

Truncating mutations of *MAGEL2* cause Prader-Willi phenotypes and autism

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Prader-Willi syndrome (PWS) is caused by the absence of paternally expressed, maternally silenced genes at 15q11-q13. We report four individuals with truncating mutations on the paternal allele of *MAGEL2*, a gene within the PWS domain. The first subject was ascertained by whole-genome sequencing analysis for PWS features. Three additional subjects were identified by reviewing the results of exome sequencing of 1,248 cases in a clinical laboratory. All four subjects had autism spectrum disorder (ASD), intellectual disability and a varying degree of clinical and behavioral features of PWS. These findings suggest that *MAGEL2* is a new gene causing complex ASD and that *MAGEL2* loss of function can contribute to several aspects of the PWS phenotype.

PWS (MIM 176270) is characterized by infantile hypotonia with poor suck and failure to thrive, followed by overeating and rapid weight gain during childhood, developmental delay, intellectual disability, hypogonadism, short stature and a characteristic behavioral profile. Comprehensive diagnostic criteria have been established by Holm *et al.*¹. PWS can result from paternal deletion of 15q11-q13 (65–75% of cases), maternal uniparental disomy 15 (20–30%), an imprinting defect (1–3%)² or possibly from rare deletions of the *SNORD116*@ cluster as discussed below. Point mutations causing PWS have not been reported so far.

The Prader-Willi domain contains five paternally expressed protein-coding genes with six ORFs (*MKRN3*, *MAGEL2*, *NDN*, *NPAP1* and *SNURF-SNRPN*) and a family of six paternally expressed small nucleolar RNA (snoRNA) genes or clusters. The search for candidate genes contributing to specific phenotypic components of PWS has not been entirely conclusive. Three affected individuals with deletions of the *SNORD116*@ snoRNA cluster manifested key characteristics of PWS^{3–5}. Paternal deletion of *MKRN3*, *MAGEL2* and *NDN* alone occurred in one affected individual and was associated with obesity and intellectual disability but not with the typical PWS phenotype⁶, leading the authors to conclude that deficiency of *MKRN3*, *MAGEL2*

and *NDN* did not result in a typical PWS phenotype. Whereas multiple PWS mouse models have been generated, including mice with targeted mutations of *Ndn*, *Magel2*, *Mkfn3*, *Snurf-Snrpn* and *Snord116* (ref. 7), no single model recapitulates the early growth deficiency and hyperphagia leading to subsequent obesity observed in humans. Numerous publications from Wevrick and colleagues argue that *Magel2*-null mice have selected biological findings similar to PWS in humans, including neonatal growth retardation, excessive weight gain after weaning, impaired hypothalamic regulation and reduced fertility^{8–10}. In conjunction, human and mouse data suggest that several genes and snoRNAs in the PWS domain may contribute to the PWS clinical phenotype, but whether PWS truly represents a ‘contiguous gene syndrome’ remains elusive.

Here we report the first individuals with point mutations in a protein-coding gene within the PWS domain. Subject 1 was enrolled in a whole-genome sequencing research study, and subjects 2–4 were identified through clinical whole-exome sequencing. In both instances, samples were submitted without prescreening criteria. Participation was based on the referring provider’s determination that the affected individual likely had a genetic cause of disease.

Subject 1 was ascertained at age 13 years with a history of a PWS-like phenotype, ASD and mild intellectual disability. Using highly accurate whole-genome sequencing with 60× coverage (developed by Drmanac *et al.*)¹¹, he was found to carry a heterozygous *de novo* c.1652delT (p.Val551fs) mutation in *MAGEL2* (NM_019066.4), one of the protein-coding genes in the PWS domain, encoding a ubiquitin ligase enhancer, which is required for endosomal protein recycling¹². Given that *MAGEL2* is only expressed from the paternal allele, we investigated whether the mutation was present on the maternal or paternal copy of chromosome 15. We performed long-fragment analysis (in part as previously reported by Peters *et al.*)¹³, which, in conjunction with parental SNP genotyping in proximity to the mutated locus, determined that the *MAGEL2* mutation was on the paternal allele (Online Methods).

On the basis of the findings in subject 1, the Baylor College of Medicine Whole-Genome Laboratory clinical whole-exome

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Table 1 Molecular and clinical phenotypes of four individuals with truncating *MAGEL2* mutations

	Subject 1	Subject 2	Subject 3	Subject 4	Summary
Sex	M	M	M	M	4 M
Age at time of diagnosis	12 years	8 years	5 years	19 years	Average of 11 years
Mutation	c.1652delT p.Val551fs	c.1802delC p.Pro601fs	c.3181_3182del p.Ile1061fs	c.3124C>T p.Gln1024*	1 nonsense, 3 frameshift indels
Inheritance	<i>De novo</i>	Not maternal	<i>De novo</i>	<i>De novo</i>	3 <i>de novo</i> , 1 not maternal
Affected allele	Paternal	Paternal	Paternal	Paternal	4 paternal
PWS major criteria^a					
Neonatal hypotonia, poor suck	+	+	-	+	3/4
Feeding problems in infancy, with need for special feeding technique	-	+	+	+	3/4
Excessive weight gain before age of 6 years	+	+	+	-	3/4
Hyperphagia, lack of satiety	-	+	-	+	2/4
Developmental delay, Intellectual disability	+	+	+	+	4/4
PWS characteristic facial features	-	+	-	-	1/4
Hypogonadism	+	+	+	-	3/4
PWS minor criteria^a					
Infantile lethargy, weak cry	+	-	+	+	3/4
Short stature	-	-	+	+	2/4
Small hands	-	-	-	+	1/4
Narrow hands	-	-	-	+	1/4
Eye abnormalities	-	+	+	+	3/4
Hypopigmentation	-	-	-	-	0/4
Thick saliva	-	-	-	-	0/4
Characteristic behavior (temper tantrums, violent outbursts, oppositional behavior, etc.)	-	-	+	+	2/4
Speech articulation defects	-	+	+	+	3/4
Skin picking	-	-	+	+	2/4
Sleep apnea	-	+	-	+	2/4
Other symptoms (non-PWS criteria)					
Autism spectrum disorder	+	+	+	+	4/4
Contractures of the proximal and distal interphalangeal joints	-	-	+	+	2/4

M, male; +, present; -, not present. Asterisk denotes nonsense substitution.

^aAccording to criteria in Holm *et al.*¹.

sequencing database was searched for probable pathogenic mutations in *MAGEL2*. Three additional subjects with nonsense or frameshift indel mutations were ascertained. A total of 1,248 whole-exome sequencing cases were reviewed.

Subject 2 was an 8-year-old boy with classic PWS, meeting the 1993 diagnostic criteria. He carried a heterozygous c.1802delC (p.Pro601fs) mutation, which was not maternally inherited (father unavailable). Subject 3 was a 5-year-old boy with a *de novo* heterozygous c.3181_3182delAT (p.Ile1061fs) mutation. Although he had a history of feeding difficulties as an infant, requiring tube feeding, and had excessive weight gain during childhood, cryptorchidism, short stature, some of

the typical behavioral phenotypes of PWS and intellectual disability, he did not meet full clinical criteria for the condition. The scenario was similar for subject 4, a 19-year-old boy, who met four of the major diagnostic criteria (five necessary): neonatal hypotonia and feeding difficulties requiring tube feeding, followed by hyperphagia and absence of satiety, and intellectual disability. He had a *de novo* c.3124C>T (p.Gln1024*) mutation. Clinical phenotypes are summarized in **Table 1**. Full clinical descriptions are provided in the **Supplementary Note**, and photographs of the subjects are shown in **Supplementary Figure 1**. Of note, all four probands had normal methylation testing for PWS and normal chromosome microarray analysis.

We then developed a test allowing the determination of the parental origin of

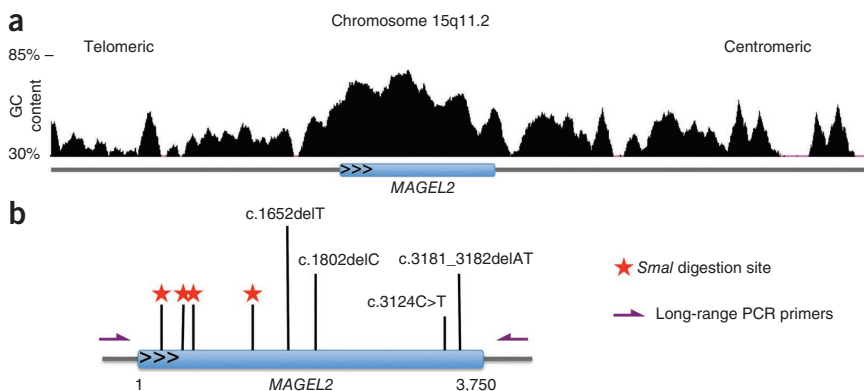


Figure 1 Truncating mutations on the paternal allele of *MAGEL2*. (a) GC content of *MAGEL2* and flanking sequence at 15q11.2 (according to the UCSC Genome Browser, hg19).

(b) Truncating *MAGEL2* mutations reported in this manuscript are indicated relative to their positions in the coding sequence of this single-exon gene. For phasing of *MAGEL2* mutations, genomic DNA was digested with the methylation-sensitive restriction endonuclease *Sma*I, which leaves only the methylated maternal *MAGEL2* allele intact. Digestion is followed by long-range PCR.

de novo *MAGEL2* mutations independent of SNP genotypes. *MAGEL2* is a relatively GC-rich single-exon gene (Fig. 1a) with CpGs methylated on the maternal chromosome 15, and it is exclusively expressed from the unmethylated paternal allele. The *MAGEL2* coding sequence contains four restriction sites (5'-CCCGGG-3') for the methylation-sensitive restriction endonuclease *Sma*I (Fig. 1b). The unmethylated allele is digested by *Sma*I, whereas the methylated allele remains intact. PCR amplification after *Sma*I digestion using oligonucleotide primers flanking one or more of the digestion sites followed by Sanger sequencing detects only the maternal allele (Online Methods). The detection of known *MAGEL2* mutations following *Sma*I digestion suggests that they are located on the maternal (inactive) allele. In contrast, a mutation undetectable by Sanger sequencing following *Sma*I digestion is located on the paternal allele and is potentially pathogenic.

Using this approach, we showed that the *MAGEL2* mutations in all four subjects were on the paternal allele (Table 2). Given the functional hemizygoty for this gene, a truncating mutation on the paternal allele leaves affected individuals without functional expressed *MAGEL2*, rendering the mutation potentially pathogenic.

In summary, this is the first report, to our knowledge, of point mutations in the imprinted *MAGEL2* gene in the 15q11-q13 domain causing classic PWS (subject 2) and PWS-like phenotypes (subjects 1, 3 and 4). This finding is particularly interesting, given previous reports^{3–5} of three individuals with small deletions of the *SNORD116* snoRNA cluster, two of which met full diagnostic criteria for PWS. There could be genetic heterogeneity underlying PWS or the phenotypic overlap between cases with *SNORD116* deletion and those with *MAGEL2* mutation might be caused by changes in higher order chromatin structure at the 15q11-q13 locus¹⁴. Many speculations are possible, but the answer is unknown at present. At this time, there does not seem to be a genotype-phenotype correlation (Supplementary Table 1).

All four subjects reported here had a diagnosis of ASD on the basis of DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) criteria and clinical evaluation by an expert. This shared feature suggests that *MAGEL2* is an additional gene in the ever-growing list of autism susceptibility genes. In a previous study investigating the comorbidity of PWS with ASD, 19% of individuals with PWS met diagnostic criteria for ASD¹⁵. Even compared to PWS generally, ASD is over-represented in our cohort of individuals with truncating *MAGEL2* mutations (four of four subjects affected), but additional individuals need to be ascertained before drawing conclusions. Nonsense or frameshifting mutations in *MAGEL2* have not been reported in exome sequencing studies of individuals with autism^{16–23}. The GC richness of the gene may impair exon capture, as well as subsequent sequencing.

The phenotypes associated with *MAGEL2* loss-of-function mutations reported herein appear consistent with data from *Magel2* knockout mouse models, which predominantly manifest poor suckling, neonatal growth retardation, excessive weight gain after weaning, impaired hypothalamic regulation and delayed onset of puberty as well as reduced fertility²⁴. Learning and memory were found to be normal in the *Magel2*-null mice, which led to the interpretation that other genes had to be responsible for these features. To our knowledge, there are no reports of *Magel2*-null mice being tested for autism-like behaviors. The latter should probably be considered, given the high prevalence of ASD among humans with *MAGEL2* loss of function, at least in this first report.

On the basis of our data, we recommend considering *MAGEL2* sequencing or exome sequencing in complex autism, especially in individuals with a history of neonatal hypotonia, feeding difficulties or hypogonadism.

Table 2 Phasing of *MAGEL2* mutations

	Sequence at mutation site without digestion	Sequence at mutation site after <i>Sma</i> I digestion	Conclusion
Subject 1	T/del	T	Mutation on paternal allele
Subject 2	C/del	C	Mutation on paternal allele
Subject 3	AT/del	AT	Mutation on paternal allele
Subject 4	C/T	C	Mutation on paternal allele

The methylation-sensitive restriction endonuclease *Sma*I cuts at four sites within the paternal (unmethylated) allele of *MAGEL2*. The inability to detect a respective mutation in *MAGEL2* by Sanger sequencing following *Sma*I restriction and long-range PCR suggests the presence of the mutation on the paternal allele.

The identification of neurological disorders caused by loss-of-function mutations in imprinted genes is particularly important, as novel therapeutic approaches might be envisioned. In another neurodevelopmental disorder, Angelman syndrome, which is caused by deletion or mutation of the paternally imprinted gene *UBE3A*, topoisomerase inhibitors could be used to unsilence the dormant *Ube3a* allele in mouse neurons²⁵. Also, antisense RNA could potentially activate the inactive, methylated allele of imprinted genes²⁶. We hope that our report will generate new research efforts to investigate the function and clinical importance of this gene with the aim of ultimately benefiting individuals with its associated disorders.

URLs. Leiden Open Variation Database (LOVD), <http://www.lovd.nl/>; *MAGEL2*-specific variations listed in LOVD, <http://databases.lovd.nl/shared/genes/MAGEL2>; Simons Foundation for Autism Research Modular Database for Autism Research (SFARI GENE), <https://gene.sfari.org/>; Complete Genomics Data File Formats, <http://www.completegenomics.com/customer-support/documentation/100357139.html>; 1000 Genomes Project, <http://www.1000genomes.org/>; dbSNP, <http://www.ncbi.nlm.nih.gov/projects/SNP/>; Exome Variant Server, <http://evs.gs.washington.edu/EVS/>.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

M.L.G.-G. and M.A.M. performed whole-genome sequencing and phase determination on subject 1. M.L.G.-G., M.A.M., B.A.P., R.D. and C.T.C. designed and analyzed the experiments for subject 1. F.X. and Y.Y. performed whole-exome sequencing and phase determination on subjects 2–4. C.P.S., F.X., Y.Y., B.Z., A.L.B. and Y.Y. designed and analyzed the experiments for subjects 2–4. L.P. and K.W.G. contributed subjects and provided detailed physical examinations. C.P.S. conceived the overall study, coordinated enrollment, supervised the experiments, wrote the manuscript and generated the figures and tables. All authors participated in the discussion and interpretation of data and results, and all participated in editing and revising the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the [online version of the paper](#).

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ONLINE METHODS

Human subjects. Subject 1 and his parents were enrolled in a whole-genome sequencing study, approved by the Institutional Review Board of Baylor College of Medicine. Enrollment in this study is not based on a particular phenotype but rather on the referring physician's determination that the enrolled subject likely has a genetic change that has led to genetic disease. Subjects 2–4 were referred to the Medical Genetics Laboratories at the Baylor College of Medicine for clinical whole-exome sequencing. Whole-exome sequencing has been offered as a clinical test at the Baylor College of Medicine Whole-Genome Laboratory since October 2011. These are consecutive, unrelated samples without prescreening criteria. The clinical whole-exome sequencing test is not a designed study. Among the cases referred for clinical whole-exome sequencing, 91.2% were pediatric, 8.1% were adult and 0.7% were prenatal. Of the referred subjects, 78% had a history of developmental delay and/or intellectual disability, and 12.2% had a history of ASD.

After the identification of truncating *MAGEL2* mutations, subjects 2–4 and their respective parents were enrolled in a research study investigating variants of unknown significance, approved by the Institutional Review Board of Baylor College of Medicine. Informed consent for all study participants was obtained. For individuals 1–3, for whom clinical photographs are shown in the **Supplementary Note**, consent was obtained specifically stating the agreement to publish these photographs in medical publications, even if the individual displayed in the picture can be recognized.

Whole-genome sequencing analysis. Family 1 comprises two healthy parents and an affected son. Their DNA was sent to Complete Genomics, Inc., for whole-genome sequence analysis. Genomics data were analyzed under a *de novo* model where we identified two high-quality private missense mutations in the affected individual, which were homozygous reference genotypes in both parents (data obtained from the masterVar file from each genomic data set; see URLs); both mutations were absent in the 1000 Genomes Project, dbSNP and the ESP6500 project. The first *de novo* mutation in gene *MYO1H* alters amino acid 83 in the protein (NP_001094891.3) from an alanine to a valine; however, there are no known human disorders associated with amino acid changes encoded in *MYO1H* (according to Online Mendelian Inheritance in Man, OMIM). The second *de novo* mutation, in gene *MAGEL2*, generates a single-base frameshift in the protein (NP_061939.3; 1,249 amino acids) starting at amino acid 551 and continuing until the new frame reaches a termination codon at amino acid 701. The *MAGEL2* mutation was validated by Sanger sequencing using PCR with primers Val1_Fw and Val1_Re (**Supplementary Table 2**). Given the fact that *MAGEL2* is located in a maternally imprinted region, 15q11.2, we proceeded to determine the phase of the mutation.

Phasing the *de novo* mutation in *MAGEL2*. Purified DNA from subject 1 was aliquotted at ~0.1 genome equivalents per well across a 384-well plate and subjected to the multiple-displacement amplification (MDA) method of whole-genome amplification as previously described¹³. At this concentration, there is a 4% probability that any two linked loci from separate DNA molecules

will be aliquotted to the same well. Furthermore, there is a 2% probability that two linked loci of different parental origin will be aliquotted to the same well. The MDA reaction was incubated at 37 °C for 19 h.

After heat inactivation of the MDA reaction, each well was diluted 13.4-fold with water. An aliquot of each well was diluted a further fivefold with water. An aliquot of this 67-fold-diluted DNA (1 µl) was used as template for quantitative PCR to identify wells containing SNP regions linked to the *de novo* deletion—one paternally inherited SNP located 12,785 bp upstream of the deletion and three maternally inherited SNPs located 506 bp upstream, 422 bp upstream and 8,032 bp downstream of the deletion. Primers used were pSNP-12785qPCRseqFw, pSNP-12785qPCRseqRe, mSNP-506-422qPCRseqFw, mSNP-506-422qPCRseqRe, mSNP+8032qPCRseqFw and mSNP+8032qPCRseqRe (**Supplementary Table 2**). Fast SYBR Green Master Mix (Applied Biosystems) was used for quantitative PCR. Quantitative PCR products of flanking SNPs were used as template for PCR with PfuTurbo Cx polymerase (Agilent Technologies) because the presence of UTP and uracil-*N*-glycosylase in the SYBR Green Master Mix makes the quantitative PCR products unstable.

Wells in which SNP regions were amplified by quantitative PCR were assumed to contain DNA fragments spanning the amplified SNP and the *de novo* deletion. A subset of these wells were chosen for PCR with primers magel11f and magel11r (**Supplementary Table 2**) using KAPA HiFi HotStart ReadyMix (Kapa Biosystems) according to the manufacturer's instructions and the cycling conditions described above to amplify the *de novo* deletion region. MDA product (~40 ng) was used as template for KAPA PCR.

KAPA HiFi and Pfu PCR products were gel purified (GeneJET Gel Extraction kit, Fermentas) and submitted to Elim Biopharmaceuticals for Sanger sequencing. Further details on phasing of the *de novo* mutation are provided in **Supplementary Figure 2**.

Copy number variation and methylation. We verified the absence of any copy number variation in the PWS region (15q11.2-q13) through analysis of the copy number variant calls generated by Complete Genomics in the copy number variation report files (see URLs) for subject 1 and by clinical chromosome microarray analysis for all four subjects²⁷.

Methylations-sensitive digestion of *MAGEL2* followed by Sanger sequencing. DNA from subjects was digested with restriction endonuclease SmaI (New England Biosystems) and amplified by long-range PCR with DNA primers LR_magel2_for and LR_magel2_rev (**Supplementary Table 2**). Specific mutation loci were amplified by nested PCR and further analyzed by capillary electrophoresis sequencing. The following pairs of DNA primers were used for nested PCR: Nested_PCR1_for, Nested_PCR1_rev; Nested_PCR2_for, Nested_PCR2_rev (**Supplementary Table 2**).

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