MUTATION IN BRIEF

Ethnically Diverse Causes of Walker-Warburg Syndrome (WWS): *FCMD* Mutations Are a More Common Cause of WWS Outside of the Middle East

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Walker-Warburg syndrome (WWS) is a genetically heterogeneous autosomal recessive disease characterized by congenital muscular dystrophy, cobblestone lissencephaly, and ocular malformations. Mutations in six genes involved in the glycosylation of α-dystroglycan (*POMT1*, *POMT2*, *POMGNT1*, *FCMD*, *FKRP* and *LARGE*) have been identified in WWS patients, but account for only a portion of WWS cases. To better understand the genetics of WWS and establish the frequency and distribution of mutations across WWS genes, we genotyped all known loci in a cohort of 43 WWS patients of varying geographical and ethnic origin. Surprisingly, we reached a molecular diagnosis for 40% of our patients and found mutations in *POMT1*, *POMT2*, *FCMD* and *FKRP*, many of which were novel alleles, but no mutations in *POMGNT1* or *LARGE*. Notably, the *FCMD* gene was a more common cause of WWS than previously expected in the European/American subset of our cohort, including all Ashkenazi Jewish cases, who carried the same founder mutation. © 2008 Wiley-Liss, Inc.

KEY WORDS: Walker-Warburg syndrome; congenital muscular dystrophy; alpha-dystroglycan; *POMT1, POMT2, FCMD, FKRP*

INTRODUCTION

Walker-Warburg syndrome (WWS; MIM# 236670) is an autosomal recessive disorder characterized by cobblestone (type II) lissencephaly, hydrocephalus, severe cerebellar and ocular malformations, and congenital

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muscular dystrophy (CMD) (Dobyns, et al., 1989; Cormand, et al., 2001; Vajsar and Schachter, 2006). It is the most severe form of CMD, leading to significant developmental delay and a life expectancy of less than 3 years.

While WWS presents with a relatively homogeneous phenotype, it is genetically heterogeneous (Beltran-Valero de Bernabe, et al., 2002; Currier, et al., 2005; Vajsar and Schachter, 2006). The most common known causes of WWS are mutations in two glycosyltransferases, protein O-mannosyltransferase 1 and 2 (*POMT1;* MIM# 607423, NM_007171; *POMT2;* MIM# 607439, NM_013382) (Beltran-Valero de Bernabe, et al., 2002; Currier, et al., 2005; van Reeuwijk, et al., 2005b). *POMT1* and *POMT2* attach the first sugar in the O-mannose-linked glycan moiety of the α-dystroglycan protein, a transmembrane glycoprotein expressed on the surface of muscle cells and neurons (Barresi and Campbell, 2006). α-dystroglycan interacts with several extracellular matrix components in the basal membrane, and disruption of its function is thought to underlie the severe defects in muscle, eye and brain development in WWS patients (Barresi and Campbell, 2006). However, *POMT1* mutations have been reported in only 10-20% of WWS cases (Currier, et al., 2005; Vajsar and Schachter, 2006), and only three WWS patients and three fetuses affected by fetal cobblestone lissencephaly have been found to carry *POMT2* mutations (van Reeuwijk, et al., 2005b; Bouchet, et al., 2007).

Disruption of α-dystroglycan glycosylation can have variable effects on muscle, eye and brain development, leading to other CMD syndromes that affect the nervous system less severely than WWS (van Reeuwijk, et al., 2005a). Additional genes in the α-dystroglycan glycosylation pathway are mutated in autosomal recessive disorders similar to WWS and grouped under the term α-dystroglycanopathies: *POMGNT1* (MIM# 606822, NM_017739) in Muscle Eye Brain disease (MEB; MIM# 253280) (Yoshida, et al., 2001), *FCMD* (MIM# 607440, NM_001079802) in Fukuyama congenital muscular dystrophy (FCMD; MIM# 253800) (Kobayashi, et al., 1998), fukutin-related protein (*FKRP*; MIM# 606596, NM_024301) in Muscular Dystrophy Congenital 1C (MDC1C; MIM# 606612) (Brockington, et al., 2001), and *LARGE* (MIM# 603590, NM_004737) in MDC1D (MIM# 608840) (Longman, et al., 2003). Further genetic analyses have revealed that these α-dystroglycanopathy genes are associated with extremely variable phenotypes ranging from mild CMD to WWS (van Reeuwijk, et al., 2005a; Barresi and Campbell, 2006; Godfrey, et al., 2007). *FCMD* mutations have been identified in four classic cases of WWS each (de Bernabe, et al., 2003; Silan, et al., 2003; Cotarelo, et al., 2008), *LARGE* mutations in two WWS cases (Godfrey, et al., 2007; van Reeuwijk, et al., 2007) and *FKRP* and *POMGNT1* mutations in one WWS case each (Beltran-Valero de Bernabe, et al., 2004, Godfrey, et al., 2007).

Such genetic heterogeneity of WWS and the variability in the genotype/phenotype correlation for each identified gene have greatly complicated the genetic diagnosis of WWS patients. Because of the rarity of the disease, a comprehensive overview of the frequency and distribution of mutations in all known genes in a carefully phenotyped and homogeneous cohort of patients had not previously been undertaken. To explore the genetic heterogeneity of WWS and provide some guidance in the choice of genetic tests in these patients, we collected a cohort of classic WWS cases of different ethnic origins and analyzed both common causes of WWS (*POMT1, POMT2*) and genes that are more rarely mutated in this disease, such as *FCMD*, *FKRP, POMGNT1* and *LARGE*.

SUBJECTS AND METHODS

Subjects

All subjects were enrolled after informed consent was obtained, and research was conducted according to protocols approved by the Institutional Review Boards of Beth Israel Deaconess Medical Center and Children's Hospital, Boston. All patients were diagnosed with classic WWS based on the following criteria: (1) brain malformation on neuroimaging characterized by cobblestone lissencephaly, severe ventriculomegaly, and cerebellar hypoplasia; (2) the presence of ocular malformations; and (3) a clinical diagnosis of congenital muscular dystrophy, usually based on severe hypotonia (Dobyns, et al., 1989; Cormand, et al., 2001). Because of the clinical severity and reduced lifespan associated with this condition, serum creatine kinase (CK) and histopathological confirmation of muscular dystrophy were not available in most cases, although CK was highly elevated in all patients tested.

Whole genome scans and sequencing analysis

Genomic DNA was prepared from peripheral blood samples from patients and available family members according to standard protocols. 600 ng of genomic DNA were used to hybridize Affymetrix 250K StyI SNP arrays at the Broad Institute of MIT and Harvard, Cambridge, MA. Genotyping data was analyzed using the loss

of heterozygosity algorithm on dChip software (Lin, et al., 2004) to identify regions of identity by descent (IBD) at known WWS loci. Sequencing of *POMT1* (NM_007171.3), *POMT2* (NM_013382.4), *POMGNT1* (NM_017739.2), *LARGE* (NM_0044737.3), *FKRP* (NM_001039885.1) and *FCMD* (NM_006731.2) coding regions was performed on PCR products after amplification of genomic DNA by SeqWright (Houston, TX). PCR primers were designed for each exon including at least 50 base pairs of flanking intronic sequences and are available upon request. DNA changes are described according to the nucleotide numbering reflects in the cDNA sequence, with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). For protein changes the initiation codon is codon 1. Whenever a putative mutation was identified, its inheritance pattern was confirmed in all available family members, and at least 96 control DNAs (192 alleles) were tested to confirm it was not a benign polymorphic change. Unfortunately, no parents were available in the four compound heterozygous cases identified in this study.

Multiplex Ligation-dependent Probe Amplification (MLPA)

MLPA analysis was performed using the SALSA MLPA EK-1 kit (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer specifications. Custom probes for each exon of the *POMT2* gene were designed following instructions from MRC-Holland and sequences are available upon request. MLPA-PCR products were run on an ABI-3130XL genetic analyzer (Applied Biosystems, Foster City, CA), and MLPA peaks were quantified on GeneMapper software (Applied Biosystems) and analyzed following protocols from MRC-Holland.

RESULTS

We collected a cohort of 43 affected individuals (from 40 families) of varying provenance. Sixteen patients from 14 families were Middle Eastern children born to parents who were first cousins (9 from Saudi Arabia, 3 from Israel, 1 from Jordan, 1 from Syria). Twenty-seven additional cases from the United States and Europe were mostly Caucasian (20, including 3 of Ashkenazi Jewish origin), with 6 Hispanic patients and 1 African-American patient.

All individuals (n=104) received genome-wide screens on Affymetrix 250K StyI SNP arrays at the Broad Institute of MIT and Harvard, Cambridge, MA. We analyzed both consanguineous and non-consanguineous families for the presence of stretches of homozygous SNPs spanning the known WWS loci (*POMT1*; *POMT2*; *POMGNT1*, *FCMD*; *FKRP* and *LARGE*), using the loss of heterozygosity algorithm in dChip software (Lin, et al., 2004). Stretches of homozygosity/identity by descent (IBD) of the order of several centiMorgans (cM) were expected in the consanguineous families in our cohort, with the mutation-bearing fragment usually larger than 5 cM for children whose parents are first cousins (Woods, et al., 2006). Smaller regions of IBD may also be present in affected children from non-consanguineous marriages subject to founder effects. Sequencing of the coding sequence of the corresponding gene was performed wherever IBD was observed. In the non-consanguineous cases, all six genes were sequenced in all affected.

In addition, the Affymetrix arrays allow for copy-number change analysis and we assessed all known WWS genes for changes, such as heterozygous or homozygous deletion and duplications, using the copy-number function in dChip (Lin, et al., 2004). No copy-number changes were identified in the six WWS loci analyzed in our cohort, however the average distance between SNPs in these arrays is approx. 10 kb and it is possible that smaller deletions/duplications may be present.

POMT1

Five Middle Eastern families and one Hispanic family showed IBD at the *POMT1* locus and upon sequencing of the *POMT1* gene all but one affected carried a homozygous mutation (Table 1, Families 1,2,4-6). All *POMT1* mutations identified were predicted to severely disrupt *POMT1* translation and function by either splicing disruption (c.280+1G>T, c.1892+1G>T), frameshift (c.1260_1261delCT, p.L421fs) or early termination (c.2163C>A, Y721X). The same mutation (c.280+1G>T) was found in two unrelated Saudi families (Table 1, Family 1, 2) carrying different haplotypes (not shown), suggesting that this mutation either originated twice in the population, or was an extremely remote founder mutation in the Saudi population.

Additional sequencing identified only one other patient carrying a *POMT1* mutation (Table 1, Patient 3). An African-American male carried two likely *POMT1* mutations: a novel missense change at cysteine 366 (c.1097G>A, p.C366Y) and a previously reported frameshift mutation (c.2167_2168insG, p.G722fs, (Beltran-

Valero de Bernabe, et al., 2002)). This patient was presumed to be a compound heterozygote, but parental samples were not available to confirm this.

Table 1. Mutations in *POMT1*, *POMT2*, *FCMD* and *FKRP*

Family	Origin	Consanguinity	Gene	Mutation
1, 2	Saudi Arabia	1st cousin	*POMT1*	homozygous c.280+1G>T (p.E94fs)
3	US (African)	No	*POMT1*	c.1097G>A (p.C366Y), c.2167_2168insG (p.G722fs)*
4	Israeli Arab	1st cousin	*POMT1*	homozygous c.1260_1261delCT (p.L421fs)*
5	Bahrain	1st cousin	*POMT1*	homozygous c.1892+1G>T (p.Q630fs)
6	US (Hispanic)	1st cousin	*POMT1*	homozygous c.2163C>A (p.Y721X)*
7	Palestinian	1st cousin	*POMT2*	homozygous c.924-2A>C (p.K307fs)
8	US (Caucasian)	No	*POMT2*	c.1006+5G>A**, c.1890A>G (splicing?)
9	Saudi Arabia	1st cousin	*POMT2*	homozygous c.1159delA (p.I387Sfs)
10	US (Caucasian)	No	*FKRP*	c.341C>G (p.A114G)*, c.437A>C (p.E146A)
11	Saudi Arabia	1st cousin	*FKRP*	homozygous c.962_970dup9 (p.A321_R323dup)
12	US (Caucasian)	No	*FCMD*	c.385delA (p.I129X), c.1176C>A (p.Y392X)
13	US (Hispanic)	Probable	*FCMD*	homozygous c.557A>G (p.H186R)
14-16	Ashkenazi	No	*FCMD*	homozygous c.1167_1168insA (p.F390fs)*

* previously identified mutations, ** novel mutations at same site of previously identified mutations
RefSeq accession number of sequences: *POMT1* NM_007171.3; *POMT2* NM_013382.4; *FKRP* NM_001039885.1; *FCMD* NM_006731.2

POMT2

POMT2 forms a heterocomplex with POMT1 to initiate glycosylation (Akasaka-Manya, et al., 2006). *POMT2* mutations have been reported in only a few WWS cases (van Reeuwijk, et al., 2005b) and more recently in at least six independent MEB cases (Godfrey, et al., 2007). Five Middle Eastern families showed IBD at the *POMT2* locus, and two mutations were found in the coding sequence of the *POMT2* gene, one in a Saudi family (c.1159delA) causing a frameshift and early termination (p.I387Sfs; Table1, Family 9) and one in a Palestinian family disrupting the splice acceptor in intron 7 (c.924-2A>C, p.K307fs, Table 1, Family 7). IBD in four families without a mutation upon sequencing was not unexpected, as the consanguineous families in our cohort often have only one affected child with multiple regions of IBD across the genome due to the high degree of consanguinity. The SNP haplotypes in the four families were all different, ruling out a common undentified mutation. We asked whether these cases carried copy-number changes, such as deletions or duplication in the *POMT2* gene. We had ruled out larger changes by copy-number analysis of the SNP data, but small copy-number changes at the single exon level would not be detected. We excluded homozygous deletion, since all exon could be amplified in all patients during PCR-based sequencing, but could not rule out homozygous duplications. Therefore, we further analyzed these patients via Multiplex Ligation-dependent Probe Amplification (MLPA) at the *POMT2* locus (Schouten, et al., 2002). No single exon deletions or duplications were identified (not shown). It is possible that non-coding mutations may cause the disorder in these four families or they may carry mutations in yet unidentified genes.

Sequencing in our non-consanguineous cohort identified one additional case carrying two heterozygous putative splicing changes in *POMT2* (Table 1, Patient 8). The first is a transition at a highly conserved guanine 5 basepairs (bp) downstream of exon 8 (c.1006+5G>A). While less universally conserved than the splice donor site, this G in intronic position +5 is an important part of the snRNP binding site (Cartegni, et al., 2002) and identical mutations at the same position have been reported to affect mRNA splicing in other CMD cases (Thi Tran, et al., 2005; Bouchet, et al., 2007). The second putative mutation is a synonymous change (c.1890A>G) located at position –2 at the end of exon 18, a position that is also highly conserved and often involved in splicing defects (Cartegni, et al., 2002). The patient is deceased and no cell lines are available for this case, therefore direct analysis of possible

splicing disruptions could not be perfomed. To confirm that these were not rare benign SNP's, we doubled the number of control DNAs tested and did not find these changes in 188 control individuals (376 chromosomes).

LARGE* and *POMGNT1

The *LARGE* gene is usually found mutated in a form of CMD (MDC1D) and the *POMGNT1* gene is responsible for Muscle-Eye-Brain disease (MEB). Recently both *LARGE* and *POMGNT1* mutations have been identified in rare WWS patients (van Reeuwijk, et al., 2007; Godfrey, et al., 2007). From our consanguineous cohort one Saudi family showed IBD at the *LARGE* locus and two Hispanic families showed IBD at the *POMGNT1* locus, but no mutation was identified upon sequence analysis. Sequencing of the *LARGE* and *POMGNT1* genes in the non-consanguineous group did not identify any potential mutations.

FKRP

FKRP mutations are associated with a wide spectrum of congenital muscular dystrophies with or without cognitive defects and brain malformations (Mercuri, et al., 2003; Louhichi, et al., 2004; Mercuri, et al., 2006). Only one case of WWS and two cases of MEB are known to carry *FKRP* mutations (Beltran-Valero de Bernabe, et al., 2004; Mercuri, et al., 2006). By homozygosity mapping, one Saudi family on our cohort showed IBD at the *FKRP* locus and carried a 9 bp duplication (c.962_970dup9) predicted to duplicate three amino acids, alanine 321 to arginine 323 (Table 1, Family 11; Figure 1A). Interestingly, the only previously reported *FKRP* mutation causing WWS is a transition affecting cysteine 318, which is close to the change in Family 11 (Figure 1C; Beltran-Valero de Bernabe, et al., 2004). Mutations at this location in the protein sequence may be particularly deleterious for brain development.

One additional WWS case carrying heterozygous *FKRP* changes was identified in the European/American non-consanguineous pool (Table 1, Patient 10). A girl from the United States presented a known mutation (c.341C>G, p.A114G, Figure 1B-C (Mercuri, et al., 2006)), accompanied by a novel missense changes, c.437A>C (p.E146A). Both changes were tested in 188 control individuals (376 chromosomes). The A114G mutation has already been described in homozygosity in one MEB case (Mercuri, et al., 2006). A114 is another residue in the FKRP protein which, when mutated, may severely perturb brain development.

FCMD

Mutations in the putative glycotransferase *FCMD* were initially described in FCMD patients in Japan, where an ancestral retrotransposon insertion in the 3' UTR of the gene is present in all affected individuals, usually in homozygosity or less commonly in heterozygosity in combination with mutations in the coding region (Figure 2C) (Kobayashi, et al., 1998; Kondo-Iida, et al., 1999). No homozygous mutations in the coding sequence were identified in FCMD patients, suggesting that such mutations may be embryonic lethal or cause a more severe disorder, such as WWS (Kondo-Iida, et al., 1999). The identification of homozygous coding mutations in the *FCMD* gene outside of Japan, in two Turkish families affected by WWS (de Bernabe, et al., 2003; Silan, et al., 2003) indicated that WWS may be allelic to FCMD. However, the relative contribution of *FCMD* mutations to the etiology of WWS has not been studied. Recently, two additional WWS patients were reported to carry *FCMD* mutations (Cotarelo, et al., 2008).

No IBD was observed at the *FCMD* locus in our consanguineous families. Due to the high resolution of our SNP screen, we wondered whether small regions of IBD with identical haplotype could be used to identify a founder mutation in non-consanguineous families with the same ethnic origin. All three Ashkenazi Jewish families in our group carried the same homozygous haplotype at the *FCMD* locus (Figure 2A). Upon sequencing of the *FCMD* gene, we found the same homozygous insertion in all three affected children (c.1167_1168insA, Table 1, Family 14-16). This insertion had been described as a *de novo* heterozygous change coupled with the 3' UTR insertion in a Japanese FCMD patient (Kondo-Iida, et al., 1999) and was concurrently identified by another group in Family 14, which was obtained from Coriell Cell Repositories, Camden, NJ (Cotarelo, et al., 2008). In our patient cohort, this mutation appears to represent an inherited founder mutation common to all Ashkenazi families (Figure 2A). We therefore analyzed 299 samples from the control Ashkenazi population in Israel to determine carrier frequency and found that 2/299 (0.7%) of the Israeli Ashkenazim carry this *FCMD* mutation in heterozygosity.

Sequencing in the remaining patients identified two additional cases carrying *FCMD* mutations. A Caucasian boy from the United States carried a deletion (c.381delA, p.I129X) and an early termination (c.1176C>A,

p.Y392X) (Table 1, Patient 11; Figure 2B) and was presumed to be a compound heterozygote, but parental samples were not available to confirm this. A Mexican boy from the United States originally reported as being born from a non-consanguineous union showed large stretches of homozygosity in his genome scan, suggesting that the parents share a common ancestor. He carried a homozygous mutation in exon 4 (c.557A>G, p.H186R) (Table 1, Patient 12; Figure 2B).

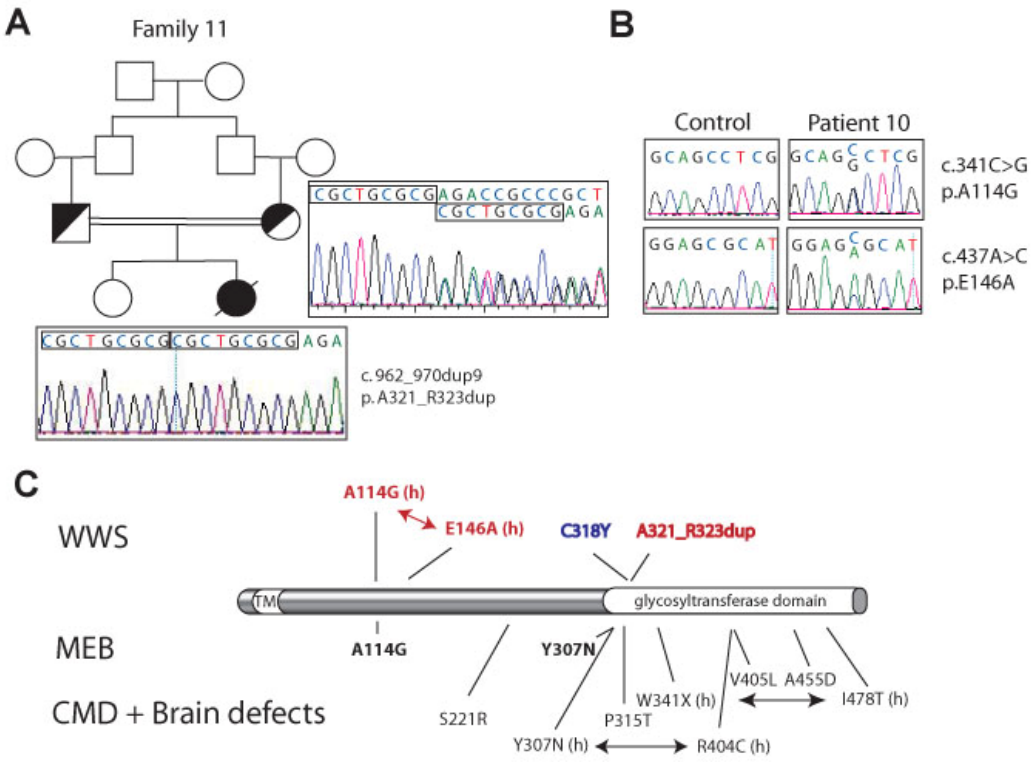

Figure 1. ***FKRP*** **mutations in classic WWS patients.** **A.** Patient 11 was born to first-cousin parents and carried a homozygous 9 basepair duplication starting at bp.962 in the *FKRP* gene. This mutation leads to a 3 amino acid duplicagtion. **B.** Two heterozygous missense mutations were identified in Patient 10. **C.** Schematic representation of the FKRP protein and mutation summary. The initiation codon is codon 1. Mutations listed above the protein cause WWS: previously reported mutations are in blue, mutations identified in this study are in red. Below the protein mutations causing MEB and CMD associated with variable brain malformations are shown. (h) means heterozygous mutations. Compound heterozygous mutations are connected by an arrow. *FKRP* mutations causing only CMD are not listed. TM: transmembrane domain.

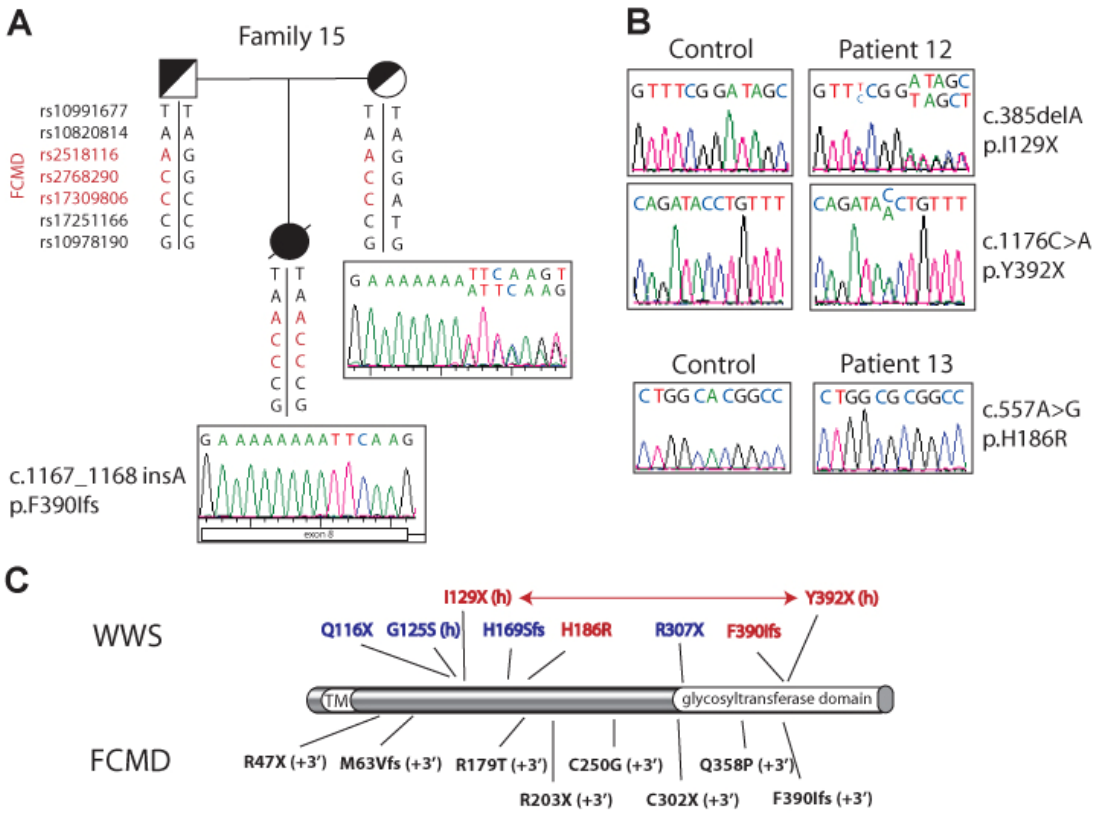

Figure 2. ***FCMD*** **mutations in classic WWS patients.** **A.** Patient 15 is of Ashkenazi Jewish origin. She carried a homozygous insertion in exon 8 of the *FCMD* gene, which was inherited from both parents. SNP genotyping results are indicated below each individual. The same mutation and the same SNP haplotype were found in two additional Ashkenazim affected in our cohort suggesting a founder effect in this population. **B.** Patient 12 carried two heterozygous nonsense mutations, while Patient 13 carried a homozygous missense mutation. **C.** Schematic representation of the fukutin protein and mutation summary. Mutations listed above the protein cause WWS: previously reported mutations are in blue (G125S was identified in heterozygosity with a small 3' UTR deletion, which is different from the classic *FCMD* mutations (Cotarelo, et al., 2008)), mutations identified in this study are in red. (h) means heterozygous mutations. Compound heterozygous mutations are connected by an arrow. Below the protein coding mutations causing FCMD are shown; (+3') next to each mutation indicates that the mutation is in heterozygosity and associated with the 3' retransposon insertion characteristic of Japanese FCMD patients. TM: transmembrane domain.

DISCUSSION

To date, there has been no comprehensive analysis of the frequency and distribution of mutations in all known WWS genes in a large group of carefully phenotyped WWS patients. Previous reports indicated that only 10-20% of WWS cases could be explained by mutations in *POMT1* and *POMT2* (Currier, et al., 2005; Vajsar and Schachter, 2006). Mutations in *FCMD*, *FKRP* and *LARGE* were reported in a few WWS patients each (Silan, et al., 2003; Beltran-Valero de Bernabe, et al., 2003 and 2004; Godfrey, et al., 2007; van Reeuwijk, et al., 2007; Cotarelo, et al., 2008), and were presumed to be extremely rare. We assembled a large cohort of patients with typical WWS, drawn from Middle Eastern consanguineous families and from consanguineous and non-consanguineous families from Europe and the Americas. We found that 40% (16/40) of patients in our cohort carried mutations in the coding sequence of *POMT1*, *POMT2*, *FKRP* or *FCMD* with no *POMGNT1* or *LARGE* mutations detected. *FCMD* and *FKRP* mutations in particular were much more common than previously

suggested and were mostly identified in non-consanguineous patients of European descent (Table 2). All Ashkenazi Jewish patients in our group shared an identical haplotype at the *FCMD* locus and the same mutation suggesting a founder effect in this population (Table 2). We identified the carrier frequency of this mutation to be 0.7% in the Ashkenazi population in Israel, which will be extremely informative for genetic testing. A striking difference was observed in the geographic distribution of mutations, as Middle Eastern families were mostly carriers of *POMT1* mutations, while the most common cause of European/American cases was *FCMD* mutations (Table 2).

Table 2. Frequency of coding mutations according to origin

	POMT1	*POMT2*	*FCMD*	*FKRP*
Middle East (14)	35.7% (5/14)	14.3% (2/14)	0.0%	7.1% (1/14)
Europe/Americas (27)	7.4% (2/27)	3.7% (1/27)	18.5% (5/27)	3.7% (1/27)
Ashkenazi (3)	0.0%	0.0%	100.0% (3/3)	0.0%
Hispanics (6)	16.7% (1/6)	0.0%	16.7% (1/6)	0.0%

Our data support the hypothesis that WWS is an extremely genetically heterogeneous disease. Analysis of the medical records and neuroimaging data available for our cohort revealed that patients carrying *POMT1*, *POMT2*, *FKRP* and *FCMD* mutations are clinically and radiologically very similar (Table 3 and Figure 3). Moreover, they are indistinguishable from WWS patients not carrying mutations in any of these genes.

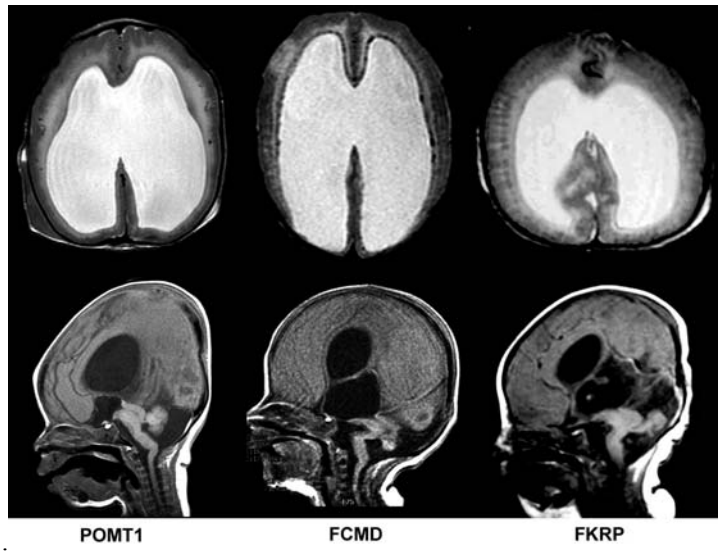

Figure 3. Brain imaging of children with WWS of varying genetic etiologies. Magnetic resonance imaging (MRI) using T2-weighted axial (top row) and T1-weighted sagittal (bottom row) sequences demonstrates that affected individuals with mutations in the *POMT1* (left), *FCMD* (middle), and *FKRP* (right) genes have indistinguishable radiological phenotypes. All demonstrate classic WWS findings including cobblestone lissencephaly with an absence of sulcation and an irregular gray-white junction, marked ventriculomegaly, and severe dysplasia of posterior fossa structures, including cerebellar hypoplasia and a "kinked" appearance of the brainstem.

Table 3. Summary of clinical findings in WWS patients carrying mutations in *POMT1*, *POMT2*, *FKRP* and *FCMD*

Pat.	Gene	Brain findings			Eye findings	Muscle findings		Other
		Cerebral Cortex	Ventricles	Cerebellum		Tone	CK (U/L)	
1	*POMT1*	cobblestone	enlarged	small, dysmorphic	bilateral glaucoma and cataracts	low	1545	seizures, congenital hip dislocation
2a	*POMT1*	cobblestone	enlarged	small, dysmorphic	retinal dysplasia, glaucoma	low	1565	
2b	*POMT1*	cobblestone	enlarged	small hemispheres, no vermis	L microphthalmia, retinal dysplasia, AC malformation, glaucoma	low	787	seizures
3	*POMT1*	lissencephaly, ctx too thin to assess	enlarged, encephalocele	small hemispheres, no vermis	microphthalmia	n/a	n/a	seizures, contractures, polydactyly
4	*POMT1*	cobblestone	enlarged	small	n/a	n/a	n/a	contractures, bilateral kidney dysplasia,
5	*POMT1*	lissencephaly, ctx too thin to assess	enlarged	small hemispheres, no vermis	shallow AC, retinal hypoplasia	low	n/a	L hydronephrosis
6	*POMT1*	cobblestone	enlarged	small	cataracts, AC malformation	n/a	5000	
7	*POMT2*	cobblestone	enlarged	small	cataracts	low	elevated	
8	*POMT2*	cobblestone	enlarged	small hemispheres, no vermis	mild ON hypoplasia	n/a	2000	tower skull deformity
9	*POMT2*	cobblestone	enlarged	small, dysmorphic	retinal dysplasia, ON hypoplasia	low	1666	hydronephrosis
10	*FKRP*	frontal PMG with cobblestone features	enlarged	small hemispheres, no vermis	detached retina	low	n/a	seizures
11	*FKRP*	cobblestone	enlarged	small, dysmorphic	bilateral cataracts	low	5941	seizures, arthrogryposis
12	*FCMD*	cobblestone	enlarged	small hemispheres, no vermis	ON hypoplasia	low	2255	seizures
13	*FCMD*	cobblestone	enlarged	small hemispheres, no vermis	ON hypoplasia	low	n/a	
14	*FCMD*	cobblestone	enlarged encephalocele	n/a	microphthalmia, microcornea, cataracts		elevated	
15	*FCMD*	cobblestone	enlarged	small, dysmorphic	microphthalmia, retinal dysplasia	n/a	39146	mild hydronephrosis
16	*FCMD*	cobblestone	enlarged	small vermis	glaucoma	low	9900	seizures

Abbreviations: R, right; L, left; AC, anterior chamber; ON, optic nerve; ctx, cortex; PMG, polymicrogyria

Since genotype/phenotype correlation does not appear to allow for the prediction of the responsible mutated gene in this disorder, systematic mutation analysis and correlation with geographical and ethnic provenance may be useful to guide a genetic diagnosis. Our data suggests that *POMT1* is more frequently mutated in Middle Eastern populations, while *FCMD* may be an additional candidate for testing in populations of European descent, in particular among Ashkenazi Jewish families. A reduced frequency of *FCMD* mutations in the Middle East may explain why *FCMD* was only identified twice by homozygosity mapping as a cause of WWS.

In addition, because the exact involvement of *FCMD*, *FKRP* and *LARGE* in α-dystroglycan glycosylation is still largely unknown, comprehensive mutation analysis in patients and genotype/phenotype correlation across the spectrum of disease caused by these genes may provide clues to gene function. Only one mutation in the putative glycosyltransferase *FKRP* has previously been identified as a cause of WWS (Beltran-Valero de Bernabe, et al., 2004). We have identified one known and two novel alleles: p.A114G was present in heterozygosity in one patient and it was previously found to cause MEB in homozygosity (Mercuri, et al., 2003), p.E146A is a novel change, and the 9bp duplication in Family 11 is a novel mutation near the site of the only previously described WWS mutation (C318Y) (de Bernabe, et al., 2003). While all other known *FKRP* mutations usually cause congenital

muscular dystrophy with mild or no brain defects (Brockington, et al., 2001; Mercuri, et al., 2006), our results indicate that changes at certain residues in the protein are extremely deleterious for brain development. Functional analyses in animal models will determine how these mutations affect the FKRP protein.

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