

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.
2. Hu H, Wienker TF, Musante L, Kalscheuer VM, Kahrizi K, Najmabadi H, Ropers HH. Integrated sequence analysis pipeline provides one-stop solution for identifying disease-causing mutations. *Hum Mutat.* 2014;35(12):1427-35.
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.