Özellikle tekrarlayıcı **Non Immun Hidrops Fetalis** vakalarında detaylı genetik araştırma çok önemlidir.
Bizim de bu konuda yaptığımız çalışmalar devam etmektedir.

A CASE STUDY : GENETIC ANALYSIS OF NON-IMMUNE HYDROPS FETALIS (NIHF) PATIENTS

GENETIKS® THE CODE OF LIFE

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INTRODUCTION

Hydrops fetalis is defined by excessive and abnormal pathologic fluid accumulation present within two or more soft tissues/body cavities detected prenatally or postnatally. Fluid collections might be in the form of ascites, pericardial effusion, pleural effusions, generalized skin oedema with skin thicker than 5mm, placental edema, polyhydramnios etc. Hydrops fetalis can be further investigated under 2 categories: (1) immune, which is mainly caused by red cell alloimmunization, (2) non-immune (NIHF), which now accounts for 90 % of the cases as broad use of Rhesus (Rh) D immune globulin dropped the prevalence of immune hydrops fetalis significantly.

With regard to structural abnormalities, variants in different genes cause various disorders associated with NIHF. In order to diagnose, karyotyping (common aneuploidy 18.3%) followed by CMA (abnormal results 3.4 %) should be done prior to WES (~30%).

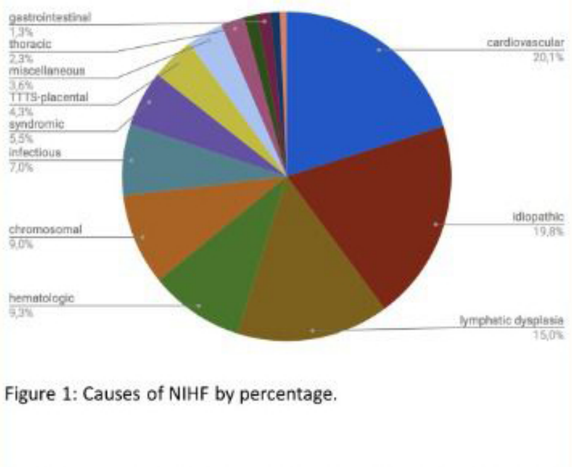

Figure 1: Causes of NIHF by percentage.

One of the most common causes of NIHF, generalized lymphatic dysplasia (GLD) is a scarce form of primary lymphedema of the extremities and systemic involvement. In a normal fluid regulation in the fetus, fluid passes from capillaries to interstitial space and is reabsorbed by the lymphatics, finally returns to the vasculature 1. However, in GLD, there is an obstruction to the lymphatic flow. Previously, variants of the genes GJC2, CALCRL, VEGFC, FLT4, LMPHM2, EPHB4, PIEZO1 and LMPHM5 have been associated with lymphoedema. PIEZO1 is a predescribed unique class of non-selective cation channels activated by physical stimuli. It exhibits important roles in cardiovascular, renal and hematopoietic systems, with a role in cell volume homeostasis in red blood cells. Also, it has a role in human lymphatic development and was implicated that it might be a genetic cause of lymphatic dysplasia. Pathogenic variants on the gene PIEZO1 have been previously related to autosomal recessive form of GLD, lymphatic malformation type VI (LMPHM6) which is frequently related to hydrops fetalis (OMIM: 616843).

Variants detectable by WES can classified under different organs and systems as follows:

- **Central nervous system** (B9D1, B9D2, CC2D2A, CEP290, MKS1, RPGRIP1L, TMEM67, TMEM216,FLVCR2 , DHCR7, PEX1 (70%)...)
- **Cardiovascular system** (SCN5A, GATA5, KMT2D, KDM6A, PIK3CA, MKKS, MID1, PIK3CA, RASA1, TSC1, TSC2...)
- **Pulmonary and thoracic** (NIPBL, FRAS1 (>50%), FREM2, GRIP1,..)
- **Gastrointestinal systems** (CTSA, GBA, NEU1, GNPTAB, GALNS, GLB1, GUSB, SMPD1, MVK, LIPA...)
- **Genitourinary and Renal systems** (SLC12A1, KCNJ1, CLCNKB, BSND, CLCNKA, CLCNKB, PKHD1, LAMB2, NPHS1, NPHS2;
- **Musculoskeletal** CLCN7 (75%), PSATI1, PSPH, FOXP3...)
- **Hematologic systems** (HBA1, HBA2, CDAN1, SEC23B, CEP, UROS, GATA1, RPL5...)
- **Lymphatic system** (PTPN11, SOS1, RAF1, RIT1, CBL, SHOC2 (RASopaties), ITGA9, PIEZO1, CCBE1, FAT4, GATA2...)
- **Skin** (SGPL1)

OBJECTIVES

Here, we present two women both with recurrent NIHF affected by novel homozygous variants on the PIEZO1.

These variants have been described as likely pathogenic according to ACMG criteria.

CASES ANALYZED

Both cases are known to be consanguineous marriages, patients married to cousins yet degree of relativity is unknown (pedigrees are depicted in Figure 2).

CASE 1

Patient I presented with recurrent NIHF with an obstetrical history of 2 intrauterine fetal demise (IUFD). In her first pregnancy in 2016, detailed ultrasonography revealed scalp oedema in fetal biometric scoring, hyperechogenic focus in the heart, bilateral hydrothorax, hyperechogenic intestinals, skin oedema and placental thickening of 82mm. Her antenatal follow up for trisomy 21 (T21) during her first trimester screening was 1/1260 and biochemistry follow ups revealed positive IgG for rubella otherwise not significant. In her routine 26 w follow up, she presented with a IUFD.

In her second pregnancy, she presented with a fetus on 18w4d with skin oedema, pleural effusion, ascites, scalp oedema resulting in hydrops fetalis. Triple screening test for T21 was 1/50, cordocentesis result showed normal karyotype. She presented with IUFD on 20w6d, autopsy confirmed non immune hydrops fetalis with autolytic changes to the organs, subcapsular hemorrhage in liver and oedema in placental villus. The couple had normal karyotyping results.

In her case pregnancy, the patient presented with hydrops fetalis diagnosed earlier on 17w of gestation. Her second trimester check- up, 23w, revealed skin oedema, placental thickness, hydrothorax, pericardial effusion confirming hydrops fetalis diagnosis. An 15.5x15mm hypoechoic cystic expansion and oedema with an internal septa suspected to be of lymphatic origin. Skin oedema resulted in 10mm around the eye area (see in Figure 2). Her microarray analysis and karyotyping did not detect any abnormalities.

On 27w3d of gestation, fetal distress was present with increased fluid around thorax; therefore, ultrasound guided thoracentesis (100cc) and paracentesis (50cc) were performed prior to a caesarean section under general anesthesia. Resuscitation was done in the operation room, a female fetus weighing 2795 gr, measuring 43 cm giving head circumference of 33.5 cm with Apgar scores of 0/2 at 1 and 5 min, respectively. The neonatal was given surfactant 200mg/kg and intratracheal adrenalin after intubation. The ventilated neonatal was then transferred into NICU. The heart rate was at 120/min but started dropping, intravenous adrenaline was given. The neonatal was connected to mechanical ventilation in SIMV mode, 100% FiO2 was given as well. Another round of thoracentesis and paracentesis was performed but was unsuccessful. Her heart rate continued dropping and demised on minute 7 of life. The autopsy was not performed upon the request of the couple.

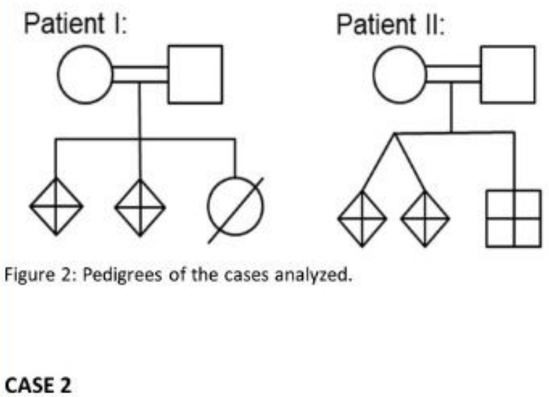

Figure 2: Pedigrees of the cases analyzed.

CASE 2

Patient II came in with a history of IUFD due to recurrent hydrops fetalis as well.

In her first pregnancy, she presented with twins on 18 weeks of gestation with fetal ascites and peripheral oedema leading to diagnosis of hydrops fetalis. Karyotyping and biochemistry analyses were normal. On 25w5d of gestation, the fetuses were presented with IUFD. No autopsy was performed honoring the wishes of the couple.

In her case pregnancy, she presented at 20w of gestation with fetal ascites, skin oedema, pericardial effusion, pleural effusion and heterogenous oedema around the neck area. Placental thickness was measured 46mm, while abdomen wall thickness was 14mm. Medial cerebral artery (MCA) and PS were 29 and 30 respectively, fetal anemia was not suspected. On 23w of gestation, amniocentesis was performed for further genetic investigation. On 24w3d, the patient was taken to the hospital as she felt no movement for 2 days. The fetus was found demised. Again, no further autopsy was done.

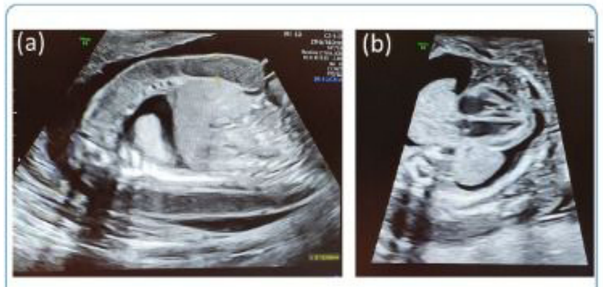

Figure 3: skin oedema reaching 10mm, patient I (a), pericardial effusion, pleural effusion, skin oedema, patient I (b).

RESULTS & DISCUSSION

For patient I, on 27w3d prior to C-section, amniotic fluid was drained. Fetal karyotyping and SNP array analysis revealed normal results. In addition, WES including CNV analysis was performed. As a result, a novel homozygous variant c.2898_2899del (p.Phe967Cysfs*99) was detected in PIEZO1 (NM_001142864.2) gene resulting in a frame shift. This frame shift has led to the formation of a stop codon and caused premature termination of the protein (1066 aa instead of 2521 aa). This variant is classified as likely pathogenic according to ACMG criteria.

Patient II's amniotic fluid was drained on 23w of gestation. Amniotic fluid was subjected to karyotyping and SNP array analysis, and the results came out normal. WES was performed, a homozygous variant c.2488del p.(Val830Cysfs*3) on PIEZO1 gene was detected, again classified as likely pathogenic according to ACMG criteria. As an incidental finding, a hemizygous variant in IGSF1 gene (c.70+1G>A, rs777462101) was also detected. The variant in PIEZO1 has led to a frame shift on the 830th codon resulting in a premature termination 3 codons after. The variant has been classified as likely pathogenic.

There are 2 human diseases associated with PIEZO1: gain-of-function mutations on this gene are a known cause of Dehydrated Hereditary Stomatocytosis (DES) with or without pseudohyperkalemia and/or perinatal oedema (OMIM:194380). On the other hand, loss-of-function variants are found to be a cause of lymphatic malformation type 6 (LMPHM6) (OMIM:616843). LMPHM6 variants in PIEZO1 appear to cause fetal onset lymphedema with common absence of seizures or intellectual disability. Up to date, 17 variants in PIEZO1 have been associated with NIHF and lymphatic dysplasia according to HGMD.

CONCLUSIONS

We report two likely pathogenic variants in the cases of PIEZO1 associated with GLD and NIHF. This sums up to a total of 19 variants associated with lymphatic dysplasia and NIHF with lymphatic dysplasia. Therefore, when a hydrops fetalis diagnosis is followed by an oedema suspected to be of lymphatic origin, like in our case, screening for PIEZO1 might be requested. Identification of an underlying genetic cause with fetal or neonatal demises due to NIHF can provide promising outcomes in the future by using reproductive options including preimplantation genetic testing and prenatal genetic testing.