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GENETIC EVALUATION AND MANAGEMENT OF FETAL CHYLOTHORAX: REVIEW AND INSIGHTS FROM A CASE OF NOONAN SYNDROME

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ABSTRACT

Fetal chylothorax is one of a very few syndromes that can be treated in utero with thoracoamniotic shunting or pleurodesis by OK-432 as two major therapeutic modalities. We report on a fetus with Noonan syndrome and a missense mutation c.182A>C (p.Asp61Ala) of PTPN11 *who responded poorly to antenatal pleurodesis by OK-432. Based on our previous publication and this case study, we propose that fetal chylothorax of a distinct genetic origin may respond poorly to OK-432 pleurodesis.*

Keywords: fetal pleural effusion, fetal chylothorax, fetal therapy, genetic counseling, hydrops fetalis, OK-432, lymphangiogenesis, Noonan syndrome, *PTPN11* mutation

Fetal chylothorax – fetal chylous pleural effusion which indicates antenatal accumulation of lymphocyte-rich pleural fluid in the pleural space – is a rare disorder with an incidence of approximately 1 in 12,000. Although the precise pathogenesis of fetal chylothorax is still unknown, it is one of a very few fetal disorders that may be treated in utero (1). Several in utero interventions are available, including maternal dietary modifications, intrapleural instillation of maternal blood, repeated thoracocentesis, thoracoamniotic shunting, and prenatal pleurodesis by OK-432 (a biologic response modifier which regulates local inflammation and induces adhesion between the parietal and visceral pleura) (2). Among these, OK-432 has been shown as a novel effective therapy with high success rate as a rescuing procedure for fetal chylothorax, even in those with bilateral and early-onset, a surprising result since both presentations have been regarded as indicators for poor prognosis (2,3). We also recently reported a subset of fetal chylothorax with *ITGA9* mutations which responded poorly to this therapeutic modality (3), although a series by Nygaard has reported a success rate of 100% (4).

Fetal chylothorax can be regarded as one manifestation of hereditary lymphedema syndromes (*Table*). The spectrum of clinical presentations reported in hereditary lymphedema syndromes include skin edema (cutaneous lymphangiectasia), chylothorax (pulmonary lymphangiectasia), chyloascites (intestinal lymphangiectasia), and even hydrops fetalis (generalized). Aberrant lymphvasculogenesis/lymphangiogenesis has

TABLE
Genetic Hereditary Lymphedema Disorders with Known Causative Loci for Chylothorax

Syndrome	OMIM	LOCUS	INHERITANCE
Milroy disease	153100	*VEGFR3*	Autosomal dominant
Lymphedema-distichiasis	153400	*FOXC2*	Autosomal dominant
Noonan syndrome	163950	*PTPN11, KRAS, RAF1, SOS1*	Autosomal dominant
Integrin α9 deficiency syndrome*	NA	*ITGA9*	Autosomal recessive

NA: not available due to novelty
*We first reported the human fetuses with *ITGA9* mutations to have chylothorax refractory to fetal therapy (4)

been proposed as the etiology underlying these syndromes (5).

Here we report a hydropic fetus whose chylothorax failed to respond to OK-432 pleurodesis. The fetus was determined to have Noonan syndrome (NS), which is just one of the genetic syndromes that may exhibit lymphedema.

CASE REPORT

A 31-year-old primigravida patient was referred to our department at 27 weeks of gestation for counseling and management because of pleural effusion associated with hydrops fetalis. The couple conceived naturally, and no anomaly was detected until progressive fetal hydrops started to appear at 25 weeks of gestation. Neither patient was a carrier of thalassemia (the most prevalent monogenic disorder in Taiwan). Serology for TORCH and parvovirus B19 were negative. No karyotyping was done because the first- and second-trimester screenings such as nuchal translucency or maternal serum quadruple test (AFP, free-ßHCG, inhibin-A, and estriol) did not reveal a necessity for invasive diagnosis. Detailed ultrasound revealed bilateral pleural effusion, ascites, hydramnios, and bulky placenta (*Fig. 1a,b*). Bilateral thoracocentesis followed by pleurodesis with OK-432 (Picibanil®, Chugai Pharmaceutical, Tokyo, Japan) was performed. 20ml (right side) and 25ml (left side) pleural fluid was removed from the fetal chest and aspirate demonstrated 98% of the cells to be lymphocytes, a feature characteristic of chylothorax. The dosage of OK-432 used in pleurodesis was 0.2 KE (right side) and 0.1KE (left side) respectively. Amnio-reduction by drainage of 800ml amniotic fluid was also performed. The pleural aspirate and the amniotic fluid were sent for genetic investigation. Subsequent ultrasound examination demonstrated evidence of intrathoracic adhesion on post-therapy Day 2 (*Fig. 1c*). This sign suggested a favorable response to pleurodesis based on our previous experience (2). However, during the hospitalization for fetal surveillance, poor variability of fetal heart beat was noted intermittently from post-therapy Day 3, and absent end-diastolic velocity (AEDV) of the umbilical artery was found from post-therapy Day 4. As the genotyping result was not yet available, no intervention was undertaken, and observation was chosen by the family after a detailed counseling. An intrauterine fetal death occurred on post-therapy Day 6, and a stillborn male infant weighing 1,140g

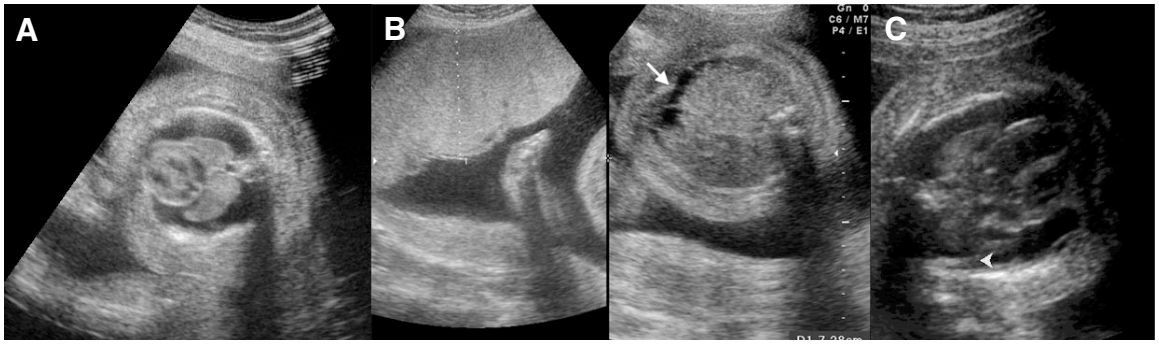

Fig. 1. (A) Transverse view at mid-thoracic level displaying bilateral hydrothorax with collapsed lungs; (B) Fetal ascites (arrow) and bulky placenta; (C) Bright band at site of adhesion following pleurodesis (arrowhead).

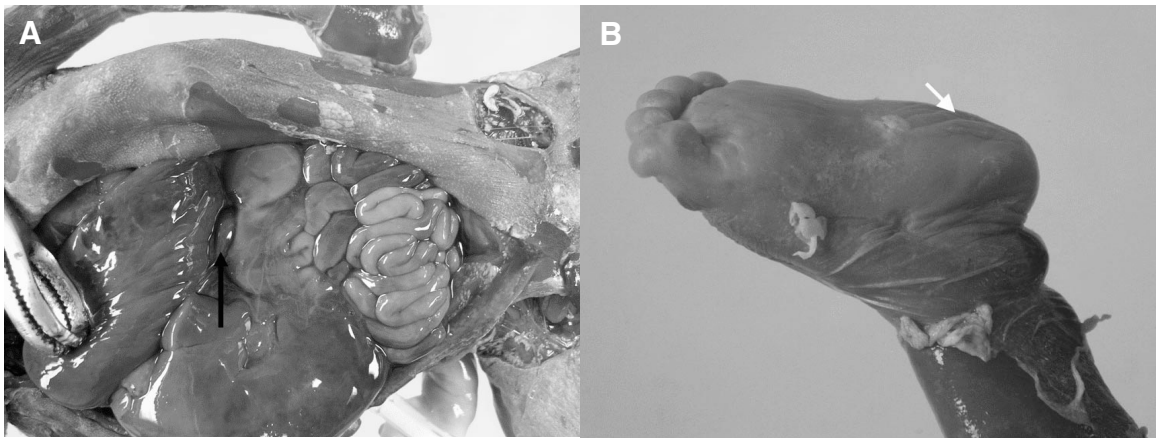

Fig. 2. Autopsy of the fetus revealed: (A) Agenesis of gall bladder. This view was exposed by using a ring forceps and the black arrow points to the pancreas in the space between liver and stomach. No gall bladder was found; (B) Left foot pad displaying thickening (white arrow).

was delivered. Autopsy revealed bilateral pleural effusion, generalized edema, pericardial effusion, agenesis of gallbladder, and bilateral thickened foot pads (*Fig. 2a,b*). Neither intracardiac anomaly nor pulmonary lymphangiectasia was noted. Karyotyping, which was completed on post-therapy Day 7, revealed 46,XY. The genotyping results of causative loci for hereditary lymphedema syndromes were not available until post-therapy Day 14. A missense mutation c.182A>C (p.Asp61Ala) at the exon 3 of *PTPN11* was found. This mutation appears to be novel (according to the Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff: http://www.hgmd.cf.ac.uk/ac/index.php). Noonan syndrome was thus confirmed to be the cause of fetal chylothorax and hydrops in this fetus.

DISCUSSION

Noonan syndrome (OMIM 163950) is a genetic disorder with autosomal dominant inheritance. Causative loci for NS include (among others) *PTPN11, SOS1, KRAS, RAF1* with half of the patients presenting with *PTPN11* mutations (6-7). Clinical manifestations of NS, which vary greatly from case to case, include distinctive facial dysmorphism, congenital heart and renal defects,

ophthalmologic abnormalities, short stature, and many others. Mental retardation is not always seen. Lymphatic vessel hypoplasia, dysplasia, or aplasia are the major findings of Noonan syndrome (20%) and chylothorax (with or without pulmonary lymphangiectasia) may occur (8). Prenatal diagnoses are rare with reported characteristics including hydrops fetalis, increased nuchal translucency, and cystic hygroma (9). *PTPN11* mutations have been detected in 16% of fetuses with cystic hygroma and 2% of fetuses with increased nuchal translucency (10). The patient described in this report failed to respond to prenatal pleurodesis. Interestingly, we had previously reported the first link between *ITGA9* mutation (a trait with autosomal recessive inheritance) and chylothorax in human fetuses and the poor response of this particular subset of patients to fetal therapy (11). The association between poor response to prenatal pleurodesis and genomic aberrations (e.g., *PTPN11* and *ITGA9* mutations) were noted. In our previous study, we also speculated that only fetuses whose chylothorax were considered to be inflammation-related responded well to prenatal pleurodesis by OK-432 (11). To determine which patients are in the genetic-related group of fetal chylothorax (poor responders of pleurodesis), a selection of candidate loci for genotyping is needed. Apart from the reported *ITGA9, VEGFR3* (OMIM 153100, Milroy disease), and *FOXC2* (OMIM 153400, lymphedema-distichiasis syndrome), NS should always be considered as a major possibility in fetuses with chylothorax (*Table*). An additional finding in this case report is "the agenesis of gallbladder," which is a novel feature and has not been previously reported (6,8-10).

We propose that a widened genetic workup (in addition to karyotyping), including candidate gene searches for *VEGFR3, FOXC2, ITGA9, PTPN11*, and even other causative loci for NS such as *SOS1, KRAS, RAF1* (7) is therefore needed prior to attempting prenatal pleurodesis treatment by OK-432. Unfortunately, genotyping of these loci may entail a considerable time delay interfering with antenatal care of fetal chylothorax. Considering this delay and the report by a French group demonstrating a prenatal case of NS which had been successfully rescued using thoracoamniotic shunting (12), drainage of the excessive fluids via the thoracoamniotic shunting may still be effective in genetic-related prenatal chylothorax despite the possible morbidity. Thoracoamniotic shunting, therefore, may have a broader range of indications than prenatal pleurodesis by OK-432 in fetal therapy with antenatal pleurodesis by OK-432 reserved for use in only those cases without genomic aberrations (particularly considering the reduced morbidity). Whether those fetuses with known genomic aberrations are candidates for fetal therapy is another difficult issue for genetic counseling.

It is obvious that more fetal studies are needed to prove our hypothesis including a randomized trial to compare the effect of pleurodesis by OK-432 or thoracoamniotic shunting to distinguish between fetuses with and without detectable genomic aberrations not only to understand underlying pathogenesis better but also help structure more effective strategies to treat this disorder in utero.

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