Is BRD7 associated with spermatogenesis impairment and male infertility in humans? A case-control study in a Han Chinese population

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Abstract

**Background:** Bromodomain-containing protein 7 (BRD7), a member of the bromodomain-containing protein family, plays important roles in chromatin modification and transcriptional regulation. A recent model of Brd7-knockout mice presented azoospermia and male infertility, implying the potential role of BRD7 in spermatogenic failure in humans. This case-control study aimed to explore the association of the BRD7 gene with spermatogenic efficiency and the risk of spermatogenic defects in humans.

**Results:** A total of six heterozygous variants were detected in the coding and splicing regions of the BRD7 gene in patients with azoospermia. For each of four rare variants predicted to potentially damage BRD7 function, we further identified these four variants in oligozoospermia and normozoospermia as well. However, no difference in the allele and genotype frequencies of rare variants were observed between cases with spermatogenic failure and controls with normozoospermia; the sperm products of variant carriers were similar to those of noncarriers. Moreover, similar distribution of the alleles, genotypes and haplotypes of seven tag single nucleotide polymorphisms (tagSNPs) was observed between the cases with azoospermia and oligozoospermia and controls with normozoospermia; associations of tagSNP-distinguished BRD7 alleles with sperm products were not identified.

**Conclusions:** The lack of an association of BRD7-linked rare and common variants with spermatogenic failure implied a limited contribution of the BRD7 gene to spermatogenic efficiency and susceptibility to male infertility in humans.

**Keywords:** BRD7, Rare variant, tagSNP, Spermatogenic failure, Male infertility
Résumé

Contexte: Le bromodomaine contenant la protéine 7 (BRD7), un membre de la famille du bromodomaine contenant des protéines, joue des rôles importants dans la modification de la chromatine et la régulation transcriptionnelle. Un modèle récent de souris Brd7-knockout présentait une azoospermie et une infertilité mâle, ce qui implique un rôle potentiel de BRD7 dans l’altération de la spermatogenèse chez l’homme. Cette étude cas-témoins visait à explorer l’association du gène BRD7 avec l’efficacité de la spermatogenèse et le risque d’altérations spermatogéniques chez l’homme.

Résultats: Un total de six variants hétérozygotes ont été détectés dans les régions de codage et d’épissage du gène BRD7 chez les patients présentant une azoospermie. Pour chacun des quatre variants rares prédits pour potentiellement endommager la fonction BRD7, nous avons en outre identifié ces quatre variants dans l’oligozoospermie et la normozoospermie. Cependant, nous n’avons observé aucune différence dans les fréquences d’allèle et de génotype des variants rares entre les cas avec altérations de la spermatogenèse et les témoins avec normozoospermie ; les produits du sperme des porteurs de variants étaient semblables à ceux des non-porteurs. Par ailleurs, on a observé une distribution semblable des allèles, des génocopes et des haplotypes de sept polymorphismes simples de nucléotide de balise (tagSNPs) entre les cas avec azoospermie ou oligozoospermie et les témoins normozoospermiques ; aucune association n’a pas été identifiée entre les allèles BRD7 tagSNP-distingués et des produits du sperme.

Conclusion: L’absence d’association des variants rares liés à BRD7 et des variants communs liés à BRD7 avec les altérations de la spermatogenèse implique une contribution limitée du gène BRD7 à l’efficacité spermatogénique et à la susceptibilité à l’infertilité masculine chez l’homme.

Mots-Clés: BRD7, Variants rares, tagSNP, Altération de la spermatogenèse, Infertilité masculine.

Background

Infertility has been a major global public health issue and causes significant psychosocial stress for couples suffering from this condition [1]. It is estimated that approximately 15% of couples suffer from infertility worldwide, and approximately half of infertility cases are caused by male factors [2]. Male infertility due to oligozoospermia (OZ) and azoospermia (AZ) is a common and complex disease. It has been postulated that the cause of infertility in 10–15% of infertile patients with AZ and severe OZ involves genetic factors, and the relevance of genetic anomalies gradually increases with decreasing sperm count [3, 4]. Both chromosomal abnormalities and monogenic mutations could be directly responsible for spermatogenic failure, in which Klinefelter’s syndrome and azoospermia factor (AZF) microdeletion are the most common cytogenetic and molecular genetic causes of spermatogenic failure, respectively [3]. However, the aetiology of approximately 40% of males with spermatogenic failure remains elusive [4], suggesting the significance of further exploring genetic causes of the protein 7 (BRD7), a member of the bromodomain-containing protein family, is highly conserved during evolution and ubiquitously distributed in various tissues with high expression in the testes of humans [5]. A recent study reported Brd7 knockout, causing AZ, and complete arrest of spermatogenesis at step 13 in mice [6]. Compared with BRD7+/+ mice, BRD7−/− mice showed a decrease in testicular size and seminiferous tubule diameter [6]. Furthermore, BRD7−/− mice had morphologically abnormal round spermatids, elongating spermatids and denatured condensed spermatids with irregular head shapes and deformed acrosomes [6]. Remarkably, BRD7 expression in the testis was reduced significantly in patients with idiopathic AZ relative to men with normozoospermia (NZ) [6]. These findings suggest a vital role of the BRD7 gene in spermatogenesis. In this case, it would be interesting to determine whether the BRD7 gene is associated with the risk of spermatogenic failure and male infertility in humans. In the present study, we detected rare and common variants of BRD7 in 315 infertile patients with spermatogenic failure and 995 men with NZ. Our results implied a limited contribution of the BRD7 gene to susceptibility to spermatogenic failure and male infertility in humans.

Materials and methods

Participants

The sample size for the case-control study was calculated using QUANTO1.2 software (Jim Gauderman and John Morrison, USA). The parameters of the type I error rate and statistical power were set at 0.05 and 0.80, respectively. The evaluated sample size was at least 314 for the case group when the size ratio of the patients and controls was 1:3. According to the sample size, we recruited 315 unrelated infertile men with idiopathic spermatogenesis impairment and 995 normozoospermic men (couple infertility due to female factors) from two affiliated hospitals of Sichuan University and Chengdu Women’s and Children’s Central Hospital between 2015 and 2020.
The diagnosis of all patients was based on standard clinical procedures, including history and physical examination, semen analysis, serum hormone analysis, ultrasound evaluation and genetic testing [7]. All of the participants underwent at least two semen analyses. Based on World Health Organization guidelines [8], AZ is defined when no sperm is found under the microscope after the semen is centrifuged (3000×g) for 15 min. OZ is defined as sperm concentration (SC) < 15 × 10^6/ml and total sperm count (TSC) < 39 × 10^6/ejaculate. NZ is defined as SC > 15 × 10^6/ml, TSC > 39 × 10^6/ejaculate and normal sperm motility and morphology. Serum hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T), were detected in individuals. Patients with alcohol or drug abuse, karyotype abnormalities, AZF microdeletions, hypogonadotropic hypogonadism, cryptorchidism, varicocele, seminal ductal obstruction, testicular trauma and tumours were excluded based on evaluation with standard clinical procedures. The case group included 142 patients with nonobstructive azoospermia (NOA) and 173 patients with OZ aged 26 to 46 years. The patients with NOA included 51 with hypospermatogenesis (8 ~ 9 scores), 30 with spermatid arrest (6 ~ 7 scores), 22 with spermatocyte arrest (4 ~ 5 scores), 16 with spermatagonia arrest (3 scores), and 23 with Sertoli cell-only syndrome (2 scores) according to the Johnsen score and predominant histopathologic pattern [9]. The controls with NZ ranged in age from 22 to 45 years old. The semen and hormonal parameters of the subjects are shown in Table 1. This study was approved by the Biomedical Research Ethics Committee of West China Hospital, Sichuan University (No. 783), and written informed consent was obtained from each participant.

| Parameters            | Patients (n = 173) | AZ (n = 142) | Controls (n = 995) |
|-----------------------|-------------------|-------------|-------------------|
| BMI (kg/m²)²         | 22.89 ± 2.25      | 22.76 ± 1.96 | 22.34 ± 2.67      |
| SC (n × 10⁶/ml)³      | 6.60 (2.55-10.86) | /           | 61.46 (40.95-91.88) |
| TSC (n × 10⁶/ejaculate)³ | 15.88 (5.87-27.13) | /           | 212.07 (133.38-317.08) |
| Total motility (PR + NP, %)³ | 38.24 (28.00-53.39) | /           | 64.56 (50.77-76.70) |
| FSH (mlU/ml)⁴        | 5.73 ± 3.69       | 8.77 ± 3.64 | 4.83 ± 1.73       |
| LH (mlU/ml)⁴         | 5.72 ± 3.47       | 6.93 ± 2.88 | 4.87 ± 2.01       |
| T (ng/ml)⁴           | 4.81 ± 1.59       | 4.52 ± 2.05 | 5.12 ± 1.63       |

OZ oligozoospermia, AZ azoospermia, NZ normozoospermia, BMI Body mass index, SC Sperm concentration, TSC Total sperm count, PR progressive motility (grades a + b), NP non-progressive motility (grade c), FSH Follicle-stimulating hormone, LH Luteinizing hormone, T Testosterone. ² Mean ± standard deviation, ³ Median and interquartile range

Detection of rare variants in the coding region and splice site of BRD7

Genomic DNA was collected from whole blood using a Blood DNA Purification Kit (BioTeke, China). The quality and concentration of DNA samples were assessed by 1% agarose gel electrophoresis. For the 142 patients with NOA, all seventeen exons of BRD7 (NG_023418) including splice sites were amplified by polymerase chain reaction (PCR), and the PCR primer information is shown in Supplementary table 1. Sanger sequencing of the PCR product was performed on a 3700XL System (Applied Biosystems, USA).

Detected variants with a minor allele frequency (MAF) < 1% in the Genome Aggregation Database (gnomAD) [10] and 1000 Genomes Project [11] were classified as ‘rare variants’. Among these variants, the influence of a missense variant on gene function was predicted by three in silico algorithms, including SIFT [12], PolyPhen-2 [13] and Mutation Taster [14], and the influence of synonymous variants and a variant in splice site on RNA splicing was predicted by two in silico algorithms, including MaxEntScan [15] and Human Splicing Finder [16]. For the rare variants predicted to potentially damage the function of BRD7 by at least two of three algorithms (SIFT, PolyPhen-2 and Mutation Taster) or one of two algorithms (MaxEntScan and Human Splicing Finder), further genotyping was conducted in 173 infertile males with OZ and 995 controls with NZ by Sanger sequencing.

Genotyping of the common variants in BRD7

The genotypes of single nucleotide polymorphisms (SNPs) within 10 kb are usually associated with the same or similar effects [17, 18], and a single tagSNP could represent the information of more SNPs in the region. Currently, tagSNP selection is mostly based on linkage disequilibrium (LD) [19]. LD, a nonrandom association of alleles at a pair of loci, is quantified by the value of D² or r² [17, 20]. The value of r² is directly related to the
The statistical power of detecting unassayed loci and disease-associated polymorphisms [17]. When the value of $r^2 \geq 0.8$, two loci are regarded as exhibiting a strong LD [19]. In the present study, we extracted BRD7 genotype data from 2 kb upstream of the transcription start site to 2 kb downstream of the transcription stop site from the 1000 Genomes Project database. The tagSNPs were screened and evaluated using Haploview 4.2 software (Broad Institute of MIT and Harvard, USA). Based on the data of Han Chinese individuals in Beijing, a total of seven tagSNPs, rs7196135, rs117164075, rs76946718, rs1062348, rs79483509, rs62029995 and rs11644238, were eventually selected with MAF > 5% and LD value of $r^2 \geq 0.8$. In theory, these tagSNPs could capture greater than 90% of the targeted BRD7 alleles at an $r^2$ threshold of 0.8.

### Table 2 The bioinformatics analysis of exonic and splicing variants detected in 142 patients with AZ

| Rs          | Variant*        | Consequence   | In silico predictive algorithm | MAF in the databases |
|-------------|-----------------|---------------|-------------------------------|-----------------------|
|             |                 |               |                               | 1000 Genomes_EAS | gnomAD_EAS |
| rs1062348   | c.846C > T      | Synonymous    | /                             | 0.497                | 0.499    |
| rs116422109 | c.537T > C      | Synonymous    | /                             | 0.005                | 0.005    |
| rs202057136 | c.592-9A > G    | Splicing      | /                             | 0.001                | 0.002    |
| rs115302634 | c.1796A > G     | Missense      | Tolerated (0.127)             | 0.003                | 0.004    |
| rs201820448 | c.1458T > C     | Synonymous    | /                             | 0.004                | 0.004    |
| rs188183810 | c.1077C > T     | Synonymous    | /                             | 0.005                | NA       |

The exons and their splice sites of bromodomain containing 7 (BRD7) were amplified by PCR and products were detected using Sanger sequencing. AZ, azoospermia, MAF, Minor allele frequency, gnomAD, Genome Aggregation Database, EAS, East Asia, NSI, No significant impact, SSC, Splice site changes. *The variants were identified in the heterozygous state in patients with AZ.

### Table 3 Comparison of allele and genotype frequencies of the rare variants between patients with OZ or AZ and controls with NZ

| Rs          | Allele/Genotype | Patients                      | Controls                      | P-values          |
|-------------|-----------------|--------------------------------|-------------------------------|-------------------|
|             |                 | Total (n = 315) | OZ (n = 173) | AZ1 + AZ2 (n = 142) | AZ1 (n = 52) | AZ2 (n = 90) | NZ (n = 995) | [1] | [2] | [3] | [4] | [5] |
|             |                 |                   |                   |                   |               |               |               |     |     |     |     |     |
| rs116422109 | T                | 625 (99.2)         | 343 (99.1)         | 282 (99.3)         | 102 (98.1)   | 180 (100)     | 1977 (99.3)  | 0.782 | 0.720 | 1.000 | 0.169 | 0.617 |
|             | C*               | 5 (0.8)           | 3 (0.9)           | 2 (0.7)           | 2 (1.9)      | 0 (0.0)       | 13 (0.7)     | 0.781 | 0.719 | 1.000 | 0.168 | 0.616 |
| rs202057136 | A                | 627 (99.5)         | 344 (99.4)         | 283 (99.6)         | 104 (100)    | 179 (99.4)    | 1981 (99.5)  | 1.000 | 0.672 | 1.000 | 1.000 | 0.580 |
|             | G*               | 3 (0.5)           | 2 (0.6)           | 1 (0.4)           | 0 (0.0)      | 1 (0.6)       | 9 (0.5)      | 0.732 | 0.361 | 0.368 | 0.542 |
| rs115302634 | A                | 627 (99.5)         | 345 (99.7)         | 282 (99.3)         | 103 (99.0)   | 179 (99.4)    | 1982 (99.6)  | 0.732 | 1.000 | 0.361 | 0.368 | 0.542 |
|             | G*               | 3 (0.5)           | 1 (0.3)           | 2 (0.7)           | 1 (1.0)      | 1 (0.6)       | 8 (0.4)      | 0.732 | 1.000 | 0.361 | 0.369 | 0.543 |
| rs188183810 | C                | 628 (99.7)         | 345 (99.7)         | 283 (99.6)         | 104 (100)    | 179 (99.4)    | 1988 (99.9)  | 0.246 | 0.382 | 0.330 | 1.000 | 0.229 |
|             | T*               | 2 (0.3)           | 1 (0.3)           | 1 (0.4)           | 0 (0.0)      | 1 (0.6)       | 2 (0.1)      | 0.246 | 0.382 | 0.330 | 1.000 | 0.229 |

P-values were calculated using Fisher’s exact test.

OZ, oligozoospermia, AZ1, azoospermia with spermatid or spermatocyte arrest, AZ2, azoospermia with hypospermatogenesis, spermatogonia arrest or Sertoli cell-only syndrome, NZ, normozoospermia. Controls vs. [1] Total patients, [2] OZ, [3] AZ1 + AZ2, [4] AZ1, [5] AZ2. *The variants were identified in the heterozygous state in the subjects.
Genotyping of the tagSNPs was performed for 315 infertile patients with impaired spermatogenesis and 995 controls with NZ using a SNPscan™ Kit (Genesky Biotechnologies, China). As described previously [21], the genotypes of the tagSNPs were identified by double-ligation and multiplex fluorescence PCR, and the results were analysed using GeneMapper 4.1 software (Applied Biosystems, USA). For quality control, 10% of the total samples were randomly selected for the second test with a concordance rate of 100%. Moreover, 5% of the samples were confirmed to have tagSNP genotypes by Sanger sequencing, producing 100% identity.

Statistical analysis
The distribution of semen parameters, including SC, TSC and motility, was analysed using the Kolmogorov-Smirnov test or descriptive statistical index in SPSS 17.0 software (SPSS Inc., USA). The Hardy-Weinberg equilibrium (HWE) test was performed for each tagSNP using PLINK 1.9 software (Shaun Purcell, USA). The genotype distributions and allele frequencies of the rare variants and tagSNPs were compared between patients and controls using Pearson’s χ2 test or Fisher’s exact test in SPSS 17.0 software. LD analysis of the tagSNPs was conducted using Haplovip 4.2 software. Haplotype analysis of the tagSNPs was performed using SHEsis software [22]. The Mann-Whitney U or Kruskal-Wallis test was performed to compare the distribution differences of SC and TSC among different genotypes of patients with NZ and fertile men. Continuous variables are presented as the mean ± standard deviation of the mean (mean ± SD) or median and interquartile range, and categorical variables are presented as frequencies (%). For all statistical tests, P < 0.05 was considered to be statistically significant. In addition, the Bonferroni method was applied to adjust for multiple testing by dividing the critical level of significance by the number of comparisons.

Results
First, we detected variants in the coding region and splice site of BRD7 in 142 patients with NOA. As a result, a total of six exonic and splicing variants were classified as heterozygous (Table 2). The properties of these variants were evaluated with publicly available population databases and in silico tools. After excluding one synonymous variant (rs201820448) without supporting evidence for its influence on RNA splicing and another (rs1062348) with MAF > 1% in the East Asian population of 1000 Genomes and gnomAD databases, the remaining four rare variants (Supplementary Fig. 1), including rs116422109, rs202057136, rs115302634 and rs188183810, were further subjected to genotyping by Sanger sequencing in 173 infertile males with NZ and 995 normozoospermic men. The four rare variants were also found in the heterozygous state in 173 patients with NZ and 995 controls. The genotype distributions of these variants were in accordance with HWE in both the patient and control groups (Supplementary Table 2). Our results showed a similar distribution of alleles and genotypes of these variants between 995 controls and 315 infertile patients (142 with NOA and 173 with OZ) (Table 3). The human BRD7 gene is mainly expressed in the nuclei of primary spermatocytes and round spermatids [6], implying that the impaired function of BRD7 may cause spermatocyte or spermatid arrest. Thus, we further compared the distribution of alleles and genotypes between the controls with NZ and NOA patients with either of the two pathological phenotypes in the tests. However, we failed to identify any significant difference in the distributions of alleles and genotypes of these variants between the two groups (Table 3). Further comparison did not reveal any difference in the sperm products between carriers of the variants and noncarriers (Table 4).

To further explore the association of the common variants of BRD7 with spermatogenesis failure, we identified seven BRD7-linked tagSNPs and performed genotyping in 315 patients with NOA or OZ and 995 controls with NZ. The genotype distributions of the seven common SNPs were in accordance with HWE in both the patient and control groups (Supplementary table 3), suggesting

| Rs             | Genotype | No. subjects | Median of SC (25th–75th percentile) (n x 10^6/ml) | P-values | Median of TSC (25th–75th percentile) (n x 10^6/ejaculate) | P-values |
|----------------|----------|--------------|-----------------------------------------------|----------|--------------------------------------------------------|----------|
| rs116422109 TT | 1152     | 49.14(18.53–79.62) | 0.899 | 159.40(52.12–274.13) | 0.902 |
| TC            | 16       | 38.63(7.74–81.67) | 1.055 | 155.42(20.28–311.51) | 0.908 |
| rs202057136 AA | 1157     | 49.09(18.36–79.96) | 0.829 | 159.40(51.94–274.89) | 0.532 |
| AG            | 11       | 47.27(25.32–71.43) | 0.445 | 159.57(51.96–275.03) | 0.745 |
| rs115302634 AA | 1159     | 49.14(18.40–79.68) | 0.445 | 159.57(51.96–275.03) | 0.745 |
| AG            | 16       | 44.46(21.31–81.98) | 1.021 | 129.40(47.00–200.77) | 0.532 |
| rs188183810 CC | 1165     | 49.19(18.55–79.89) | 0.273 | 166.14(64.62–279.00) | 0.902 |
| CT            | 3        | 27.28(7.58–56.64) | 0.715 | 71.67(5.02–271.81) | 0.902 |

P-values were calculated using Mann-Whitney U test. SC Sperm concentration, TSC Total sperm count.
that the study sample is representative of the population. As shown in Table 5, the distribution of alleles and genotypes of the seven tagSNPs was similar between patients with NOA or OZ and fertile male controls.

Typically, a haplotype composed of SNPs may lead to a larger joint effect on complex traits compared with that noted for single-marker analysis [23]. Therefore, we next conducted pairwise LD analysis of the tagSNPs using Haploview 4.2 software. The results showed that five of the seven tagSNPs, rs62029995, rs76946718, rs79483509, rs1062348 and rs117164075, formed a haplotype block that exhibited a strong LD in both patients and controls (Fig. 1). Haplotype analysis with SHEsis software predicted six haplotypes of the haplotype block.

Table 5 Comparison of allele and genotype frequencies of the tagSNPs between patients with OZ or AZ and controls with NZ

| TagSNPs | Allele/ Genotype | Patients | Controls | P-values |
|---------|-----------------|----------|----------|----------|
|         | Total (n = 315) | OZ (n = 173) | AZ (n = 142) | NZ (n = 995) | [1] | [2] | [3] |
| rs7196135 | G | 176 (27.9) | 98 (28.3) | 78 (27.5) | 578 (29.0) | 0.592 a | 0.785 a | 0.582 a |
|         | A | 454 (72.1) | 248 (71.7) | 206 (72.5) | 1412 (71.0) | 0.423 a | 0.766 a | 0.442 a |
| rs117164075 | T | 75 (11.9) | 40 (11.6) | 35 (12.3) | 268 (13.5) | 0.311 a | 0.333 a | 0.596 a |
|         | C | 555 (88.1) | 306 (88.4) | 249 (87.7) | 1722 (86.5) | 0.716 a | 0.903 a | 0.472 a |
| rs76946718 | T | 54 (8.6) | 32 (9.2) | 22 (7.7) | 180 (9.0) | 0.716 a | 0.903 a | 0.472 a |
|         | C | 576 (91.4) | 314 (90.8) | 262 (92.3) | 1810 (91.0) | 0.683 a | 0.862 a | 0.646 a |
| rs1062348 | G | 306 (48.6) | 172 (49.7) | 134 (47.2) | 993 (49.9) | 0.561 a | 0.948 a | 0.392 a |
|         | A | 324 (51.4) | 174 (50.3) | 150 (52.8) | 997 (50.1) | 0.832 a | 0.862 a | 0.646 a |
| rs79483509 | C | 134 (21.3) | 71 (20.5) | 63 (22.2) | 446 (22.4) | 0.547 a | 0.434 a | 0.931 a |
|         | T | 496 (78.7) | 275 (79.5) | 221 (77.8) | 1544 (77.6) | 0.547 a | 0.434 a | 0.931 a |
| rs62029995 | G | 130 (20.6) | 76 (22.0) | 64 (22.5) | 424 (21.3) | 0.626 a | 0.783 a | 0.637 a |
|         | A | 400 (62.5) | 210 (61.1) | 190 (61.1) | 1056 (54.8) | 0.742 a | 0.942 a | 0.316 a |

P-values were calculated using Chi-squared test a or Fisher’s exact test b. OZ oligozoospermia, AZ azoospermia, NZ normozoospermia, SNP Single nucleotide polymorphism. Controls vs. [1] Total patients, [2] OZ, [3] AZ

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with a frequency of greater than 0.03. However, we did not identify any significant difference in the distribution of these haplotypes between patients with spermatogenesis failure and controls with NZ (Table 6).

Furthermore, we investigated the correlation between BRD7 and sperm products, including SC and TSC. The results showed that men with any BRD7 alleles distinguished by the seven tagSNPs presented similar SC and TSC (Table 7), further implying the absence of the association of BRD7 tagSNPs with susceptibility to spermatogenic failure.

Discussion
Spermatogenesis is a complex process involving approximately 2000 genes [4, 24]. By studying human patients with spermatogenic failure, some autosome-linked gene variants have been demonstrated to cause central hypogonadism, monomorphic teratozoospermia or asthenozoospermia [4]. In recent years, the reproductive investigation of gene-knockout mice has suggested more candidate genes for spermatogenic failure [25], providing an additional clue for the aetiological study of spermatogenic failure in humans. In this case, it is encouraged to clarify the contribution of these genes to spermatogenic failure and male infertility in humans when considering the similarity of function between mouse and human genes [26].

BRD7 plays various roles in cellular biological processes, such as transcriptional regulation, chromatin modification and cell cycle control [27–29]. As a catalytic subunit of the switch/sucrose nonfermenting (SWI/SNF) complex, brahma-related gene 1 (BRG1) facilitates DNA double-strand break repair and recombination during meiosis in the male germline [30], and BRD7 is a subunit of polybromo-associated BRG1-associated factor-specific SWI/SNF and is essential for the activation and repression of target genes in embryonic stem cells [31]. In addition, as a protein recognition module, the bromodomain can bind acetyllysine residues on the histone tail, which is a pivotal mark of epigenetic regulation [32, 33]. Remarkably, BRD7 is highly expressed in the pachytene stage to the round spermatid stage during mouse spermatogenesis, which is similar to that in humans, and Brd7−/− male mice present AZ and male infertility [6]. Interestingly, a study reported that whole-body BRD7 knockout in mice caused embryonic lethality

Table 6 Comparison of the haplotype frequencies between patients with OZ or AZ and controls with NZ

| Haplotypes | Patients | Controls | P-values |
|-----------|----------|----------|----------|
|            | Total (n = 315) | OZ (n = 173) | AZ (n = 142) | NZ (n = 995) | [1] | [2] | [3] |
| CCCGC      | 0.096    | 0.098    | 0.092    | 0.092    | 0.824 | 0.763 | 0.990 |
| CCCGT      | 0.123    | 0.121    | 0.126    | 0.134    | 0.506 | 0.519 | 0.740 |
| CCTAC      | 0.496    | 0.507    | 0.483    | 0.506    | 0.630 | 0.939 | 0.490 |
| CCTGC      | 0.076    | 0.088    | 0.059    | 0.07     | 0.701 | 0.280 | 0.513 |
| GCTGC      | 0.121    | 0.098    | 0.151    | 0.108    | 0.422 | 0.564 | 0.051 |
| GTTGC      | 0.086    | 0.088    | 0.084    | 0.084    | 0.860 | 0.814 | 0.991 |

The haplotypes were reconstructed by Haploview software. P-values were calculated using Chi-squared test. OZ oligozoospermia, AZ azoospermia, NZ normozoospermia. Controls vs. [1] Total patients, [2] OZ, [3] AZ
male infertility. Moreover, another bromodomain is sufficient to allow knockout mice to survive and cause \( \alpha \text{Cre/EII} \) controlled by the EII \( \alpha \) promoter may lead to low spermatogenesis, and it is highly possible that only a small number of patients with AZ are likely to carry two pathogenic alleles of \( \text{BRD7} \). These patients may not be detected in the limited number of AZ samples. Thus, the spermatogenic phenotype of complete loss of \( \text{BRD7} \) function could not be assessed in humans. (ii) The detection of rare variants in patients with OZ was not performed, and the patients could carry different rare variants than those carried by patients with AZ. (iii) The selected tagSNPs captured 90% of the target alleles with \( r^2 > 0.8 \) and MAF > 0.05, but they were not representative of all target alleles. (iv) Testicular \( \text{BRD7} \) levels of patients with severe spermatogenic impairment were not assessed due to ethical reasons. Our results require

| TagSNPs   | Genotype | No. subjects | Median of SC (25th-75th percentiles) (n × 10⁶/ml) | P-values | Median of TSC (25th-75th percentiles) (n × 10⁶/ejaculate) | P-values |
|-----------|----------|--------------|-------------------------------------------------|----------|-------------------------------------------------|----------|
| rs7196135 | GG       | 94           | 53.35 (11.60–80.74)                              | 0.473    | 154.77 (28.35–266.32)                             | 0.556    |
|           | GA       | 488          | 50.96 (19.08–80.80)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
|           | AA       | 586          | 46.95 (16.21–79.32)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
| rs117164075 | TT     | 17           | 48.07 (27.36–82.11)                              | 0.062    | 105.16 (26.14–171.25)                             | 0.523    |
|           | TC       | 274          | 53.85 (23.49–84.83)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
|           | CC       | 877          | 47.38 (16.05–78.88)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
| rs76946718 | TT     | 7            | 50.67 (23.97–70.07)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
|           | TC       | 198          | 50.58 (19.10–85.73)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
|           | CC       | 963          | 48.61 (17.86–79.03)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
| rs1062348 | AA       | 283          | 48.73 (21.87–79.68)                              | 0.756    | 164.74 (63.86–282.19)                             | 0.514    |
|           | AG       | 599          | 47.66 (16.23–79.96)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
|           | GG       | 286          | 49.63 (18.42–78.68)                              | 0.146    | 134.81 (25.88–237.52)                             | 0.183    |
| rs79483509 | CC     | 52           | 49.59 (20.45–82.81)                              | 0.220    | 142.04 (31.08–269.80)                             | 0.532    |
|           | CT       | 413          | 53.44 (20.92–81.19)                              | 0.220    | 142.04 (31.08–269.80)                             | 0.532    |
|           | TT       | 703          | 46.95 (16.23–79.11)                              | 0.220    | 142.04 (31.08–269.80)                             | 0.532    |
| rs62029995 | GG     | 46           | 47.16 (16.95–84.6)                               | 0.406    | 149.14 (31.68–275.15)                             | 0.237    |
|           | GC       | 356          | 45.53 (18.36–78.54)                              | 0.406    | 149.14 (31.68–275.15)                             | 0.237    |
|           | CC       | 766          | 51.33 (22.09–80.64)                              | 0.406    | 149.14 (31.68–275.15)                             | 0.237    |
| rs11644238 | CC     | 53           | 44.72 (15.85–83.19)                              | 0.331    | 146.31 (45.10–283)                                | 0.288    |
|           | CA       | 394          | 47.18 (1603–77.81)                               | 0.331    | 146.31 (45.10–283)                                | 0.288    |
|           | AA       | 721          | 50.56 (20.42–81.64)                              | 0.331    | 146.31 (45.10–283)                                | 0.288    |

P-values were calculated using Kruskal-Wallis test. SNP Single nucleotide polymorphism, SC Sperm concentration, TSC Total sperm count

at mid-gestation, suggesting a pivotal role for \( \text{BRD7} \) during growth and development [34]. This discrepancy between the two studies was probably due to the different knockout systems. The former \( \text{BRD7} \)-knockout mice were obtained using the Cre/loxP and flp/FRT recombination systems, which both conditionally and globally destroyed \( \text{BRD7} \) [6]. In the Cre/loxP system, Cre expression is controlled by the EII \( \alpha \) promoter, and minor leakage of the Cre/EII \( \alpha \) promoter may lead to low \( \text{BRD7} \) expression that is sufficient to allow knockout mice to survive and cause male infertility [35, 36]. Moreover, another bromodomain protein, bromodomain testis-specific protein (BRDT), has been reported to be involved in susceptibility to spermatogenesis impairment in humans [37]. These findings imply that \( \text{BRD7} \) may be a potential candidate gene for human spermatogenesis impairment.

To explore the association of \( \text{BRD7} \) with human spermatogenic failure, we comprehensively investigated the influence of rare and common variants of \( \text{BRD7} \) on the spermatogenic phenotype in 315 infertile patients with AZ or OZ and 995 males with NZ in the present study. However, we did not identify any rare variants of \( \text{BRD7} \) that could impair sperm production to influence the risk of spermatogenic failure in our population. Regarding the common variants of \( \text{BRD7} \), we failed to obtain any evidence for the association of their alleles with spermatogenic efficiency and susceptibility to spermatogenic failure. Collectively, our findings imply a limited contribution of \( \text{BRD7} \) to human male infertility. This observation may be reasonable when considering that \( \text{BRD7} \) may have partial functional redundancy with other genes during spermatogenesis in humans; thus, it is potentially nonessential for spermatogenesis in humans [38, 39].

Several limitations of the present study should be noted: (i) Approximately 2000 genes play a role in spermatogenesis, and it is highly possible that only a small number of patients with AZ are likely to carry two pathogenic alleles of \( \text{BRD7} \). These patients may not be detected in the limited number of AZ samples. Thus, the spermatogenic phenotype of complete loss of \( \text{BRD7} \) function could not be assessed in humans. (ii) The detection of rare variants in patients with OZ was not performed, and the patients could carry different rare variants than those carried by patients with AZ. (iii) The selected tagSNPs captured 90% of the target alleles with \( r^2 > 0.8 \) and MAF > 0.05, but they were not representative of all target alleles. (iv) Testicular \( \text{BRD7} \) levels of patients with severe spermatogenic impairment were not assessed due to ethical reasons. Our results require
further validation in a larger cohort considering the limited number of participants in this study.

Conclusions
In summary, this study is the first to investigate the association of the BRD7 gene with spermatogenic failure and male infertility in humans. We failed to obtain any rare or common variant-based evidence for the significant influence of BRD7 on spermatogenic efficiency and susceptibility in men, implying a limited contribution of the autosomal-linked gene to spermatogenic failure and male infertility in humans.

Abbreviations
BRD7: Bromodomain-containing protein 7; SNP: Single nucleotide polymorphisms; AZF: Azoospermia factor; AZ: Azoospermia; OZ: Oligozoospermia; NZ: Normozoospermia; NOA: Nonobstructive azoospermia; SC: Sperm concentration; TSC: Total sperm count; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; T: Testosterone; PCR: polymerase chain reaction; gnomAD: Genome Aggregation Database; MAF: Minor allele frequency; HWE: Hardy-Weinberg equilibrium; LD: Linkage disequilibrium; SWI/SNF: Switch/sucrose nonfermenting; BRG1: Brahma-related gene 1; BRDT: Bromodomain testis-specific protein

Supplementary Information
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Authors’ contributions
TRH, MHL, XYL, ZKW, YWZ, YQL, DCT, XYZ, XLT and YY contributed to writing-original draft. TRH and YY supervised the study and provided financial support. All authors and MHL contributed to conceptualization. TRH, MHL and XYL designed and performed the study. TRH and MHL, XYL, ZKW, YWZ, YQL, DCT, XYZ, XLT and YY contributed to data analysis. TRH and SYX contributed to software. TRH and MHL analysed the data. TRH, MHL and XYL contributed to writing-review and editing.

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