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Association of UHRF1 gene polymorphisms with oligospermia in Chinese males

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Abstract

Background UHRF1 plays an important role in maintaining DNA methylation patterns during spermatogenesis. This study was performed to evaluate the association between UHRF1 gene variations and infertility in males with oligozoospermia in a Chinese population.

Methods In this case-control study of 735 Chinese men, single-nucleotide polymorphism (SNP) genotypes and alleles in the UHRF1 gene were assessed by direct sequencing. The effects of the mutations on UHRF1 transcription were investigated using a dual-luciferase reporter gene assay.

Results We identified 24 SNPs, including nine SNPs in the promoter region, three in the 5′ untranslated region, five in introns, and seven in exons. Interestingly, the genotype frequencies of SNP rs2656927 ($P = 0.014$) and rs8103849 ($P < 0.001$) significantly differed between men with oligozoospermia in case group 1 and normozoospermic men. Moreover, four variants (three were novel) were detected only in the patient group, with two in introns and the others in the promoter region. The results of the luciferase assay showed that the -1615C>T-C and -1562A>G-A alleles increased luciferase activity compared with the -1615C>T-T and -1562A>G-G alleles.

Conclusions We detected two SNPs in the UHRF1 gene showing a significant difference between the case and control groups. Two screened SNPs affected UHRF1 promoter activity, improving the understanding of the pathophysiology of oligozoospermia.

Keywords UHRF1 · Polymorphism · Promoter · Oligozoospermia

Introduction

Infertility affects approximately 15% of couples worldwide [1]. Numerous genes have been suggested to be potentially associated with male infertility [2–4]. However, these genes can only explain a small proportion of male infertility, and the pathophysiology is not well-understood. Determining the exact etiology and pathophysiology of infertility remains a critical challenge. Infertility is thought to be related to epigenetic factors such as sperm DNA methylation, chromatin remodeling, histone modifications, and RNAs, which may deteriorate spermatogenesis either alone or in combination with environmental factors [5].

As the most widely studied type of epigenetic modification, DNA methylation exhibits dynamic changes during spermatogonial stem cell (SSC) formation and differentiation [6] and is thought to play a fundamental role in male germline

Weiqiang Zhu and Jing Du are similar in author order.

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development [7]. DNA methylation in the male germline plays an important role in preventing retrotransposons from participating in meiotic recombination [8], and in regulating the expression of germ cell-specific genes [9]. Altered DNA methylation has been widely identified in the spermatozoa of infertile men [10, 11]. For instance, the paternally expressed human gene MEST is demethylated in the fetus and remains unmethylated throughout sperm development in human spermatogenesis [12]. In contrast, in male germ cells, the H19 gene is methylated prior to meiosis at the spermatogonial stage of development [12]. Both H19 hypomethylation and MEST hypermethylation have been detected in the sperm of infertile men [13]. However, the origins of these errors are poorly understood.

Genetic variations in DNA-methylation-regulating genes including DNA methyltransferase I (DNMT1) have been found to be associated with male infertility [14–17]. DNMT1, the most abundant DNMT in mammalian cells, is considered as the key maintenance methyltransferase during cell division [18, 19]. Knockout of DNMT1 in mouse embryonic stem cells resulted in a lack of DNA methylation, preventing genomic imprinting which was followed by apoptosis of spermatogonial cells [20, 21]. DNMT1 predominantly methylates hemimethylated CpG dinucleotides in the genome. The hemimethylated-CG-binding protein UHRF1 (ubiquitin-like-containing plant homeodomain and ring finger 1) plays a critical role in maintaining CG methylation by recruiting DNMT1 to hemimethylated CG sites [22].

UHRF1 is a multi-domain nuclear protein, containing a ubiquitin-like domain, Tudor domain, plant homeodomain, SET domain, and RING-associated domain and RING finger domain. It is mostly expressed in proliferating cells, whereas it is not found in fully differentiated tissues [23]. Many studies have validated UHRF1 as a powerful diagnostic and prognostic tool for differentially diagnosing cancer and predicting therapeutic responses and for assessing the risk of tumor progression and recurrence [24]. The oncogenic role of UHRF1 is largely associated with DNA hypermethylation in the promoters of tumor suppressor genes [25–27].

Additionally, UHRF1 is highly expressed in mouse testis tissues [28], not only in proliferating spermatogonia but also in meiotic spermatocytes and differentiating spermatids that are not proliferating [29]. A mechanism study showed that ablation of UHRF1 interfered with differentiation and viability only after spermatogonia became Kit-positive (a marker of differentiating spermatogonia) [30]. However, whether mutations in UHRF1 are related to male infertility is unclear. The association of polymorphisms in UHRF1 and human diseases has not been reported. In the current study, we aimed to identify single-nucleotide polymorphisms (SNPs) in UHRF1 and evaluate their effects in 393 idiopathic male infertility cases and 342 normal controls to explore the association between UHRF1 and the pathophysiology of oligozoospermia.

Material and methods

Study samples

This study was approved by the Ethics Committee of Shanghai Institute of Planned Parenthood Research (SIPPR) on Human Research. All participants were of Han Chinese ancestry and lived in the Shanghai area. And all participants were collected with a written informed consent form in accordance with the Declaration of Helsinki.

(1) Case group: A total of 393 infertile patients with oligozoospermia (semen count less than 20×10^6/mL), and mean age 31.95 ± 3.21, were recruited from the Shanghai Institute of Planned Parenthood Research Hospital. All patients undertook World Health Organization (WHO) standard semen analysis at least twice and were excluded from diseases such as urinary tract infection, spermaduct obstruction, testicular tumors or orchitis, and no Y chromosome microdeletion. Two groups of infertile patients were generated according to their sperm density: case group 1 (289 idiopathic infertile men with sperm concentration $< 15 \times 10^6$/mL) and case group 2 (104 idiopathic infertile men with sperm concentration $15–20 \times 10^6$/mL).
(2) Control group: 342 normozoospermia without any history of primary or secondary infertility and with semen count more than 20×10^6/mL, and mean age 32.16 ± 3.26, were randomly enrolled from the same hospital in this case-control study. All controls were healthy men with normal reproductive function and confirmed to have healthy babies 6–8 months later.

Semen assessments

Each male donated 1 mL (case cohort) or 0.5 mL (control cohort) of semen that was obtained by masturbation after at least 4 days of abstinence; this abstinence period was based on the standard protocol used in the collaborating infertility clinic. Each ejaculate was subjected to a classical seminal analysis approximately 30 min after liquefaction based on the recommendations and semen evaluation protocols and standards of the World Health Organization (2010); parameters analyzed included sperm concentration and sperm motility.

Isolation of DNA, SNP selection, and genotyping

All 735 participants donated a 3–5-mL blood sample and genomic DNA was isolated from samples using the phenol/chloroform method. We used resequencing to carry out systematic screening in the promoter and all the exon regions of the UHRF1 gene. The sequences of the PCR primers are given

in Additional file 1: Table S1. PCR was carried out in a 15-μL reaction mixture containing 10 ng of DNA, 10 pmol of each primer, 2.5 mM $MgCl_2$, 0.2 mM dNTP and 0.25 U Taq DNA polymerase. All reactions had an initial denaturation step of 3 min at 94 °C, followed by 35 cycles of 94 °C for 30 sec denaturation, annealing at the specific annealing Tm for 30 sec and 72 °C for 1 min, and finally at 72 °C for 10 min on a Gene Amp PCR system 9700 (Applied Biosystems, Foster City, CA). Preparation of DNA for sequencing included incubation of PCR products with 0.1 U of shrimp alkaline phosphatase (Roche, Basel, Switzerland) and 0.5 U of exonuclease I (New England Biolabs Inc., Beverly, MA) at 37 °C for 45 min, followed by heat inactivation at 85 °C for 20 min to remove primers. Cycle sequencing was carried out using a volume of 10 mL containing 50–100 ng of concentrated PCR product DNA purified in a 96-well filtration plate (Millipore, Bedford, USA) with 3.2 pmol of either sense or antisense primer in the GeneAmp 9700 thermocycler (PE Applied Biosystems, Perkin-Elmer) and a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Perkin-Elmer). The sequences were analyzed in an ABI PRISM model 3700 DNA Sequencer (PE Applied Biosystems, Perkin-Elmer) to determine the genotypes of variation at the same position on both forward and reverse sequences.

Plasmid constructs: promoter reporter vectors

The wild-type 1742-bp fragment of the human UHRF1 promoter region (− 2091 to − 349, relative to the transcriptional start codon) and its two mutant forms were generated by de novo chemical synthesis. Each mutant form contains one SNP (-1615C>T-C, and -1562A>G-A). The constructions including the SNPs were confirmed by sequencing. Then, the three fragments were amplified using the following polymerase chain reaction (PCR) primers: the Kpn I site-linked primer, 5′-TTTCTCTATCGATAGGTACCGAGACTGAGTTTTGCTTTTGTTGC-3′, as a forward primer and the Xho I site-linked primer, 5′-CTTAGATCGCAGATCTCGAGGGGAGTTGCGGGGCCGCAGGAG-3′, as a reverse primer. Finally, these fragments were cloned to the GV238 luciferase vector (GeneChem).

Transfection and luciferase activity analysis

The 293T cells were cultured in Dulbecco Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C under 5% CO_2. The cells were seed on 96-well plates 24 hours before transfection.

Luciferase assay was performed in order to study the effects of SNPs on the UHRF1 promoter activity. These wild type or mutated constructs were transiently transfected into 293T cells using X-tremeGENE HP transfection reagent (Roche, Sweden) according to the technical guide of product. The cells were collected 48 h after transfection and assayed for firefly and Renilla luciferase expression using the Dual-Luciferase Reporter Assay System (Promega) to detect the relative luciferase activity and reflect the transcriptional activity of the promoter. Renilla luciferase activities were normalized to firefly luciferase activity.

Prediction of the functional elements of the 5′-flanking region

The JASPAR database (http://jaspar.genereg.net/) and Promoter Scan (http://www-bimas.cit.nih.gov/molbio/proscan/) were used to predict transcription factors (TFs).

Statistics

All experiments were repeated at least three times. Data from the luciferase assay are expressed as the mean ± standard deviation. Statistical analyses were carried out using SPSS version 22.0 software (SPSS, Inc., Chicago, IL, USA). Genotypic or allele frequencies between cases and controls were compared using the χ^2 test. A haplotype block was inferred from analysis of linkage disequilibrium [25] between polymorphisms using Haploview 4.2, assuming a scheme based on the representation of the confidence interval of the normalized linkage disequilibrium coefficient (D'). The Chi-squared test was used to analyze the frequencies of the obtained haplotypes between groups [31]. The odds ratio (OR) and their 95% confidence intervals (CI) were estimated for the effects of the alleles. Analysis of variance was used to compare differences in means between the variants and their controls. $P < 0.05$ was considered to indicate a statistically significant difference. Data were log-transformed before analysis of variance to satisfy the equal variances assumption.

Results

Identification of UHRF1 variants in patients

Twenty-four SNPs in the UHRF1 gene were identified in 342 normozoospermic men and 393 oligozoospermia patients by sequencing. The locations of these 24 variants within the UHRF1 are presented in Fig. 1. The allele and genotype frequencies determined in the analysis of UHRF1 genetic variants are shown in Table 1. The 24 SNPs, including nine SNPs in the promoter region, three SNPs in 5′ untranslated regions, five SNPs in introns, and seven SNPs in exons were identified. Moreover, eight novel SNPs were detected. Four were in the UHRF1 promoter region and the other four were in introns and have not been reported as genetic polymorphisms in the public database dbSNP135 (www.ncbi.nlm.nih.gov/projects/SNP/) or

1000 Genome Project dataset (www.internationalgenome.org/home). Additionally, as shown in Table 1, seven synonymous variants were detected in four exons.

To analyze the combined effect of UHRF1 SNPs on oligozoospermia development, we investigated linkage disequilibrium (LD) [25] for all possible two-way comparisons among all SNPs in UHRF1 (Fig. 2). However, we found that the genotypic distribution of rs2602713 was not in Hardy-Weinberg equilibrium. When analyzing the LD and common haplotype patterns of all 15 SNPs (among all 24 SNPs, 8 were only found in healthy controls or patients, and rs2602713 was not tested), to search for systematically novel associations and validate our results for the variants within UHRF1, we identified one haplotype block in which rs4807663, rs4807664, rs2656927, rs375487487, rs8103849, a new SNP (chr19:4909640), and rs557510777 in the UHRF1 gene showed strong relationships ($|D'| > 0.8$) (Fig. 2). Particularly, the CTCCCCC ($P = 0.012$) and CTCCGCC ($P = 0.012$) haplotypes were associated with oligozoospermia.

In the case-control study, we evaluated the genotype frequencies of 15 SNPs in allele and genotype frequency analysis in 342 normal men and 393 patients with oligozoospermia in the UHRF1 gene (Table 2). Adjusted ORs and 95% CIs for the associations between idiopathic male infertility and 15 SNPs are presented in Table 2. The distribution of polymorphisms was in Hardy–Weinberg equilibrium. rs2656927 ($P = 0.014$) and rs8103849 ($P < 0.001$) were found to significantly differ between case group 1 and fertile men. Genotypes and alleles of the other SNPs in the UHRF1 gene showed no significant association with infertility.

Association of gene polymorphisms with semen quality variables

The semen density among fertile subjects was $62.22 \pm 1.36 \times 10^6$/mL, while that in case group was $10.43 \pm 0.27 \times 10^6$/mL. As shown in Table 2, we found a significant difference only between case group 1 and fertile men. Thus, we analyzed the effects of the 15 genetic variations on semen quality variables in case group 1 using SPSS software (Table 3). We compared the association of sperm parameters (sperm density, progressively motile sperm (PMS), non-progressively motile sperm (NMS), and PMS + NMS) in patients with oligozoospermia with 15 SNPs genotypes. The results showed patients with the CG genotype of rs8103849 had significantly higher sperm density ($P < 0.05$) than those with the wild-type genotypes. Additionally, the CG genotype of rs8103849 carriers showed more progressively motile sperm ($P = 0.049$), non-progressively motile sperm ($P = 0.012$), and PMS + NMS ($P = 0.034$) compared with controls.

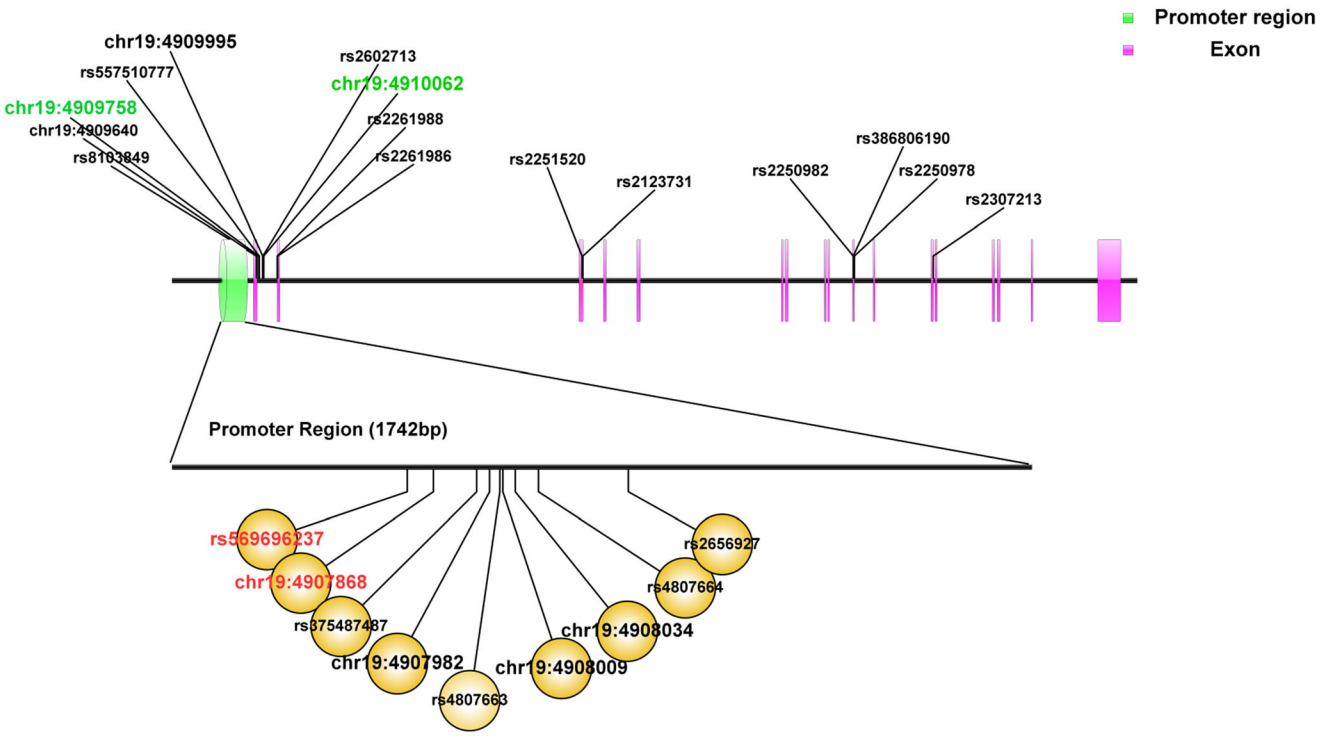

Fig. 1 Genomic structure and locations of polymorphic sites in the human UHRF1 gene. This gene is on chromosome 19p13.3 and contains 17 exons. We carried out a systematic study of the promoter, exon, and intron regions of UHRF1 by direct sequencing and detected 24 SNPs. We selected 16 SNPs with high allele frequencies (> 5%) for allele and genotype frequency analysis. Eight SNPs (highlighted) were only detected in cases or controls, 2 SNPs (marked with red color) were only detected in patients and were screened and tested in the luciferase assay, and 2 SNPs (marked in green color) in intron regions were only detected in patients and were not further tested

Table 1 Summary of genetic variations detected in the UHRF1 gene

Position	NG_033256.2position	dbSNP	Sequence variants	Codon change	Amino acid change
Promoter region	Chr19:4907815	rs569696237	-1615C>T		
Promoter region	Chr19:4907868	New	-1562A>G		
Promoter region	Chr19:4907956	rs375487487	-1474C>A		
Promoter region	Chr19:4907982	New	-1448G>A		
Promoter region	Chr19:4908003	rs4807663	-1427C>T		
Promoter region	Chr19:4908009	New	-1421G>A		
Promoter region	Chr19:4908034	New	-1396C>G		
Promoter region	Chr19:4908081	rs4807664	-1349T>G		
Promoter region	Chr19:4908263	rs2656927	-1167C>T		
5′UTR	Chr19:4909617	rs8103849	-49C>G		
5′UTR	Chr19:4909640	New	-26C>T		
Intron	Chr19:4909758	New	-11+103C>T		
Intron	Chr19:4909790	rs557510777	-11+135C>T		
Intron	Chr19:4909995	New	-11+340C>T		
Intron	Chr19:4910009	rs2602713	-11+354A>C		
Intron	Chr19:4910062	New	-11+407C>G		
5′UTR	Chr19:4910877	rs2261988	-9G>T		
Exon 2	Chr19:4910912	rs2261986	c.27C>T	GAC>GAT	Asp9Asp
Exon 3	Chr19:4929401	rs2251520	c.333T>C	GGT>GGC	Gly111Gly
Exon 3	Chr19:4929461	rs2123731	c.393A>G	GAA>GAG	Glu131Glu
Exon 10	Chr19:4945902	rs2250982	c.1347A>C	ATA>ATC	Ile449Ile
Exon 10	Chr19:4945902-4945905	rs386806190	c.1347_1350delACA CinsCCAT	ACAC>CCAT	Ile449_His450=
Exon 10	Chr19:4945962	rs2250978	c.1407T>C	GAT>GAC	Asp469Asp
Exon 12	Chr19:4950758	rs2307213	c.1665C>T	TAC>TAT	Tyr555Try

Effect of SNPs on binding of transcriptional factors

As shown in Table 1, four variants were detected only in patients, with two in the promoter region and two in introns. We predicted that the two patient-only mutants in the promoter affect TF binding and UHRF1 expression. The TFs which bind the UHRF1 promoter region (− 1921 to + 145) were previously reported [32]. We evaluated whether the SNPs in the UHRF1 promoter region affect the interaction of TFs and *cis*-elements in the UHRF1 promoter. According to our analysis based on the JASPAR program, these two patient-only SNPs may bind to 21 TFs (Additional file 2: Table S2). Many

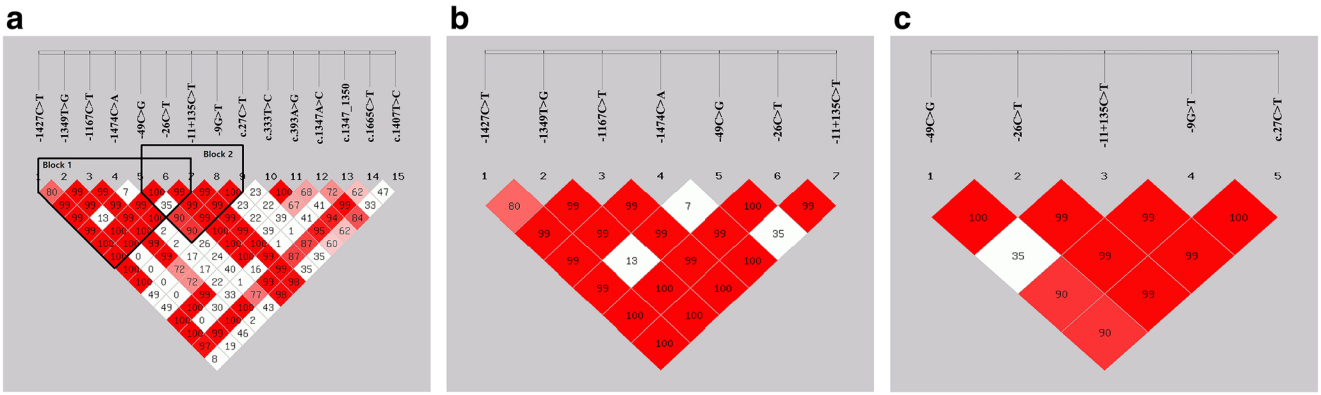

Fig. 2 Estimates of linkage disequilibrium (LD) [25] statistics between all pairs of markers. The numbers in the diamond-shaped cells are values (× 100). The shades of pink colors from white to red in the diamond-shaped cells correspond to values from 0 to 1. **a** All detected SNPs in LD in cases (sperm density < 20 × 10^6/mL) and controls (sperm density ≥ 20 × 10^6/mL). **b** Block of -1427C>T, -1349T>G, -1167C>T, -1474C>A, -49C>G, -26C>T, and -11+135C>T in LD in cases (sperm density < 20 × 10^6/mL) and controls (sperm density ≥ 20 × 10^6/mL). **c** Block of -49C>G, -26C>T, -11+135C>T, -9G>T, and c.27C>T in LD in cases (sperm density < 20 × 10^6/mL) and controls (sperm density ≥ 20 × 10^6/mL)

Table 2 Allele and genotype frequencies in UHRF1 genetic variant analysis in men with oligozoospermia (n = 393) and healthy controls (n = 342)

NG_033256.2 position	RS ID	Group	Number of genotypes and genotype frequency (Freq.)	P value	Number of alleles and allele frequency (Freq.)	OR (95% CI)
Chr19:4908003	rs4807663		CC CT		C T	
		Case 1[a]	283 (0.979) 6 (0.021)	0.557	572 (0.990) 6 (0.010)	0.70 [0.213–2.312]
		Case 2[b]	104 (1.000) 0 (0.000)	0.215	208 (1.000) 0 (0.000)	-
		Control	337 (0.985) 5 (0.015)		679 (0.993) 5 (0.007)	
Chr19:4908081	rs4807664		GT TT		G T	
		Case 1[a]	20 (0.069) 269 (0.931)	0.117	20 (0.035) 558 (0.965)	1.72 [0.859–3.427]
		Case 2[b]	3 (0.029) 101(0.971)	0.573	3 (0.014) 205 (0.986)	0.70 [0.196–2.470]
		Control	14 (0.041) 328 (0.959)		14 (0.020) 670 (0.980)	
Chr19:4908263	rs2656927		CC CT		C T	
		Case 1[a]	289 (1.000) 0 (0.000)	*0.014*	578 (1.000) 0 (0.000)	-
		Case 2[b]	103 (0.990) 1 (0.010)	0.465	207 (0.995) 1 (0.005)	2.14 [0.262–17.498]
		Control	335 (0.980) 7 (0.020)		677 (0.990) 7 (0.010)	
Chr19:4907956	rs375487487		AC CC		A C	
		Case 1[a]	14 (0.048) 275 (0.952)	0.209	14 (0.024) 564 (0.976)	1.67 [0.737–3.780]
		Case 2[b]	3 (0.029) 101 (0.971)	0.983	3 (0.014) 205 (0.986)	0.99 [0.269–3.618]
		Control	10 (0.029) 332 (0.971)		10 (0.015) 674 (0.985)	
Chr19:4909617	rs8103849		CC CG		C G	
		Case 1[a]	273 (0.945) 16 (0.055)	*< 0.001*	562 (0.972) 16 (0.028)	3.01 [1.704–5.320]
		Case 2[b]	88 (0.846) 16 (0.154)	0.921	192 (0.923) 16 (0.077)	1.03 [0.575–1.839]
		Control	288 (0.842) 54 (0.158)		630 (0.921) 54 (0.079)	
Chr19:4909640	New		CC CT		C T	
		Case 1[a]	289 (1.000) 0 (0.000)	0.065	578 (1.000) 0 (0.000)	-
		Case 2[b]	104 (1.000) 0 (0.000)	0.268	208 (1.000) 0 (0.000)	-
		Control	338 (0.988) 4 (0.012)		680 (0.994) 4 (0.006)	
Chr19:4909790	rs557510777		CC CT		C T	
		Case 1[a]	284 (0.983) 5 (0.017)	0.171	573 (0.991) 5 (0.009)	0.336 [0.065–1.738]
		Case 2[b]	104 (1.000) 0 (0.000)	0.434	208 (1.000) 0 (0.000)	-
		Control	340 (0.994) 2 (0.006)		682 (0.997) 2 (0.003)	
Chr19:4910877	rs2261988		GG GT TT		G T	
		Case 1[a]	110 (0.873) 15 (0.119) 1 (0.008)	0.636	235 (0.933) 17 (0.067)	1.31 [0.748–2.292]
		Case 2[b]	75 (0.798) 17 (0.181) 2 (0.021)	0.556	167 (0.888) 21 (0.112)	0.75 [0.445–1.275]
		Control	288 (0.845) 47 (0.138) 6 (0.018)		623 (0.913) 59 (0.087)	
Chr19:4910912	rs2261986		CC CT TT		C T	
		Case 1[a]	110 (0.873) 15 (0.119) 1 (0.008)	0.636	235 (0.933) 17 (0.067)	1.31 [0.748–2.292]
		Case 2[b]	75 (0.798) 17 (0.181) 2 (0.021)	0.556	167 (0.888) 21 (0.112)	0.75 [0.445–1.275]
		Control	288 (0.845) 47 (0.138) 6 (0.018)		623 (0.913) 59 (0.087)	
Chr19:4929401	rs2251520		CC CT TT		C T	
		Case 1[a]	4 (0.032) 19 (0.151) 103 (0.817)	0.444	27 (0.107) 225 (0.893)	0.957 [0.601–1.523]
		Case 2[b]	1 (0.011) 15 (0.160) 78 (0.830)	0.721	17 (0.090) 171 (0.910)	0.79 [0.456–1.377]
		Control	6 (0.018) 64 (0.188) 271 (0.795)		76 (0.111) 606 (0.889)	
Chr19:4929461	rs2123731		AA AG GG		A G	
		Case 1[a]	103 (0.817) 19 (0.151) 4 (0.032)	0.444	225 (0.893) 27 (0.107)	1.05 [0.656–1.664]
		Case 2[b]	79 (0.840) 14 (0.149) 1 (0.011)	0.598	172 (0.915) 16 (0.085)	1.35 [0.766–2.372]
		Control	271 (0.795) 64 (0.188) 6 (0.018)		606 (0.889) 76 (0.111)	
Chr19:4945902	rs2250982		AA AC		A C	
		Case 1[a]	125 (0.992) 1 (0.008)	0.929	251 (0.996) 1 (0.004)	1.11 [0.115–10.711]
		Case 2[b]	94 (1.000) 0 (0.000)	0.362	188 (1.000) 0 (0.000)	-
		Control	338 (0.991) 3 (0.009)		679 (0.996) 3 (0.004)	

Table 2 (continued)

NG_033256.2 position	RS ID	Group	Number of genotypes and genotype frequency (Freq.)	*P* value	Number of alleles and allele frequency (Freq.)	OR (95% CI)
Chr19:4945902-4945905	rs386806190		AA CC		A C	
		Case 1[a]	17 (0.135) 109 (0.865)	0.237	34 (0.135) 218 (0.865)	1.46 [0.936–2.264]
		Case 2[b]	6 (0.064) 88 (0.936)	0.322	12 (0.064) 176 (0.936)	0.64 [0.336–1.204]
		Control	33 (0.097) 308 (0.903)		66 (0.097) 616 (0.903)	
Chr19:4945962	rs2250978		CC CT TT		C T	
		Case 1[a]	123 (0.976) 0 (0.000) 3 (0.024)	0.172	246 (0.976) 6 (0.024)	0.36 [0.116–1.139]
		Case 2[b]	94 (1.000) 0 (0.000) 0 (0.000)	0.573	188 (1.000) 0 (0.000)	-
		Control	337 (0.988) 2 (0.006) 2 (0.006)		676 (0.991) 6 (0.009)	
Chr19:4950758	rs2307213		CC CT TT		C T	
		Case 1[a]	55 (0.437) 57 (0.452) 14 (0.111)	0.350	167 (0.663) 85 (0.337)	1.23 [0.905–1.660]
		Case 2[b]	43 (0.457) 38 (0.404) 13 (0.138)	0.198	124 (0.660) 64 (0.340)	1.21 [0.861–1.696]
		Control	124 (0.364) 172 (0.504) 45 (0.132)		420 (0.616) 262 (0.384)	

[a] Case 1 (case group 1): idiopathic infertile men with sperm concentration < 15×10^6/mL

[b] Case 2 (case group 2): idiopathic infertile men with sperm concentration (15–20) × 10^6/mL

binding sites for transcription factors, including SP1, Meis1, GATA2, TFAP2A, and sterol regulatory element-binding transcription factor 2 (SREBF2), among others, were predicted to be present in these two SNP regions. These results suggest that the SNPs affect UHRF1 expression by disturbing the interactions between the promoter and diverse TFs.

Effects of SNPs on UHRF1 promoter activity

To investigate whether the two mutants (-1615C>T-C and -1562A>G-A) in the promoter region affect UHRF1 promoter activity, the -1615C>T-C and -1562A>G-A promoter regions and UHRF1 promoter were synthesized and cloned into the GV238 vector, resulting in a GV238-promoter, -1615C>T-C, and -1562A>G-A, which were transfected into 293T cells. The results indicated that the luciferase activity of the plasmid GV238-promoter was greater than that of plasmids -1615C>T-C and -1562A>G-A in 293T cells ($P < 0.05$; Fig. 3). These results indicate that the two mutations in the promoter region affected the promoter activity of the Chinese male UHRF1 gene by repressing TF interactions.

Discussion

In this study, we investigated whether genetic variations in UHRF1 were associated with oligozoospermia. We scored genetic variations in a large case-control sample (393 oligozoospermia cases, 342 healthy controls). A total of 24 SNPs in the UHRF1 gene were detected. Both rs2656927 and rs8103849 were more frequent in control subjects, suggesting that they protect against infertility. Consistent with this hypothesis, rs8103849 was found to be associated with sperm density, progressively motile sperm, non-progressively motile sperm, and PMS + NMS. Importantly, two SNPs (-1615C>T-C and -1562A>G-A allele) were detected only in patients. In vitro analysis revealed that the two variants reduced UHRF1 promoter activity. Given that deficiency of the UHRF1 gene severely damages mouse spermatogenesis [30], our results strongly suggest that the two genetic variations in UHRF1 result in oligozoospermia by lowering UHRF1 expression. Studies of the SNPs in UHRF1 genes have not been reported previously.

Aberrant sperm DNA methylation likely occurs because of low expression of UHRF1, as UHRF1 is currently the only protein identified to recruit DNMT1 to hemimethylated CG sites. Knockout of UHRF1 significantly reduced the overall level of DNA methylation in male germ cells [30], oocytes [33], and diverse somatic cells, whereas DNA methylation at imprinting control regions is particularly sensitive to UHRF1 deficiency [33]. Therefore, the inheritance of these SNPs in UHRF1 may result in hypomethylation of the genome when the epigenetic pattern is established in early development. The persistence of altered DNA methylation patterns in the germline may, therefore, increase the risk of infertility. The association between these genetic variations in UHRF1 and the risk of aberrant methylation of imprinted genes should be further evaluated in oligozoospermia. Although genetic variations in DNMT1 are associated with male infertility [14, 17], no studies have evaluated whether the SNPs in DNMT1 are associated with altered DNA methylation. However, Louie et al. recently

Table 3 Association between different genotypes of UHRF1 and semen quality traits of Chinese infertile males with sperm density < 15×10^6/mL (mean ± standard error)

SNP	Genotype	Case group 1[a]			
		Sperm density (million/mL)	Progressively motile sperm (%)	Non-progressively motile sperm (%)	PMS + NMS (%)[b]
rs4807663	CC	8.23 ± 0.25	5.72 ± 0.38	8.72 ± 0.40	14.35 ± 0.76
	CT	7.03 ± 1.80	4.60 ± 1.78	7.80 ± 2.33	12.40 ± 4.03
rs4807664	TT	8.12 ± 0.26	5.64 ± 0.40	8.59 ± 0.41	14.15 ± 0.78
	GT	9.31 ± 0.89	6.42 ± 1.23	10.21 ± 1.45	16.63 ± 2.58
rs2656927	CC	8.20 ± 0.25	5.70 ± 0.38	8.70 ± 0.40	14.32 ± 0.75
	CT	0.00	0.00	0.00	0.00
rs375487487	CC	8.19 ± 0.26	5.75 ± 0.39	8.76 ± 0.41	14.42 ± 0.78
	AC	8.43 ± 1.13	4.71 ± 1.35	7.64 ± 1.45	12.36 ± 2.67
rs8103849	CC	7.98 ± 0.26	5.47 ± 0.38	8.43 ± 0.40	13.86 ± 0.76
	CG	12.13 ± 0.35[A]**	9.73 ± 1.90[A]*	13.47 ± 2.06[A]*	21.75 ± 3.84[A]*
chr19:4909640	CC	8.20 ± 0.25	5.70 ± 0.38	8.70 ± 0.40	14.32 ± 0.75
	CT	0	0	0	0
rs557510777	CC	8.20 ± 0.25	5.70 ± 0.38	8.68 ± 0.40	14.29 ± 0.76
	CT	8.44 ± 3.02	5.80 ± 1.80	9.80 ± 3.25	15.60 ± 4.98
rs2250982	AA	11.80 ± 0.14	7.45 ± 0.66	10.29 ± 0.66	17.52 ± 1.27
	AC	14.00	2.00	4.00	6.00
rs386806190	CC	11.76 ± 0.15	7.58 ± 0.74	10.26 ± 0.71	17.67 ± 1.39
	AA	12.18 ± 0.35	6.35 ± 1.23	10.13 ± 1.70	15.88 ± 2.88
rs2261988	GG	11.72 ± 0.15	6.92 ± 0.68	9.74 ± 0.67	16.56 ± 1.29
	GT	12.40 ± 0.36	11.43 ± 2.24	14.07 ± 2.30	23.80 ± 4.47
	TT	14.00	4.00	10.00	14.00
rs2261986	CC	11.72 ± 0.15	6.92 ± 0.68	9.74 ± 0.67	16.56 ± 1.29
	CT	12.40 ± 0.36	11.43 ± 2.24	14.07 ± 2.30	23.80 ± 4.47
	TT	14.00	4.00	10.00	14.00
rs2251520	TT	11.83 ± 0.16	7.00 ± 0.72	9.77 ± 0.70	16.68 ± 1.37
	CT	11.89 ± 0.38	8.69 ± 1.54	12.00 ± 2.08	19.47 ± 3.50
	CC	11.00 ± 0.00	12.75 ± 4.19	15.00 ± 2.89	27.75 ± 7.08
rs2123731	AA	11.83 ± 0.16	7.00 ± 0.72	9.77 ± 0.70	16.68 ± 1.37
	AG	11.89 ± 0.38	8.69 ± 1.54	12.00 ± 2.08	19.47 ± 3.50
	GG	11.00 ± 0.00	12.75 ± 4.19	15.00 ± 2.89	27.75 ± 7.08
rs2250978	CC	11.82 ± 0.14	7.45 ± 0.67	10.20 ± 0.66	17.42 ± 1.28
	CT	0	0	0	0
	TT	11.67 ± 0.88	6.00 ± 3.00	11.67 ± 4.41	17.67 ± 7.31
rs2307213	CC	11.82 ± 0.22	7.52 ± 1.12	9.85 ± 1.04	17.37 ± 2.08
	CT	11.88 ± 0.21	7.52 ± 0.95	10.56 ± 0.96	17.75 ± 1.86
	TT	11.57 ± 0.34	6.57 ± 1.00	10.54 ± 1.61	16.36 ± 2.57

Results are presented as the mean ± SD. *PMS*, progressively motile sperm; *NMS*, non-progressively motile sperm

[a] Idiopathic infertile men with sperm concentration < 15×10^6 /mL

[b] Grade of sperm movement according to WHO [2010] criteria

[A] Variant vs. wild type

*$P < 0.05$

**$P < 0.001$

reported that the MTHFR C677T genotype, which reduces synthesis of the methyl donor, was not predictive of altered DNA methylation in spermatozoa [34].

Considering that the DNA-methylation-maintaining role of DNMT1-UHRF1 is completed during S phase of the cell cycle [35], high expression of UHRF1 not only in proliferating

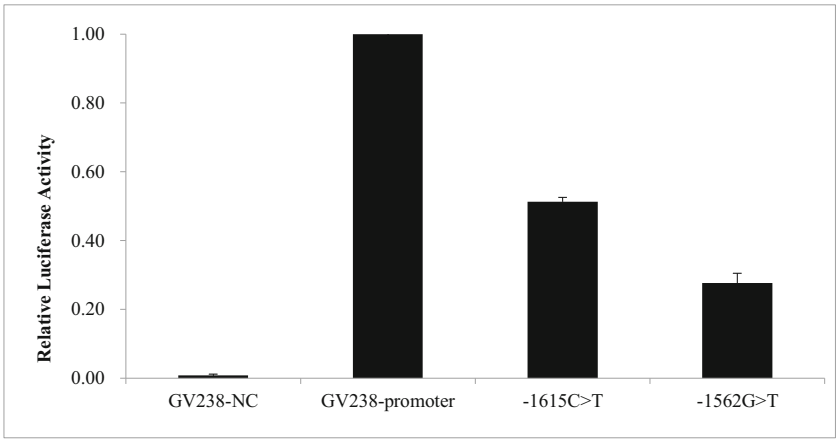

Fig. 3 Function of two SNPs in the UHRF1 promoter region. Transactivation activity of WT and two UHRF1 mutant promoters. The GV238 and WT or mutant UHRF1 promoters were co-transfected into 293T cells. UHRF1 promoter activity was analyzed by luciferase assay. NC: GV238 empty vector was transfected into 293T cells as a negative control. Fold change is shown as the ratio of WT or mutants to the average of NC

spermatogonia but also in meiotic spermatocytes and differentiating spermatids which were not proliferating [29] suggests that UHRF1 has different functions throughout spermatogenesis. Accordingly, these genetic variations of UHRF1 may contribute to the development of male infertility via other mechanisms besides DNA methylation, given that the protein contains multiple functional domains. For example, UHRF1 regulates DNA repair [36, 37], which depends on intrinsic E3 ligase activity in the Ring domain of UHRF1 [37]. Impaired DNA repair has been widely found to damage mouse spermatogenesis [38–40] and has been detected in oligozoospermia [41, 42]. Therefore, we predict that the fraction of sperm cells bearing double-strain breaks is significantly increased in the UHRF1-SNPs-positive oligozoospermic semen compared with that in semen from normozoospermic men. The hypothesis requires further analysis.

Currently, it remains unknown how these SNPs affect UHRF1 promoter activity. While numerous studies have been conducted to evaluate the function of UHRF1, the transcriptional regulation of UHRF1 has not been widely examined. The UHRF1 promoter region contains 5 YY1-binding motifs, and the occupation of YY1 at these sites represses transcription of the UHRF1 gene [32]. Interestingly, YY1 likely plays a role in maintaining the unmethylated status of these DMRs during spermatogenesis [43], supporting the hypothesis that YY1 regulates DNA methylation by down-regulating UHRF1.

Our analysis based on JASPAR suggested that the two SNPs (-1615C>T-C and -1562A>G-A) can bind to 21 TFs. Although the roles of most of these factors have not been reported in spermatogenesis, five (Meis1, GATA2, TFAP2A, SP1, and SREBF2) of these factors likely play important roles in SSCs [44–47]. Meis1 is a major transcriptional regulator controlling SSC-specific gene expression [48]. SP1 was reported to regulate the expression of SOHLH1 [49], which is required for the differentiation of SSC. NFIX silencing led to enhanced cell proliferation and DNA synthesis and reduced the early apoptosis of human SSCs [50]. These transcriptional factors may make different contributions to the self-renewal and/or differentiation of SSCs via regulating expression of UHRF1. Interestingly, p63 and p73, two members of p53 the family, are important for controlling the expression multiple genes throughout spermatogenesis [51, 52]. They likely contribute to the high expression of UHRF1 which was previously reported in differentiating spermatids [29]. Our study indicates that mutations in the UHRF1 gene are associated with male infertility, providing insight into the origin of widely altered DNA methylation in sperm from infertile men. Considering that some endocrine-disrupting chemicals exert male reproductive toxicity by affecting DNA methylation [53, 54], studies are needed to explore whether mutations in UHRF1 mediate the differential response of humans to exposure to diverse environmental factors.

Conclusion

We found that two SNPs in the UHRF1 gene may be associated with oligozoospermia and two patient-only SNPs in the 5′ untranslated region may affect UHRF1 promoter activity. Our findings suggested that the UHRF1 gene is involved in the pathology of male infertility with oligozoospermia.

Authors' contributions WZ was responsible for the statistical analysis and wrote the manuscript. RL and JD contributed in conducting the study, designing the experiments, and writing, reviewing, and revising the manuscript. HS, BW, and JX acquired and managed the patients, collected the samples, and DNA preparation. TL, FZ, QC, and YB were responsible for DNA sequencing and analysis of promoter activity. All authors read and approved the final manuscript.

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Data availability The dbSNP and UCSC datasets generated and analyzed during the current study are available at http://genome.ucsc.edu/ and https://www.ncbi.nlm.nih.gov/snp/?term=. Other data generated and/or analyzed during this study are included in this article and its supplementary information files or are available on reasonable request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Abbreviations SSC, spermatogonial stem cell; DNMT1, DNA methyltransferase I; PHD, plant homeodomain; UHRF1, ubiquitin-like containing plant homeodomain (PHD) and Ring Finger 1; SIPPR, Shanghai Institute of Planned Parenthood Research; WHO, World Health Organization; TF, transcription factor; SPSS, Statistical Package for the Social Sciences; OR, odds ratio; CI, confidence intervals; ANOVA, analysis of variance; LD, linkage disequilibrium; PMS, progressively motile sperm; NMS, non-progressively motile sperm; SNP, single-nucleotide polymorphism; UTR, untranslated region; SP1, specificity protein 1; SREBF2, sterol regulatory element-binding transcription factor 2

References

1. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. Fertil Steril. 2002;77(5):873–82.
2. O'Flynn O'Brien KL. A.C. Varghese, and A. Agarwal, The genetic causes of male factor infertility: a review. Fertil Steril. 2010;93(1): 1–12.
3. Qin H, Wang R, Pang X, Wei Y, Yang F, Wang J. Association of GRP78 promoter polymorphisms and serum GRP78 level with risk of asthenozoospermia. J Assist Reprod Genet. 2018;35(12):2223–31.
4. Sato Y, Hasegawa C, Tajima A, Nozawa S, Yoshiike M, Koh E, et al. Association of TUSC1 and DPF3 gene polymorphisms with male infertility. J Assist Reprod Genet. 2018;35(2):257–63.
5. Giacone, F., et al., Epigenetics of male fertility: effects on assisted reproductive techniques. World J Mens Health, 2018.
6. Kubo N, et al. DNA methylation and gene expression dynamics during spermatogonial stem cell differentiation in the early postnatal mouse testis. BMC Genomics. 2015;16:624.
7. Rajender S, Avery K, Agarwal A. Epigenetics, spermatogenesis and male infertility. Mutat Res. 2011;727(3):62–71.
8. Zamudio N, Barau J, Teissandier A, Walter M, Borsos M, Servant N, et al. DNA methylation restrains transposons from adopting a chromatin signature permissive for meiotic recombination. Genes Dev. 2015;29(12):1256–70.
9. Pan B, Chao H, Chen B, Zhang L, Li L, Sun X, et al. DNA methylation of germ-cell-specific basic helix-loop-helix (HLH) transcription factors, Sohlh2 and Figlalpha during gametogenesis. Mol Hum Reprod. 2011;17(9):550–61.
10. Camprubi C, et al. Semen samples showing an increased rate of spermatozoa with imprinting errors have a negligible effect in the outcome of assisted reproduction techniques. Epigenetics. 2012;7(10):1115–24.
11. McSwiggin HM, O'Doherty AM. Epigenetic reprogramming during spermatogenesis and male factor infertility. Reproduction. 2018;156(2):R9–R21.
12. Marques CJ, João Pinho M, Carvalho F, Bièche I, Barros A, Sousa M. DNA methylation imprinting marks and DNA methyltransferase expression in human spermatogenic cell stages. Epigenetics. 2011;6(11):1354–61.
13. Santi D, de Vincentis S, Magnani E, Spaggiari G. Impairment of sperm DNA methylation in male infertility: a meta-analytic study. Andrology. 2017;5(4):695–703.
14. Cheng P, Chen H, Zhang RP, Liu SR, Zhou-Cun A. Polymorphism in DNMT1 may modify the susceptibility to oligospermia. Reprod Biomed Online. 2014;28(5):644–9.
15. A, Z.C., et al., Single nucleotide polymorphism C677T in the methylenetetrahydrofolate reductase gene might be a genetic risk factor for infertility for Chinese men with azoospermia or severe oligozoospermia. Asian J Androl, 2007. 9(1): p. 57–62.
16. Huang JX, Scott MB, Pu XY, Zhou-Cun A. Association between single-nucleotide polymorphisms of DNMT3L and infertility with azoospermia in Chinese men. Reprod Biomed Online. 2012;24(1): 66–71.
17. Tang Q, et al. Idiopathic male infertility and polymorphisms in the DNA methyltransferase genes involved in epigenetic marking. Sci Rep. 2017;7(1):11219.
18. Gowher H, Jeltsch A. Mammalian DNA methyltransferases: new discoveries and open questions. Biochem Soc Trans. 2018;46(5): 1191–202.
19. Kurihara Y, Kawamura Y, Uchijima Y, Amamo T, Kobayashi H, Asano T, et al. Maintenance of genomic methylation patterns during preimplantation development requires the somatic form of DNA methyltransferase 1. Dev Biol. 2008;313(1):335–46.
20. Laurentino S, Borgmann J, Gromoll J. On the origin of sperm epigenetic heterogeneity. Reproduction. 2016;151(5):R71–8.
21. Qi S, Wang Z, Li P, Wu Q, Shi T, Li J, et al. Non-germ line restoration of genomic imprinting for a small subset of imprinted genes in ubiquitin-like PHD and RING finger domain-containing 1 (Uhrf1) null mouse embryonic stem cells. J Biol Chem. 2015;290(22):14181–91.
22. Liu X, et al. UHRF1 targets DNMT1 for DNA methylation through cooperative binding of hemi-methylated DNA and methylated H3K9. Nat Commun. 2013;4:1563.
23. Hopfner R, Mousli M, Jeltsch JM, Voulgaris A, Lutz Y, Marin C, et al. ICBP90, a novel human CCAAT binding protein, involved in the regulation of topoisomerase IIalpha expression. Cancer Res. 2000;60(1):121–8.
24. Ashraf W, Ibrahim A, Alhosin M, Zaayter L, Ouararhni K, Papin C, et al. The epigenetic integrator UHRF1: on the road to become a universal biomarker for cancer. Oncotarget. 2017;8(31):51946–62.
25. Abu-Alainin W, Gana T, Liloglou T, Olayanju A, Barrera LN, Ferguson R, et al. UHRF1 regulation of the Keap1-Nrf2 pathway in pancreatic cancer contributes to oncogenesis. J Pathol. 2016;238(3):423–33.
26. Gao SP, Sun HF, Li LD, Fu WY, Jin W. UHRF1 promotes breast cancer progression by suppressing KLF17 expression by hypermethylating its promoter. Am J Cancer Res. 2017;7(7): 1554–65.
27. Obata Y, Furusawa Y, Endo TA, Sharif J, Takahashi D, Atarashi K, et al. The epigenetic regulator Uhrf1 facilitates the proliferation and maturation of colonic regulatory T cells. Nat Immunol. 2014;15(6): 571–9.
28. Fujimori A, Matsuda Y, Takemoto Y, Hashimoto Y, Kubo E, Araki R, et al. Cloning and mapping of Np95 gene which encodes a novel nuclear protein associated with cell proliferation. Mamm Genome. 1998;9(12):1032–5.
29. Uemura T, Kubo E, Kanari Y, Ikemura T, Tatsumi K, Muto M. Temporal and spatial localization of novel nuclear protein NP95 in mitotic and meiotic cells. Cell Struct Funct. 2000;25(3):149–59.
30. Shirakawa T, Yaman-Deveci R, Tomizawa S, Kamizato Y, Nakajima K, Sone H, et al. An epigenetic switch is crucial for spermatogonia to exit the undifferentiated state toward a Kit-positive identity. Development. 2013;140(17):3565–76.
31. Barrett, J.C., Haploview: visualization and analysis of SNP genotype data. Cold Spring Harb Protoc, 2009. 2009(10): p. pdb ip71.

32. Kim KB, Son HJ, Choi S, Hahm JY, Jung H, Baek HJ, et al. H3K9 methyltransferase G9a negatively regulates UHRF1 transcription during leukemia cell differentiation. Nucleic Acids Res. 2015;43(7):3509–23.
33. Maenohara S, Unoki M, Toh H, Ohishi H, Sharif J, Koseki H, et al. Role of UHRF1 in de novo DNA methylation in oocytes and maintenance methylation in preimplantation embryos. PLoS Genet. 2017;13(10):e1007042.
34. Louie K, Minor A, Ng R, Poon K, Chow V, Ma S. Evaluation of DNA methylation at imprinted DMRs in the spermatozoa of oligozoospermic men in association with MTHFR C677T genotype. Andrology. 2016;4(5):825–31.
35. Zhang J, Gao Q, Li P, Liu X, Jia Y, Wu W, et al. S phase-dependent interaction with DNMT1 dictates the role of UHRF1 but not UHRF2 in DNA methylation maintenance. Cell Res. 2011;21(12):1723–39.
36. Mistry H, Tamblyn L, Butt H, Sisgoreo D, Gracias A, Larin M, et al. UHRF1 is a genome caretaker that facilitates the DNA damage response to gamma-irradiation. Genome Integr. 2010;1(1):7.
37. Zhang H, et al. A cell cycle-dependent BRCA1-UHRF1 cascade regulates DNA double-strand break repair pathway choice. Nat Commun. 2016;7:10201.
38. Pan H, et al. Ndrg3 gene regulates DSB repair during meiosis through modulation the ERK signal pathway in the male germ cells. Sci Rep. 2017;7:44440.
39. Forand A, Dutrillaux B, Bernardino-Sgherri J. Gamma-H2AX expression pattern in non-irradiated neonatal mouse germ cells and after low-dose gamma-radiation: relationships between chromatid breaks and DNA double-strand breaks. Biol Reprod. 2004;71(2):643–9.
40. Wu S, Hu YC, Liu H, Shi Y. Loss of YY1 impacts the heterochromatic state and meiotic double-strand breaks during mouse spermatogenesis. Mol Cell Biol. 2009;29(23):6245–56.
41. Derijck AA, van der Heijden G, Ramos L, Giele M, Kremer JA, de Boer P. Motile human normozoospermic and oligozoospermic semen samples show a difference in double-strand DNA break incidence. Hum Reprod. 2007;22(9):2368–76.
42. Zhang X, Ding M, Ding X, Li T, Chen H. Six polymorphisms in genes involved in DNA double-strand break repair and chromosome synapsis: association with male infertility. Syst Biol Reprod Med. 2015;61(4):187–93.
43. Kim JD, Kang K, Kim J. YY1's role in DNA methylation of Peg3 and Xist. Nucleic Acids Res. 2009;37(17):5656–64.
44. Carlomagno G, van Bragt M, Korver CM, Repping S, de Rooij DG, van Pelt A. BMP4-induced differentiation of a rat spermatogonial stem cell line causes changes in its cell adhesion properties. Biol Reprod. 2010;83(5):742–9.
45. Ehlermann J, Pfisterer P, Schorle H. Dynamic expression of Kruppel-like factor 4 (Klf4), a target of transcription factor AP-2alpha during murine mid-embryogenesis. Anat Rec A Discov Mol Cell Evol Biol. 2003;273(2):677–80.
46. Thomas K, Wu J, Sung DY, Thompson W, Powell M, McCarrey J, et al. SP1 transcription factors in male germ cell development and differentiation. Mol Cell Endocrinol. 2007;270(1–2):1–7.
47. Wang H, San Agustin JT, Witman GB, Kilpatrick DL. Novel role for a sterol response element binding protein in directing spermatogenic cell-specific gene expression. Mol Cell Biol. 2004;24(24):10681–8.
48. Sisakhtnezhad S, Heshmati P. Comparative analysis of single-cell RNA sequencing data from mouse spermatogonial and mesenchymal stem cells to identify differentially expressed genes and transcriptional regulators of germline cells. J Cell Physiol. 2018;233(7):5231–42.
49. Toyoda S, Yoshimura T, Mizuta J, Miyazaki J. Auto-regulation of the Sohlh1 gene by the SOHLH2/SOHLH1/SP1 complex: implications for early spermatogenesis and oogenesis. PLoS One. 2014;9(7):e101681.
50. Zhou F, Yuan Q, Zhang W, Niu M, Fu H, Qiu Q, et al. MiR-663a stimulates proliferation and suppresses early apoptosis of human spermatogonial stem cells by targeting NFIX and regulating cell cycle. Mol Ther Nucleic Acids. 2018;12:319–36.
51. Wang H, Yuan Q, Niu M, Zhang W, Wen L, Fu H, et al. Transcriptional regulation of P63 on the apoptosis of male germ cells and three stages of spermatogenesis in mice. Cell Death Dis. 2018;9(2):76.
52. Inoue S, Tomasini R, Rufini A, Elia AJ, Agostini M, Amelio I, et al. TAp73 is required for spermatogenesis and the maintenance of male fertility. Proc Natl Acad Sci U S A. 2014;111(5):1843–8.
53. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet. 2007;8(4):253–62.
54. Miao M, Zhou X, Li Y, Zhang O, Zhou Z, Li T, et al. LINE-1 hypomethylation in spermatozoa is associated with Bisphenol A exposure. Andrology. 2014;2(1):138–44.