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Founder *Fukutin* mutation causes Walker-Warburg syndrome in four Ashkenazi Jewish families†

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Abstract

Objective—Walker-Warburg syndrome (WWS) is a genetically heterogeneous congenital muscular dystrophy caused by abnormal glycosylation of α-dystroglycan (α-DG) that is associated with brain malformations and eye anomalies. The *Fukutin* (*FKTN*) gene, which causes autosomal recessively inherited WWS is most often associated with Fukuyama congenital muscular dystrophy in Japan. We describe the clinical features of four nonconsanguinous Ashkenazi Jewish families with WWS and identify the underlying genetic basis for WWS.

Method—We screened for mutations in *POMGnT1*, *POMT1*, *POMT2*, and *FKTN*, genes causing WWS, by dideoxy sequence analysis.

Results—We identified an identical homozygous c.1167insA mutation in the *FKTN* gene on a common haplotype in all four families and identified 2/299 (0.7%) carriers for the c.1167insA mutation among normal American Ashkenazi Jewish adults.

Conclusion—These data suggest that the c.1167insA *FKTN* mutation described by us is a founder mutation that can be used to target diagnostic testing and carrier screening in the Ashkenazi Jewish population.

Keywords

genetic screening; muscle-eye-brain disease

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‡[Correction made here after initial online publication].

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INTRODUCTION

Walker-Warburg syndrome (WWS; OMIM 236 670) is one of several autosomal recessively inherited, genetically heterogeneous disorders including muscle-eye-brain (MEB) disease (OMIM 253 280) and Fukuyama congenital muscular dystrophy [(FCMD); OMIM 253 800] (Walker, 1942; Warburg, 1976; Warburg, 1978; Dobyns *et al*., 1989; Dobyns and Truwit, 1995; Muntoni and Voit, 2004; Vajsar and Schachter, 2006) characterized by a triad of developmental defects involving congenital muscular dystrophy (CMD), brain malformations, and eye abnormalities (Dobyns and Truwit, 1995; Toda *et al*., 2000; Muntoni and Voit, 2004, 2005; Vajsar and Schachter, 2006). These diseases are caused by the hypoglycosylation of α-dystroglycan (α-DG) through genetic defects in known or putative glycosyltransferase enzymes that act on α-DG and are known as the dystroglycanopathies (or sometimes secondary dystroglycanopathies) (Muntoni and Voit, 2004, 2005; van Reeuwijk *et al*., 2005a; Godfrey *et al*., 2007). FCMD, the most common form of CMD in Japan, clinically presents with a combination of generalized muscle weakness, congenital brain malformations with mental retardation, seizures, decreased vision, and cardiomyopathy (Fukuyama *et al*., 1960; Fukuyama and Ohsawa, 1984; Toda *et al*., 2000; Muntoni and Voit, 2004). The gene responsible for FCMD was identified to be *Fukutin* (*FKTN*) on chromosome 9q31 (Toda *et al*., 1993; Kobayashi *et al*., 2001; Silan *et al*., 2003). In 87% of FCMD patients, a founder insertion of a 3kb retrotransposon composed of $(TCTCCC)_{41}$ is located within the 3′ noncoding region of the cDNA (Colombo *et al*., 2000; Toda *et al*., 2000; Watanabe *et al*., 2005). Homozygosity for this retrotransposon insertion is associated with a milder phenotype, but compound heterozygotes carrying this mutation on one allele and a point mutation of *FKTN* on the other allele may have more severe phenotypes including WWS (Kondo-Iida *et al*., 1999; Toda *et al*., 2000; Muntoni and Voit, 2004; Muntoni and Voit, 2005).

WWS is the most severe phenotype on the spectrum of the dystroglycanopathies, with a life expectancy of less than 3 years (Dobyns *et al*., 1989; Toda *et al*., 2000; Muntoni and Voit, 2004; van Reeuwijk *et al*., 2005a; Vajsar and Schachter, 2006). Characteristic clinical features include muscle weakness, hypotonia, feeding difficulties, seizures, blindness (from anterior and posterior chamber eye malformations), and male genital anomalies (Dobyns *et al*., 1989; Muntoni and Voit, 2004; Vajsar and Schachter, 2006). Characteristic brain malformations include cobblestone lissencephaly, kinking of the brain stem, flattening of the pons, and cerebellar hypoplasia. Absence of the corpus callosum and septum pellucidum, and presence of a posterior encephalocele have also been reported (Dobyns *et al*., 1989; Muntoni and Voit, 2004; Vajsar and Schachter, 2006).

WWS is known to be genetically heterogeneous and is caused by mutations in at least six different genes. Among the known genes, mutations in *Protein O-Mannosyltransferase 1* (*POMT1*) were identified as the underlying genetic cause in 7-20% of WWS cases, and *Protein O-Mannosyltransferase 2 (POMT2)* mutations were found in 7% of patients with WWS (Beltrán-Valero de Bernabé *et al*., 2002; Beltran-Valero de Bernabé *et al*., 2003; van Reeuwijk *et al*., 2005b, 2006; Currier *et al*., 2005). Mutations in *O-mannose Beta-1,2-N-acetylglucosaminyltransferase* (POMGnT1), Acetylglucosaminyltransferase-like Protein (*LARGE*), *FKTN*, and *Fukutin-Related Protein* (*FKRP*) have less frequently been identified in patients with WWS (Beltran-Valero de Bernabé *et al*., 2004; Muntoni *et al*., 2004; Barresi and Campbell, 2006; Godfrey *et al*., 2007; van Reeuwijk *et al*., 2007). From the largest cohort studies that include a total of 85 families (Bouchet *et al*., 2007; Manzini *et al*., 2008), it is estimated that *POMT1*, *POMT2*, *POMGnT1*, *FKTN*, *LARGE*, and *FKRP* account for only approximately 42% of WWS cases.

Genetic heterogeneity increases the complexity and cost of clinical molecular diagnosis in patients with WWS. Here, we describe five offspring with WWS from four nonconsanguinous

Ashkenazi Jewish families who presented prenatally with congenital brain and eye anomalies and myopathy. All five cases carried an identical homozygous mutation in the *FKTN* gene on a common haplotype, suggesting this is a founder mutation that can be used to target diagnostic testing and carrier screening in the Ashkenazi Jewish population in which we determined the carrier frequency to be 0.7%.

CASE REPORTS

Family A

Patient A-1—The mother of A-1 presented prenatally as a 25-year-old G2P1000 woman at 20 weeks' gestation with ultrasonographic evidence of hydrocephalus and cataracts in the fetus (Figure 1A and B). The family history was significant for a previous female child who had died at the age of 9 months with muscular dystrophy, bilateral cataracts, and multiple congenital central nervous system anomalies, which were consistent with WWS. The parents were nonconsanguinous, Ashkenazi Jewish of Eastern-European origin, with no other family history of congenital malformations, neonatal death, or mental retardation.

A-1 was born at term via cesarean delivery due to failure to progress, hydrocephalus, and fetal tachycardia. At birth, the patient was hypotonic and bradycardic, with Apgars of 5, 5, and 7 at 1, 5, and 10 min respectively. Her birth weight was 2630 g (10th percentile), her length was 49 cm (50th percentile), and her head circumference was 37.5 cm (>99th percentile). Physical examination showed a dysmorphic infant with macrocephaly, bilateral cataracts, low-set ears, and micrognathia. Neurological exam was significant for persistent general hypotonia and frog-leg positioning.

Axial magnetic resonance imaging (MRI) imaging of the brain demonstrated marked ventriculomegaly, cobblestone lissencephaly, diffuse cerebral hypomyelination, and an absent septum pellucidum (Figure 1C and D), as well as an extremely hypoplastic cerebellar vermis, and cerebellar dysplasia including polymicrogyria (not shown). Kinking of the midbrain, flattening of the pons, and a thin but intact corpus callosum were also demonstrated on sagittal MR imaging. Her serum creatine kinase level was elevated to 9180 U/l (normal range is 39-238 U/l) on day 3 of life.

A-1 remained in the neonatal intensive care unit for 4 weeks due to poor feeding, and a gastrostomy tube was placed at 1 month of age to assist with feedings. At 3 months of age, A-1 received a ventriculoperitoneal shunt for persistent hydrocephalus, and she was admitted to the hospital several times over the following few months for fever and was treated for multiple urinary tract infections. Renal ultrasound demonstrated mild unilateral hydronephrosis, and a voiding cystourethrograms was normal. At 5 months of age, A-1 began having generalized seizures characterized by total body stiffening, arm flexion, back arching, and apneic periods of 15-30 s. Electroencephalogram confirmed constant generalized seizures during sleep and wakefulness. A-1 died at 39 months of age of aspiration pneumonia.

Patient A-2—A-1 was the sister of A-2. Prenatal ultrasonographic examination at 20 weeks' gestation demonstrated intrauterine growth restriction, thinning of the corpus callosum, and markedly dilated ventricles with little brain cortex (Figure 2A and B). By ultrasound, the head circumference was 24.5 cm (28%), abdominal circumference was 18.5 cm (<5%), and estimated fetal weight was 737g (<3%). The patient was born at 27 weeks of gestation via emergent repeat cesarean delivery to a 30-year-old G3P2000 mother due to preterm labor. The patient was delivered with a nuchal cord and was resuscitated. She was limp and cyanotic, with Apgars of 3, 5, and 7 at 1, 5, and 10 min, respectively. Her birth weight was 805 g (25th percentile), her length was 34 cm (25th percentile), and her head circumference was 25cm

(35th percentile). On physical exam, she had left microophthalmia, bilateral corneal opacification, abnormal sclera, small low-set ears, and hypotonia.

Her serum creatine kinase was elevated at 881 U/l (normal range is 39-238 U/l) on the first day of life. An ultrasound of the head showed marked hydrocephalus involving the lateral and third ventricles. Patient A-2 was admitted to the neonatal intensive care unit and remained incubated. On day 13 of life, she had a spontaneous gastrointestinal perforation and was managed surgically. She developed acute renal failure on day 19 of life, and fungal and bacterial urinary tract infections on day 25 of life. Renal ultrasound demonstrated normal anatomy but bilaterally increased echogenicity. On day 62 of life, CT of the head showed increasing hydrocephalus (Figure 2C) and a markedly hypoplastic cerebellum and small posterior fossa (Figure 2D). An ileostomy was placed on day 73 of life, and on day 78 of life, a right ventricular subgaleal shunt was placed. A-2 died at 3 1/2 months of *Escherichia coli* sepsis that led to bradycardia and asystole.

Family B

Patient B-1—The mother of B-1, a 27-year-old G1P000 woman of Ashkenazi Jewish descent, presented prenatally at 34 weeks' gestation for evaluation of intrauterine growth restriction and ultrasonographic evidence of brain malformations including meningocele, hydrocephalus, subependymal cyst in the right lateral ventricle, cerebellar hypoplasia, vermis abnormality, lissencephaly, micrognathia, hypotelorism, cataract of the left eye, and a dilated renal pelvis. B-1 was born at 43 weeks via spontaneous vaginal delivery. At birth, the patient was cyanotic, pale and apneic immediately after birth, with Apgars of 7 and 8 at 1 and 5 min, respectively. Her birth weight was 2840 g (10% percentile), her length was 49.0 cm (25-50th percentile), and her head circumference was 31.0 cm (<10th percentile). Physical examination showed an ill-appearing infant with a 1.5 × 1.5 inch meningocele in the occipital area, small deep-set eyes with no opening of the right eye, a highly arched palate, and a beaked nose.

MRI of the brain showed a bony defect in the midline occipital area with a cerebrospinal fluid (CSF)-containing membranous sac projecting posteriorly through the bony defect consistent with meningocele, with posterior angulation of the midbrain and tethering of the superior cerebellum superiorly and posteriorly toward the meningocele. The MRI also noted aplasia of the cerebellar vermis with the fourth ventricle communicating with a CSF-containing space in the midline posterior to the cerebellum consistent with a cerebellar hypoplasia. Dilatation of the bilateral temporal horns, the lateral ventricles, and third ventricle was consistent with communicating hydrocephalus or aqueductal stenosis, and germinal matrix hemorrhage with intraventricular hemorrhage in the bilateral occipital horns. The MRI showed a small shrunken right globe with subacute hemorrhage and retinal detachment. Her serum creatine kinase level was elevated, measuring 27.01 ng/mL (normal range 0.7-6.0 ng/mL) on day 4 of life.

Family C

Patient C-1—This male patient was diagnosed with WWS and was previously described by Cotarelo *et al.* (2008). The parents were nonconsanguinous and of Ashkenazi Jewish descent. The patient was described to have ventriculomegaly with lissencephaly, occipital bone defects with extrusion of the CNS, microcornea, microphthalmia, cataracts, and elevated serum creatine kinase levels.

Family D

Patient D-1—The mother of fetus D-1, a 30-year-old G6P1051 woman of Ashkenazi Jewish descent, presented following pregnancy termination of her fourth pregnancy at 20 3/7 weeks for ultrasound diagnosis of bilateral ventriculomegaly (1.3 and 1.9 cm), dilated 3rd ventricle, dilated bowel, and bilateral pelviectasis. Amniotic fluid chromosome analysis revealed a

normal female karyotype of 46, XX. Given the severity of the brain anomalies on prenatal sonography, she elected to terminate the pregnancy. Pathological examination of the products of conception was significant for hydrocephalus and imperforate anus associated with a dilated distal colon with hyperplastic smooth muscle (ganglion cells identified). There was no family history of central nervous system abnormalities. The patient was counseled that this constellation of findings may represent an atypical form of VACTERL association. Approximately 1 year later, the patient presented at 13 weeks of gestation for reassessment of a suspected brain abnormality identified through ultrasound examination performed prior to chorionic villus sampling at 12 weeks of gestation. On ultrasound, the ventricles appeared prominent, measuring 8 mm on the right and 7 mm on the left. Bilaterally, the choroid plexi were anteriorly displaced. The cerebellum appeared flattened in some images. On follow up ultrasound, the ventricular atria measured approximately 10 mm bilaterally with ‘dangling’ choroids. A 7-mm posterior fossa cyst and markedly abnormal posterior fossa were also noted (Figure 3). Fetal karyotype was 46, XX. on the basis of the ultrasound findings, the patient elected to terminate the pregnancy at 15 2/7 weeks. Pathological examination of the products of conception was significant for gross evidence of marked hydrocephaly, borderline microophthalmia, retinal hyperplasia (with possible focal dysplasia), and thickened cardiac valvular tissue. Because these findings were consistent with WWS, histologic and molecular genetic analyses were performed. A skeletal muscle specimen was sent to the Department of Pathology at The University of Iowa Hospitals and Clinics for immunostaining. The muscle appeared dystrophic and the abnormal immunostaining pattern observed was suggestive of alpha-dystroglycan hypoglycosylation, a finding characteristic of WWS (Figure 4). Furthermore, there also appeared to be a severe, secondary deficiency of merosin (data not shown) similar to that commonly seen in WWS.

The mother of fetus D-1 also had a history of two first trimester spontaneous abortions of unknown etiology, one chemical pregnancy, and one uncomplicated vaginal delivery of a son who is healthy and developmentally appropriate for age. Following diagnosis of D-1, she had a chorionic villus sample taken in a subsequent pregnancy that identified a genetically unaffected fetus.

METHODS

All studies were approved by the Columbia University Institutional Review Board and were conducted in accordance with the ethical standards of the Helsinki Declaration. Blood samples were obtained from Patients A-1, A-2, B-1, their parents, the parents of fetus D-1, and 299 adult healthy American Ashkenazi Jewish controls. Genomic DNA was isolated from the peripheral blood lymphocytes using a Qiagen purification kit according to the manufacturer’s instructions (Qiagen, Valencia, CA). Genomic DNA was isolated from fetus D-1. Genomic DNA from Patient C-1 and his parents was obtained through Coriell Cell Repositories in Camden, New Jersey (Family 1165: NA10180, NA10181, NA10182).

Informed consent for genetic analysis was obtained, and the studies were approved by the Columbia University Institutional Review Board.

Genetic analysis

On the basis of clinical findings, Patients A-1, A-2, B-1, and D-1 were clinically diagnosed with WWS and were screened for mutations in *POMGnT1*, *POMT1*, *POMT2*, and *FKTN* by dideoxy sequence analysis.

To genotype the patients and parents for the common c.1167insA *FKTN* mutation, which was independently identified in each of the four families, polymerase chain reactions were performed for exon 9 of *FKTN* using the primers FKTN9F (5′ -

TATTTCCTTTGTTTGTTTCAGTGTG-3′), and FKTN9R (5′ -CTTCTTTATTTCTACCTCCTG-3′) to amplify a 210 bp polymerase chain reaction (PCR) product. PCR amplifications were performed in a 20-μL reaction mix, containing 40 ng of genomic DNA, 10 units of GoTaq DNA polymerase (Promega, Madison, WI), 4 pmol of each primer, and 4 umol of dNTPs in 10× Green GoTaq Reaction Buffer (Promega, Madison, WI). After an initial denaturation step of 4 min at 94 °C, 35 cycles of amplification (94 °C for 20 s; 58 °C for 30 s; 72 °C for 20 s) were performed, followed by a final elongation step of 5 min at 72 °C. After amplification, PCR products were sequenced at Genewiz (www.genewiz.com) by dideoxysequencing. Totally, 299 unrelated, healthy adult Ashkenazi Jewish subjects were similarly genotyped for the c.1167insA *FKTN* mutation to estimate the carrier frequency in the Ashkenazi population.

Haplotype analysis

Haplotype analysis was performed in subjects A-1, A-2, B-1, C-1, D-1, and in their parents to determine whether this mutation represents a single founder mutation or a recurrent mutation. *FKTN* is located on chromosome 9q31. Single nucleotide polymorphisms (SNPs) for 11 intragenic and flanking markers (rs10820812, rs1960023, rs2518128, rs2768294, rs2518131, rs2518108, rs885954, rs583973, rs7874737, rs7867775, and rs6477450) to *FKTN*, spanning a total of 73.5 kilobases, were located using dbSNP Build 128 (http://www.ncbi.nlm.nih.gov/projects/SNP/). Primer sequences were selected using Primer3 (http://frodo.wi.mit.edu/) and were confirmed to be unique with BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) and are available upon request. Locations of SNPs were confirmed with BLAT (http://genome.ucsc.edu/). Patients and parents were genotyped at each SNP by dideoxysequencing.

RESULTS

Patient A-1

In exon 9 of *FKTN*, a homozygous 1-base pair insertion (c.1167insA) was identified. This is predicted to result in a frameshift at Phe390 and a premature termination of translation at codon 403 after the addition of 13 novel residues (p.Phe390IlefsX14). Both parents were heterozygous for the insertion (Figure 5).

Patient A-2

Patient A-2, the sibling of Patient A-1, was homozygous for the same 1-base pair insertion (c. 1167insA) in exon 9 of the *FKTN* gene mutation that was identified in her sister.

No pathogenic mutations were detected in the coding regions or splice sites of the *POMT2, POMGnT1*, or *POMT1*.

Patients B-1, C-1, and D-1

Patients B-1, C-1, and D-1 were also found to be homozygous for the same c.1167insA mutation in exon 9 of *FKTN*, and parents of Patients B-1, C-1, and D1 were found to be heterozygous for the c.1167insA mutation.

Haplotype analysis

Haplotype analysis demonstrated that the parents of Patients A-1, A-2, B-1, C-1, and D-1 all shared a common haplotype (Figure 6) of eight intragenic and *FKTN*-flanking SNPs from rs10820812 to rs583973. Patients A-1, A-2, B-1, C-1, and D-1 were homozygous for the same haplotype. The haplotype of the father of A-1 and A-2 at rs7874737, rs7867775, and rs6477450

demonstrates evidence of a recombination in a previous generation between rs583973 and rs7874737.

Carrier frequency analysis

Two out of the 299 unrelated normal adult American Ashkenazi Jewish patient samples were found to be carriers for the c.1167insA *FKTN* mutation.

DISCUSSION

We describe four unrelated, nonconsanguinous Ashkenazi Jewish families with an identical c.1167insA mutation in *FKTN* associated with WWS and a consistently severe phenotype. This c.1167insA mutation is located within a repeat of eight As and is likely the result of slippage of DNA polymerase. The c.1167insA mutation was previously described by Manzini *et al*. in three Ashkenazi Jewish families that had the same homozygous insertion in three affected children. Further analysis showed a carrier frequency of 2/299 (0.7%) in a control Ashkenazi population in Israel (Manzini *et al*., 2008). This c.1167insA mutation has also been previously described in combination with other *FKTN* mutations. A Japanese patient with severe FCMD had the 3kb retrotransposon insertion allele along with c.1167insA on the other allele (Watanabe *et al*., 2005). Two families diagnosed with a novel form of limb girdle muscular dystrophy (LGMD2L) without brain abnormalities were compound heterozygous for the c.1167insA mutation (Godfrey *et al*., 2006). Two siblings were compound heterozygotes for the c.1167insA mutation and a novel missense mutation c.920G > Ain *FKTN*. In another family, a child was heterozygous for the c.1167insA mutation and 1363delG. The siblings were children of Jewish-Indian origin, and the other family was from Israel. It is likely that the c.1167insA mutation in these last two families represents the same founder mutation we describe. In contrast, all five patients in our report were homozygous for the c.1167insA mutation, and all had a severe clinical presentation of prenatal onset. Thus, we conclude that homozygosity for the c.1167insA mutation is associated with a consistently severe WWS phenotype. The prenatal phenotype seen across the cases included hydrocephalus, cerebellar hypoplasia, cobblestone lissencephaly, intrauterine growth restriction, cataracts, and other eye malformations (Table 1).The differential diagnosis for this set of manifestations includes MEB disease, FCMD, CMD 1C with structural brain involvement, and other lissencephalies (MillerDieker syndrome, X-linked lissencephaly with agenesis of the corpus callosum, and Norman Roberts syndrome). However, in WWS, the most consistent findings that are prenatally observable are the characteristic brain malformations, which should prompt testing for the c.1167insA mutation in Jewish families.

We demonstrated by haplotype analysis that the c.1167insA mutation is a founder Ashkenazi Jewish mutation common to all four unrelated families. Our observed carrier frequency of the c.1167insA *FKTN* allele was 2/299 (0.7%) in unrelated normal American Ashkenazi Jewish subjects and was similar to the carrier frequency determined in Ashkenazi Jews in Israel. The identification of this Ashkenazi Jewish mutation for such a genetically heterogeneous condition should greatly simplify molecular genetic testing in this population and allow for prenatal diagnosis after identification of ultrasound findings which should be present in the second trimester. Although the carrier frequency for the c.1167insA mutation is low, it should be considered for addition to the Ashkenazi Jewish carrier panel given the severity of the disease and lack of effective intervention.

CONCLUSION

We describe four nonconsanguinous Ashkenazi Jewish families with WWS, all caused by an identical homozygous c.1167insA mutation in the *FKTN* gene on a common haplotype. This

suggests that the mutation we describe is a founder mutation that can be used to target diagnostic testing and carrier screening in the Ashkenazi Jewish population.

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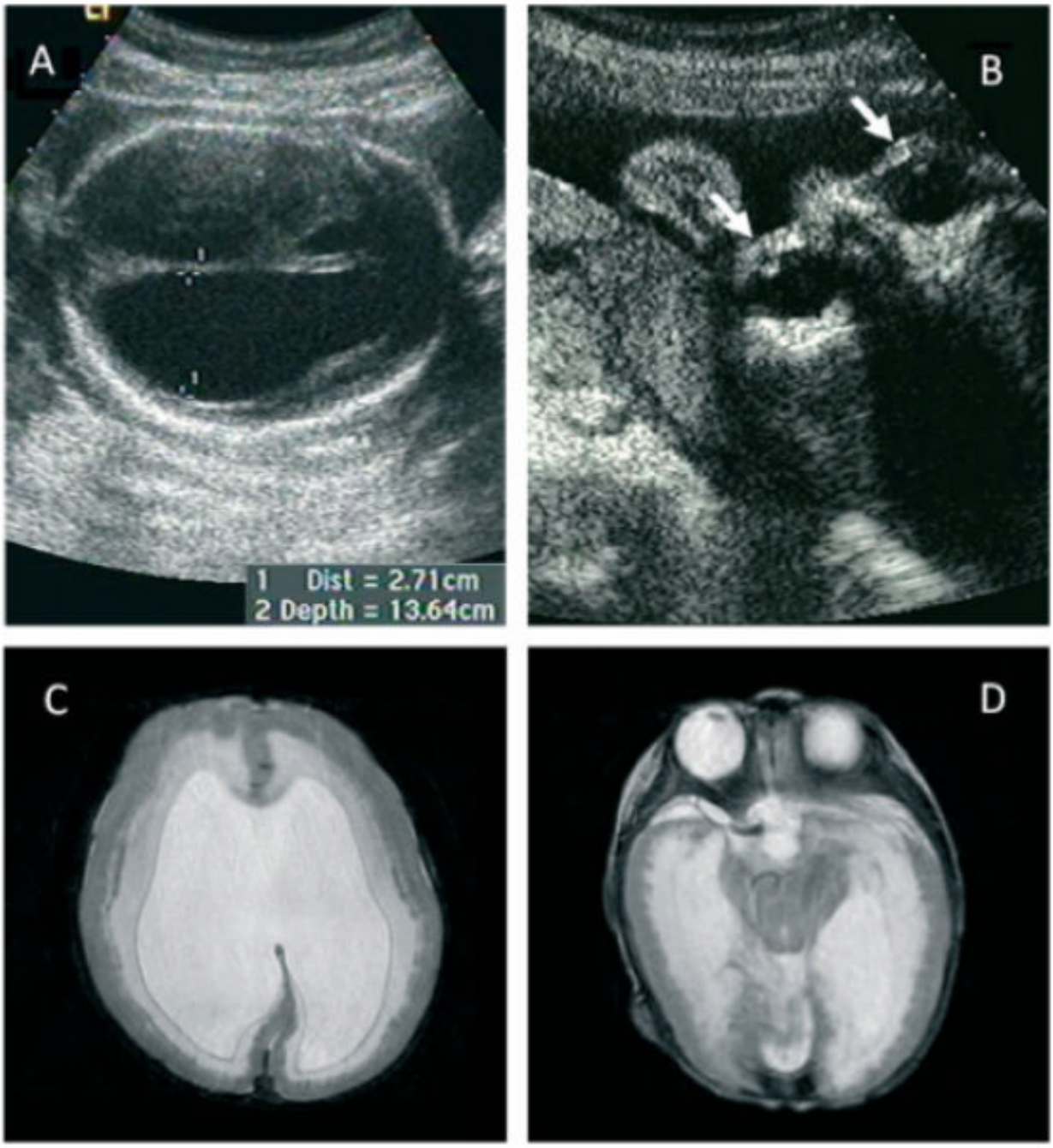

Figure 1.

(A) Prenatal ultrasound imaging for Patient A-1 at 26 weeks' gestation shows hydrocephalus and (B) bilateral cataracts, with the right lens larger than the left. (C) Postnatal axial T2W brain MR imaging of Patient A-1 at 26 weeks of age shows persistent hydrocephalus, diffuse hypomyelination of white matter, extensive cobblestoning, and (D) bilateral cataracts, with a large dense lens on the right and a hypoplastic lens on the left

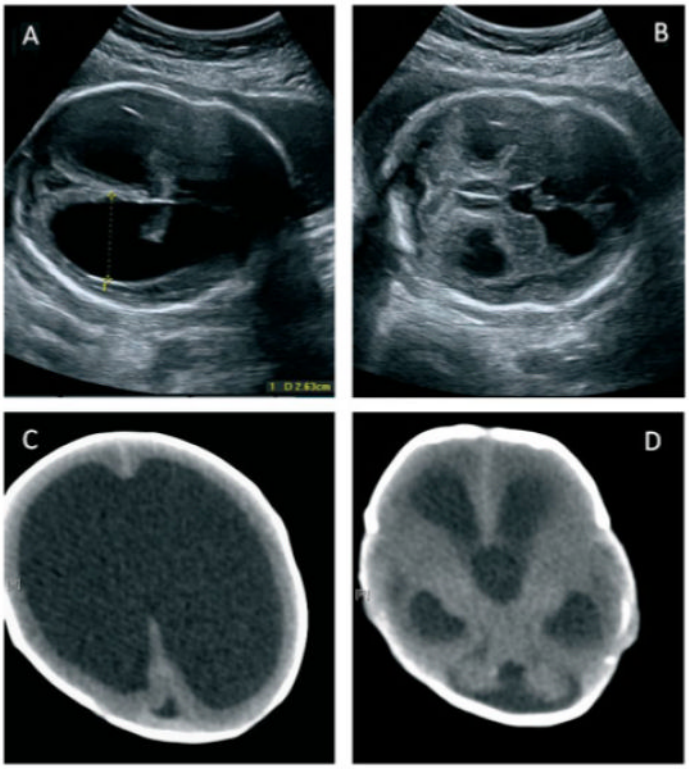

Figure 2.
(A) Prenatal axial head ultrasound imaging for Patient A-2 at 26 weeks' gestation demonstrates bilateral ventriculomegaly on axial view of the fetal head and (B) dilatation of the third ventricle the anterior and posterior horns of the lateral ventricles. (C) Postnatal axial head CT imaging of Patient A-2 at 8 weeks of age shows increased ventriculomegaly, abnormal residual cortex, partial absence of the septum pellucidum, and (D) a small posterior fossa containing a hypoplastic cerebellum without evidence for a vermis

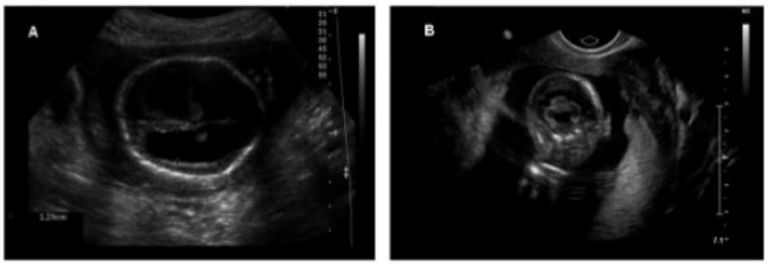

Figure 3.
(A) Prenatal axial head ultrasound imaging for Patient D-1 shows ventriculomegaly and (B) an abnormal posterior fossa

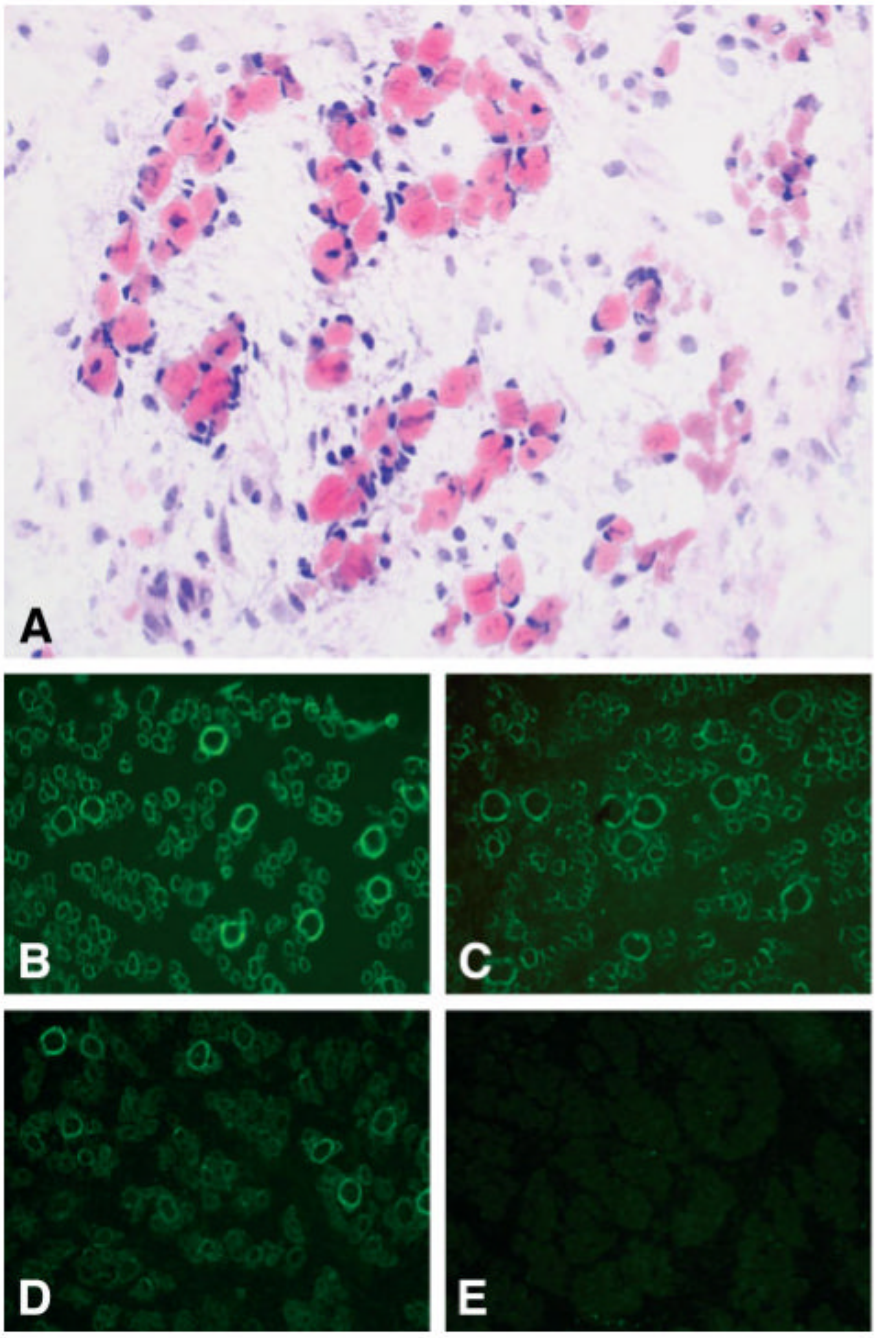

Figure 4.

Histopathology and immunostaining. The quadriceps muscle from the 15-week fetus was frozen for routine histology and immunofluorescence staining. (A) Routine hematoxylin and eosin shows wide variation in fiber size, myonecrosis, and muscle fiber regeneration. (B) Anti-dystrophin immunostaining with a rod domain antibody appears normal. Alpha-dystroglycan (C) and beta-dystroglycan (D) are both present; the alpha-dystroglycan antibody in panel C is goat 20, which detects the core protein. No immunostaining is detected with glycoepitope, anti-alpha-dystroglycan antibodies; IIH6 is shown here (E). All photomicrographs were taken at 40×

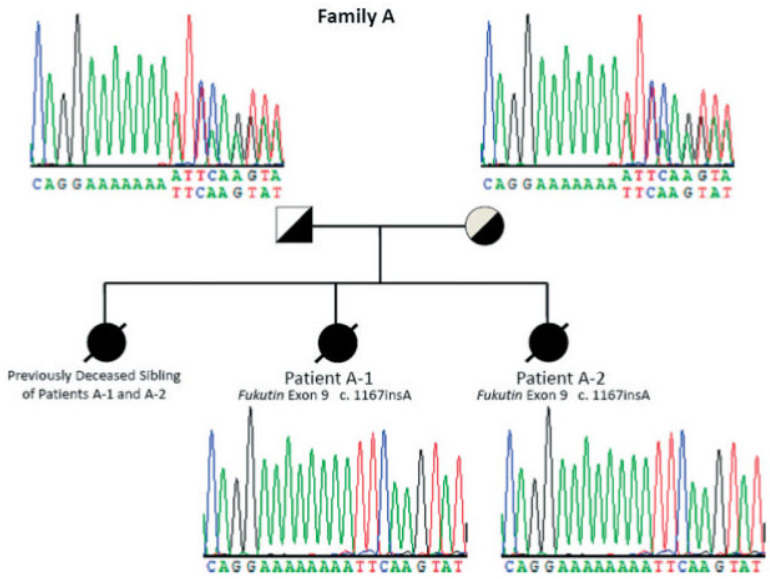

Figure 5.

Point mutation of the *FKTN* gene in Family A. Patients A-1 and A-2 were homozygous for the c.1167insA mutation in exon 9 of *FKTN*, resulting in a frameshift mutation and premature termination of translation. The parents in Family A were heterozygous for the point mutation

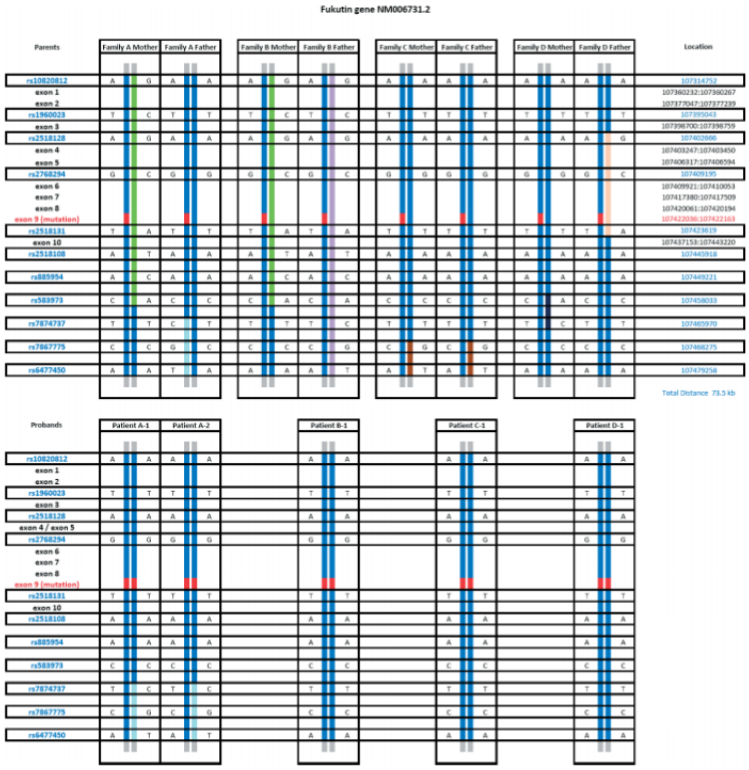

Figure 6.

Haplotype analysis of Patients A-1, A-2, B-1, C-1, D-1, and their respective parents. The parents of Patients A-1, A-2, B-1, C-1, and D-1 share a common haplotype. Patients A-1, A-2, B-1, C-1, and D-1 are homozygous for the same haplotype. Recombination that occurred in a previous generation between rs583973 and rs7874737 is shown in the haplotype of the father of A-1 and A-2, which was subsequently inherited by both A-1 and A-2

Table 1

Summary of clinical findings in WWS case reports. Cases A-1, A-2, B-1, and D-1were all diagnosed prenatally through ultrasonographic imaging techniques. Patients A-1, A-2, B-1, C-1, and D-1 all had brain malformations, ophthalmologic abnormalities, and myopathy, which is summarized below

Patient	**A-1**	**A-2**	**B-1**	**C-1**	**D-1**
Gestational Age at Diagnosis	20 weeks	20 weeks	34 weeks	Unknown	13 weeks
Brain Malformation	Hydrocephalus Thin corpus callosum	Hydrocephalus Absence of the corpus callosum	Hydrocephalus Vermis abnormality	Hydrocephalus Cobblestone lissencephaly	Hydrocephalus Abnormal posterior fossa with posterior fossa cyst
	Cobblestone lissencephaly	Midline fusion of the thalamus	Cerebellar hypoplasia	Occipital bone defects with extrusion of the CNS	Flattened cerebellum
	Absent septum pellucidum Small dysplastic cerebellar vermis	Small cerebellum and fossa posterior	Cobblestone lissencephaly Subependymal cyst in right lateral ventricle		Anteriorly displaced choroid plexus
	Cerebellar hypoplasia		Meningocele		
Ophthalmologic Findings	Bilateral cataracts	Left microophthalmia Bilateral corneal opacification Abnormal sclera	Cataract of left eye	Microcornea	Retinal hyperplasia
			Hypotelorism	Microophthalmia	Microophthalmia
			Shrunken right globe	Cataracts	
			Retinal detachment		
Evidence of Myopathy	Persistent general hypotonia	Persistent general hypotonia			Necrotizing myopathy, hypoglycosylation of α-dystroglycan, and secondary deficiency of merosin in skeletal muscle
	CK: 9180 U/L (normal range 39-238 U/L)	CK: 881 U/L (normal range 39-238 U/L)	CK: 27.01 ng/mL (normal range 0.7-6.0 ng/mL)	CK: elevated	
Outcome	Death at 39 months of aspiration pneumonia	Death at 3 months of *E.coli* sepsis	Death at 4 months	Unknown	Pregnancy terminated at 15 weeks