+ pump to maintain osmotic balance is outweighed by the gain of function in the PIEZO1 channel, causing loss of cell water and decrease in volume. This change in cell membrane permeability causes the red blood cell to dehydrate. PIEZO1 channels play a crucial role in cardiovascular development and adult cardiovascular function, and are activated by fluid shear stress. The ability of PIEZO1 to sense shear stress suggests a role connecting mechanotransduction and lymphovascular development and function.

Introduction

Piezos are large mechanosensitive ion channel proteins. PIEZO1 is mainly expressed in non-sensory tissues while PIEZO2 is predominantly found in sensory tissues (Wu *et al.* 2017). Many different roles are being assigned to PIEZO1, e.g. contributing to the regulation of urinary osmolarity (Martins *et al.* 2016), controlling blood pressure (Wang *et al.* 2016), or acting as a sensor of epithelial cell crowding and stretching (Gudipaty *et al.* 2017). PIEZO1 is also expressed in developing blood vessels and plays a key role in blood vessel formation (Li *et al.* 2014; Ranade *et al.* 2014). The Online Mendelian Inheritance of Man database (OMIM) lists two phenotypes associated with variants in the *PIEZO1* gene. One is the autosomal dominant disorder, dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal oedema (DHS; OMIM 194380) caused by gain-of-function mutations. The other is autosomal recessive generalised lymphatic dysplasia of Fotiou, with non-immune fetal hydrops (GLDF; OMIM 616843) caused by biallelic, loss-of-function mutations. There are examples of allelic disorders in the literature where defects in a single gene are implicated in different phenotypes (Zhu *et al.* 2014). For example, biallelic loss-of-function mutations in *SCN9A*

cause autosomal recessive congenital insensitivity to pain (Cox *et al.* 2006), whereas gain-of-function, heterozygous mutations in the same gene cause autosomal dominant primary erythromelalgia, where there is burning pain of the lower limbs (Cummins *et al.* 2004; Yang *et al.* 2004). However, the paradox here is that both GLDF and DHS have reported cases of perinatal oedema, so it appears that both loss-of-function and gain-of-function mutations in *PIEZO1* can produce similar phenotypic features. In this Symposium Review, the two conditions caused by *PIEZO1* mutations are presented, and overlapping features between the two phenotypes are discussed. We highlight key questions that remain to be answered to improve our current understanding of the role of PIEZO1 in health and disease.

DHS: dehydrated hereditary stomatocytosis – a gain-of-function phenotype

Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal oedema (DHS; OMIM 194380) is an autosomal dominant haemolytic anaemia. The condition is characterized by mild to moderate haemolysis with varying numbers of stomatocytes on peripheral blood smears. There can also be an

elevated MCHC (mean corpuscular haemoglobin concentration) and decreased osmotic fragility. Sometimes high MCV (mean corpuscular volume) and reticulocyte counts are reported. Probably the most sensitive test for DHS is osmotic gradient ektacytometry, which measures red blood cell (RBC) membrane deformability as a function of osmolality. The ektacytometry profile of patients with DHS is shifted to the left indicating RBC dehydration (Fig. 1). Another indicator of DHS is stomatocytic RBCs, where cells can have the appearance of 'coffee beans' (Fig. 2). Other frequent clinical findings in DHS patients associated with the haemolytic anaemia are splenomegaly (resulting from increased red cell trapping in the spleen), cholelithiasis (due to elevated bilirubin levels), infantile hepatitis, jaundice and iron overload, which can lead to hemosiderosis or more widely distributed haemosiderosis. Some patients are asymptomatic, others have transient anaemia, which can go undetected for years. In patients with mild disease, stomatocytes are scarce on blood film, and often DHS patients only come to medical attention because of the unusual presentation of hemosiderosis (Syfuss *et al.* 2006).

Occasionally, pseudohyperkalemia is also observed in the blood samples of DHS patients. This is another indicator of defective cell membrane properties. It can also be a transient finding, as shown in a review of routine plasma potassium measurements over a 7-year period in one patient, where values fluctuated between high-normal and elevated (Syfuss *et al.* 2006).

Some individuals with DHS also present with perinatal oedema which can vary from ascites to severe hydrops, including pericardial or pleural effusions and/or subcutaneous oedema (including hands and feet). The perinatal oedema can be so severe that it is lethal, and *in utero*

death due to generalised oedema has been reported. Some are stillborn or die soon after birth. The pregnancies are frequently complicated by polyhydramnios. If the baby survives the pregnancy, ventilation is often required at birth. The hydrops sometimes resolves *in utero*, at other times it resolves spontaneously within weeks or months after birth and never recurs. Some individuals present with chylothoraces upon the commencement of feeding. Entezami and colleagues treated a case of intra-uterine anaemia but they were not able to cure the oedema, suggesting that the anaemia was not the cause of the oedema (Entezami *et al.* 1996). It has been suggested that the ascites can be of hepatic origin (Rees *et al.* 2004). A persistent cystic hygroma, reported in one DHS case, suggests the oedema could be lymphatic in origin (Ami *et al.* 2009). The mechanism by which perinatal oedema and ascites develop, and then spontaneously resolve in some fetal DHS cases, has not yet been elucidated. No consistent dysmorphic features are associated with DHS.

The age of onset varies widely. A family history is not essential for the diagnosis of DHS, but should there be one, there can be great inter-familial and intra-familial variability in expression. Because of the highly variable expression, DHS can be very difficult to diagnose, ranging from the absence of clinical symptoms to lethal perinatal oedema. For example, one report on intra-familial variation described how one member only had haematological symptoms, while her cousin had perinatal oedema but no signs of haemolytic anaemia (Grootenboer *et al.* 2000). Several families have not been aware of their diagnosis until the occurrence of fetal oedema (Grootenboer *et al.* 2001).

The condition was first reported in 1971, where Miller and colleagues described a large family with over 50 affected members (Miller *et al.* 1971). Later, heterozygous missense mutations, segregating with the disease in DHS families (including the family described by Miller *et al.*), were identified in the *PIEZO1* gene (Zarychanski *et al.* 2012). This finding was corroborated by additional studies of familial and sporadic cases (Albuisson *et al.* 2013; Andolfo *et al.* 2013). Interestingly, upon sequencing of the gene in the family described by Miller *et al.*, three of the affected siblings were found to be homozygous for the variant, whereas the rest of the affected family members were heterozygous (Zarychanski *et al.* 2012). It was reported that the homozygotes had severe haemolytic anaemia with reticulocytosis, stomatocytosis and hyperbilirubinaemia, while the heterozygotes showed only cholelithiasis and intermittent jaundice. A number of cis-missense variants have been identified, but which of those contribute to the DHS phenotype is unclear (Andolfo *et al.* 2013).

Human *PIEZO1* is a highly polymorphic gene with many coding variants reported in the 1000 Genomes Project database, including variants predicted

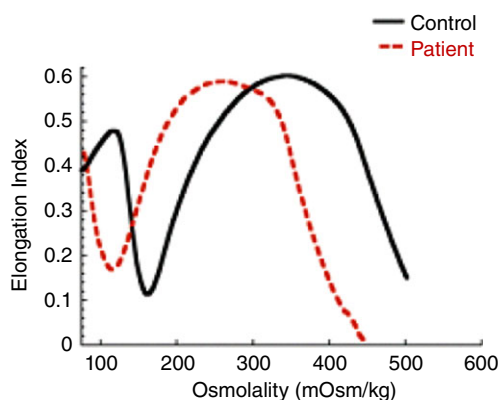


Figure 1. Ektacytometry curves in DHS

This idealised graph shows RBC membrane deformability (using the elongation index) as a function of osmolality. The ektacytometry osmotic deformability profile of a DHS patient (dashed red line) is shifted to the left of the healthy control (continuous black line) indicating high osmotic resistance and RBC dehydration.

as deleterious. A recent case report suggests that the c.7505A>G variant (dbSNP: rs34830861) can cause a DHS phenotype (Del Orbe Barreto *et al.* 2016). Although the patient showed an abnormal curve shifted to the left on ektacytometry, and other blood findings supported the diagnosis of DHS, the rs34830861 SNP has been reported in 97 heterozygous cases in ExAC. Therefore, work to determine whether this mutation alters PIEZO1 channel activity is warranted to confirm that it is pathogenic. Until then, this finding should be interpreted with caution. Electrophysiological studies have been carried out for several DHS-associated PIEZO1 mutations and whole-cell patch clamp demonstrated that the tested mutations could be defined as gain of function (Albuisson *et al.* 2013; Bae *et al.* 2013). The experiments were done in transfected HEK293 cells, and showed that mutations in PIEZO1 slowed the channel inactivation rate. This could explain the red blood cell (RBC) dehydration seen in

PIEZO1 mutation-positive DHS patients. Mechanistically, the slower inactivation of mutant channels causes an excessive calcium (Ca^{2+}) influx into the RBC, which promotes potassium (K^+) efflux through KCa3.1 channels accompanied by osmotic efflux of water, with subsequent cell dehydration (Cahalan *et al.* 2015). A recent paper has shown that the RBC phenotype seen in DHS patients is not just related to delayed channel inactivation; a range of pathophysiological mechanisms are also contributing to the altered channel kinetics (Glogowska *et al.* 2017).

GLDF: generalised lymphatic dysplasia of Fotiou – a loss-of-function phenotype

Autosomal recessive generalised lymphatic dysplasia of Fotiou (GLDF; OMIM 616843) is characterised by a widespread lymphoedema that can affect all segments of the body. Like DHS, GLDF has a high incidence of

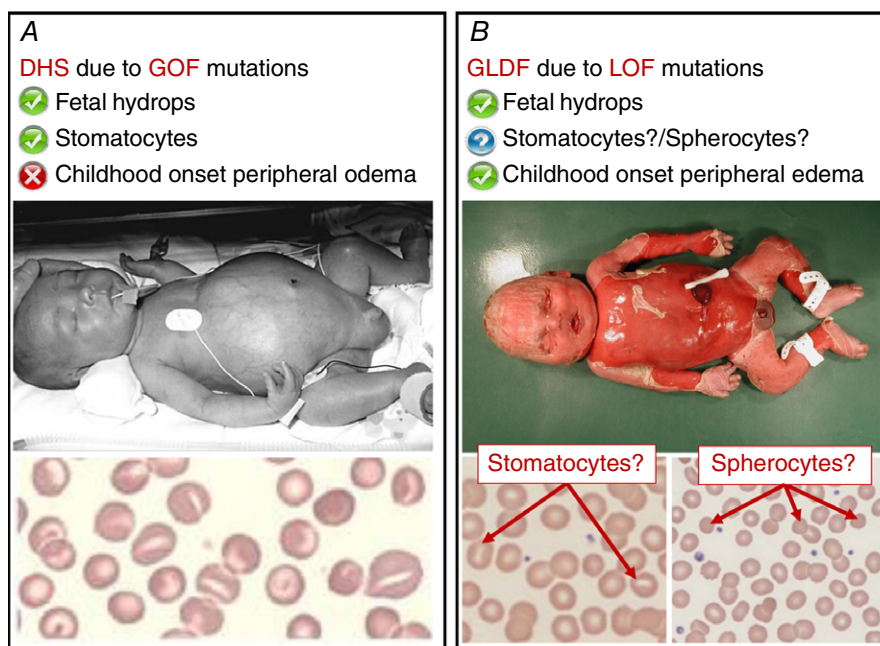


Figure 2. Contrasting DHS and GLDF

A, patients with DHS can present with fetal hydrops. The blood film of a DHS patient is characterised by mouth-shaped (stomatocytic) red blood cells. The red blood cell dehydration seen in DHS patients is due to altered permeability of the RBC membrane which causes a loss of cation content due to the gain-of-function (GOF) mutations in *PIEZO1*. B, patients with GLDF can also present with fetal hydrops. In contrast to DHS, the GLDF patients often develop childhood onset peripheral oedema, indicating that in GLDF the hydrops could be due to an underlying lymphatic problem. Is the fetal hydrops due to the same underlying disease mechanism in the two conditions? Do all GLDF patients present with stomatocytes or spherocytes on their blood films? Not enough cases have been investigated to make a firm conclusion. Will the loss-of-function (LOF) mutations in GLDF patients lead to overhydrated red blood cells? Many questions remain unanswered, thus there is a clear need for more studies into the functional role of *PIEZO1*. Figure 2A upper panel reproduced from Archives of Disease in Childhood: Fetal & Neonatal, Dehydrated hereditary stomatocytosis with transient perinatal ascites, A P Basu, P Carey, T Cynober, M Chetty, J Delaunay, G W Stewart, S Richmond, 88(5), F438-9, Copyright 2003 with permission from BMJ Publishing Group Ltd. Figure 2A lower panel reprinted from Blood Cells, Molecules, and Diseases, 56(1), Lydie Da Costa, Ludovic Suner, Julie Galimand, Amandine Bonnel, Tiffany Pascreau, Nathalie Couque, Odile Fenneteau, Narla Mohandas, Diagnostic tool for red blood cell membrane disorders: Assessment of a new generation ektacytometer, 9-22, Copyright 2015 with permission from Elsevier.

non-immune fetal hydrops, i.e. presenting with persistent bilateral pleural effusions, ascites and subcutaneous oedema, with either fetal death or a complete resolution of the hydrops postnatally. The pregnancies are frequently complicated by polyhydramnios. The babies are often hydropic at birth with generalised oedema and pleural effusions requiring ventilation for several weeks. After introduction of milk feeds, the pleural effusions become chylous.

Sometimes lymphoedema of the peripheries recurs in early childhood. This is mainly lower limb lymphoedema, but can also involve the hands, arms and face (which may be intermittent). Genital oedema, which may be intermittent, is frequent in males, sometimes with thickened scrotum, oedema of the foreskin, and hydroceles. Many patients have multiple episodes of cellulitis in their legs, and several of the patients have also had intermittent, severe facial swelling due to recurrent facial cellulitis. The facial cellulitis is a striking feature, and is rarely seen in other forms of primary lymphoedema. Four cases with severe, recurrent facial cellulitis and significant morbidity have been reported (Fotiou *et al.* 2015). There was high pyrexia and respiratory distress that led to admission to intensive care with ventilation. In addition to facial swelling, some of the patients had mild dysmorphic features (cupped simple ears, mild telecanthus due to epicanthic folds, and micrognathia). Some patients were also reported to have varicose veins, papillomatosis, verrucas and warts. Many of the affected children had systemic involvement with chylothoraces and/or pericardial effusions – but intestinal lymphangiectasia is rare (S. Mansour, personal observation).

It was noted that GLDF patients and unaffected carriers were also found to have mild changes (occasional stomatocytes and/or spherocytes) on their blood film, but with no history of haemolytic anaemia. It is questionable whether observations on the blood film are comparable to that of the DHS patients, as more work is needed in this area.

Through whole exome sequencing, Fotiou and colleagues identified biallelic, homozygous and compound heterozygous mutations in the *PIEZO1* gene (Fotiou *et al.* 2015). The mutations segregated with the phenotype in the families. An independent study by Lukacs and colleagues confirmed this finding (Lukacs *et al.* 2015). A mix of missense, stop-gain (nonsense), frameshift and splice site variants have been identified. Sanger sequencing of cDNA from carriers of a splice site mutation showed that intron 13 was not spliced out in the mutant protein. None of the variants, except for 2 found in cis, were found in the dbSNP, or 1000 Genomes Project databases or in 900 in-house control samples (Fotiou *et al.* 2015).

Western blot analysis of RBCs from GLDF patients showed a complete absence or reduction of *PIEZO1* protein expression compared to a healthy control (Fotiou

et al. 2015). Analysis of RBCs from a patient with biallelic mutations in *PIEZO1* showed decreased *PIEZO1* function (Lukacs *et al.* 2015). This was mainly due to a reduced abundance of *PIEZO1* channels in the RBC plasma membrane. The absence of symptoms in the parents carrying just one variant each argues against a dominant negative effect of the variants. Where the DHS-associated *PIEZO1* mutations have been shown to lead to dehydration of RBCs due to excessive calcium influx, the GLDF associated *PIEZO1* mutations give rise to decreased *PIEZO1* function with no calcium influx. As a consequence, reduced influx of calcium may cause fluid retention in the RBCs leading to ‘hyper-hydration’ (Cahalan *et al.* 2015).

Piezo1 is expressed in embryonic endothelial cells and Piezo1 deficiency causes the disruption of vascular development in the mouse embryo. Loss of *PIEZO1* results in reduced mechanosensitivity of the endothelial cells in terms of alignment and organization in the direction of flow in response to shear stress. Mechanistically, Piezo1 channel activity is stimulated by shear stress leading to calcium (Ca^{2+}) entry in the endothelial cell, increased calpain activity, and modification of the actin cytoskeleton and the focal adhesions required for cell reorganization (Li *et al.* 2014; Ranade *et al.* 2014). Mechanotransduction is also a critical regulator of lymphatic vascular development, and shear stress triggers signalling pathways that promote lymphatic vessel maturation and lymphatic valve formation (Sabine *et al.* 2012; Cha *et al.* 2016). The loss of *PIEZO1* function in GLDF patients, and consequent defects in mechanical stimulation and downstream signalling pathways, could disrupt embryonic lymphatic vascular development. This could result in the progression of hydrops or childhood onset peripheral oedema.

Discussion

In summary, we have presented two conditions caused by mutations in the *PIEZO1* gene, autosomal recessive GLDF caused by loss of function, and autosomal dominant DHS where the disease is a gain of function. In disorders with phenotypic heterogeneity, where different mutations in the same gene give rise to different phenotypes, mutations usually have distinct effects on biochemical and cellular activities. Furthermore, disease specific mutations tend to cluster in key regions. For example, missense mutations in Dynamin2 (*DNM2*) have been described in association with four different phenotypes, depending on which domain of the *DNM2* gene the mutation is located in (Brown *et al.* 2017). *PIEZO1* missense variants are common in both GLDF and DHS, but a one domain–one disease pattern is not clear. The missense variants show a wide distribution across the gene, and the GLDF missense variants are interspersed among the DHS

missense variants (Fotiou *et al.* 2015). Therefore, the clinical phenotype may depend on the nature of the conformational change produced by a disease specific mutation at the individual genomic position in *PIEZO1*. Interestingly, centronuclear myopathy (CNM), one of the *DNM2*-associated phenotypes, is usually caused by mutations in the PH domain, but a few missense mutations have been identified interspersed with Charcot-Marie-Tooth (CMT) neuropathy-associated missense mutations in the middle domain of the *DNM2* gene. Chin and colleagues demonstrated that the mutations had different effects on protein function and that CMT was caused by loss-of-function mechanisms and CNM was caused by gain-of-function mechanisms (Chin *et al.* 2015).

The mutations in *PIEZO1* have also been shown to have distinct effects. The gain-of-function mutations were reported to increase calcium influx into RBCs leading to their dehydration. The loss-of-function mutations were reported to yield no calcium influx, which may lead to the over-hydration of RBCs. According to the literature, ektacytometry is the most effective method for diagnosing DHS, rather than direct observation of blood films alone. None of the patients of Fotiou *et al.* underwent ektacytometry, and it would be interesting to study this further, to see if the ektacytometry curves in the GLDF patients show a shift to the right, in contrast to the shift to the left observed in DHS patients. Could ektacytometry consequently be an equally suitable method for the diagnosis of GLDF?

Although caused by different disease mechanisms, the two disorders at first sight show some overlapping features. For example, fetal hydrops has been reported for both disorders (Fig. 2). Fetal hydrops is not the cause of disease, but rather an outcome of an underlying developmental problem (Bellini *et al.* 2015). Therefore, we need to understand better the fetal hydrops in both DHS and GLDF cases, to determine whether they have the same cause. The GLDF hydrops seems most likely to be lymphatic in origin, but is this also true in DHS? Or could it be hepatic in origin as previously suggested (Rees *et al.* 2004)? It is also unclear why the hydrops resolves at birth. Are there changes that occur then which could compensate for the defective function of *PIEZO1*?

Interestingly, in some GLDF patients, the fetal hydrops resolved soon after birth, but persistent peripheral oedema appeared later in childhood. This does not seem to be the case in DHS patients; at least this has not been reported. If, as suggested above, the hydrops is due to different disease mechanisms, the underlying fault is then not necessarily producing the same effect later in life. This could explain the development of peripheral oedema in one disorder and not the other. It is not clear whether the DHS patients have been examined for peripheral oedema later in life. Particularly those who presented with hydrops (if they survived). Perhaps the fetuses with

perinatal oedema associated with the DHS phenotype, have a second, but as yet unidentified, hit or variant in *PIEZO1* or a modifying gene.

The fact that the fetal hydrops in both conditions are able to fully resolve in the few weeks after birth suggests a physiological change at birth that is having a major effect on the oedema. If this could be understood, the potential for treatment of this, and other lymphatic related hydrops, is tangible. There is clearly a need for more work to be carried out, in order to fully understand the role of *PIEZO1* in the two disorders presented here.

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Additional information

Competing interests

The authors have no competing interests.

Author contribution

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