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Short Report

A homozygous *FKRP* start codon mutation is associated with Walker–Warburg syndrome, the severe end of the clinical spectrum

van Reeuwijk J, Olderode-Berends MJW, van den Elzen C, Brouwer OF, Roscioli T, van Pampus MG, Scheffer H, Brunner HG, van Bokhoven H, Hol FA. A homozygous *FKRP* start codon mutation is associated with Walker–Warburg syndrome, the severe end of the clinical spectrum. Clin Genet 2010; 78: 275–281. © John Wiley & Sons A/S, 2010

Dystroglycanopathies are a heterogeneous group of disorders caused by defects in the glycosylation pathway of α -dystroglycan. The clinical spectrum ranges from severe congenital muscular dystrophy with structural brain and eye involvement to a relatively mild adult onset limb-girdle muscular dystrophy without brain abnormalities and normal intelligence. Mutations have been identified in one of six putative or demonstrated glycosyltransferases. Many different *FKRP* mutations have been identified, which cover the complete clinical spectrum of dystroglycanopathies. In contrast to the other known genes involved in these disorders, genotype–phenotype correlations are not obvious for *FKRP* mutations. To date, no homozygous or compound heterozygous null mutations have been identified in *FKRP*, suggesting that null mutations in *FKRP* could result in embryonic lethality. We report a family with two siblings carrying a homozygous mutation in the start codon of *FKRP* that is likely to result in a loss of functional FKRP protein. The clinical phenotype of the patients was consistent with Walker–Warburg syndrome, the most severe disorder in the disease spectrum of dystroglycanopathies.

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Defective O-linked glycosylation of the peripheral membrane protein α -dystroglycan is the common pathophysiological mechanism in a group of disorders, referred to as dystroglycanopathies. The clinical phenotypes range from adult onset limb-girdle

muscular dystrophy (LGMD2I; MIM 607155) to early lethal Walker–Warburg syndrome (WWS; MIM 236670). WWS is characterized by a combination of congenital muscular dystrophy (CMD), and structural brain and eye abnormalities (1, 2).

A specific type of glycosylation, *O*-linked man-nose glycosylation is a prerequisite for binding of α -dystroglycan to ligands such as laminin- α 2, agrin, and perlecan in muscle, and neurexin in the brain. The only known target for this type of glycosylation is α -dystroglycan, and together with other proteins of the dystrophin–glycoprotein complex it forms a link between extracellular matrix proteins and actin cytoskeleton (3). Mutations have been reported in six putative or demonstrated glycosyl-transferases; *FKRP*, *FKTN*, *LARGE*, *POMGnT1*, *POMT1*, and *POMT2*. Initially, each gene was associated with one syndrome: *FKRP* mutations giving rise to congenital muscular dystrophy type 1C (MDC1C; MIM 606612); *FKTN* mutations in patients with Fukuyama congenital muscular dystrophy (FCMD; MIM 253800); *LARGE* mutations in a patient with congenital muscular dystrophy type 1D (MDC1D; MIM 608840); *POMGnT1* mutations in patients with muscle–eye–brain disease (MEB; MIM 253280); and *POMT1* and *POMT2* mutations in patients with WWS (4–9). Subsequently, mutation analysis in patients with milder or more severe syndromes within the dystroglycanopathy spectrum demonstrated allelic heterogeneity for different mutations in each of the dystroglycanopathy genes (10). A wide clinical spectrum is most evident for the *FKRP* mutations that were first reported in patients with congenital or late-onset muscular dystrophies (MDC1C and LGMD2I), and later also in patients with CMD and mild structural brain involvement, and in patients with severe structural brain and eye involvement (MEB and WWS) (1, 7, 11–13). Mutations have been identified throughout the *FKRP* coding sequence (http://www.dmd.nl/fkrp_seqvar.html). Most mutations result in amino acid substitutions. Only a few mutations resulting in a premature termination codon have been reported and prior to this report, were almost exclusively identified in compound heterozygous form with an amino acid substitution. A genotype–phenotype correlation is not obvious for most of these mutations of which the functional consequences are difficult to predict owing to a lack of knowledge about the function of the *FKRP* protein (2, 13). There is some evidence that the degree of loss of high-molecular-weight α -dystroglycan, detected by immunocytochemistry and Western blot analysis in patients with MDC1C and LGMD2I, correlates with the clinical severity (13–15). Also, patients with FCMD, MEB, or WWS, caused by mutations in one of the other genes, produce a hypoglycosylated form of α -dystroglycan, which is less capable of binding to laminin- α 2 (16, 17).

Recently, a patient with MDC1C was reported to carry a homozygous *FKRP* stop codon mutation, c.1378C>T, Gln460X (13). Our group previously reported a homozygous missense mutation (c.953G>A, Cys318Tyr) in a patient with WWS, which is clinically the most severe case reported with *FKRP* mutations (12). On the basis of these observations, and the absence of evident loss-of-function mutations in *FKRP*, it was hypothesized that *FKRP* null mutations are not compatible with life (12, 13, 15, 18). We report for the first time the association of a homozygous *FKRP* start codon mutation, which likely results in loss of functional *FKRP* protein, with WWS in two siblings.

Patients and methods

Patient 1

The oldest affected sibling, the first child of consanguineous Caucasian parents was born at 37 + 1 weeks after an elective Caesarean section with Apgar scores of 6 and 7 after 3 and 5 min, respectively. Severe hydrocephalus had been diagnosed at 34 weeks of gestation by ultrasonography, performed because of reduced fetal movements. Examination after birth showed a macrocephalic boy with a birth weight of 3120 g and a head circumference of 43 cm ($\gg +2$ SD). The anterior fontanel was large and bulging with palpable separation of the cranial sutures. He had limited spontaneous limb movements and depressed tendon reflexes. He had no sucking reflex, and grasping was present in the hands, but was absent in both feet. An ophthalmologic examination showed asymmetric pupils (right eye 3 mm and left eye 2 mm) and the right eye appeared smaller (cornea diameter right eye 8 mm and left eye 10 mm). The pupillary light reflexes were bilaterally absent. Blood vessels in the iris were dilated. There were bilateral cataracts, more pronounced in the right eye. Fundi and intraocular vessels appeared normal.

Postnatal brain magnetic resonance imaging (MRI) (Fig. 1a,b) showed extreme enlargement of the lateral ventricles and the third ventricle with a small malformed fourth ventricle consistent with aqueduct stenosis. Interpretation of the cortical morphology was difficult due to severe hydrocephalus. Both cerebellar hemispheres, the vermis and the pons, were small with abnormal kinking of the brainstem. The MRI confirmed microphthalmia and detected persistent hyperplastic primary vitreous of the right eye.

Spine radiography showed no abnormalities. Neonatal hearing screening was abnormal. Serum

Fig. 1. Cerebral magnetic resonance imaging of patient 1. (a) Sagittal T1 showing severe hydrocephalus, hypoplastic brainstem and cerebellar vermis (arrows), and absent corpus callosum. (b) Axial T2 showing enlarged ventricles. (c) Ultrasound examination of patient 2 at 17 + 5 weeks of gestation. Typical position of plexus choroideus (arrow), normally a 45° angle, indicating extreme hydrocephalus.

creatine kinase (CK) could not be measured due to blood sampling limitations. A muscle biopsy from the quadriceps muscle showed a dystrophic pattern with atrophic and hypertrophic muscle fibers, extensive necrosis of fibers and increase of central nuclei. Immunohistochemistry showed reduced staining of dystrophin 1 with normal staining of sarcoglycans and merosin. Postnatal chromosomal analysis showed a normal male karyotype.

As the prognosis was severe, it was decided not to pursue active treatment and the child died at the age of 6 days due to respiratory failure. Consent was not given for autopsy.

Patient 2

Severe hydrocephalus was diagnosed in the younger affected female sibling at 17 + 5 weeks of gestation (Fig. 1c). Chromosomal analysis after amniocentesis showed a normal karyotype. At

Fig. 2. (a) Family pedigree indicating the first cousin relationship of the parents. (b) 10K SNP-array analysis identified 10 regions of homozygosity (≥ 10 cM or with ≥ 20 consecutive homozygous SNPs) with identical genotypes for both affected siblings. The *FKRP* locus resides in the largest region of 33 cM and 66 homozygous SNPs on chromosome 19 (arrow). (c) Sequence analysis of the *FKRP* gene revealed a start codon mutation (c.1A>G), homozygous in the two patients, heterozygous in the parents.

23 + 3 weeks of gestation, the pregnancy was terminated. Her birth weight was 530 g. No visible eye abnormalities were seen. Consent was not given for autopsy.

Genetic analysis

DNA was extracted from peripheral blood lymphocytes using standard methods. 10K SNP-array analysis (GeneChip® Mapping 10K 2.0 Array, Affymetrix, Santa Clara, CA) was carried out at our linkage facility. Regions ≥ 10 cM or with ≥ 20 consecutive homozygous SNPs, and identical genotypes for both affected siblings were defined as regions of homozygosity. Mutation analysis for *FKRP* (NM_024301.4) was performed as described previously (12).

Results

The affected siblings were born to consanguineous parents (Fig. 2a). Both siblings showed extreme hydrocephalus as shown in postnatal brain MRI for patient 1 (Fig. 1a,b) and ultrasound examination of patient 2 at 17 + 5 weeks of gestation (Fig. 1c). Patient 1 showed clinical features consistent with a diagnosis of Walker–Warburg syndrome (Table 1). In order to map the disease-causing mutations, we performed genome-wide homozygosity mapping by 10K SNP-array analysis (Fig. 2b). We identified a homozygous region of 66 contiguous SNP markers on chromosome 19 with identical genotypes in both affected siblings. This region of 33 cM in size encompasses the *FKRP* locus. By direct sequencing of the *FKRP* coding DNA, we identified a homozygous mutation in the start codon

Table 1. Clinical features of patients 1 and 2

Feature	WWS ^a	Patient 1
Brain		
Cortical abnormalities	Y+++	Y+++
Encephalocele	Y	N
Hydrocephalus	Y+++	Y+++
White matter abnormalities	Y+++	ND
Fusion of hemispheres	Y+	N
Septum hypoplasia	Y+++	Y+++
Absence corpus callosum	Y	Y
Cerebellar cortex hypoplasia	Y+++	Y++
Cerebellar vermis hypoplasia	Y+++	Y+++
Speech (words)	N	ND
Mental retardation	Y+++	ND
Eye		
Microphthalmia	Y++	Y++
Myopia	Y	ND
Retinal dysplasia	Y++	ND
Congenital cataract	Y	Y
Anterior chamber abnormalities	Y+++	N
Muscle		
Serum CK (normal value: 200 U/l)	>5x	ND
Muscular dystrophy	Y+++	Y++
Other		
Able to walk	N	ND
Age	<3 years	Deceased at 6 days postpartum

WWS, Walker–Warburg syndrome; Y, observed; N, not observed; +, mild; ++, moderate; +++, severe; ND, no data; CK, creatine kinase.

^aClinical features of patients with WWS as previously reported (19, 20).

(c.1A>G, Met1Val) in both affected siblings. Both parents were heterozygous carriers of this mutation (Fig. 2c).

Discussion

The combination of abnormalities of brain, eye and muscle in patient 1 was consistent with a dystroglycanopathy. The differentiation between MEB and WWS was difficult due to significant clinical overlap. MEB patients are however predominantly diagnosed in the Finnish population and mostly die after early childhood (20), whereas most patients with WWS die in their first year of life (19). The clinical phenotype of patient 1, showing extreme prenatal hydrocephalus, severe brain malformation with neuronal migration defect, presence of severe eye abnormalities with microphthalmia and cataract, CMD, and the early lethal course were most consistent with a diagnosis of WWS (20). The younger female sibling was diagnosed at a gestational age of 17 weeks by ultrasonography, which showed hydrocephalus, consistent with the same diagnosis.

Mutations in the *FKRP*, *FKTN*, *LARGE*, *POMT1*, and *POMT2* genes are associated with WWS in approximately one-third of the patients with WWS in our research cohort. The genetic cause for the majority of patients with WWS remains unknown. *FKRP* is one of the six known genes (including *POMGnT1*, which is not associated with WWS) in which mutations result in defective glycosylation of α -dystroglycan (7). *FKRP* mutations have been reported to cause a broad spectrum of clinically related muscular dystrophies with severe neurological impairment at the severe end of the spectrum. *FKRP*-associated disorders include LGMD2I, MDC1C, CMD with mild structural brain involvement, MEB, and WWS (1, 7, 11, 13). Immunocytochemical labeling and Western blot analysis of muscle biopsies from these patients with antibodies that recognize the *O*-glycan moiety of α -dystroglycan (VIA4-1, I1H6) show a variable reduction of α -dystroglycan labeling, which suggests an association between the reduced labeling and the clinical presentation resulting from *FKRP* mutations (13–15).

One of the most common Mendelian mutations in any gene is the heterozygous *FKRP* missense mutation, c.826C>A (Leu276Ile). The mutation is of European origin, but is found in patients throughout the world (21). In homozygous state, this mutation causes the mildest phenotype observed for *FKRP* mutations, a relative mild form of late-onset LGMD without visible neurological or eye involvement (LGMD2I). Patients who show compound heterozygosity for the Leu276Ile mutation show either a slightly more severe LGMD phenotype or an early onset muscular dystrophy (Fig. 3). These ‘partner’ mutations with Leu276Ile are therefore suggested to be more detrimental for *FKRP* function than that in homozygous form (12, 15). Indeed, compound heterozygosity for the c.919T>A (Tyr307Asn) and Leu276Ile mutations have been reported in a patient with a severe LGMD2I phenotype who died in his late teens. The severe effect of the Tyr307Asn mutation was confirmed by its presence in homozygous form in an patient with MEB. The homozygous Met1Val mutation identified in this study is likely to result in a loss of functional *FKRP* protein and represents the severe end of the clinical spectrum associated with *FKRP* mutations. This is consistent with two patients carrying the Leu276Ile mutation in compound heterozygous form with the start codon mutation Met1Val. These two patients showed no abnormalities at birth and developed a Duchenne-like phenotype with high-serum CK from the first months and from the age of 1.5 years, respectively. No brain MRI was

Fig. 3. Schematic representation of the FKRP protein and the phenotype associated with different mutations in *FKRP*. **(a)** The coding DNA sequence of the *FKRP* gene is contained in one exon. The homozygous Met1Val mutation, identified in a WWS family in this study, is shown in bold. Use of an in frame alternative translational start site (Met143) will result in loss of the N-terminal part of the FKRP protein including the signal peptide, which is important for its targeting to the Golgi (23). LicD (Pfam domain PF04991) is found in protein involved in phosphorylcholine metabolism. **(b)** Different mutations in homozygosity or compound heterozygosity give rise to a range of dystroglycanopathies.

reported. They showed a similar severe LGMD2I phenotype as for the Tyr307Asn/Leu276Ile combination (15, 22). Hence, it is not surprising that a homozygous mutation Met1Val is associated with the severe WWS phenotype in two patients from the WWS family presented here. This start codon mutation may result in the use of an alternative start codon downstream of the coding sequence. The next two potential translational start sites are out of frame. The first alternative in frame translational start site is located 143 codons downstream of the original start codon, resulting in the loss of N-terminal residues important for Golgi targeting and the retention of the FKRP protein (23). Thus, we predict that the Met1Val mutation creates a null-allele.

The only other *FKRP* mutation associated with WWS predicts an amino acid substitution Cys318Tyr (12). The evolutionary conserved cysteine residue appears to be required to form disulfide bridges for protein folding, which are crucial for the function of FKRP. Only one homozygous nonsense mutation, c.1378C>T (Gln460X), has been reported for a patient with MDC1C (13), suggesting that this homozygous nonsense mutation is not as severe as the *FKRP* mutations identified in patients with MEB and WWS. This may be explained by residual activity of the corresponding mutant FKRP protein, which lacks the last 36 amino acids. Clearly, the Met1Val and Cys318Tyr mutations are more disruptive and

likely represent null-alleles. To our knowledge, FKRP-knockout mice have not been reported. Mice that are homozygous for an FKRP knock-down allele die soon after birth (24).

Null mutations in *FKTN*, *LARGE*, *POMT1* and *POMT2* are associated with WWS (4, 9, 25–27). Only for *POMGnT1*, null alleles are associated with a slightly milder phenotype, MEB. We and others previously hypothesized that *FKRP* null mutations are not compatible with life (12, 13, 15, 18). This study shows that null mutations in *FKRP* as well as in other genes involved in the O-linked glycosylation of α -dystroglycan, such as *FKTN*, *POMGnT1*, *POMT1*, *POMT2* and *LARGE*, are associated with the most severe end of the clinical spectrum of dystroglycanopathies.

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Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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