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GENETICS

The NEMP family supports metazoan fertility and nuclear envelope stiffness

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Human genome-wide association studies have linked single-nucleotide polymorphisms (SNPs) in NEMP1 (nuclear envelope membrane protein 1) with early menopause; however, it is unclear whether NEMP1 has any role in fertility. We show that whole-animal loss of NEMP1 homologs in *Drosophila*, *Caenorhabditis elegans*, zebrafish, and mice leads to sterility or early loss of fertility. Loss of Nemp leads to nuclear shaping defects, most prominently in the germ line. Biochemical, biophysical, and genetic studies reveal that NEMP proteins support the mechanical stiffness of the germline nuclear envelope via formation of a NEMP-EMERIN complex. These data indicate that the germline nuclear envelope has specialized mechanical properties and that NEMP proteins play essential and conserved roles in fertility.

INTRODUCTION

Reproductive life span in women is dictated by the finite number of germ cells created during fetal life and loss during aging (1). Premature ovarian insufficiency (i.e., menopause before age of 40) affects ~1 to 2% of women and is a leading cause of infertility in the Western world. Examination of published human genome-wide association studies data revealed that single-nucleotide polymorphisms (SNPs) near the NEMP1 (nuclear envelope membrane protein 1) locus show an association with reduced age at menopause (2, 3). We analyzed an additional cohort of 106,353 women from the UK Biobank study (4) and confirmed an association between NEMP1 and age of menopause, with the most significant association in a region ~300 kb away from NEMP1 (rs2277339, $P = 6.7 \times 10^{-51}$) (fig. S1A). Published Hi-C data from human ovaries indicate that rs2277339 physically interacts with the NEMP1 locus (fig. S1B), with chromatin changes consistent with the possibility that this is a distant enhancer element for NEMP1. In addition, we found an association ($P = 6.7 \times 10^{-3}$)

with a previously unknown variant identified in the Genotype-Tissue Expression (GTEx) study (rs114352356) (5). This SNP encodes a rare nonsynonymous disruptive allele, which lies in the NEMP1 coding region, and is associated with 3.64-year (standard error 1.23) earlier onset of menopause (fig. S1A).

RESULTS

NEMP1 is a poorly understood, multi-transmembrane nuclear envelope (NE) protein with homologs found in all metazoans (Fig. 1A). No genetic models exist for Nemp function in any system. We first examined *Drosophila*, which has a single Nemp gene (CG9723). We found that dNemp is ubiquitously expressed in all examined larval and pupal tissues (fig. S2). Super-resolution microscopy revealed that dNemp localizes to inner nuclear membranes (INMs) (Fig. 1B to 1D), as does *Xenopus* and human NEMP1 (6, 7).

We generated two independent dNemp mutants and found that dNemp^{-/-} males and females are sterile (Fig. 1E to 1G and fig. S3A). Loss of dNemp also resulted in ~40% pupal lethality (Fig. 1H). Bacterial artificial chromosome (BAC) rescue constructs containing the genomic region of dNemp (Fig. 1I) or tubulin-Gal4-driven expression of dNemp complementary DNA (cDNA) (Fig. 1E and 1J) fully restored fertility and viability, confirming that these phenotypes are caused by the loss of dNemp. Human NEMP1 could rescue viability and partially rescue dNemp^{-/-} sterility, suggesting that the role of NEMP in fertility may be conserved (Fig. 1E and 1J, and fig. S3B).

dNemp^{-/-} mutants had extremely small testes and ovaries (Fig. 1F and 1G). Germline dNemp knockdown led to fewer germ cells in testes (Fig. 1I) and ovaries (Fig. 1K). Somatic knockdown also caused defects in gonad structure and infertility (fig. S3, C and D). Thus, dNemp is required by both somatic and germline cells for fertility.

In dNemp^{-/-} testes, the stem cell niche and surrounding self-renewing germline and somatic stem cells appeared normal (figs. S3, E to H, and S4, A and B). However, subsequent development was disrupted, with a markedly reduced number of Vasa⁺ germ cells (fig. S4, C and D), reduced proliferation (fig. S4, E to G), and disorganization

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