Supplemental information

Supplemental methods

Genetic analysis. Whole exome sequencing (WES) procedures were performed on HiSeq4000 deep sequencer (Illumina, CA, USA) with a 150 bp paired-end protocol after enrichment in the coding regions by the SureSelect Human All Exon V5 kit (Agilent, CA, USA). The average coverage of WES for DNA samples of all three affected individuals reached more than 600X. The generated raw sequences were processed by the MERAP package for alignment, quality control, calling single nucleotide variant (SNV), insertion and deletion (Indel), structural variation (SV), and copy number variation (CNV), the variant annotation and prioritization. The variant prioritization and evaluation of etiologic involvement were performed by the two pipelines MERAP and ANNOVAR.(1, 2) In brief, MERAP pipeline first filters all identified variants through comparison with the disease-associated variants in the Human Gene Mutation Database (HGMD, 2020.2) and the Online Mendelian Inheritance in Man (OMIM) to collect those known disease-causing variants.(3) In principle, candidate variants causing recessive traits should not occur in healthy controls as homozygotes, and the frequency of respective heterozygotes should not exceed 0.1%. Changes destroying conventional splice sites or introducing novel splice sites are identified by MERAP's module called SSFinder. To assess the pathogenicity of missense mutations, MERAP generates a single score integrating the results of seven different algorithms, including the Grantham score, PhyloP, GERP, SIFT, PolyPhen2, Mutation-Taster, and the Conserved Domains Database (CDD, http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). With empirical false discovery rate cutoffs, this score serves as dichotomized pathogenicity predictions even if any two of the seven algorithms might not coincide. MERAP rules out candidate genes reported to harbor homozygous loss-of-function (LOF) variants in healthy individuals. For novel candidate genes without prior link to disease, MERAP offers information on their interaction with known disease genes, based on data from Biogrid (http://thebiogrid.org/) and IntAct

(http://www.ebi.ac.uk/intact/), assuming that genes implicated in clinically similar disorders tend to cluster in gene or protein interaction networks. Variants were considered de novo if neither parent had the variant, and candidate variants were selected by segregation analysis. The pathogenic and likely pathogenic variants were defined according to the standards and guidelines of ACMG.(3) The ANNOVAR pipeline was also used, with additional considerations from the Residual Variation Intolerance Score (RVIS) and the Combined Annotation-Dependent Depletion (CADD) score. The former ranks genes in terms of intolerance to functional genetic variation and the latter integrates several well-known tools. We empirically set a cutoff RVIS score of 50th percentile for known and novel genes and a cutoff CADD score of 20 for novel candidate genes. When defining likely causal variants, we followed the guidelines designated by ACMG. WES results were confirmed by Sanger sequencing. Primers for the amplification of the exons carrying variants were designed by using the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast), which uses Primer3 and BLAST.

Table S1. MCM-related disorders.

MCM COMPONENT	OMIM #	DISEASE	PHENOTYPE	REFERENCE
MCM2	116945	Non-syndromic sensorineural hearing loss	Deafness	Gao et al., 2015
MCM4	602638	Natural killer cell and glucocorticoid deficiency with DNA repair defect	Immunodeficiency	Gineau et al., 2012
MCM5	602696	Meier–Gorlin Syndrome	Meier–Gorlin Syndrome	Vetro et al., 2017
MCM6	601806	Lactase persistence/ non-persistence	Lactase persistence/ non-persistence	Enattah et al., 2002 Bersaglieri et al, 2004
MCM8	608187	Premature ovarian failure	Premature ovarian failure	AlAsiri et al., 2015
MCM9	610098	Ovarian dysgenesis	Ovarian dysgenesis	Fauchereau et al., 2016
ORC1	601902	Meier-Gorlin Syndrome	Meier–Gorlin Syndrome	Bicknell et al., 2011

Table S2. List of primers.

Primer	Sequence (5'-3')	Species	Application
hMCM7-F	ggggTTTTgCTgCTCTTTTCT	human	Sanger Sequencing for Genetic Analyses
hMCM7-R	TgCCTAgggAgggAAggTTg	human	Sanger Sequencing for Genetic Analyses
mMCM7-qPCR-F	ACAAggAgAAggAggTggTg	mouse	qPCR
mMCM7-qPCR-R	TACTggTTTTgggggTTCCg	mouse	qPCR
mMCM7-ISH-F	ATgAAgATTCAAgAgCATAgTgACC	mouse	ISH
mMCM7-ISH-R	CCTCTAAAgTCAgTTCTCCACTCAC	mouse	ISH
hMCM7-mut-F	TCCAggCTgggTAATCCTTgTgTTCTCTCCTTC	human	Site directed mutagenesis
hMCM7-mut-R	gAAggAgAgAACACAAggATTACCCAgCCTggA	human	Site directed mutagenesis
hMCM7-F1	ATggCACTgAAggACTACgC	human	Sanger Sequencing for site directed mutagenesis
hMCM7-F2	AgCATCggCTAATgATggAg	human	Sanger Sequencing for site directed mutagenesis
hMCM7-F3	AgCTgAggCAAATTgCAgAg	human	Sanger Sequencing for site directed mutagenesis
hMCM7-F4	ACCgAgACAATgACCTACgg	human	Sanger Sequencing for site directed mutagenesis
hMCM7-F	ggACTTTCCAAAATgTCg	human	Sanger Sequencing for site directed mutagenesis (OriGene)

Table S3. **List of antibodies.**

Antibody	**Source**	**Catalog No.**	**Host**	**Dilution**	**Application**
MCM7	Thermo Scientific	MA5-14291	mouse	1:250	ICC
NeuN	Millipore	ABN78	rabbit	1:200	ICC
Sox2	Abcam	ab59776	rabbit	1:200	ICC
Oct4	Abcam	ab19857	rabbit	1:500	ICC
Mcm7	Invitrogen	PA5-79651	rabbit	1:500	Western blot
Myc-Tag	Cell Signaling Technology	2276	mouse	1:1000	Western blot
Actin	Millipore	MAB1501	mouse	1:1000	Western blot

Table S4. Prediction of variants.

Gene	Variant	Grantham	phyloP	GERP	SIFT	PolyPhen2	Mutation Taster	Logit
MCM7 (NM_005916.4)	g.7:99695841C>T, c.793G>A,p.A265T	58	5,059	4,45	Damaging (0.04)	Possibly damaging (0.63)	Disease causing (0.9987)	3,058

References

1. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164.
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3. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.