A mutation in the nucleoporin-107 gene causes aberrant Dpp/BMP signaling and XX gonadal dysgenesis

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Background: Though the genes and signalling pathways involved in sexual development have only been partially elucidated, it is known that their disruption can result in disorders of sexual development (DSD). XX ovarian dysgenesis (XX-OD) is a rare, genetically heterogeneous disorder characterized by underdeveloped and dysfunctional ovaries. We previously identified a novel missense mutation in Nucleoporin107 (Nup107, c.1339G>A, p.D447N), an essential component of the nuclear pore complex, as the cause of XX-OD in a consanguineous family. We then utilized Drosophila, a powerful genetic tool sharing gene orthologues and fundamental pathways with humans, specifically in gonadogenesis, to model the human mutation.

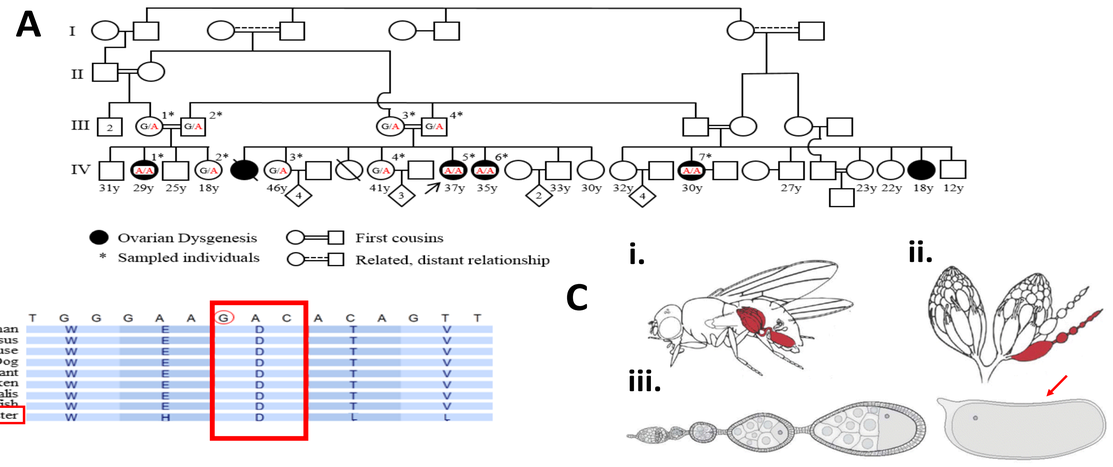

Figure 1. Consanguineous family affected with XX Ovarian Dysgenesis.
(A) Affected individuals are indicated by filled-in circles, with WT (G, black) and variant (A, red) nucleotides indicated. Segregation is consistent with autosomal recessive inheritance. **(B)** The aspartic amino acid (D) is conserved in all species including in *Drosophila.* **(C)** i) Drawing of *Drosophila* with ovary pair labeled in red. ii) Ovaries are made up of 16-20 individual ovarioles, labeled in red. iii) Individual *Drosophila* ovariole contains developing egg chambers at multiple stages, culminating in a mature egg (arrow).

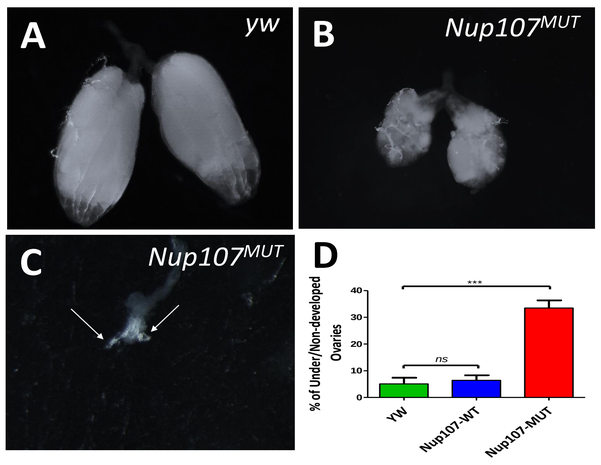

Figure 3. Do Nup107 mutant Drosophila reciprocate the human model of underdeveloped ovaries? Yes!
Imaging of wild-type, **(A)** normal ovaries, and mutant **(B)** under-developed and **(C)** non-developed (arrows) *Drosophila* ovaries. **(D)** Quantification revealed 35% of NUP107^{MUT} flies possessed under or non-developed ovaries.

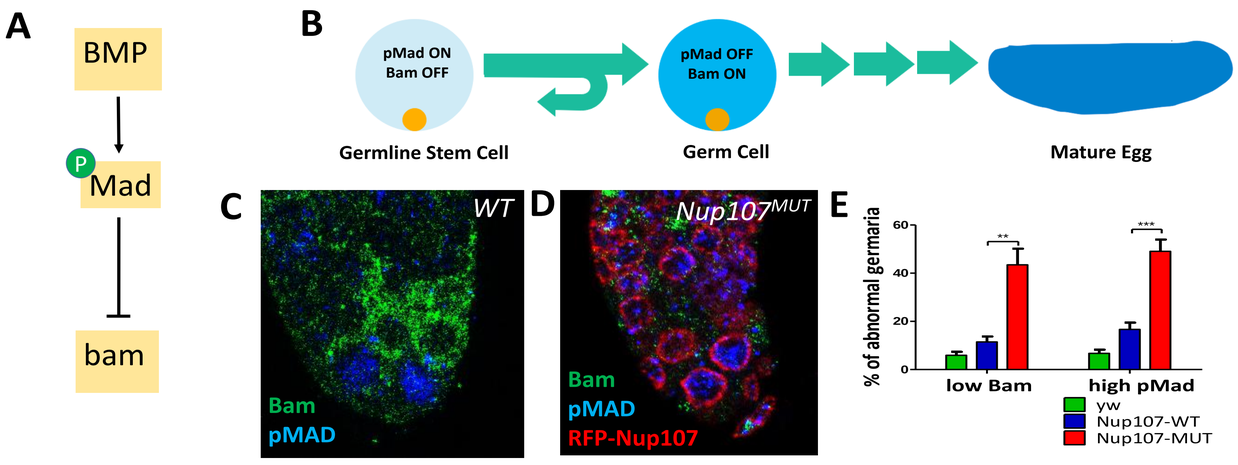

Figure 5. Do the excess PGCs in Nup107^{MUT} gonads differentiate normally? No!
(A) In germline stem cells (GSCs), BMP signals lead to phosphorylation of Mad, which represses expression of differentiation gene bam and allows maintenance of "stem-ness". **(B)** pMad specific expression in GSC, but not later, enables elevation of bam levels in daughter germ cells which eventually differentiate into mature eggs, as seen in **(C)** WT ovarioles. **(D)** Nup107^{MUT} ovarioles fail to downregulate pMad (blue) in daughter cells, consequently resulting in lack of bam (green), as quantified in **(D)**.

Conclusion: Our genetic and cellular analyses strongly suggest that ovarian failure resulting from Nup107 mutation is mediated through the BMP pathway. This concept has implications for clinical evaluation as the human BMP signalling pathway and specifically BMP15 and BMP receptor BmprIB have been implicated in subfertility, and have been shown to be essential for female reproductive function. Testing of this pathway may be indicated in clinical cases of XX-DSD and premature ovarian failure.

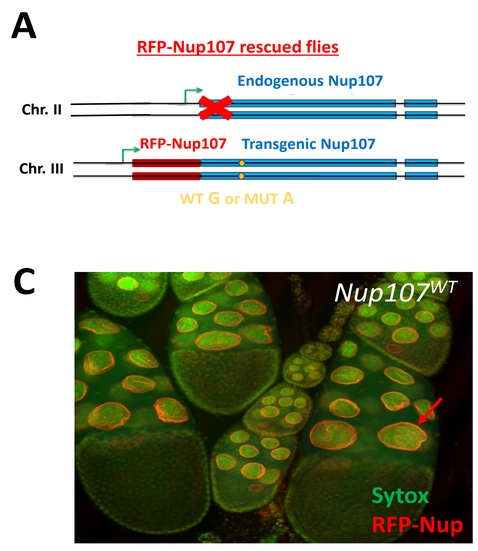

Figure 2. Are Nup107-mutant flies fertile?
(A) We recapitulated the familial mutation in the fly genome by deleting the endogenous gene from chromosome II and inserting either RFP-Nup107^{WT} or RFP-NUP107^{MUT} on chromosome III. **(B)** Nup107^{MUT} flies lay ~50% of the eggs laid by WT rescued transgenic females (blue), and their progeny count is 5 times lower (red). **(C)** Nup107^{WT} ovarioles show normal oocyte formation, nuclear envelope (arrow, red) and nuclear DNA (green), with no evidence of apoptosis. **(D)** Nup107^{MUT} ovarioles show envelope disintegration (red), condensed and punctuated nuclear chromatin (green), and high levels of cleaved caspase-3 (blue), indicating extensive apoptosis. ^

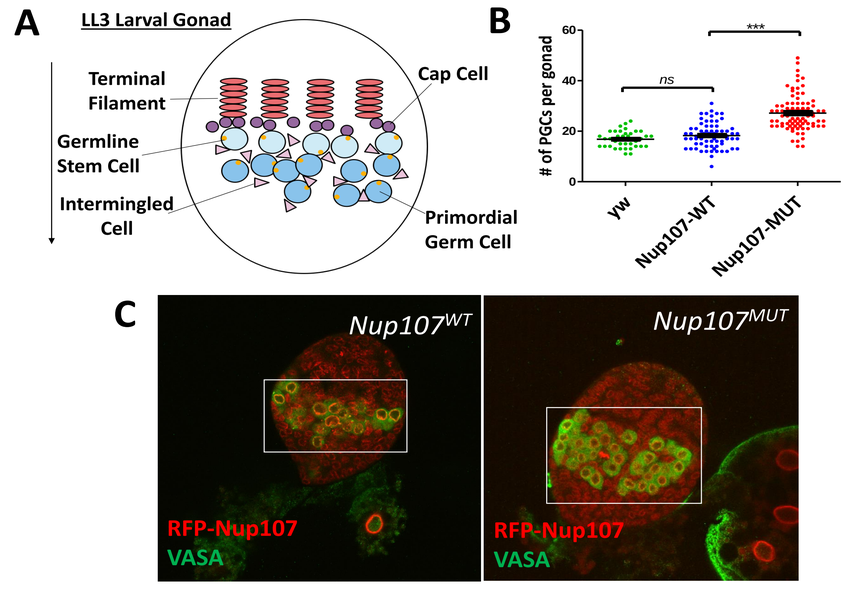

Figure 4. At what stage of development does ovarian dysgenesis originate?
(A) Schematic drawing of the larval (fetal) gonad development from germline stem cell to primordial germ cell (PGC), with surrounding supportive somatic cells. Nup107^{MUT} gonads show a 55% greater PGC count than Nup107^{WT}, as quantified in **(B)** and illustrated in **(C).**

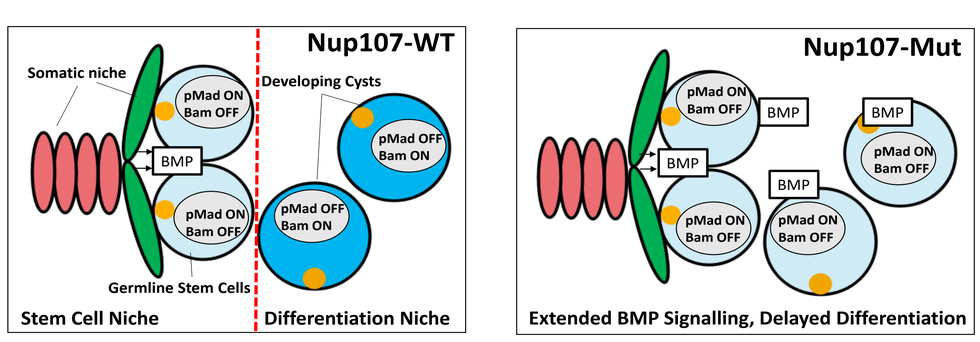

Figure 6. Mutation in Nup107 results in *Drosophila* ovarian failure due to delayed differentiation resulting from abnormal continuous BMP signaling in later developmental stages, which represses the differentiation factor Bam.

*equal contribution

^Weinberg-Shukron, A. *et al.* A mutation in the nucleoporin-107 gene causes XX gonadal dysgenesis. *J Clin Invest* **125**, 4295-4304, doi:10.1172/JCI83553 (2015).