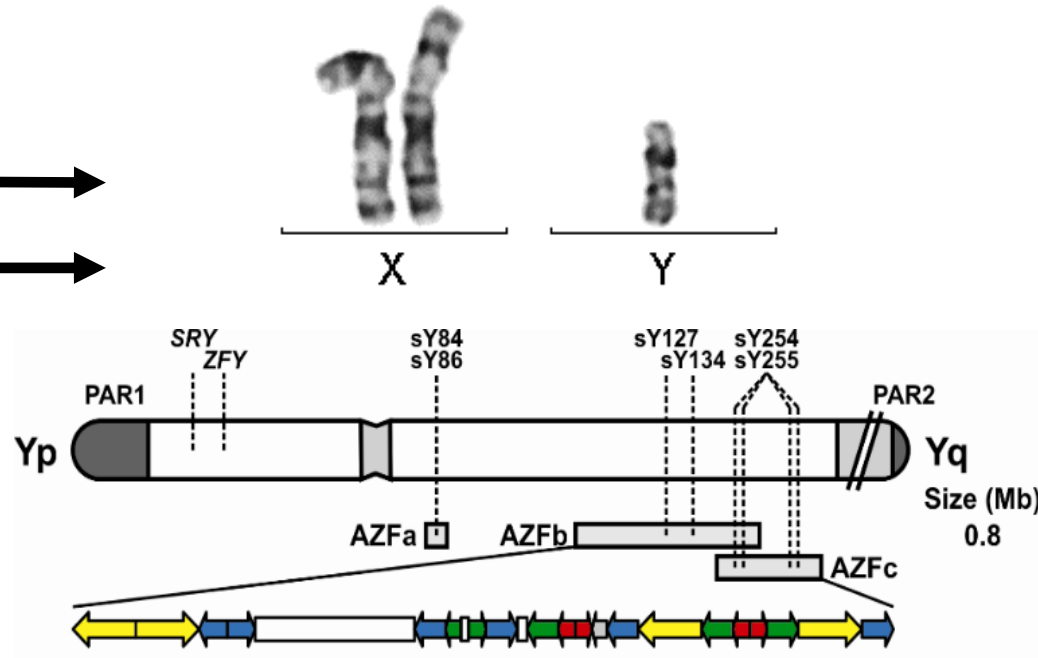
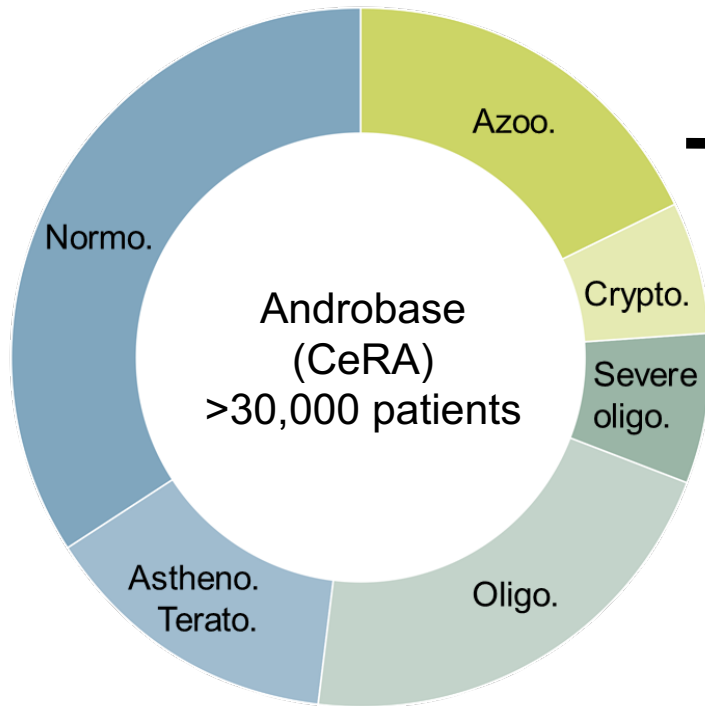


Inherited defects of piRNA biogenesis cause transposon de-repression, impaired spermatogenesis, and human male infertility

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Azoospermia/cryptozoospermia: Genetic analyses workflow



What about
monogenic
causes?

CFTR (1989)

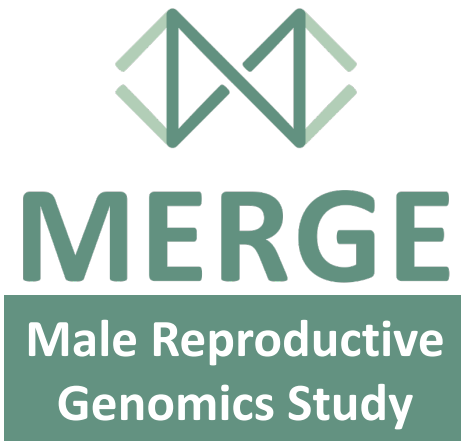
Do also monogenic causes contribute to spermatogenic failure?

- ▶ 77% of all human genes are expressed in the testis.
- ▶ 1992 genes show an elevated expression in the testis compared to other tissue types
- ▶ 582 genes are only expressed in the testis
- ▶ 118 genes are linked to spermatogenic failure in mice knockout models

The Male Reproductive Study

Hypothesis: Monogenic causes for SPGF are underdiagnosed

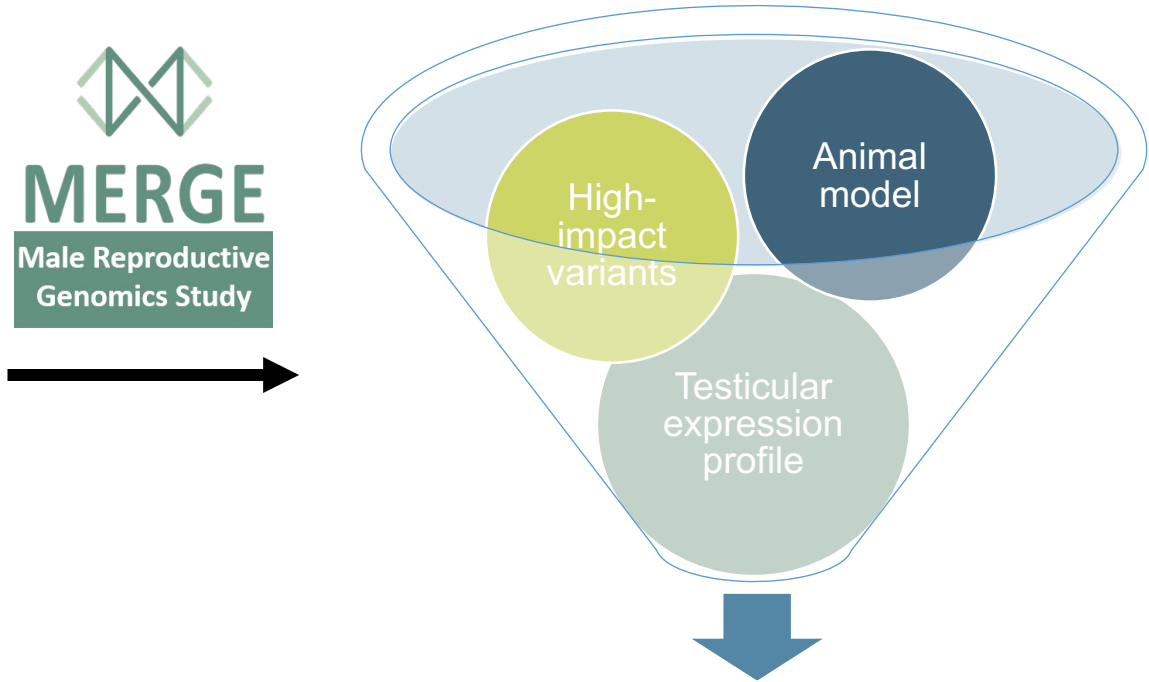
MERGE cohort (12/2024)



- ▶ Exome/genome sequencing data of 2,973 infertile men
- ▶ Most cases were recruited at the CeRA in Münster
- ▶ 2,616 cases with severely decreased sperm count (azoo-, crypto-, and severe oligozoospermia)
- ▶ Testicular biopsies are available for 1,044 samples

Azoospermia: A heterogeneous disease

Identification and functional characterisation of high-impact variants in >15 disease (candidate) genes (2017-2023)



TEX11, TEX15, TEX14, MSH4, MSH5, M1AP, TERB1, TERB2, SHOC1, SYCP3, ZMYND15, GCNA, DDX3Y, RXFP2, INSL3

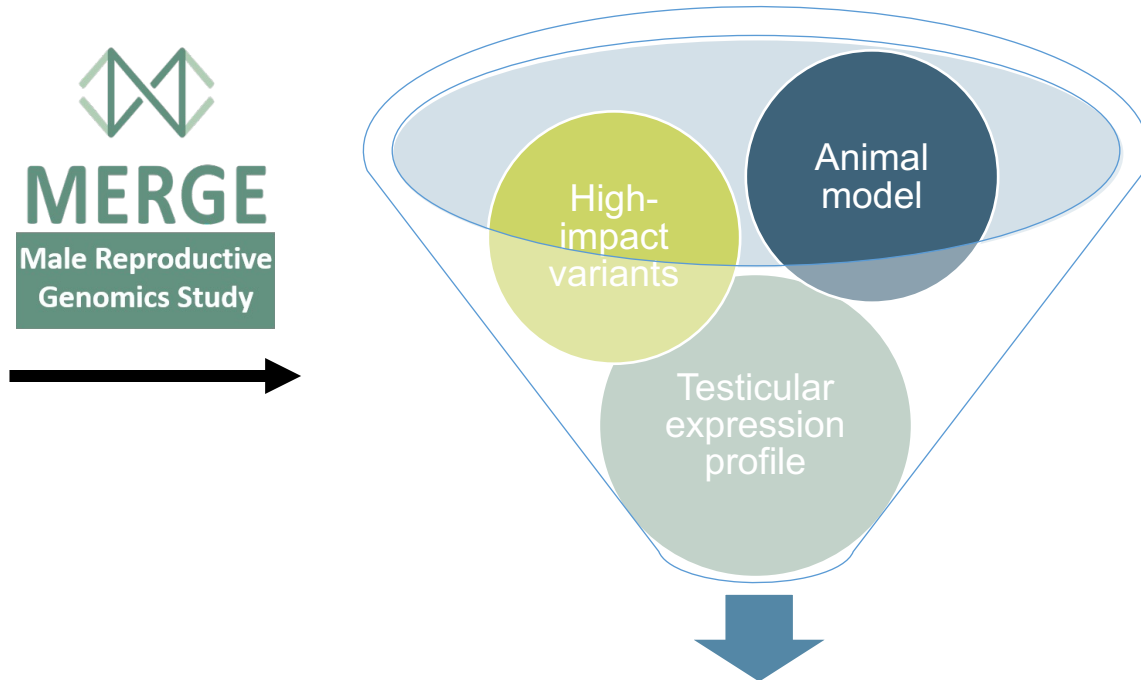


Oligo-/Azoospermia

Oligozoospermia	NOA		OA
FANCM	ADAD2	MSH4	ADGRG2
	AR	MSH5	CFTR
	C14orf39	NR5A1	
	DDX3Y	PNLDC1	
	DMC1	RAD21L1	
	DMRT1	SHOC1	
	FKBP6	SPO11	
	GCNA	STAG3	
	HFM1	STRA8	
	KASH5	SYCE1	
	KCTD19	TDRD9	
	M1AP	TERB1	
	MCM8	TERB2	
	MCM9	TEX11	
	MCMD2	TEX14	
	MEI1	TEX15	
	MEIOB	ZMYND15	
	MLH3	ZSWIM7	

Azoospermia: A heterogeneous disease

Identification and functional characterisation of high-impact variants in >15 disease (candidate) genes (2017-2023)



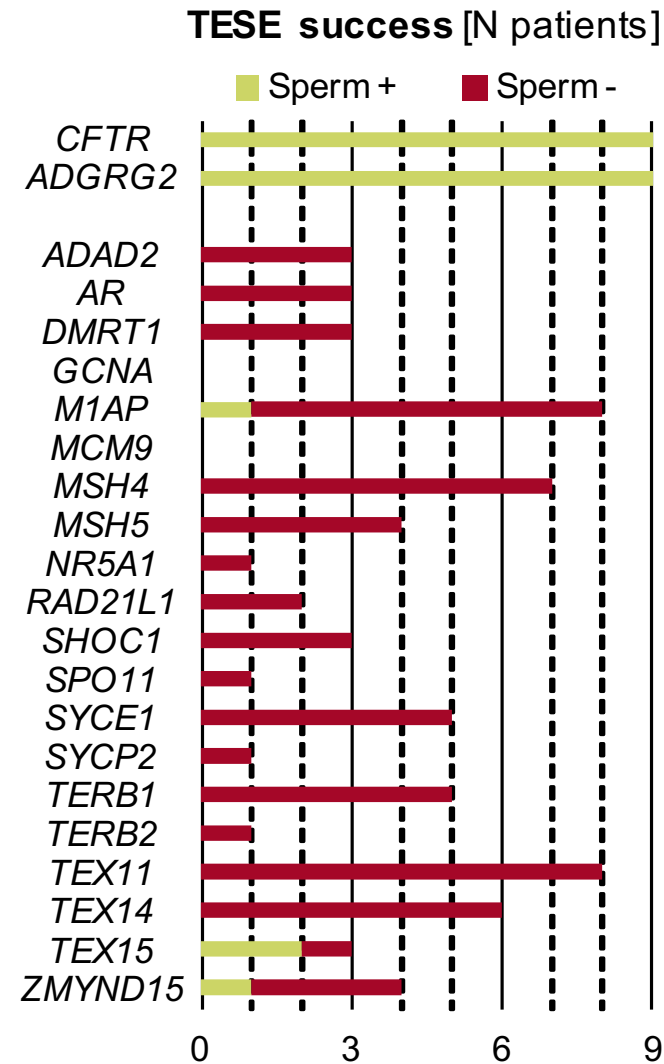
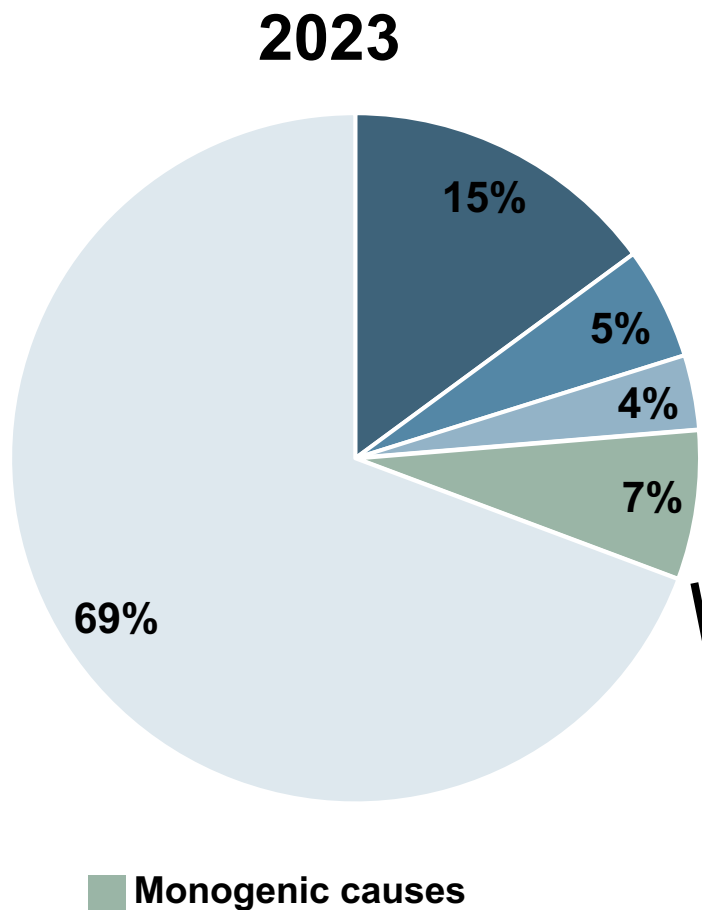
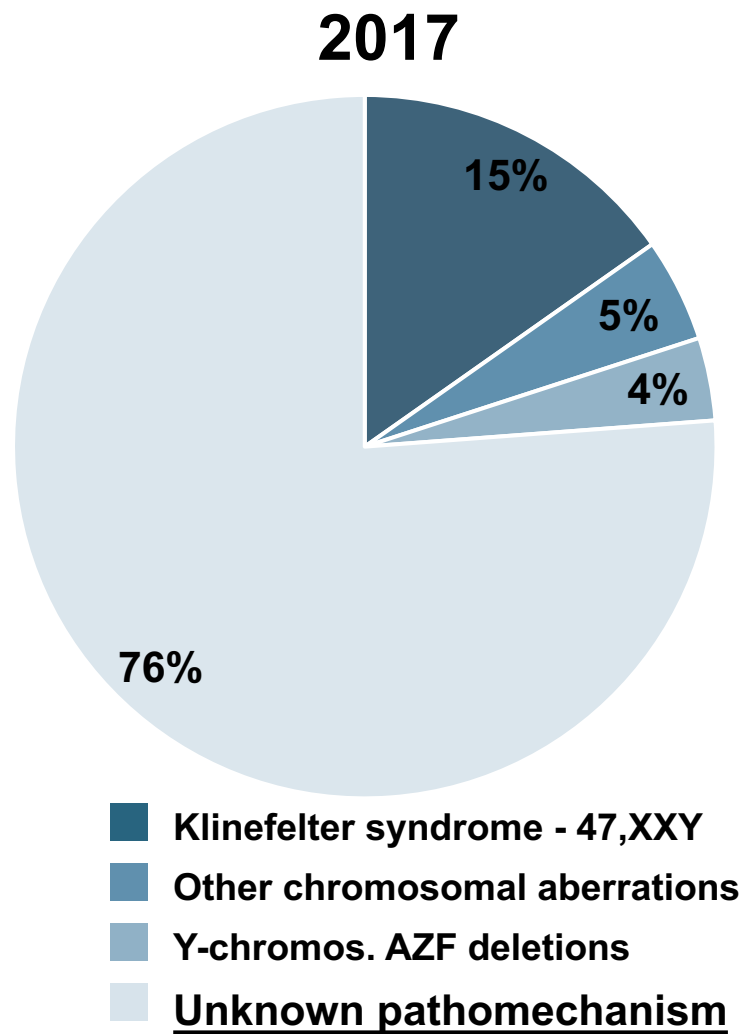
TEX11, TEX15, TEX14, MSH4, MSH5, M1AP, TERB1, TERB2, SHOC1, SYCP3, ZMYND15, GCNA, DDX3Y, RXFP2, INSL3



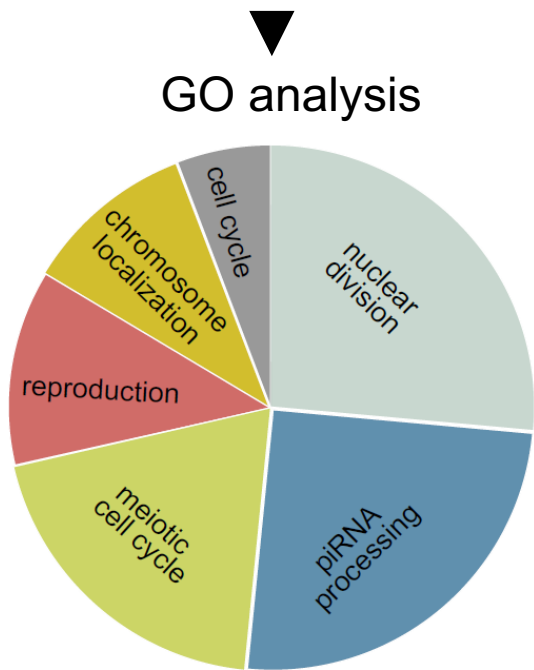
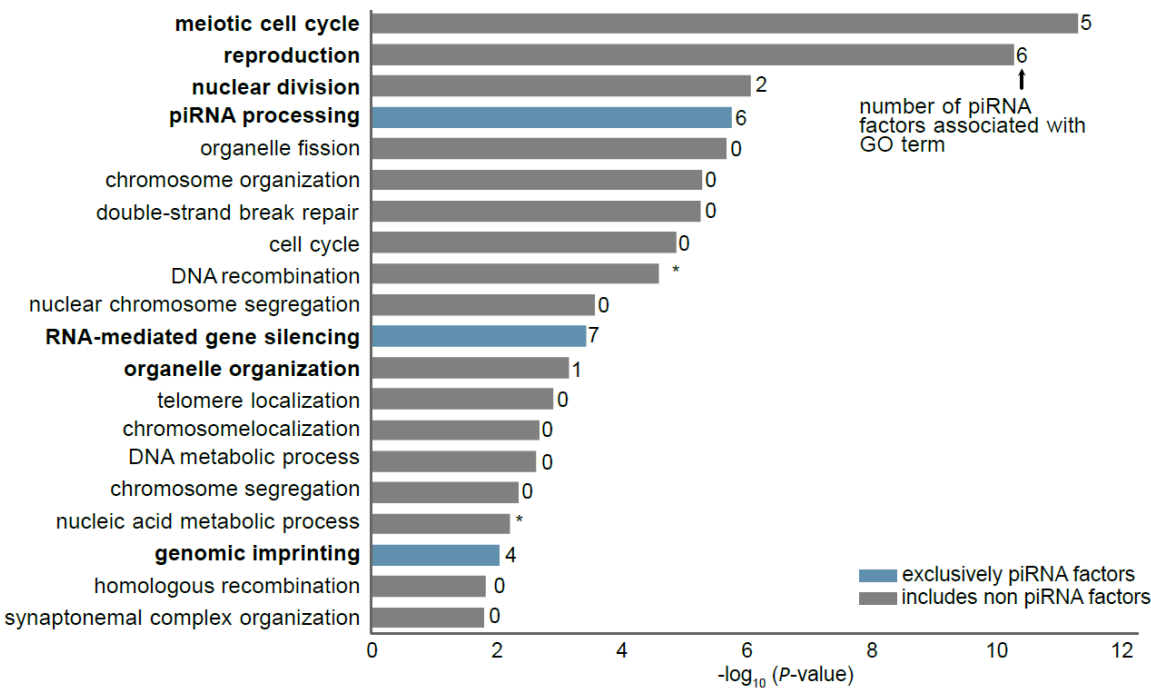
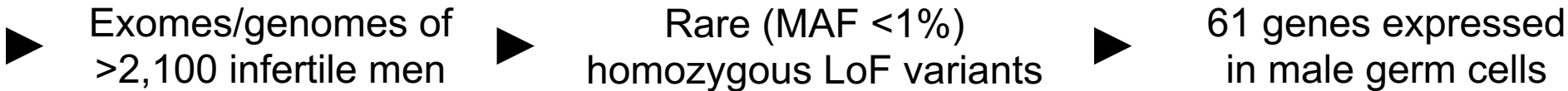
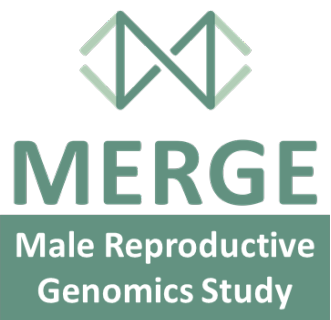
Oligo-/Azoospermia

Oligozoospermia	NOA		OA
FANCM	ADAD2★ AR★ C14orf39★ DDX3Y★ DMC1★ DMRT1★ FKBP6★ GCNA★ HFM1★ KASH5★ KCTD19★ M1AP★ MCM8★ MCM9★ MCMDC2★ MEI1★ MEIOB★ MLH3★	MSH4★ MSH5★ NR5A1★ PNLDC1★ RAD21L1★ SHOC1★ SPO11★ STAG3★ STRA8★ SYCE1★ TDRD9★ TERB1★ TERB2★ TEX11★ TEX14★ TEX15★ ZMYND15★ ZSWIM7	ADGRG2★ CFTR★

Azoospermia: Diagnostic yield

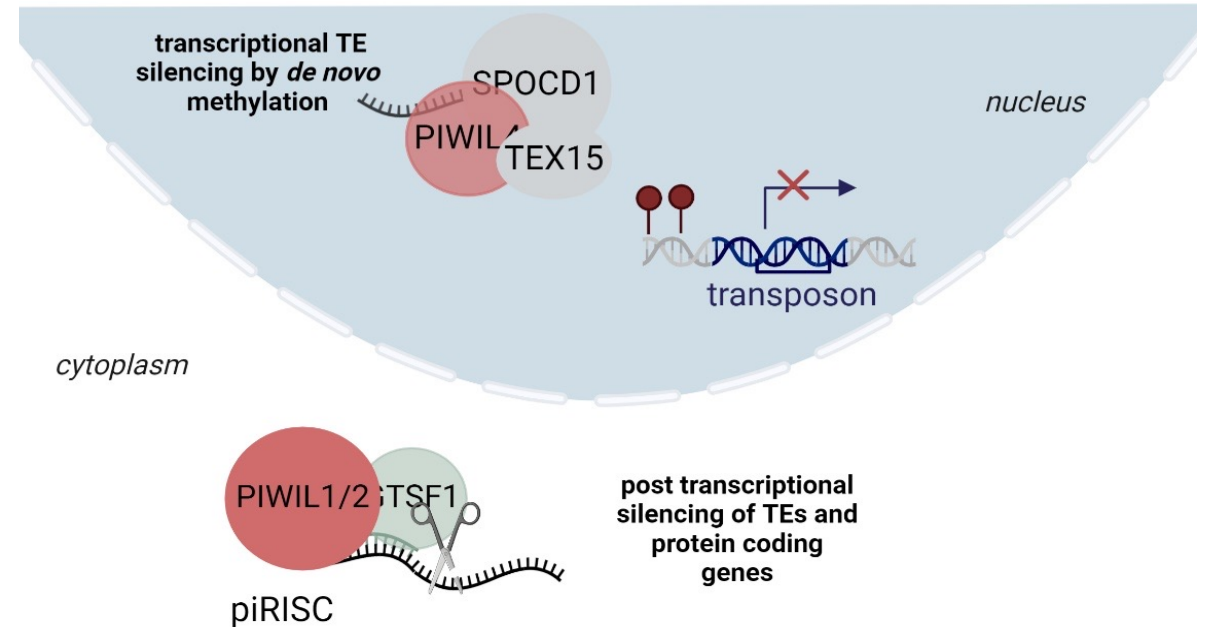


Elucidating novel disease pathways for SPGF



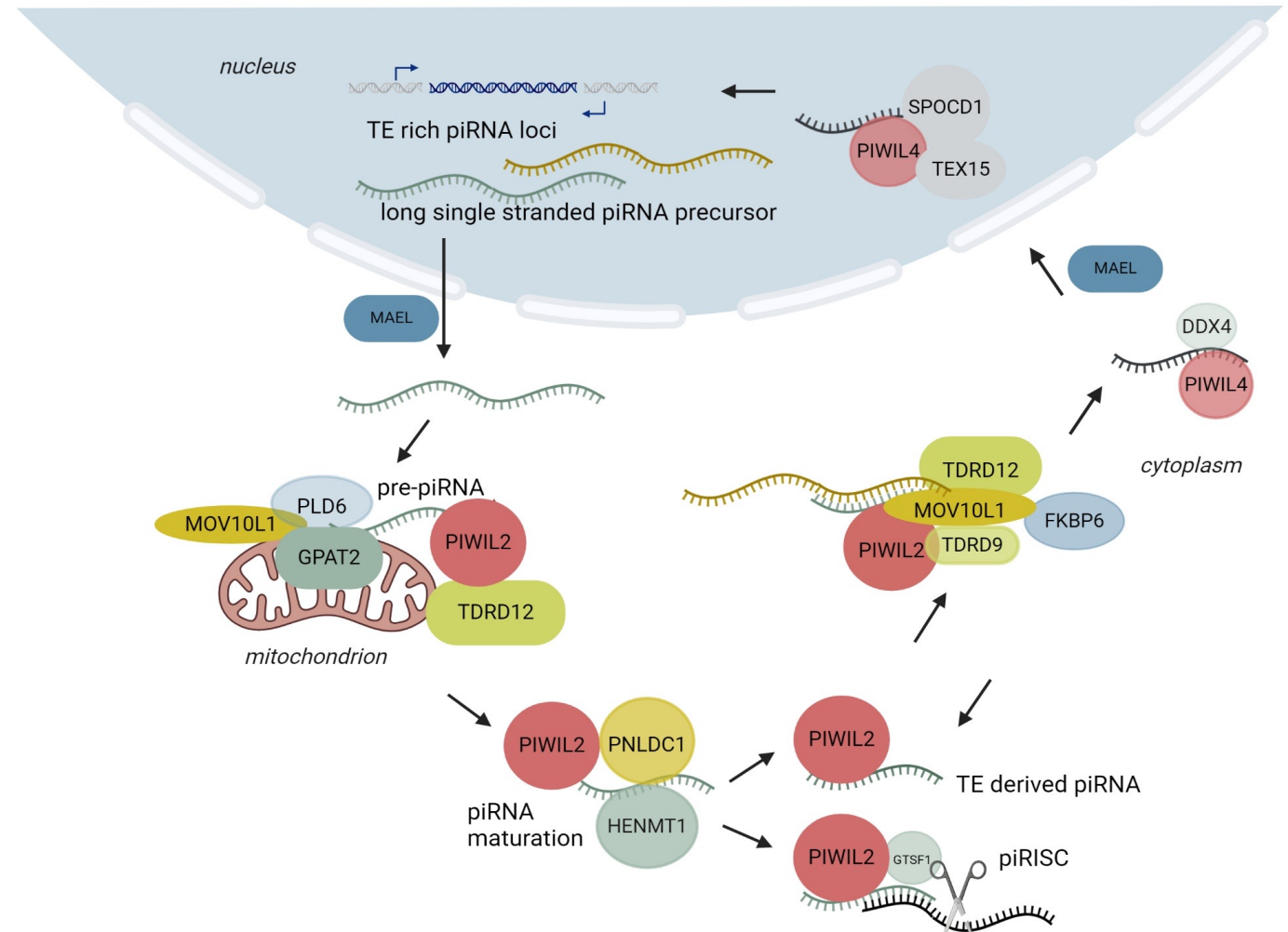
Function of piRNAs in male germ cells

- ▶ Most abundant small non-coding RNAs in the testis
- ▶ Bind to PIWI subfamily of Argonaute proteins (*PIWIL1-4*)
- ▶ Involved in transposon silencing by
 - ▶ post transcriptional inactivation
 - ▶ de novo methylation
- ▶ Involved in the regulation of gene expression in spermatocytes

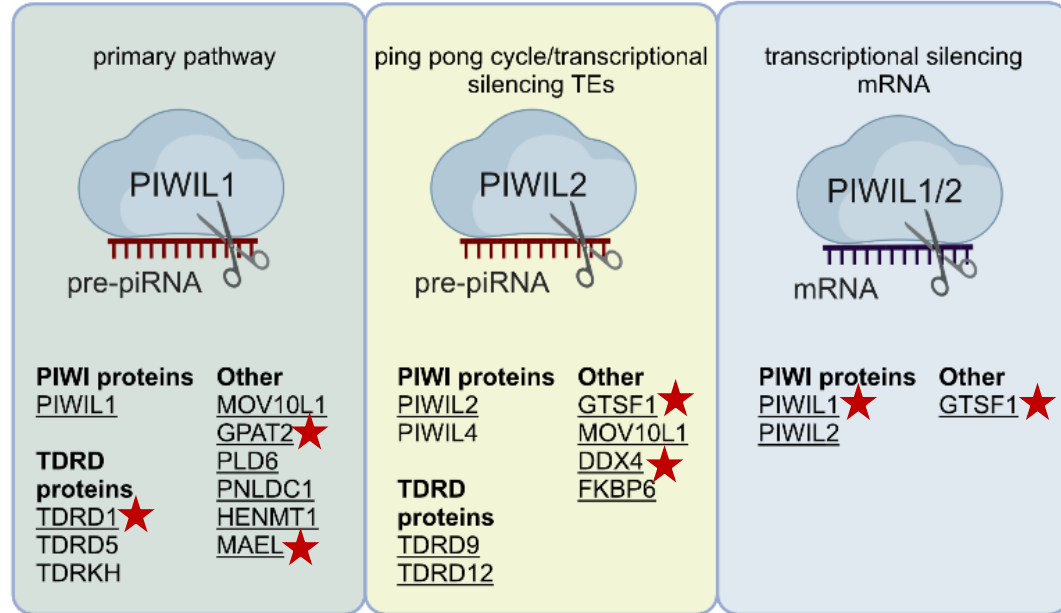


piRNA pathway

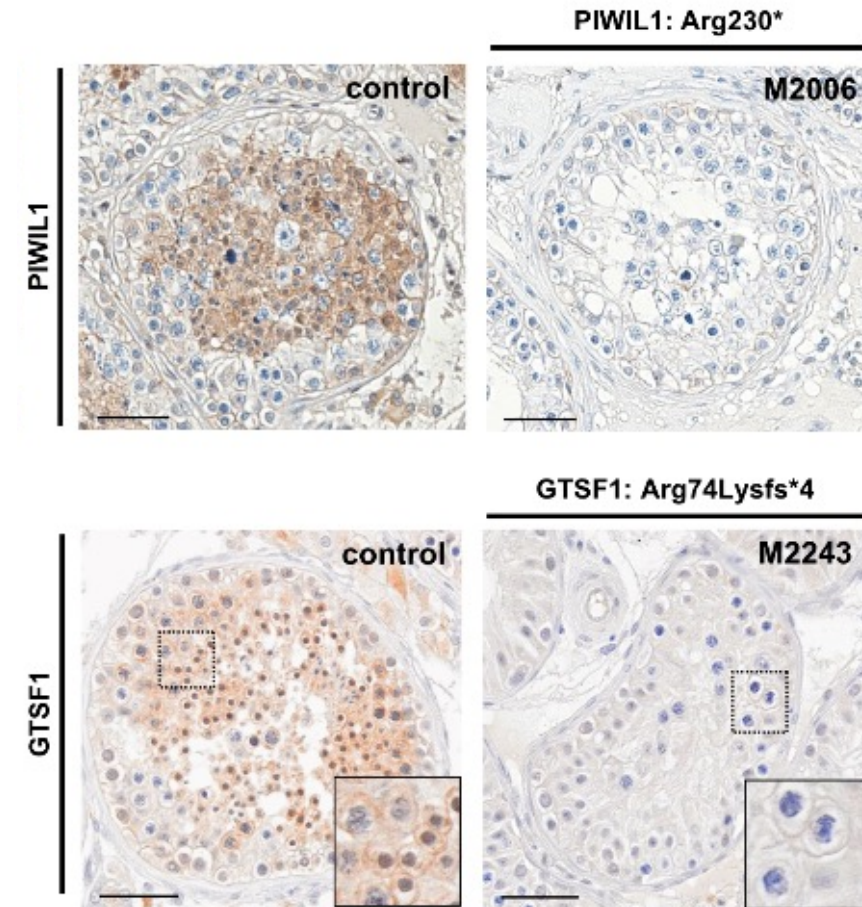
- ▶ Biogenesis is highly complex
- ▶ Disruption of piRNA biogenesis genes in mice leads to
 - Impaired spermatogenesis
 - Male infertility
 - Transposon de-silencing in spermatocytes



Disturbed piRNA biogenesis in human male germ cells

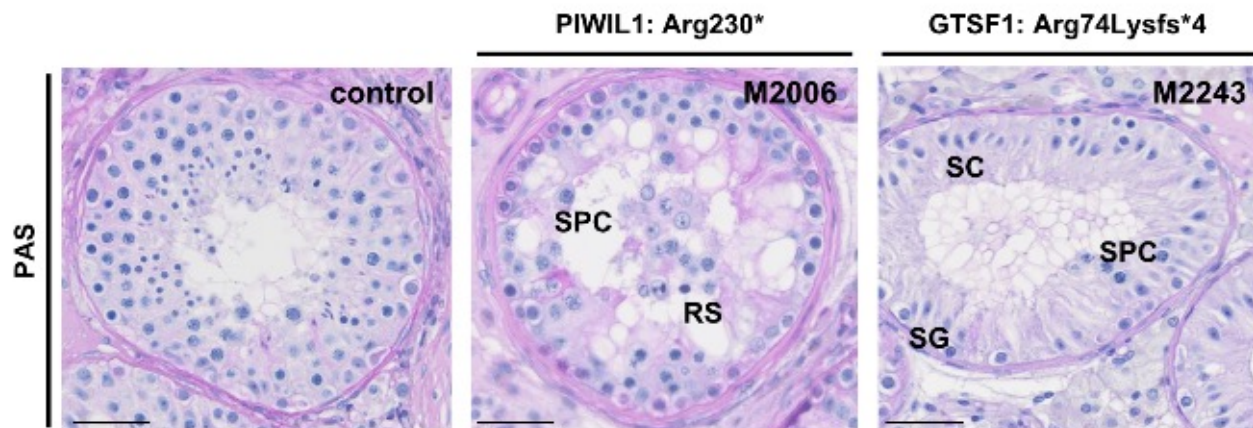


- ▶ MERGE: 31 infertile men carrying biallelic variants in 14 piRNA pathway genes
- ▶ 6 novel disease genes
- ▶ 12 biallelic LoF variants (human knockouts)



Clinical phenotypes of variant carriers

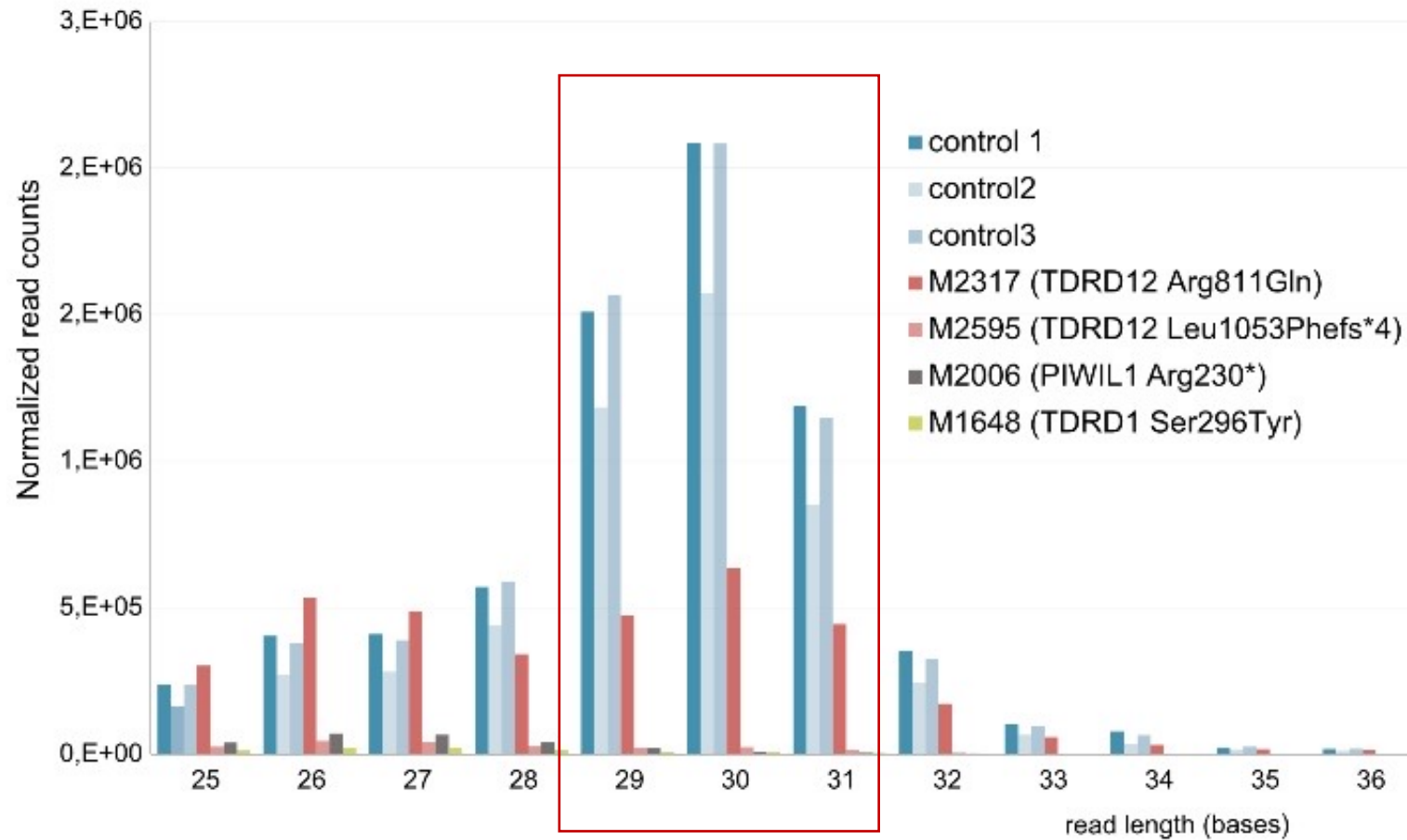
- ▶ Patients are mainly affected by azoospermia
- ▶ Spermatogenic failure, ranging from Sertoli cell-only to hypospermatogenesis
- ▶ Close genotype-phenotype correlation
- ▶ Human gene-specific phenotypes repeatedly differ from the phenotypes observed in knockout mice



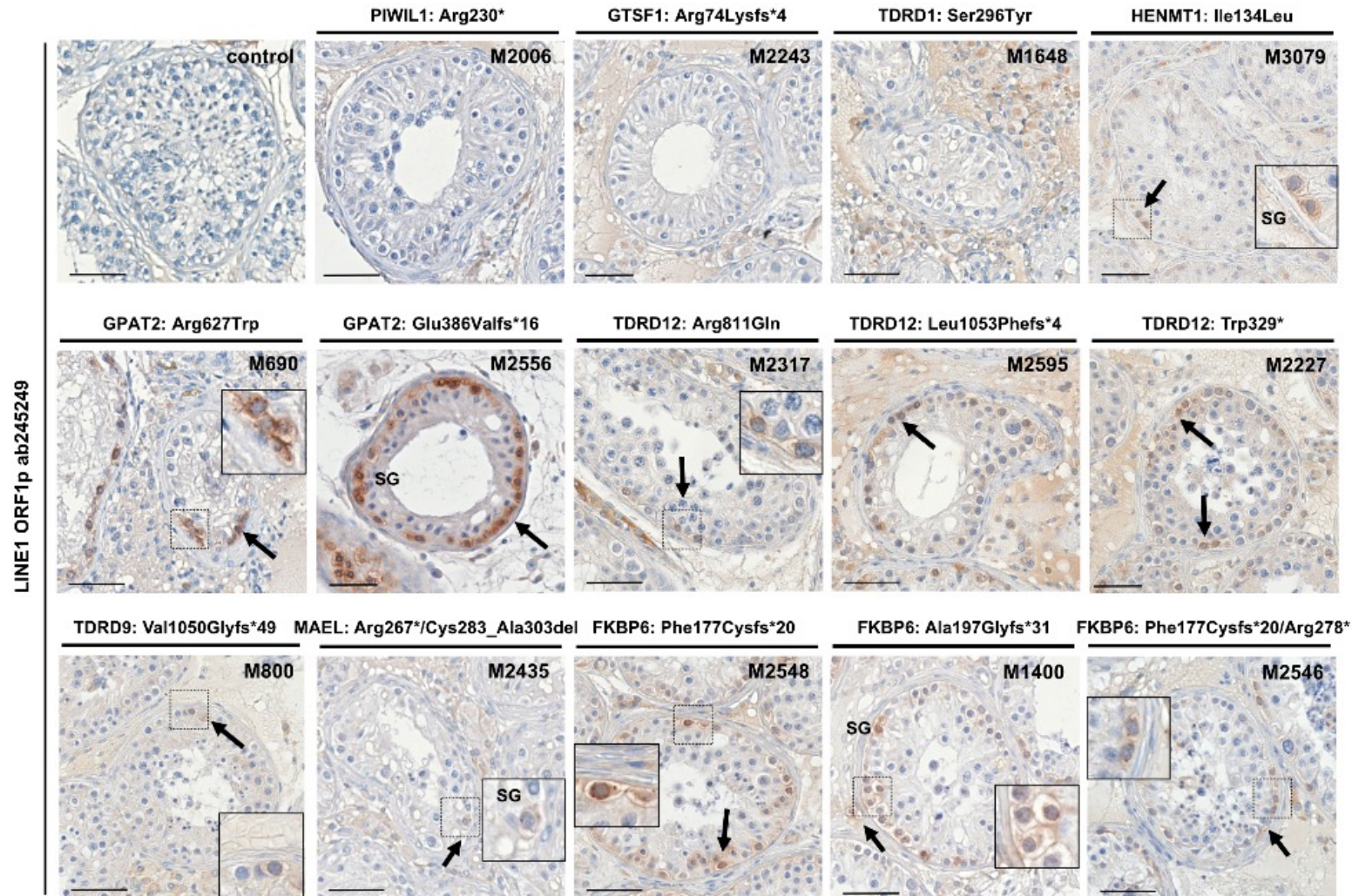
	SCOSpgA	MeiA	RsA	ES+	
PIWIL1					Mouse Human
PIWIL2					Mouse Human
GTSF1					Mouse Human
MOV10L1					Mouse Human
PLD6					Mouse Human
GPAT2					Mouse Human
PNLDC1					Mouse Human
HENMT1					Mouse Human
FKBP6					Mouse Human
MAEL					Mouse Human
DDX4					Mouse Human
TDRD1					Mouse Human
TDRD9					Mouse Human
TDRD12					Mouse Human

■ Arrest type observed in at least one tubule
■ Arrest type not observed

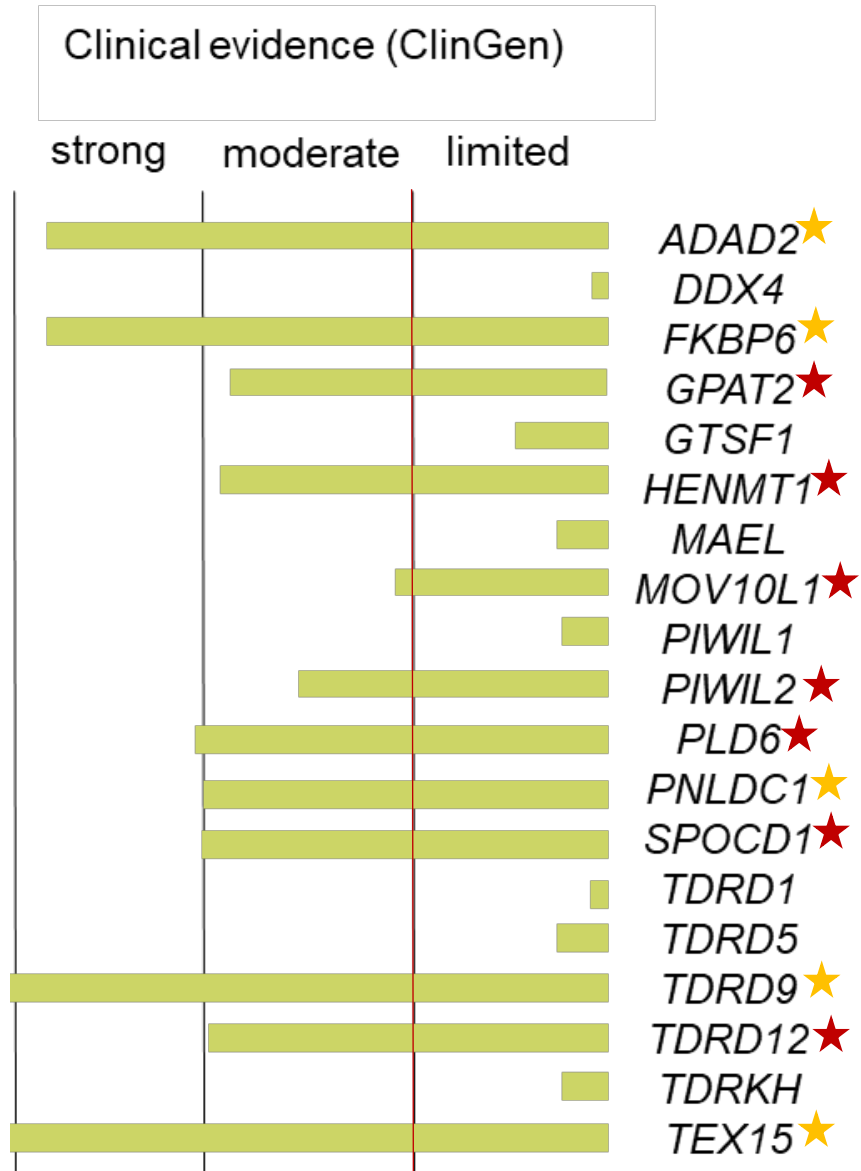
Decreased piRNA levels in variant carriers



Gene-specific transposon de-silencing in spermatogonia



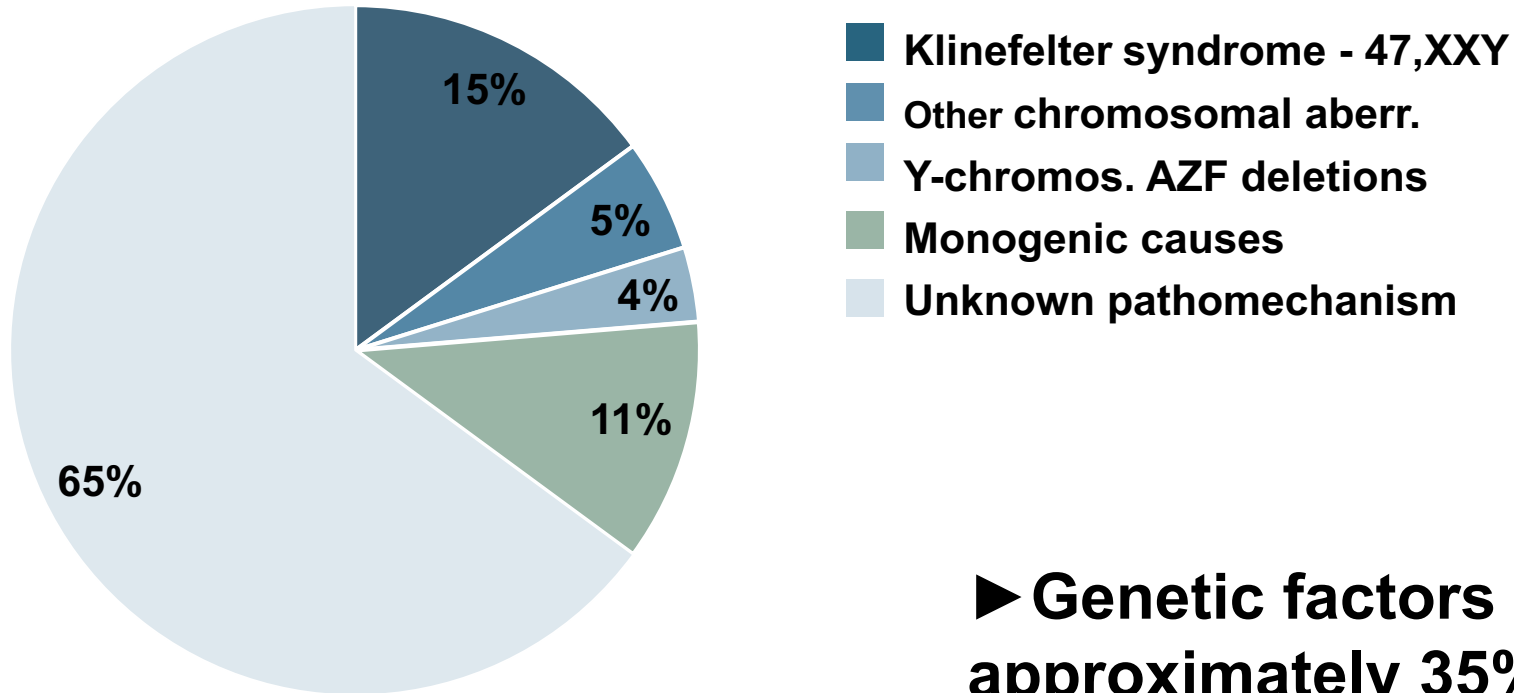
Translation into the clinic



- ▶ 12 piRNA pathway genes reach sufficient validity to be included in diagnostics
- ▶ TESE success rates are virtually zero

Translation into the clinic

2025



► Genetic factors account for approximately 35% of azoospermia

Thank you for your attention!



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