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Clinical report

ISPD gene homozygous deletion identified by SNP array confirms prenatal manifestation of Walker–Warburg syndrome

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ABSTRACT

Walker–Warburg syndrome (WWS) is a rare form of autosomal recessive, congenital muscular dystrophy that is associated with brain and eye anomalies. Several genes encoding proteins involved in abnormal α-dystroglycan glycosylation have been implicated in the aetiology of WWS, most recently the *ISPD* gene. Typical WWS brain anomalies, such as cobblestone lissencephaly, hydrocephalus and cerebellar malformations, can be prenatally detected through routine ultrasound examinations. Here, we report two karyotypically normal foetuses with multiple brain anomalies that corresponded to WWS symptoms. Using a SNP-array examination on the amniotic fluid DNA, a homozygous microdeletion was identified at 7p21.2p21.1 within the *ISPD* gene.

Published data and our findings led us to the conclusion that a homozygous segmental intragenic deletion of the *ISPD* gene causes the most severe phenotype of Walker–Warburg syndrome. Our results also clearly supports the use of chromosomal microarray analysis as a first-line diagnostic test in patients with a foetus with one or more major structural abnormalities identified on ultrasonographic examination.

1. Introduction

Walker–Warburg syndrome (WWS) is an autosomal recessive, multisystem disorder characterised by major neurological deficits, visual and muscular impairments, and a rapidly fatal outcome [Dobyns et al., 1989]. Classically, syndromes with cerebral ocular, and muscular dystrophy were attributed to aberrant α-dystroglycan glycosylation [Roscioli et al., 2012]. α-dystroglycan and β-dystroglycan are central components of the dystrophin-glycoprotein complex, which forms a link between the cytoskeleton and the basal lamina [Buysse et al., 2013]. Mutations in 18 genes encoding putative or confirmed glycosyltransferases or other proteins involved in the α-dystroglycan glycosylation pathway have been identified in the dystroglycanopathies: *POMT1*, *POMT2*, *POMGNT1*, *FKTN*, *FKRP*, *LARGE*, *ISPD*, *GTDC2* (*POMGT2*), *DAG1*, *TMEM5*, *B3GALNT2*, *SGK196* (*POMK*), *B3GNT1* (*B4GAT1*), *GMPPB*, *DOLK*, *DPM1*, *DPM2 and DPM3, as reviewed by* [Ohtsuka et al., 2015]. Recently, bi-allelic loss-of function mutations in the isoprenoid synthase domain-containing gene (*ISPD*, OMIM614631), which maps to chromosome 7p21, were identified as a second most common cause of WWS [Roscioli et al., 2012; Willer et al., 2012].

While the WWS phenotype of the affected individuals with *ISPD* mutations was described for postnatal cases [Cirak et al., 2013; Czeschik et al., 2013; Roscioli et al., 2012; Willer et al., 2012], only few prenatal cases were published [Vuillaumier-Barrot et al., 2012]. We report two prenatally detected *ISPD* gene deletions in foetuses with multiple brain anomalies that corresponded to the WWS phenotype.

2. Clinical report

A 22-year-old woman underwent a second trimester screening at our clinic during her third gravidity. With the same partner, a 22-year-old healthy male, she had a 4-year-old healthy daughter and a history of one miscarriage during the first trimester (3 years before this gravidity). The woman was referred for amniotic fluid sampling because of positive biochemical screening (increased alpha-fetoprotein, neural tube defect risk 1:2) on the 15th week of

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gestation (WG). Ultrasound examination during the 17th WG showed intrauterine growth restriction and gastroschisis (Foetus 1/F1) (Fig. 1a). Karyotyping and array examination were performed. A later ultrasound on the 21st WG confirmed a 24 × 35 × 19 mm gastroschisis formed by the small intestine. The woman continued the pregnancy, and the daughter was born at 37 weeks of gestation with weight 2400 g. She underwent successful surgery for the gastroschisis immediately after her delivery: reposition of the small intestine and the stomach into abdominal cavity and suture of the abdominal wall. On 6th day the enteral intake was started and progressive per oral nutrition was successively tolerated. The girl was dismissed from the hospital on 33rd day after surgery with weight 2888 g. Sonography of the brain did not reveal any abnormalities.

11 months later, ultrasound examination in the 20th WG of her fourth gravidity with normal biochemical screening (Foetus 2/F2) showed a 12 × 10 mm occipital meningocele, hydrocephalus, obliteration of the cisterna magna (banana sign), corpus callosum agenesis, dilated III cerebral ventricle, and a multicystic left kidney (Fig. 1b–d). Amniotic fluid sampling, followed by karyotyping and array examination, was performed. Clinical features and laboratory results (including SNP array data) were discussed with the couple, and the parents decided to terminate pregnancy during the 21st WG. The autopsy confirmed female foetus with weight of 350 g and length 170 mm, occipital subcutaneous protrusion with 15 mm diameter, absent brain gyrification, enlarged lateral ventricles, corpus callosum agenesis and multicystic dysplasia of the left kidney.

6 months later, the woman underwent a second trimester ultrasound examination in the 17th WG of her fifth gravidity (Foetus 3/F3). The findings included hydrocephalus, cerebellar vermis agenesis, and a dilated IV cerebral ventricle (Fig. 1e–f). Amniotic fluid sampling for array was performed. The poor prognosis of the brain structural anomalies together with SNP array data were discussed with the couple, and the pregnancy was terminated during the 18th WG. Unfortunately, the autopsy report is not available.

In addition to the classic karyotype, SNP array examination (whole genome genotyping of 298,649 SNPs) was performed on the DNA isolated from the amniotic fluid samples of foetuses F1, F2, F3 and on the DNA isolated from the lymphoblastoid cell line of both parents; the genotyping analysis was performed using the Illumina HumanCytoSNP-12v2.1 platform (Illumina, Inc., San Diego, CA). All the data were analysed using the GenomeStudio v2011.1 software. RefSeq genes and base positions were annotated according to the UCSC Human genome assembly GRCh37/hg19.

Karyotypes of the three foetuses F1, F2, and F3 were normal (F1/46, XX; F2/46, XX, and F3/46, XY). SNP array analysis of all of the samples, including the parents and the three foetuses, revealed a total of 12 copy number variations (CNVs). The summary of all of the CNVs, their clinical interpretation and their parental origin are shown in Table 1. Taking into account the similar ultrasound findings for F2 and F3, we searched for an identical abnormality. We found a 360 kb long homozygous microdeletion at 7p21.2p21.1 that spanned 9 out of the 10 exons of the *ISPD* gene (isoprenoid synthase domain-containing protein, OMIM 614631). Recessive mutations in the *ISPD* gene were recently identified in individuals with Walker–Warburg syndrome [Roscioli et al., 2012; Willer et al., 2012]. The multiple brain ultrasound anomalies were consistent with the postnatal phenotype of WWS, and the homozygous 7p21.2p21.1 microdeletion was considered pathogenic. The mother, father and foetus F1/girl with gastroschisis carried the same heterozygous microdeletion.

4. Discussion

Brain anomalies are among the most prevalent sonographic indications for second trimester prenatal microarray analysis for foetuses with normal karyotypes; 12% of the sonographic anomalies observed in the Kleeman study were brain anomalies [Kleeman

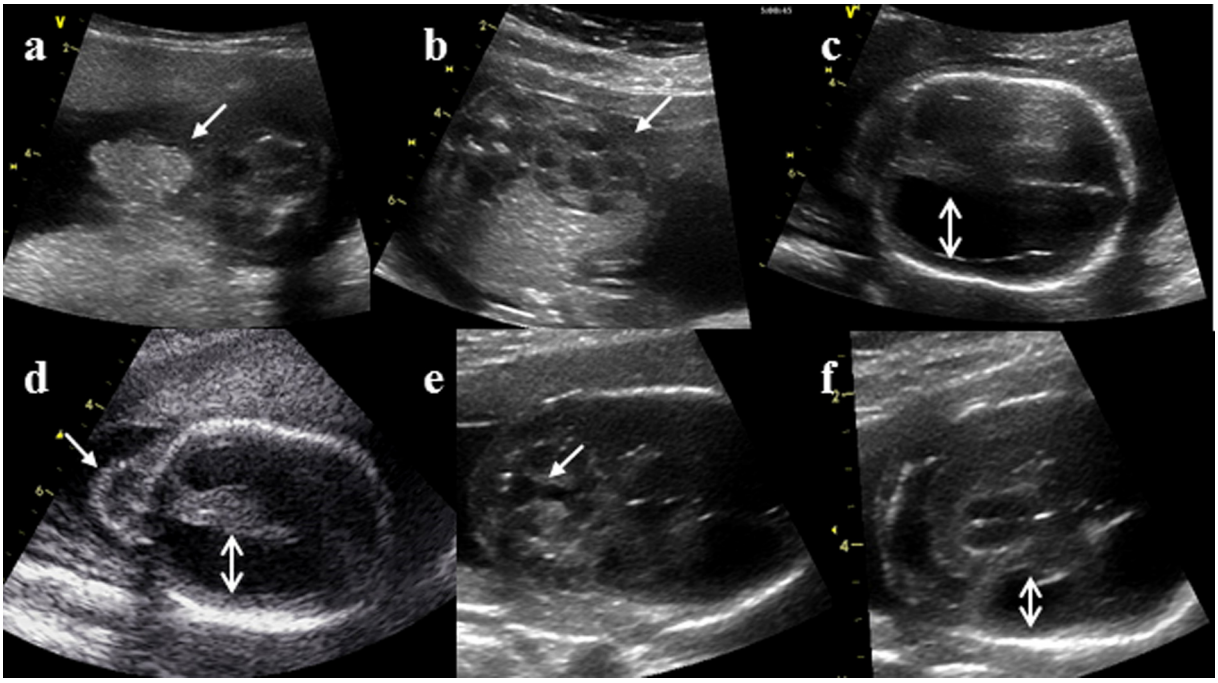

Fig. 1. Ultrasound findings in three foetuses of one family. (Ultrasound anomalies are indicated by the white arrows). a: gastroschisis formed by the small intestine in foetus 1 (F1). b: multicystic left kidney in foetus 2 (F2). c: ventriculomegaly in foetus 2 (F2). d: occipital meningocele in foetus 2 (F2). e: cerebellar vermis agenesis, dilated IV cerebral ventricle in foetus 3 (F3), f – ventriculomegaly in foetus 3 (F3).