REPORT

Truncating Mutations of *MAGEL2*, a Gene within the Prader-Willi Locus, Are Responsible for Severe Arthrogryposis

Dan Mejlachowicz,[1] Flora Nolent,[1] Jérome Maluenda,[1] Hanitra Ranjatoelina-Randrianaivo,[2] Fabienne Giuliano,[3] Ivo Gut,[4] Damien Sternberg,[5] Annie Laquerrière,[6] and Judith Melki[1,*]

Arthrogryposis multiplex congenita (AMC) is characterized by the presence of multiple joint contractures resulting from reduced or absent fetal movement. Here, we report two unrelated families affected by lethal AMC. By genetic mapping and whole-exome sequencing in a multiplex family, a heterozygous truncating *MAGEL2* mutation leading to frameshift and a premature stop codon (c.1996delC, p.Gln666Serfs*36) and inherited from the father was identified in the probands. In another family, a distinct heterozygous truncating mutation leading to frameshift (c.2118delT, p.Leu708Trpfs*7) and occurring de novo on the paternal allele of *MAGEL2* was identified in the affected individual. In both families, RNA analysis identified the mutated paternal *MAGEL2* transcripts only in affected individuals. *MAGEL2* is one of the paternally expressed genes within the Prader-Willi syndrome (PWS) locus. PWS is associated with, to varying extents, reduced fetal mobility, severe infantile hypotonia, childhood-onset obesity, hypogonadism, and intellectual disability. *MAGEL2* mutations have been recently reported in affected individuals with features resembling PWS and called Schaaf-Yang syndrome. Here, we show that paternal *MAGEL2* mutations are also responsible for lethal AMC, recapitulating the clinical spectrum of PWS and suggesting that *MAGEL2* is a PWS-determining gene.

Arthrogryposis multiplex congenita (AMC) has an overall incidence of one in 3,000 live births.[1,2] Some non-genetic factors, such as mechanical limitation of fetal movements or maternal autoimmune myasthenia, might cause AMC. Isolated AMC is the direct consequence of fetal akinesia and/or hypokinesia sequence. Isolated AMC is genetically heterogeneous and caused by mutations of genes encoding components required for the formation or the function of the neuromuscular junction, including *CHRNG* (MIM: 100730), *CHRNA1* (MIM: 100690), *CHRND* (MIM: 100720), *CHRNB1* (MIM: 100710), *DOK7* (MIM: 610285), *RAPSN* (MIM: 601592), and *CHAT* (MIM: 118490); or the skeletal muscle, including *TPM2* (MIM: 190990), *MYH2* (MIM: 160740), *MYH3* (MIM: 160720), *MYH8* (MIM: 160741), *TNNI2* (MIM: 191043), *TNNT3* (MIM: 600692), *MYBPC1* (MIM: 160794), *DMPK* (MIM: 605377), *ACTA1* (MIM: 102610), *NEB* (MIM: 161650), *RYR1* (MIM: 180901), *SYNE1* (MIM: 608441), *PIEZO2* (MIM: 613629), and *TTN* (MIM: 188840); or the survival of motor neurons or myelination of peripheral nerves, including *GLE1* (MIM: 603371), *PIP5K1C* (MIM: 606102), *ERBB3* (MIM: 190151), *SMN1* (MIM: 600354), *TRPV4* (MIM: 605427), *ADCY6* (MIM: 600294), *CNTNAP1* (MIM: 602346), *ECEL1* (MIM: 605896), and *GPR126* (MIM: 612243). The difficulty in establishing a genetic diagnosis for AMC-affected individuals is due to high genetic heterogeneity and/or to the existence of some not-yet-identified disease-associated genes.

The parents of all affected individuals provided written informed consent for genetic analysis of their children or fetuses and themselves in accordance with the ethical standards of our institutional review boards. In family 1, three affected fetuses were born to non-consanguineous healthy parents (Figure 1). According to ultrasound examination, the first fetus displayed reduced mobility detectable from 22 weeks of gestation (w.g.) and associated with polyhydramnios and unilateral club foot. Karyotype analysis was 46, XX. Fetal death was observed at 24 w.g. Fetopathological examination revealed severe micrognathia associated with short palpebral fissures, hypertelorism, a short and large neck, bilateral club foot, flexion of elbows and knees, and camptodactyly of the fingers (Figure S1). For the second fetus, ultrasound examination revealed bilateral club foot associated with polyhydramnios and bilateral camptodactyly at 23 w.g. Fetal death was observed at 24 w.g. Pathological examination revealed an identical phenotype in this male fetus. Morphological examination of the brain, spinal cord, and the neuromuscular system did not reveal any specific defect. The third fetus, a male, was also affected with an identical phenotype and died at 27 w.g. Genetic mapping of disease loci was performed in the family with Affymetrix GeneChip Human Mapping 250K microarrays. Multipoint linkage analysis of SNP data was performed with the Alohomora[3] and Merlin softwares.[4] Whole-exome sequencing (WES) of the first fetus was performed with the Exome Capture

[1]UMR-1169, INSERM and University of Paris South, Le Kremlin Bicêtre, 94276, France; [2]Medical Genetic Unit, South University Hospital, St Pierre-La Réunion, 97448, France; [3]Reference Center of Developmental Anomalies and Malformations, University Hospital of Nice, Nice, 06000, France; [4]Centro Nacional de Análisis Genómico, Barcelona, 080028, Spain; [5]Service de Biochimie Métabolique and UMR 1127-7225, Hôpitaux Universitaires Pitié-Salpêtrière, Paris, 75651, France; [6]Pathology Laboratory and NeoVasc Region-INSERM Team ERI28, Institute of Research for Innovation in Biomedicine, University of Rouen, Rouen, 76031, France
*Correspondence: judith.melki@inserm.fr
http://dx.doi.org/10.1016/j.ajhg.2015.08.010.

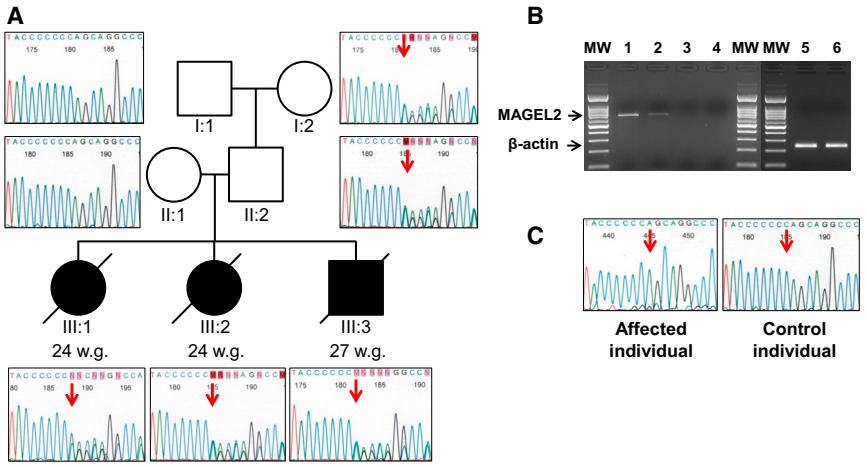

Figure 1. Truncating Mutation of Paternal *MAGEL2* Allele in Family 1 and RNA Studies

(a) Sequencing of DNA showing the *MAGEL2* c.1996delC mutation in family 1 (I:2; II:2; III-1 to III-3). Arrows indicate mutant nucleotide position.

(b) PCR analysis of *MAGEL2* (1–4, 660 bp) and β-actin (5 and 6, 250 bp) in affected individuals (2, 4, and 6) and control individuals (1, 3, and 5) after reverse transcription (RT; 1 and 2) or without RT of RNA (3 and 4). Note the marked reduction of *MAGEL2* in the affected individual (2) with respect to the control individual (1) and β-actin (5 and 6). MW, molecular weight.

(c) Sequencing of the *MAGEL2* RT-PCR products showing the paternally mutated allele only in the affected individual.

Agilent SureSelect XT V5 Kit for library preparation and exome enrichment as previously described.[5] Sequencing was performed on a Genome Analyzer IIx Illumina instrument with paired-end 75 bp reads. The median coverage of the WES was 72. Reads were aligned to the human reference genome sequence (NCBI Genome browser build 37.3) via the Burrows-Wheeler Aligner program.[6] Variants were selected with the SAMtools[7] then annotated with ANNOVAR.[8] Variants in coding regions (including nonsynonymous and nonsense mutations), intron-exon junctions, or short coding insertions or deletions were selected when the minor allele frequency was less than 0.003. Variants mapping to the candidate regions, as determined by linkage analysis, were selected. Under the hypothesis of autosomal-recessive inheritance, no candidate variants were identified. Under the hypothesis of paternally autosomal-dominant inheritance, a heterozygous 1 bp deletion in *MAGEL2* (c.1996delC [GenBank:NM_019066.4]), leading to frameshift and a premature stop codon (p.Gln666Serfs*36; Figure 1), was identified within the 15q11.2 region, one of the linked disease loci (Figure 1). Sanger sequencing of PCR products via primers flanking the mutation (Table S1) confirmed the mutation in the three affected fetuses (III:1 to III:3; Figure 1) and showed that it was inherited from the unaffected father (II:2; Figure 1). Analysis of the father's parents indicated that the mutation was inherited from the unaffected grandmother (I:2; Figure 1). Via both WES and microarray data, a total of 240 SNPs were identified in this family at the Prader-Willi locus at an average distance of 6.4 Kb. In 140 of them, heterozygosity was observed in the affected fetuses. At the SNORD116 snoRNA cluster, 79 out of 87 SNPs were found to be heterozygous (data not shown, available on request). These data exclude a large heterozygous deletion or maternal uniparental disomy at this locus. Given that *MAGEL2* is only expressed from the paternal allele, *MAGEL2* RNA analysis was performed from skeletal muscle of an affected fetus. Because *MAGEL2* is a single-exon gene, and to avoid contamination of RNA by DNA, RNA was treated with DNase RNase-Free (Macherey-Nagel). Then, *MAGEL2* PCR analysis was performed from RNA before or after reverse transcription (SuperScript III Reverse Transcriptase, Invitrogen, Table S1). In comparison to β-actin expression in control RNA, a marked reduction of *MAGEL2* expression was observed in affected individuals (Figure 1). Importantly, sequence analysis of RT-PCR product revealed the paternally mutated allele, only indicating the absence of maternally expressed *MAGEL2* allele in the affected individuals (Figure 1 and Table S1).

Sanger sequencing of *MAGEL2* in additional affected individuals or fetuses with arthrogryposis and/or reduced fetal mobility (n = 84) of unknown origin allowed the identification of a distinct truncating mutation of *MAGEL2* in another affected individual (II:1, family 2; Figure 2). In this family, the female affected individual was born to non-consanguineous healthy parents. The abnormal phenotype was discovered at birth and was characterized by microretrognathia, a short neck, bilateral camptodactyly of the third and fourth fingers, and bilateral club foot. The newborn displayed severe hypotonia with respiratory distress from birth and died at postnatal day 2. Pathological examination of the brain, spinal cord, and other organs was normal. Array comparative genomic hybridization was normal, as well as Sanger sequencing of *CHRNG*, *CHRNA1*, *CHRNB1*, *CHRND*, *RAPSN*, *DOK7*, and *CHAT*. The *DMPK* locus was normal. Paternal deletion of 15q11–15q13 and maternal uniparental disomy 15 were excluded. Through sequencing of *MAGEL2*, a heterozygous 1 bp deletion in *MAGEL2* (c.2118delT [GenBank: NM_019066.4]), leading to frameshift and a stop codon (p.Leu708Trpfs*7; Figure 2 and Table S1), was identified in this affected individual (II:1). Analysis of both parents showed that the mutation had occurred de novo. To determine whether the de novo *MAGEL2* mutation occurred on the paternal or maternal allele, *MAGEL2* RNA analysis was performed from affected-individual fibroblasts. Sequence analysis of RT-PCR was performed via primers flanking the frameshift deletion as well as a SNP (rs2233070) located in *MAGEL2* and inherited from the mother (I:2; Figure 2

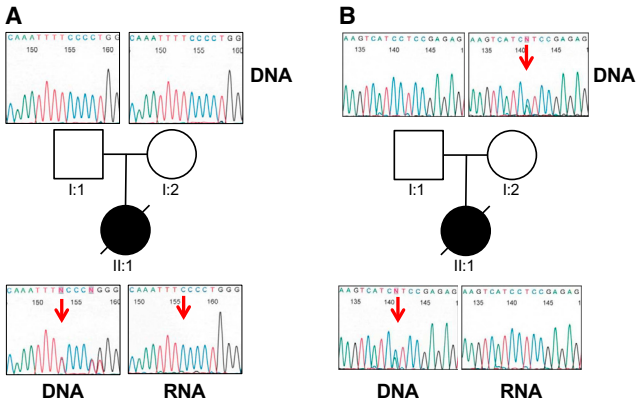

Figure 2. De Novo Truncating Mutation Occurring on the Paternal *MAGEL2* Allele in Family 2 and RNA Studies
(a) Sequencing of DNA showing the de novo *MAGEL2* c.2118delT mutation in affected individual II:1. Arrows indicate mutant nucleotide position. Sequencing of *MAGEL2* RT-PCR products (RNA) from affected individual II:1 revealed the mutated allele only.
(b) Sequencing of DNA showing the rs2233070 polymorphism inherited from the mother (I:2 and II:1, arrow). Sequencing of *MAGEL2* RT-PCR products (RNA) from affected individual II:1 revealed the paternal allele only.

and Table S1). Importantly, sequence analysis of RT-PCR product revealed the frameshift deletion allele only and the lack of the maternal SNP allele, indicating that the deleterious de novo *MAGEL2* mutation occurred on the paternal allele (II:1; Figure 2). Marked reduction of *MAGEL2* expression was observed in the affected individual (data not shown). These two truncating mutations were found neither in the current Exome Variant Server database (ESP6500SI-V2) nor in the Single Nucleotide Polymorphism Database (dbSNP v.144).

MAGEL2 is one of the paternally expressed genes located within the Prader-Willi syndrome (PWS) locus. PWS is associated with, to varying extents, reduced fetal mobility, distal joint contractures, neonatal hypotonia with respiratory distress, early-childhood-onset obesity, intellectual disability, hypogonadotropic hypogonadism, and short stature. PWS can result from paternal deletion of 15q11–15q13 (in 65%–75% of affected individuals), maternal uniparental disomy 15 (in 20%–30% of affected individuals), or, more rarely, an imprinting defect or microdeletion including the SNORD116 cluster (reviewed in Cassidy et al.[9]). The Prader-Willi locus contains five paternally expressed genes (*MKRN3*, *MAGEL2*, *NDN*, *NPAP1*, and *SNURF-SNRPN*) and a family of six paternally expressed snoRNA genes or clusters.[9] PWS has been suggested as being a contiguous gene syndrome.

PWS has been reported in fetuses with polyhydramnios associated with reduced fetal mobility and distal arthrogryposis.[10–13] In these cases, paternal 15q11–15q13 deletion or maternal uniparental disomy of 15q11.2–15q12 were identified, and it was suggested that PWS should be considered in fetuses with polyhydramnios and/or abnormal fetal position, especially if diminished fetal movements are reported. Here, we report four affected individuals from two unrelated families who have a phenotype very similar to those previously reported[10–13] and who carry heterozygous truncating mutations on the paternal allele of *MAGEL2*. These mutations are either inherited from or occurred de novo on the paternal allele, indicating that truncating mutations in *MAGEL2* are responsible for this condition. Appropriate genetic information from other members of the family might need to be provided, especially when a paternally mutated allele identified in affected individuals is transmitted silently from the grandmother.

Recently, truncating mutations of *MAGEL2* have been identified on the paternal allele of four affected individuals with features resembling PWS and called Schaaf-Yang syndrome (MIM: 615547).[14,15] However, another affected individual carrying a large paternally inherited deletion of 3.9 Mb that includes *MAGEL2*, but not the SNRPN-SNORD116 locus, was reported as not showing intellectual disability at 3 years of age, but did show transient neonatal hypotonia associated with a slight delay of fine motor skills.[16]

These data revealed that intragenic mutations or deletion of the paternal allele of *MAGEL2* result in a large clinical spectrum, ranging from a severe fetal phenotype characterized by polyhydramnios, reduced fetal mobility and distal joint contractures (our report), and syndromic intellectual disability or autism (Schaaf-Yang syndrome)[14,15] to an abnormal position of distal joints with a slight delay of fine motor skills without intellectual disability.[16] Of note, three out of four affected individuals reported by Schaaf et al.[14] displayed neonatal hypotonia and feeding problems with the need for special feeding techniques, and in two out of four affected individuals, contractures of the proximal and distal interphalangeal joints were reported. These data strongly support the view that *MAGEL2* intragenic mutations recapitulate the PWS phenotype.

Recently, Matarazzo and Muscatelli (2013)[17] detected *Magel2* transcripts in the brains of mice deleted for the paternal allele of *Magel2*, indicating an incomplete silencing of the maternal allele. Interestingly, paternally *Magel2*-null mice have reduced embryonic viability (in 10% to 30% of embryos) but otherwise normal embryonic growth in survivors, followed by post-natal growth retardation and excessive weight gain, recapitulating aspects of the PWS phenotype.[18,19] Similarly, Guo et al.[20] have reported an imprinting variance of *Magel2* in pigs during fetal development. These data suggest that the imprinting pattern of *MAGEL2,* as well as other genes at the PWS locus, could be regulated during development by *trans* effects on the maternal allele. We hypothesize that the clinical expression of PWS might depend on the temporal or spatial variation in expression of the maternal *MAGEL2* allele and that a complete lack of maternal *MAGEL2* allele expression is responsible for early-onset and very severe

disease phenotypes. However, we cannot exclude a role of other genes linked or not to the PWS locus in the clinical expression of PWS.

During mouse embryogenesis, *Magel2* expression is restricted to the neural tube, forebrain, midbrain, and hypothalamus, as well as to the dorsal root ganglia and peripheral neurons innervating limb and trunk muscles.[19] In non-neuronal tissues, *Magel2* expression is confined to the genital tubercle, midgut region, and placenta. In affected individuals with PWS, neuromuscular studies including muscle biopsy are generally normal or show non-specific signs suggesting that the motor deficit is of central origin. The expression pattern of *MAGEL2* in the CNS during development and the fetal onset of motor defects associated with truncating mutations of *MAGEL2* reported here strongly suggest that *MAGEL2* has an essential role in neuronal development. The expression pattern of maternal *MAGEL2* warrants further investigation in the nervous system during development.

Altogether, these data strongly suggest that *MAGEL2* is a PWS-determining gene. In the absence of paternal deletion of 15q11–15q13 or maternal uniparental disomy 15, a search for intragenic mutations on the paternal allele of *MAGEL2* should be proposed for fetuses with reduced movements, polyhydramnios, and distal arthrogryposis, newborns with severe undiagnosed central hypotonia, or children for whom PWS is clinically suspected.

Supplemental Data

Supplemental Data include one figure and one table and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2015.08.010.

Acknowledgments

This work was supported by grants from the French Ministry of Health (PHRC 2010, AOM10181), the Association Française contre les Myopathies (AFM, DAJ1891), and INSERM (to J.M.), and was sponsored by the Assistance Publique- Hôpitaux de Paris (AP-HP). The authors would like to thank the Clinical Research Unit of Bicêtre Hospital, the Direction of Clinical Research and Development (AP-HP), and the NHLBI GO Exome Sequencing Project and its ongoing studies that produced and provided exome variant calls for comparison.

Received: July 20, 2015
Accepted: August 24, 2015
Published: September 10, 2015

Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, http://www.1000genomes.org/
dbSNP, http://www.ncbi.nlm.nih.gov/snp/
NCBI Gene, http://www.ncbi.nlm.nih.gov/gene
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
OMIM, http://www/omim.org/

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