A double homozygous mutation in the *POMT1* gene involving exon skipping gives rise to Walker-Warburg syndrome in two Spanish Gypsy families

To the Editor :

Alpha-dystroglycanopathies are a large group of autosomal recessive muscular dystrophies that involve defects in *α*-dystroglycan (*α*-DG) Oglycosylation.
Walker–Warburg syndrome (WWS, OMIM 236670) is the most severe alpha-dystroglycanopathy, and affected infants rarely survive past the first year of life. The phenotype mainly involves muscle, eye and brain abnormalities associated
with anomalous neuronal migration, and it overlaps with Fukuyama congenital muscular dystrophy (FCMD, OMIM 253800) and muscleeye-brain (MEB) disease (OMIM 253280), other severe alpha-dystroglycanopathies. Mutations in six genes are known to cause alphadystroglycanopathies, all encoding known or putative glycosyltransferases: *POMT1*, *POMT2, POMGnT1, fukutin, FKRP* and *LARGE*.
Mutations in *POMT1* are responsible for approximately 25% of WWS cases and its targeted disruption in mice provokes embryonic lethality. Murine *Pomt1* is predominantly expressed in the tissues most severely affected in WWS patients, this expression persisting in the muscles, eyes, brain and cerebellum.
Here, we describe a double homozygous mutation in the *POMT1* gene in two unrelated Gypsy families, reflecting the often higher incidence of recessive diseases in endogamic populations.
Family1. Two affected male siblings, patients 1.1 and 1.2, were born to second-degree cousins in a Spanish Gypsy family. Patient 1.1 had profound muscular hypotonia at birth and a creatine kinase (CK) value of 8,400 IU/l. Muscular dystrophy was later confirmed by biopsy although merosin appeared normal. The infant showed retromicrognatia, low implanted ears, microphthalmia, cataract, microcornia and retinal dysplasia. Brain MRI identified type II lyssencephaly, hydrocephalus, agenesis of the corpus callosum, hypomyelination, cerebellar and brain stem hypoplasia, and Dandy–Walker malformation.
He died at 4 months. Patient 1.2 displayed similar symptoms and had a similar appearance to his brother, but there are no biological samples and his age of death was not determined.
Family2. This affected male sibling, patient 2.1, was born to double first-degree cousins in a Spanish Gypsy family. The patient had profound paralysing muscle hypotonia and a CK value of 40,851 IU/L at birth. Muscle biopsy confirmed muscular dystrophy although merosin appeared normal. Ocular examination revealed microphthalmia and bilateral retinal detachment. The patient also exhibited hypertelorism, frontal bossing and low implanted ears. Brain MRI showed lissencephaly, hydrocephalus, agenesis of the septum
pellucidum and of the posterior corpus callosum, and cerebellar hypoplasia. The patient died 10 days after birth.
Analysis of the complete coding region of the *POMT1* gene revealed a double homozygous mutation in both patients in reference to the most common *POMT1* splice variant (NM_001077 365.1) (13). The first homozygous mutation, g.3553G>T (NC_000009.10), was found at the beginning of intron 4 at the exon–intron boundary
(Fig. 1Aa). Indeed, the mutation produced the loss of the entire exon 4 in mRNA transcripts from patients 1.1 and 2.1. As this loss of 17 amino acids did not alter the reading frame, the ensuing protein has 708 instead of 725 amino acids. Exon 4 is located at Loop 1 of the POMT1 protein, within the conserved pfam02366 domain (protein mannosyltransferase, PMT) that potentially influences the protein's catalytic activity. This exon is highly conserved, with significant amino acid sequence identity, supporting information online), and it is the same size in all studied organisms.
The second homozygous mutation found, c.1545C>G, predicts the p.Ser515Arg substitution in exon 16 of *POMT1*, a serine only conserved from birds to humans. The same substitution has already been described in heterozygosity in an American WWS

patient, although no additional mutations were found in this patient to account for the WWS phenotype. Recently, this mutation was found in heterozygosity in an Italian patient diagnosed with congenital muscular dystrophy and mental retardation who also carried another mutation in the *POMT1* coding region, raising the possibility that this is a hot-mutation site in the gene.

Western blotting revealed a complete lack of glycosylated *α*-DG, which was confirmed by laminin overlay assay, while *β*-DG detection was normal (data not shown). Immunohistochemical analysis of muscle biopsies with VIA-4 antibody corroborated the alpha-dystroglycanopathy in both patients. However, the *α*-DG core protein was correctly located at the plasma membrane as assessed by the p*α*DAG antibody and distributed within the cell, probably undergoing post-translational modifications. Immunolabelling of *β*-DG and laminin was normal.

POMT1 is an integral endoplasmic reticulum (ER) membrane protein distributed in a similar pattern to the ER-marker calnexin. In muscle cells, the sarcoplasmic reticulum (SR) forms a network around the transverse T tubules that is continuous with the plasma membrane. In controls, POMT1 and calnexin were detected in the SR as part of this network within the muscle cell, as well as in the subsarcolemic space. While the distribution of calnexin did not appear to change in patients 1.1 and 2.1, POMT1 was only located in certain parts of the subsarcolemma and diffusely within the cell.

The mutation g.3553G>T removes exon 4 from the *POMT1* transcript of patients 1.1 and 2.1 while preserving the open reading frame and the epitope recognized by the anti-POMT1 antibody in Loop 5. Nevertheless, the distribution of POMT1 is altered, and it no longer co-localizes with calnexin, implying it has lost its ER-localization. This could be due to conformational changes in the POMT1 protein or to the degradation of the mutated protein inside the muscle cell. Staining for calnexin dismisses any major alterations to the SR in these patients.

Of these two mutations, the one causing exon 4 skipping is likely to be the most relevant for the WWS phenotype. In the second mutation, the substituted Ser515 is not very conserved and patients carrying this mutation do not provide much information about its relevance as none are homozygous carriers. Thus, although disease causing, this mutation might be responsible for milder phenotypes if not accompanied by a more severe change.

Founder effects have been reported for genes causing dystroglycanopathies in close-knit populations and less so in families with no defined inbreeding. We recently reported a potential founder mutation in the *fukutin* gene in the Ashkenazi Jewish population that was subsequently confirmed in a larger study. Since both our subjects carrying the same homozygous mutations belong to unrelated Gypsy families, the mutations identified here could reflect a possible founder effect in the *POMT1* gene within the Spanish Gypsy population. Patients 1.1 and 2.1 came from families with a high degree of consanguinity and thus, it is likely that the parents of both patients were heterozygous carriers of the mutations. Unfortunately, no samples from the parents were available for studies. Inbreeding is very common in the Gypsy population, manifesting a high prevalence of phenotypes caused by recessive mutations and the transmission of these mutations.

Further studies will be necessary to determine if this double mutation in the *POMT1* gene is widespread among the Spanish Gypsy population.

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