ABSTRACT

Infertility affects approximately 15% of couples worldwide, with genetic factors often underlying its etiology. NANOS1, an RNA-binding protein that regulates germ cell development post-transcriptionally through target mRNA 3′UTRs, has emerged as a critical fertility determinant from flies up to humans. A specific p.[Pro34Thr; Ser78del] variant (MUT-NANOS1) in NANOS1 is implicated in the absence of germ cells in the seminiferous tubules of infertile male patients, indicating a possible pathological mechanism. Preliminary studies in a seminoma-derived cell line showed that overexpression of MUT-NANOS1 substantially reduced cell viability, whereas wild-type (WT-) NANOS1 exerted anti-apoptotic effects.

To investigate how this variant influences early human primordial germ cell (hPGC) development, we employed a well-characterized hESC model offered by Prof Azim Surani from the University of Cambridge. Under defined in vitro conditions, transient mesendoderm-like precursors (pre-me) aggregate into embryoid bodies and differentiate into primordial germ cell–like cells (PGCLCs) at 10–40% efficiency under BMP stimulation. The W15 (46, XY) hESC line, equipped with a NANOS3-tdTomato fluorescent reporter, enabled real-time PGCLC tracking. We generated doxycycline-inducible WT- and MUT-NANOS1 lines and performed enhanced cross-linking immunoprecipitation (eCLIP) coupled with RNA-sequencing to identify bound and differentially expressed mRNAs.

Our data reveal that the p.[Pro34Thr; Ser78del] NANOS1 variant exerts a premature, repressive gain-of-function effect by downregulating canonical WNT signalling in pre-me cells, consequently impairing regulators such as EOMES and diminishing PGC competence. Notably, in PGCLCs, MUT-NANOS1 insufficiently represses WNT/TGF-β components, leading to aberrant WNT pathway activation, EMT-like transitions, and a shift toward mesendoderm/mesoderm fates. This disruption is further compounded by potential MUT-NANOS1 binding at the 5′UTR of NANOG and the 3′UTR of OCT4, suppressing these core pluripotency factors and reducing germ cell markers, including SOX17, TFAP2C, and NANOS3. Together, these alterations help explain the diminished *in vitro* PGC pool and gonadal germ cell depletion observed in patients carrying MUT-NANOS1. Notably, WNT inhibition partially restores pluripotency and germ cell markers while reducing BMP4 levels, highlighting the vital interplay among BMP, WNT, and TGF-β in safeguarding germ cell fate.

This study underscores how MUT-NANOS1 disturbs the transcriptome and signalling dynamics necessary for early germ cell specification, offering mechanistic insight into male infertility associated with NANOS1 p.[Pro34Thr; Ser78del] mutation.