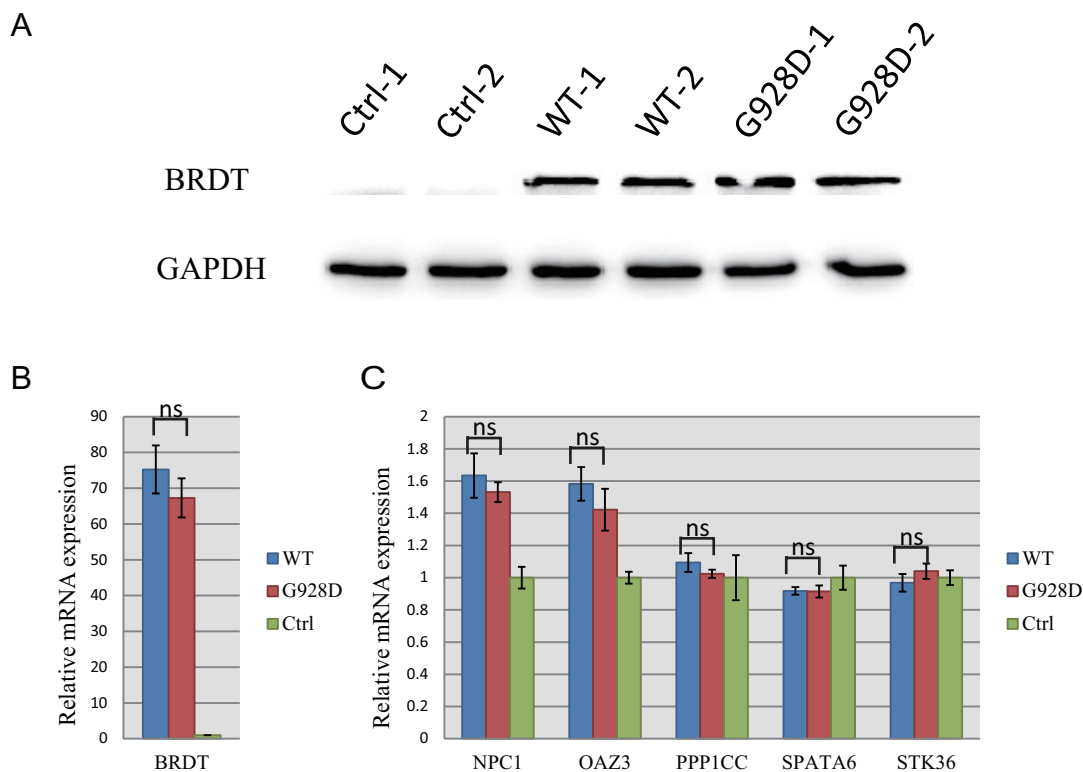


Whole-exome sequencing identified a homozygous *BRDT* mutation in a patient with acephalic spermatozoa

SUPPLEMENTARY FIGURE AND TABLES



Supplementary Figure 1: **A.** Western blot analysis of BRDT protein expression. 293FT cells were transiently transfected with GFP (Ctrl), WT or G928D BRDT expression vector. GAPDH is the house keeping protein used in this western blot as a loading control. **B** and **C.** Quantitative real-time PCR (q-PCR) analysis of gene expression in WT, G928D and Ctrl cells. NTERA-2 cells were infected with WT, G928D BRDT or GFP (Ctrl) lentivirus and selected with blasticidin (to kill the uninfected cells) for 3 days. The mRNA expression is relative to *GAPDH*. Experiments were performed in triplicates. ns, not significant.

Supplementary Table 1: Homozygous sequence variants were found by whole-exome sequencing

Gene	Change	Annotation
<i>ADAMTS7</i>	nonsynonymous_SNV	NM_014272:exon21:c.G4496A:p.R1499Q,
<i>ADAMTSL4</i>	nonsynonymous_SNV	NM_019032:exon17:c.G2839A:p.V947M,
<i>BCAP31</i>	nonsynonymous_SNV	NM_005745:exon2:c.T53C:p.V18A,
<i>BRDT</i>	nonsynonymous_SNV	NM_207189:exon19:c.G2783A:p.G928D,
<i>DMD</i>	nonsynonymous_SNV	NM_004009:exon17:c.C2084G:p.A695G,
<i>GABPB2</i>	nonsynonymous_SNV	NM_144618:exon6:c.C701T:p.A234V,
<i>GAPVD1</i>	nonsynonymous_SNV	NM_015635:exon19:c.G3232A:p.A1078T,
<i>LOC100507003</i>	nonsynonymous_SNV	NM_001195256:exon1:c.C67T:p.P23S,
<i>MYBBP1A</i>	nonsynonymous_SNV	NM_014520:exon1:c.T104C:p.F35S,
<i>PHKG1</i>	nonsynonymous_SNV	NM_006213:exon8:c.C773T:p.S258L,
<i>PIP5K1A</i>	nonsynonymous_SNV	NM_003557:exon1:c.C68A:p.S23Y,
<i>SLC12A7</i>	nonsynonymous_SNV	NM_006598:exon19:c.C2498T:p.S833L,
<i>TMEM26</i>	nonsynonymous_SNV	NM_178505:exon1:c.G25T:p.A9S,
<i>CHRNA3</i>	nonsynonymous_SNV	NM_000743:exon5:c.C1112T:p.P371L,
<i>GPR144</i>	nonsynonymous_SNV	NM_001161808:exon7:c.G1301A:p.R434H,
<i>HKDC1</i>	nonsynonymous_SNV	NM_025130:exon14:c.A1984C:p.M662L,

Supplementary Table 2: DNA Primers used in this study

Gene	Forward Primer	Reversed Primer	Experiment
<i>GAPDH</i>	TGTTGCCATCAATGACCCCTT	CTCCACGACGTACTCAGCG	Q-PCR
<i>BRDT</i>	TCCCTTGAACGTGGTACAGG	GCAGGAGTTGTTGTATCTGCT	Q-PCR
<i>NPC1</i>	GTCCAGCGCAGGTGTTTTTC	GCCGAACATCACAAACAGAGAC	Q-PCR
<i>OAZ3</i>	TATCCCATATCAGGCCTTGG	ACCTCTGTCTGTTCCGATCAT	Q-PCR
<i>PPP1CC</i>	GCGATGGCGGATTTAGATA	GCTGGACATTCTTACCAGGC	Q-PCR
<i>SPATA6</i>	CATCAGGCTCATCAAAGACATT	GGGTCACTGTCATAGGCAGA	Q-PCR
<i>STK36</i>	CGGAGCACTGATGTAGTGGA	AATAGGCACAGGCTCAGTGG	Q-PCR
BRDT PCR with NotI or AscI restriction enzyme sites	gactgcgccgaccATGTCTCTGCC AAGTCGACAAACAG	gactggcgccTTAATCAAAGTT GTTTTCAAACATTGTCATA	Vectors Construction
BRDT-G928D overlap PCR	GAGAAGCAATGGaGGGTACCA TTGATATGA	TCATATCAATGGTAcca CCATTGCTTCTC	Vectors Construction

Supplementary Table 3: Antibodies used in Western Blot

Primary/ Secondary antibodies	Source	Western Dilution	Cat no.
BRDT	Rabbit polyclonal	1:1000	AP7115a, ABGENT
GAPDH	Mouse monoclonal	1:2000	60004-1-Ig, Proteintech Group
Goat Anti-Rabbit IgG (H+L)		1:1000	ZB-2301, ZSGB-BIO
Goat Anti-Mouse IgG (H+L)		1:1000	ZB-2305, ZSGB-BIO

Supplementary Table 4: Expression levels of RefSeq genes in G928D-BRDT or WT-BRDT over-expression cells

See Supplementary File 1