An association study of the single-nucleotide polymorphism c190C>T (Arg64Cys) in the human testis-specific histone variant, H3t, of Japanese patients with Sertoli cell-only syndrome

Toshinobu Miyamoto¹, Masashi Iijima³, Takeshi Shin³, Gaku Minase¹, Hiroshi Ueda¹, Yasuaki Saijo⁴, Hiroshi Okada¹, Kazuo Sengoku¹

Asian Journal of Andrology (2018) 20, 527–528; doi: 10.4103/aja.aja_66_17; published online: 6 February 2018

Dear Editor,

Approximately 20% of men with nonobstructive azoospermia (NOA) are diagnosed with infertility caused by genetic defects.¹ These include chromosomal abnormalities, Y-chromosome microdeletions, and several specific gene mutations/deletions, such as in DAZ, RBMY, USP9Y, SYCP3, HSF2, PLK4, and TEX1.¹ Several histones have been detected in mammalian testes, and testis-specific variants are specifically and highly expressed during spermatogenesis.¹ Recently, histone H3 variants of human and mouse genomes have been identified by in silico hybridization screening.² The mouse H3t histone has a human counterpart, H3T (H3.4), and shares a common chaperon recognition motif with H3.1 and H3.2.³ Knockout mice for H3t were first generated in 2017; both male and female H3t null mice were viable and healthy, but the male mice were sterile.⁴ H3t deficiency leads to azoospermia because of the loss of haploid germ cells.⁵ The phenotype of H3t null male mice is identical to that of Sertoli cell-only syndrome (SCOS) in humans. Therefore, we analyzed human H3T in genomic DNA from Japanese patients with SCOS.

This study was approved by the Ethics Committee of Asahikawa Medical University, Japan. Written informed consent was obtained from each participant. Patients with azoospermia secondary to SCOS with no chromosomal abnormalities were recruited from three national hospitals in Japan between 2001 and 2017. Those with defective spermatogenesis caused by infections, seminal tract obstruction, pituitary gland dysfunction, and other causes of testicular disorder were excluded from the study. A total of 178 Japanese patients with SCOS, mainly from Kanazawa, Osaka, and Tokyo, were included, together with 110 fertile Japanese men as normal controls. All patients underwent testicular microdissection with sperm extraction; however, no spermatozoa were present in their testes. A final diagnosis of SCOS was performed by two pathologists. All fathers of the patients were fertile, and none of their brothers suffered from azoospermia.

Direct sequencing of the H3T coding region from chromosome 1 was performed on PCR-amplified fragments using peripheral leukocyte DNA and gene-specific primers: H3T-cds1-Fw (5′-CCACAGGCATGAATATAAG-3′) and H3T-cds1-Rv (5′-ACCTAATCAGAAGTAGGTA-3′). Fisher’s exact test was used to evaluate the statistical significance of H3T variants in patients. Hardy–Weinberg equilibrium (HWE) was tested for the variants using SNPAlyze software (Dynacom, Chiba, Japan). Linkage disequilibrium of all possible two-way single-nucleotide polymorphism (SNP) combinations was tested by calculating absolute correlation coefficient values. Haplotype frequencies were estimated by the maximum likelihood method based on the expectation-maximization algorithm under the assumption of HWE. Linkage disequilibrium and haplotype frequency were tested using SNPAllyze. All P values were determined by Chi-square approximation, with significance assumed at P < 0.05. The potential pathogenicity of H3T mutations was predicted by in silico analysis using three different software packages: MutationTaster (http://www.mutationtaster.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2), and SIFT (http://sift.jcvi.org/).

The H3T coding region was sequenced in all 178 patients with SCOS. Seven variants were detected in this patient group (Table 1) – SNP1: c15G>A, Chr1:228613012, rs199672652; SNP2: c88G>A, Chr1:228612939, rs531385963; SNP3: c109A>C, Chr1:228612918, rs201151997; SNP4: c135C>T, Chr1:228612892, rs56336130; SNP5: c158G>A, Chr1:228612869, rs201904037; SNP6: c189A>C, Chr1:228612838, rs2230656; and SNP7: c190C>T, Chr1:228612837, rs201294185. All seven SNPs have been reported previously; however, we found no information about their frequencies in the Japanese population.

| SNP | Chromosome | Position | Ref alleles | Alt alleles | Minor allele frequency |
|-----|------------|----------|-------------|-------------|-----------------------|
| SNP1 | Chr1:228613012 | rs199672652 | c15G | A | 0.0478 (0.0465: alleles) |
| SNP2 | Chr1:228612939 | rs531385963 | c88G | A | 0.0478 (0.0465: alleles) |
| SNP3 | Chr1:228612918 | rs201151997 | c109A | C | 0.0478 (0.0465: alleles) |
| SNP4 | Chr1:228612892 | rs56336130 | c135C | T | 0.0478 (0.0465: alleles) |
| SNP5 | Chr1:228612869 | rs201904037 | c158G | A | 0.0478 (0.0465: alleles) |
| SNP6 | Chr1:228612838 | rs2230656 | c189A | C | 0.0478 (0.0465: alleles) |
| SNP7 | Chr1:228612837 | rs201294185 | c190C | T | 0.0478 (0.0465: alleles) |

A significant association with SCOS was observed only for SNP7 (P = 0.0465: genotypes and P = 0.0478: alleles). We also found that the distributions of SNP7 (c190C>T [Arg64Cys]) genotypes and allele frequencies...
differed significantly between patients and controls. Seven patients carried the T allele at SNP7, but this was absent from all 110 controls. Therefore, this T allele might have been inherited from their mothers. The SNP7 change was predicted to be “deleterious” and “disease causing” in an in silico analysis using SIFT and MutationTaster; however, it was predicted to be “benign” by PolyPhen-2. Haplotype analysis revealed similar estimated haplotype frequencies for all seven SNPs ($P = 0.2595–1.0000$). Haplotype estimation and linkage disequilibrium analysis also revealed no statistically significant critical differences between groups ($P > 0.05$).

We hypothesized that mutations or polymorphisms in H3T may be associated with SCOS. An earlier study demonstrated that human nucleosome assembly protein 2 (hNap2) catalystizes the formation of H3t-containing nucleosomes in vitro. Previous mutational analyses using recombinant H3t revealed that its Val111 residue plays an essential role in hNap2-mediated nucleosome formation. However, the SNPs identified in the present study do not change the Val111 residue of H3t, indicating that they do not have an impact on nucleosome formation.

This study had a number of limitations. First, the sample size was not determined before the start. Second, the number of patients analyzed was not sufficient to allow a definitive conclusion to be drawn. However, retrospective power calculations demonstrated that this study had 80% power to detect an increased genotype prevalence of 23.4% in cases against a control of 10%, and 86% power to detect an increased genotype prevalence of 9% against a control of 1%. Third, all patients were from Kanazawa, Osaka, or Tokyo, so were not representative of all areas of Japan. The H3t null male mice are sterile, but the patients we examined and diagnosed the patients and collected DNA samples. TM, YS, and HO wrote and revised the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS
All authors declared no competing interests.

ACKNOWLEDGMENTS
This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

REFERENCES
1 Miyamoto T, Minase G, Okabe K, Ueda H, Sengoku K. Male infertility and its genetic causes. J Obstet Gynaecol Res 2015; 41: 1501–5.
2 Song SH, Chiba K, Ramasamy R, Lamb DJ. Recent advances in the genetics of testicular failure. Asian J Androl 2016; 18: 350–5.
3 Kwak HG, Dohmae N. Proteomic characterization of histone variants in the mouse testis by mass spectrometry-based top-down analysis. Biosci Trends 2016; 10: 357–64.
4 Maehara K, Harada A, Sato Y, Matsumoto M, Nakayama KI, et al. Tissue-specific expression of histone H3 variants diversified after species separation. Epigenetics Chromatin 2015; 8: 35.
5 Chauhan S, Mandal P, Tomar RS. Biochemical analysis reveals the multifactorial mechanism of histone H3 clipping by chicken liver histone H3 protease. Biochemistry 2016; 55: 5464–82.
6 Ueda J, Harada A, Urahama T, Machida S, Maehara K, et al. Testis-specific histone variant H3 gene is essential for entry into spermatogenesis. Cell Rep 2017; 18: 593–600.
7 Tachiwana H, Osakabe A, Kimura H, Kurumizaka H. Nucleosome formation with the testis-specific histone H3 variant, H3T, by human nucleosome assembly proteins in vitro. Nucleic Acids Res 2008; 36: 2208–18.