KIF18A MUTATIONS LEAD TO CELL DIVISION ERRORS : INSIGHT INTO DIMINISHED FERTILITY IN YOUNG WOMEN

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BACKGROUND

- Female fertility depends on coordinated organ systems, hormonal regulation, and genetics.
- Egg quality—especially chromosome number (euploidy)—is a key factor that declines with maternal age.
- Currently, maternal age is the only clinical predictor of egg euploidy, with quality decreasing in the mid 30s and fertility nearly lost by the mid-40s
- Elevated egg aneuploidy (EEA) drives reproductive aging, but its mechanisms remain under investigation
- The kinesin motor protein KIF18A plays a critical role in oocyte meiosis and ensuring euploidy
- Mutations of KIF18A, including S126L and T273A, have been linked to increased EEA in both animal models and young women undergoing infertility treatment (1, 2).

OBJECTIVE

To determine how KIF18A mutations affect the following functions of the protein:

1. Chromosomal alignment
2. Spindle length
3. Spindle polarity
4. Mitotic index

METHODS

- HeLa Kyoto cell lines with GFP tagged KIF18A S126L and T273A were created using transformation and transfection via lipofectamine and electroporation techniques
- Doxycycline was used to drive expression of GFP tagged KIF18A WT, KIF18A S126L, and KIF18A T273A
- siRNA was used to knockout endogenous KIF18A
- Cells were fixed and stained for fluorescence microscopy
- Mitotic timing was assessed via 24-hour live imaging at 40x
- Alignment of chromosomes, spindle length, mitotic index, and polarity were assessed via fixed fluorescence microscopy at 40x and 60x
- To quantify mitotic timing, the time-point at which cells underwent rounding and cytokinesis was determined and the difference was calculated.
- To assess chromosomal alignment, a line spanning the spindle poles was used to measure kinetochore distribution with the Plot Profile tool in ImageJ/Fiji. Signal intensity was normalized to its maximum and plotted along the pole-to-pole axis using a custom MATLAB macro. Gaussian fitting was applied, and the Full Width at Half Maximum (FWHM) and spindle length were reported for each cell.

ACKNOWLEDGEMENTS AND CONTACTS

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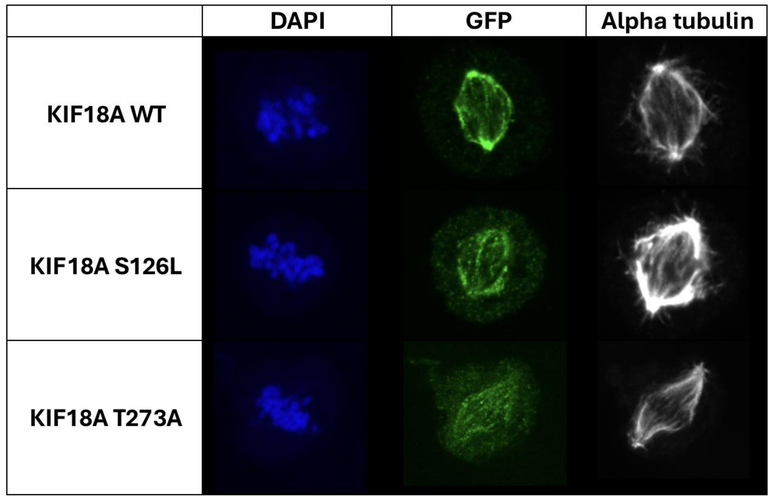
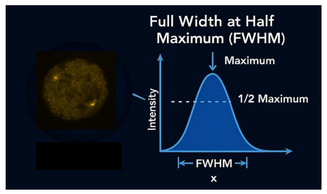
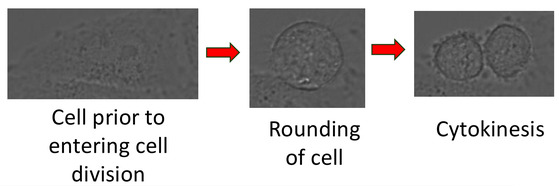
VARIANT EXPRESSION IN MITOTIC CELLS



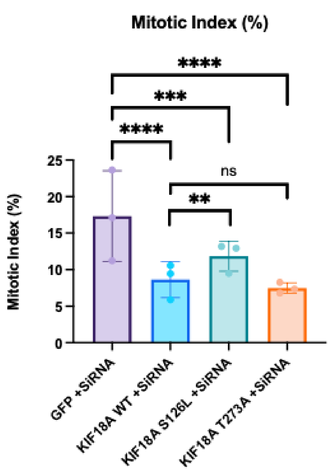


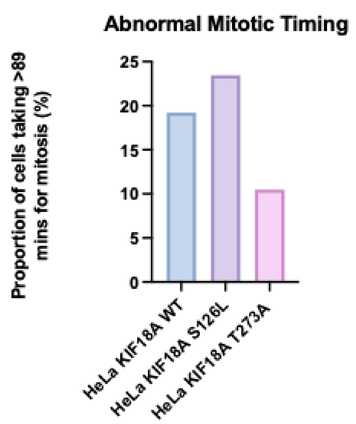


RESULTS

Mitotic Index
The mean mitotic index was 17.3% for GFP control, 8.6% for WT ($p < 0.0001$), 11.86% for S126L ($p = 0.0006$), and 7.47% for T273A ($p < 0.0001$). Compared to WT, S126L had a statistically higher mitotic index ($p = 0.003$), indicative of mitotic delay caused by loss of KIF18A.

Mitotic Timing
Mean percentage of cells with abnormal mitotic timing were 19.2% for WT, 23.5% for S126L, and 10.5% for T273A.



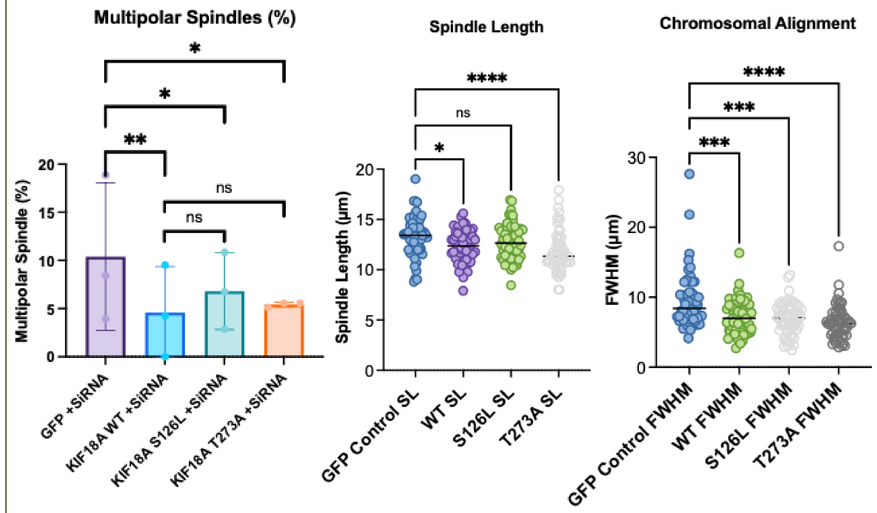


RESULTS

Spindle Polarity
Mean percentage of cells with multipolar spindles were 10.4% for GFP control, 4.6% for WT ($p = 0.002$), 6.8% for S126L ($p = 0.048$), and 5.4% for T273A ($p = 0.02$). Differences between WT and the variants were not statistically significant.

Spindle Length
Median spindle lengths were 13.4 µm for GFP control, 12.4 µm for WT, 12.7 µm for S126L, and 11.3 µm for T273A. Differences were statistically significant between GFP control and both WT and T273A ($p < 0.0001$).

Chromosomal Alignment
Chromosome alignment, measured by determining the full width at half maximum (FWHM) for chromosomes in metaphase, was 8.4 µm for GFP control), 7.0 µm for WT, 7.0 µm for S126L, and 6.2 µm for T273A. FWHM was significantly lower in all KIF18A variants vs GFP control: WT ($p = 0.0008$), S126L ($p = 0.0002$), and T273A ($p < 0.0001$).



CONCLUSIONS

- Mitotic index, spindle length, and mitotic timing were significantly affected by the S126L mutation compared to WT.
- Spindle bipolarity and chromosomal alignment were not significantly affected by either mutation.
- These findings indicate that S126L but not T273A significantly reduces KIF18A function in promoting mitotic progression and spindle length control.
- These results suggest a mechanism to explain how infertility associated KIF18A mutations can lead to egg aneuploidy and diminished fertility in younger women.

REFERENCES

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