Reclassifying DNMT3A VUS Variants from Tatton-Brown-Rahman Syndrome-Associated Leukemias in Drosophila assays

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1. Goal

We aim to test the utility of a transgenic *Drosophila* model as a versatile platform to perform large-scale clinical interpretation of rare human DNMT3A variants. *Drosophila*'s efficient molecular genetic tractability allows us to perform inexpensive, reproducible *in vivo* testing for hundreds of human variants identified in many disorders [1-3]. Our group has pioneered the use of known clinical variants to calibrate assays to ACMG guidelines for clinical interpretation of variants of unknown significance (VUS).

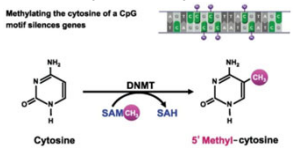
DNMT3A is a *de novo* DNA cytosine methyltransferase; it adds methyl groups to cytosines in DNA. Cytosine methylation does not occur naturally in *Drosophila*, but transgenic expression of DNMT3A in *Drosophila* cells leads to *de novo* DNA cytosine methylation – which makes *Drosophila* cells very sick.

Aberrant transcriptional patterns that result from *de novo* ectopic 5mC marks cause quantifiable deficits in *Drosophila* form and function which match the extent of DNMT3A protein function.

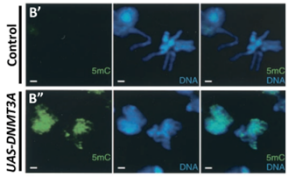
These include early lethality when DNMT3A is expressed at high levels in the whole animal.

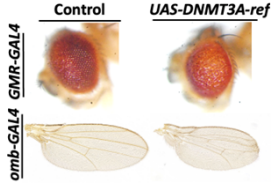
When expressed in the eye or the developing wing tissue, DNMT3A causes reduced cell number and consequent tissue abnormalities.

DNMT3A cytosine methyltransferase



***Drosophila* chromatin methylated by DNMT3A**





2. Objective

***Aim 1:* Allelic series of *DNMT3A* VUS in *Drosophila*.** We will generate *Drosophila* strains with inducible *UAS-DNMT3A* variants integrated into the *attP2* genomic locus for heritable transmission and to ensure equivalent expression of all variants in our assays. We will generate a minimum of 10 identified VUS, and also calibrating transgenes for three Gain-of-Function (GoF) variants causal for Heyn-Sproul-Jackson syndrome, three LoF variants causal for TBRS and an acute myeloid leukemia (AML), and five benign variants. All variants are within the methyl transferase domain, and will be tested alongside DNMT3A-ref and a negative control[1-3], with the pathogenic and benign variants used as clinical calibrating variants as per ACMG guidelines.
(i) We will drive expression of variants using GAL4 drivers that are ubiquitous (*Act5c-GAL4*), eye-specific (*GMR-GAL4*) or wing-specific (*omb-GAL4*). We will compare phenotypes (pupal lethality, rough eye, small wing) generated by variants in each assay (see Fig) to provide quantitative assessment of DNMT3A's functional impact. We will also confocal image immunoreactivity to 5mC in late larval salivary glands, developing wing and eye tissues to assess DNMT3A's enzymatic activity and its triggering of chromatin compaction. These data will be used to create an allelic series of function in order to rank-order mutations in order of severity, to inform screening recommendations for patients with risk of AML and other cancers.

3. Study Methods

Variant Selection.

[redacted]

[redacted]

4. Pilot Results

[redacted]

[redacted]

5. Impacts / Outcome

[redacted]

6. Project timelines – start and end date

June 2026: Assay optimization
September 2026: Complete wing dataset
October 2026: Cytosine DNA methylation assay complete

December 2026: Testing additional TBRS variants of interest that we have generated - through the above assays.

[redacted]