P-529 Whole-genome methylation analysis of testicular germ cells from cryptozoospermic men points to recurrent and functionally relevant DNA methylation changes

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Study question: Do DNA methylation changes occur in testicular germ cells (TGCs) from patients with impaired spermatogenesis?
Summary answer: TGCs from men with cryptozoospermia exhibit altered DNA methylation levels at several genomic regions, many of which are associated with genes involved in spermatogenesis.
What is known already: In the last 15 years, several studies have described DNA methylation changes in sperm of infertile men. More recently, using whole genome bisulfite sequencing (WGBS) we were able to refute these findings by demonstrating that somatic DNA contamination and genetic variation confound methylation studies in swim-up purified sperm of severely oligozoospermic men. However, it remains unknown whether altered DNA methylation plays a role during the development of the germ cells in the testes of these patients.
Study design, size, duration: For identifying DNA methylation differences associated with impaired spermatogenesis, we compared the TGC methylomes of men with cryptozoospermia (CZ) and men with obstructive azoospermia (n=4 each), who had normal spermatogenesis and served as controls (CTR). Study participants were selected among an age-matched cohort of 24 CTR and 10 CZ. The selection was based on similar composition of the TGC suspension evaluated by ploidy analysis and absence of somatic DNA.
Participants/materials, setting, methods: TGCs were isolated from biopsies after short-term cell culture. Presence of somatic DNA was evaluated by analyzing the DNA methylation levels of *H19*, *MEST*, *DDX4* and *XIST*. WGBS was performed at ~14× coverage. Bioinformatic tools were used to compare global DNA methylation levels, identify differentially methylated regions (DMRs) and functionally annotate the DMRs. Single-cell RNA sequencing (scRNA-seq) was used to associate the DNA methylation changes to gene expression.
Main results and the role of chance: We could not identify any difference in the global DNA methylation level or at imprinted regions between CZ and CTR samples. However, using stringent filters to identify group-specific methylation differences, we detected 271 DMRs, 238 of which were hypermethylated in CZ (binominal test, p<2.2×10-16). The DMRs are associated with 132 genes, 61 of which are known to be differentially expressed at various stages of spermatogenesis according to scRNA-seq studies. Almost all of the DMRs associated with the 61 genes are hypermethylated in CZ (63/67, p=1.107×10-14). As assessed by scRNA-seq, 13 DMR-associated genes, which were mainly expressed during meiosis and spermiogenesis, show a significantly different pattern of expression in CZ patients. In four of these genes, the promoter was hypermethylated in CZ men, which correlates with a lower expression level in these patients. In the other nine genes, most of which downregulated in CZ, germ cell-specific enhancers may be affected.
Limitations, reasons for caution: The small sample size constitutes a limitation of this study. Furthermore, even though the cellular composition of samples was similar by ploidy analysis, we cannot rule out that the observed DNA methylation changes might be due to differences in the relative proportion of different germ cell types.
Wider implications of the findings: Impaired spermatogenesis is associated with DNA methylation changes in testicular germ cells at functionally relevant regions of the genome, which points to an important role of DNA methylation in normal spermatogenesis. The DNA methylation changes may contribute to premature abortion of spermatogenesis and therefore not appear in mature sperm.
Trial registration number: N/A