

A homozygous nonsense mutation in the *Fukutin* gene causes a Walker-Warburg syndrome phenotype

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J Med Genet 2003;40:845–848

Neuronal migration is a key process in the development of the cerebral cortex. During neocortex lamination new sets of neurones proliferate at the subventricular zone and migrate alongside specialised radial glial fibres to occupy their final destinations in an “inside-out” fashion.¹ More than 25 neuronal migration disorders resulting in death or improper positioning of the cortical neurones have been described in humans.² In the cobblestone neocortex the postmitotic neurones do not respond to their stop signals, and, crossing through the neocortex, bypass the glia limitans and invade the subarachnoid space. The resulting cortex is chaotically structured, consisting of an irregular lissencephalic surface and absence of lamination.

Cobblestone lissencephalies are often seen in association with additional features, such as eye malformations and congenital muscular dystrophy. Walker-Warburg syndrome (WWS, OMIM:236670), muscle-eye-brain (MEB, OMIM:253280), and Fukuyama congenital muscular dystrophy (FCMD, OMIM:253800) are the three major entities of this group. Patients are classified into these three entities on the

basis of the severity of the phenotype and the presence of syndrome specific symptoms (table 1). WWS is the most severe syndrome of the group, especially with regard to the brain phenotype. The WWS brain manifests cobblestone lissencephaly with agenesis of the corpus callosum, fusion of hemispheres, hydrocephalus, dilatation of the fourth ventricle, cerebellar hypoplasia, hydrocephalus, and sometimes encephalocele.^{3,4}

Causative genes for WWS (*POMT1*)⁵, FCMD (*Fukutin*)⁶ and MEB (*POMGnT1*)⁷ have been identified. WWS is genetically heterogeneous⁵, and approximately 20% of the patients show *POMT1* mutations. We hypothesised that severe mutations in *Fukutin* could give rise to a WWS phenotype.

The genotype-phenotype correlations found in FCMD patients favour this hypothesis.^{6,8,9} *Fukutin* mutations have been found only in Japanese FCMD patients.^{6,8,10} The vast majority present at least one copy of the same mild *Fukutin* mutation, a 3 kb insertion in the 3'UTR. Patients homozygous for this insertion show a milder phenotype than do compound heterozygotes, carrying the insertion in combination with a missense or nonsense mutation on the other allele (fig 1B). We therefore postulated that homozygous nonsense mutations would give rise to a more severe phenotype. This hypothesis is supported by the fact that *Fukutin* deficient chimeric mice show a severe phenotype which closely resembles WWS.¹¹

Based on this premise, we did a linkage study in 30 consanguineous WWS families. These patients have been diagnosed as WWS according to Cormand *et al.*³ In brief, patients with WWS die before the age of three years and have a thin cerebral mantle, a smooth cerebral surface, severe cerebellar/vermis hypoplasia, absent corpus callosum, and fusion of hemispheres. Two markers flanking the *FCMD* locus were selected: D9S1784, located 0.30 Mb centromeric to *FCMD*, and D9S172, located 0.27 Mb telomeric. Homozygosity was observed in three of the 30 patients, each of them with different haplotypes: patient 1, D9S1784:180/180, D9S172:299/299; patient 2, D9S1784:186/186, D9S172:303/303; and patient 3, D9S1784:192/192, D9S172:303/303.

Amplification of each of the 10 exons and flanking intron sequences of the *FCMD* gene and mutation analysis was carried out by direct sequencing.⁸ A homozygous mutation was found in one of the three patients linked to the *FCMD* locus. Patient 1 is homozygous for a novel dinucleotide substitution c.345_346GC→CT that creates a Gln116Stop mutation in exon 4 (fig 1). The mendelian segregation of the mutation Gln116Stop was confirmed by direct sequencing of family members. The Gln116Stop mutation was not

Key points

- Three rare autosomal recessive syndromes form a group of related diseases: muscle-eye-brain disease (MEB), Walker-Warburg syndrome (WWS), and Fukuyama congenital muscular dystrophy (FCMD). All share the combination of congenital muscular dystrophy and brain malformations, including a neuronal migration defect.
- The genes underlying these three disorders have been implicated in O-linked protein glycosylation: *Fukutin* (FCMD), *POMGnT1* (MEB), and *POMT1* (WWS). WWS is genetically heterogeneous, and mutations in the *POMT1* gene account for approximately 20% of WWS patients.
- A candidate gene strategy was used to elucidate the genetic defect in the remaining WWS patients. A homozygous nonsense mutation in the *Fukutin* gene was identified in one Turkish patient. So far, *Fukutin* mutations have been restricted to Japanese FCMD patients, whose phenotype is clinically much less severe than WWS. The Japanese FCMD patients are predicted to maintain residual *fukutin* activity. In contrast, the homozygous nonsense mutation identified in the present WWS patient is predicted to cause a total loss of *fukutin* activity.
- This result establishes a genotype-phenotype correlation for *Fukutin* mutations, which may also apply to other disorders involving defective O-linked glycosylation.

Abbreviations: FCMD, Fukuyama congenital muscular dystrophy; MEB, muscle-eye-brain disease; WWS, Walker-Warburg syndrome

