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Genetic variations of antioxidant genes and their association with male infertility in Vietnamese men

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Abstract

Background: Antioxidant genes, such as superoxide dismutase (*SOD*), catalase (*CAT*), and nitric oxide synthase (*NOS*), play critical roles in spermatogenesis and sperm functions. Polymorphisms of antioxidant genes have been shown to be strongly associated with sperm quality which affects male fertility.

Methods: To investigate the association of antioxidant gene polymorphisms to male infertility in Vietnamese men, in this case–control study, using Sanger sequencing, we genotyped four variants *SOD1*:7958G>A, *SOD2*:c.47T>C, *CAT*:-262C>T, and *NOS3*:-786C>T.

Results and Conclusions: We identified *SOD1*:7958GA genotype and *NOS3*:-786CT genotype in the infertility group were significantly higher than in the control with OR = 2.191 (95% CI: 1.226–3.915, $p = 0.004$) and OR = 3.135 (95% CI: 1.591–6.180, $p < 0.001$), respectively. We also detected that the frequency of the *SOD2*:c.47TC genotype was significantly higher in the male infertility group than in fertile men (OR = 1.941, 95% CI: 1.063–3.595, $p = 0.029$). Gene–gene interactions between the SNPs of *SOD1*, *SOD2*, and *CAT* might increase the risk of male infertility patients. In particular, patients carrying the *SOD1*:GA+AA, *SOD2*:TC+CC, and *CAT*:CT/TT genotype pattern have an increased risk of male infertility (OR = 7.614, $p = 0.007$). To our knowledge, this is the first study to evaluate the association between the *SOD1*:7958G>A polymorphism and male infertility. Further studies with larger sample sizes and more genes are needed to better assess the association between variants of antioxidant genes and male infertility.

KEYWORDS
catalase, male infertility, nitric oxide synthase, superoxide dismutase, Vietnamese men

1 | INTRODUCTION

Infertility is the failure to conceive after frequent, unprotected sexual intercourse of couples for at least 12 months.[1] Infertility affects 8–12% of couples globally, of which male infertility accounts for approximately 50%.[2,3] There are several factors involved in the underlying causes of male infertility including environmental, nutritional, behavior, and genetic factors.[4]

Reactive oxygen species (ROS) can lead to plasma membrane destruction, DNA breakage, and reduced sperm quality.[5] Therefore,

ROS must be eliminated for normal spermatogenesis and fertilization. Enzymes involved in antioxidant processes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and nitric oxide synthase (NOS, EC 1.14.13.39) are abundant in seminal plasma and sperm cells. Polymorphisms of these antioxidant enzymes have been reported to be associated with male infertility in humans.[6,7]

It has been identified that the SOD isozyme family has been identified that contains CuZn-SOD (SOD1), Mn-SOD (SOD2), and extracellular SOD (SOD3).[8] SOD activity in semen is positively correlated with sperm concentration and motility.[6,7,9] In semen, genetic polymorphisms of the *SOD* genes play a crucial role in SOD enzyme activity, which could affect sperm quality. Several studies suggested the association between the *SOD2* Val16Ala variant (rs4880) and male infertility. Faure et al. reported that the Ala-MnSOD allele (rs4880) significantly increased the risk of male infertility. In addition, the *SOD2* rs4880 CC variant decreased SOD activity in infertile men, thereby increasing the risk of male infertility.[6,10] The Val16Ala variant of SOD2 also increases the risk of depression,[11] stroke,[12] and epilepsy.[13]

The CAT enzyme, encoded by the *CAT* gene, converts H_2O_2 to H_2O. In seminal plasma, H_2O_2 can be found directly as the consequence of the leukocyte infiltration or indirectly as a product of ROS detoxification by superoxide dismutase. Polymorphism in this gene was demonstrated to be correlated with diminished CAT activity.[14]

NOSs are a family of enzymes that include neuronal NOS (NOS1), inducible NOS (NOS2), and endothelial NOS (NOS3), which are responsible in catalyze the conversion of L-arginine to nitric oxide (NO).[15] At low concentrations, NO is considered an antioxidant, aiding to remove ROS.[16–18] The role of NO in sperm motility and its impact on fertility has been reported.[19] In the testis, NOS3 is responsible for NO production during spermatogenesis. Genetic variants of *NOS3* may be potential risk factors for defective spermatogenesis.[20] In various ethnic populations, polymorphisms of *NOS3* have been determined to be associated with sperm defects.[8,21–23] *NOS3*–786C allele was associated with the risk for poor semen parameters in Iranian men[23] or significantly associated with higher levels of sperm DNA fragmentation as well as increased risk for male infertility in Chinese.[20,24]

Although polymorphisms of antioxidant genes have been shown to be strongly associated with sperm quality and affect male fertility, no study on the effect of these polymorphisms on male infertility patients in Vietnam has been conducted. In this case–control study, we evaluated the association between the polymorphism of antioxidant genes and male infertility.

2 | MATERIALS AND METHODS

2.1 | Subjects

In our study, 107 diagnosed infertile patients (ranging from 21 to 50 years old) and 85 control men (ranging from 23 to 43 years of age) were recruited from Hanoi Medical University Hospital, between the years of 2018 to 2020. All of the participants consented to participate and signed informed consent. The study was approved by the Ethics Review Board of the Hanoi Medical University (76/HMU-IRB). A standard medical examination including physical examination, semen analysis, and cytogenetic and genetic tests were performed for all patients to exclude chromosome aberrations and Y chromosome microdeletion. Infertile men with with fertility-related diseases, such as prostate cancer, cryptorchidism, and varicocele were excluded from this study. The control group included of healthy men who showed normal reproductive function and confirmed to have healthy babies. Sperm concentration, sperm motility, sperm vitality, and sperm morphology of patient and control samples were analyzed with CASA instrument according to 2010 World Health Organization criteria.[25]

3 | METHODS

3.1 | SNP genotyping

Peripheral blood from all study subjects were collected into EDTA-K2 containing tubes (at least 2 ml for each subject). Genomic DNA was then extracted from leukocytes using Exgene™ Blood SV (GeneAll Biotechnology) following standard protocol. DNA quantitation and qualification were conducted by using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and 0.8% agarose gel electrophoresis.

In the present study, we selected four SNPs, including rs4998557, rs4880, rs1001179, and rs2070744, of *SOD1*, *SOD2*, *CAT*, and *NOS3*, respectively. Three of them (rs4880, rs1001179, and rs2070744) have been reported to be influential on male infertility. Meanwhile, the variant rs4998557 is a polymorphism of the *SOD1* gene that encodes the enzyme SOD1, which is relatively abundant in semen. Sanger sequencing was used for genotyping all of the polymorphisms. Specific primers (Table S1) were designed to amplify the sequence surrounding all SNP's positions, using primer3plus.[26] PCR reaction was performed with a total volume of 20 μl containing: 10 ng of total genomic DNA, 0.8 pmol of each primer, 1X Neb Master mix (New England Biolabs), and deionized water. The thermocycling was as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 58°C for 30 s, 68°C for 20 s, and a final extension at 68°C for 5 min. All amplicons were later purified by Multiscreen PCR 96 Filter Plate (Merck-Millipore, Burlington) following to the manufacturer's protocol. Bi-directions sequencing were subsequently performed using ABI Prism BigDye Terminator Cycle Sequencing Kit Version 3.1 (Applied BioSystems) on ABI 3500 Genetic Analyzer (Applied Biosystems).

Raw sequence data were manipulated by Sequencing Analysis Software (Applied Biosystems) and then aligned to reference sequences by SeqScape 3.0 software (Applied Biosystems).

3.2 | Statistical analysis

All statistical analyses were performed using R packages. Pearson's Chi-squared (χ^2) test was used to evaluate the association between

categorical variables (genotype distribution, smoking, and drinking status of patient and control groups) and was used to test for Hardy–Weinberg equilibrium of allele frequency of genetic polymorphisms. Differences between the mean age, body mass index (BMI), and semen parameters were assessed by Wilcoxon rank-sum test. The Analysis of variance (ANOVA) test was used to determine the difference between genotypes of cases and controls. Pairwise comparison between the genotype of patients was performed by using Wilcoxon rank-sum test. A multinomial logistic regression model was used to evaluate the association of genetic polymorphisms and infertility risk based on odds ratios (OR) value and 95% confidence interval (95% CI). Bonferroni correction was used to adjust the significance of *p*-value.

4 | RESULTS

4.1 | Characteristics of the study samples

Demographic characteristics and semen parameters of the study samples were summarized in Table 1. There was no significant difference in mean age between the infertile patients (mean ± SD: 31.93 ± 6.3 years) and the fertile men (31.96 ± 4.87 years) ($p = 0.92$). Similarly, we did not find a significant difference in the smoking status of the infertile patients compared with the control group ($p = 0.55$). In terms of alcohol intake, however, there was a marginally significant difference between the patient and control groups ($p = 0.034$). The body mass index (BMI) was also significantly higher in the patient group than in the control group ($p < 0.001$).

For the semen parameters, as expected, there was a significant difference between the infertile patients and the controls (Table 1) with respect to semen concentration ($53.17 \pm 42.77 \times 10^6$/ml vs. $100.84 \pm 56.51 \times 10^6$/ml, $p =$ 3.00E-10), total sperm count ($150.35 \pm 126.85 \times 10^6$ vs. $273.73 \pm 192.47 \times 10,^6$ $p =$ 3.40E-7), vitality (81.25 ± 5.09% vs. 87.02 ± 2.72%, $p =$ 2.00E-16), motility (30.41 ± 7.06% vs. 47.19 ± 5.32%, $p =$ 2.00E-16), and morphology (7.46 ± 3.69% vs. 11.59 ± 3.09%, $p =$ 2.30E-14), for case versus control, respectively. The semen volume at ejaculation was not significantly different between two groups ($p = 0.213$).

4.2 | Genotype distribution and their association with male infertility

In our study, four SNPs 7958G>A (rs4998557), c.47T>C (rs4880), -262C>T (rs1001179), and -786C>T (rs2070744) of the *SOD1*, *SOD2*, *CAT*, and *NOS3* genes, respectively, were genotyped (Figure S1). The genotype distribution of four SNPs is shown in Table 2. Both patient's and healthy's samples did not deviate the Hardy–Weinberg equilibrium for all SNPs with $p > 0.05$.

4.3 | SOD1 7958G>A (rs4998557)

We identified 23 GG genotypes (21.50%), 66 GA genotypes (61.68%), and 18 AA genotypes (16.82%) in the patient group. Meanwhile, in the control group, 26 individuals carried the GG genotype (30.59%), 36 individuals had the GA genotype (42.35%), and 18 individuals had the AA genotype (27.06%).

TABLE 1 Demographic characteristics and semen parameters of the study samples

Characteristics	Patients ($n = 107$)	Controls ($n = 85$)	*p*-Value*
Demographic characteristics			
Age (Mean ± SD) (Years)	31.93 ± 6.3	31.96 ± 4.87	0.920
BMI (Mean ± SD) (Kg/m^2)	24.84 ± 2.31	23.53 ± 2.55	**<0.001**
Smoking status			
Yes (%)	65 (60.75)	48 (56.47)	0.550
No (%)	42 (39.25)	37 (43.53)	
Drinking status			
Yes (%)	103 (96.26)	75 (88.23)	**0.034**
No (%)	4 (3.74)	10 (11.77)	
Semen parameters (Mean ± SD)			
Volume (ml)	2.88 ± 1.55	3.15 ± 1.37	0.213
Concentration (10^6/ml)	53.17 ± 42.77	100.84 ± 56.51	**2.20E-10**
Total sperm count (10^6)	150.35 ± 126.85	273.73 ± 192.47	**6.80E-07**
Vitality (%)	81.25 ± 5.09	87.02 ± 2.72	**2.00E-16**
Motility (% progressive)	30.41 ± 7.06	47.19 ± 5.32	**2.00E-16**
Morphology (% normal)	7.46 ± 3.69	11.59 ± 3.09	**8.10E-13**

Abbreviations: BMI, Body Mass Index; SD, Standard deviation.

**p-value* was derived from the χ^2 test for categorical variables (Smoking and Drinking status) and Wilcoxon rank-sum test for the other variables (Age, BMI, and semen parameters). Bold formats represent statistical significance ($p < 0.05$).

There was a difference in genotypic distribution between the male infertility patients and the fertile men ($p = 0.027$), but the allele frequency between the two groups was not significantly different ($p = 0.649$). Our data showed that the GA genotype in the group of cases was significantly higher than in control men with OR = 2.059 (95% CI: 1.208–4.163, $p = 0.037$) (Table 2).

4.4 | SOD2 c.47 T > C (rs4880)

Homozygous TT genotypes in the group of patients and controls were 51 (47.66%) and 56 (65.88%), respectively. Similarly, the number of heterozygous TC genotypes in the disease group was 48 (44.86%) and 27 (31%) in the control. Only 7.48% of patients in the case group and 2.35% in the control group were homozygous CC genotypes.

We found that there was a difference in both genotype distribution ($p = 0.026$) and allele frequency ($p = 0.019$) between the patient and control groups. The frequency of TC and TC + CC genotypes in the patient group was significantly higher than that in the control group with OR = 1.941 (95% CI: 1.063–3.595, $p = 0.029$) and OR = 2.108 (95%CI: 1.175–3.822, $p = 0.010$), respectively. At the allele level, we found that T allele carriers had a lower risk of disease (OR = 0.565, 95% CI: 0.349–0.912, $p = 0.010$) than C allele carriers (OR = 1.771, 95% CI: 1.096–2.862, $p = 0.010$) (Table 2).

4.5 | CAT - 262C>T (rs1001179)

For variant *CAT* -262C>T, CC genotype dominated, accounting for 82.24% and 88.24% in the disease and control groups. Heterozygous CT genotype was found in 16.82% of the infertile male group and

TABLE 2 Genotypes distribution of four SNPs (*SOD1* 7958G>A, *SOD2* c.47T>C, *CAT* -262C>T, and *NOS3*–786C>T) in infertile patients and fertile men

Gene	Patients *n* (%)	Controls *n* (%)	OR	95% CI	*p*-Value (OR)	*p*-Value (χ^2)
SOD1 7958G>A (rs4998557)						
GG	23 (21.50)	26 (30.59)	Ref	NA	NA	**0.027**
GA	66 (61.68)	36 (42.35)	2.059	1.028–4.163	**0.037**	
AA	18 (16.82)	23 (27.06)	0.886	0.380–2.055	0.773	
GA + AA	84 (78.50)	59 (71.76)	1.604	0.833–3.108	0.151	
Allele G	112 (52.34)	88 (51.76)	1.098	0.734–1.643	0.325	0.649
Allele A	102 (47.66)	82 (48.24)	0.911	0.609–1.363	0.325	
SOD2 c.47T>C (rs4880)						
TT	51 (47.66)	56 (65.88)	Ref	NA	NA	**0.026**
TC	48 (44.86)	27 (31.76)	1.941	1.063–3.595	**0.029**	
CC	8 (7.48)	2 (2.35)	4.124	0.952–31.140	0.0504	
TC+CC	56 (52.34)	29 (36.47)	2.108	1.175–3.822	**0.011**	
Allele T	150 (70.09)	139 (81.76)	0.565	0.349–0.912	**0.010**	**0.019**
Allele C	64 (29.91)	31 (18.24)	1.771	1.096–2.862	**0.010**	
CAT – 262C>T (rs1001179)						
CC	88 (82.24)	75 (88.24)	Ref	NA	NA	0.401
CT	18 (16.82)	10 (11.76)	1.521	0.668–3.648	0.311	
TT	1 (0.93)	0 (0)	NA	NA	NA	
CT + TT	19 (17.76)	10 (11.76)	1.604	0.711–3.827	0.249	
Allele C	194 (90.65)	160 (94.12)	0.611	0.265–1.323	0.209	0.209
Allele T	20 (9.35)	10 (5.88)	1.635	0.755–3.767	0.209	
NOS3 –786C>T (rs2070744)						
CC	2 (1.87)	1 (1.18)	Ref	NA	NA	**0.003**
CT	43 (40.19)	15 (17.65)	1.507	0.045–19.888	0.774	
TT	62 (57.94)	69 (81.18)	0.480	0.020–8.354	0.700	
CT+TT	105 (98.13)	84 (98.82)	0.665	0.056–7.011	0.352	
Allele C	47 (21.96)	17 (10)	2.513	1.405–4.692	**0.002**	**0.002**
Allele T	167 (78.04)	153 (90)	0.397	0.213–0.711	**0.002**	

Note: Bold formats represent statistical significance ($p < 0.05$).

Abbreviations: *CAT*, Catalase; CI, Confidence interval; NA, Not available; *NOS3*, endothelial nitric oxide synthase; OR, Odds ratio; *SOD1*, Superoxide dismutase 1; *SOD2*, Manganese superoxide dismutase.

TABLE 3 The association between semen parameters and genotype of the samples

SNP Semen parameters	Genotype			*p*-Value*	*p*-Value**
***SOD1* 7958G>A (rs4998557)**	**GG**	**GA**	**AA**		
Concentration (10^6/ml)					
Control	114.80	104.10	78.84	0.070	0.360
Patient	46.87	57.03	50.72	0.590	
Total sperm count (10^6)					
Control	273.93	295.81	235.22	0.500	0.950
Patient	127.82	159.98	150.16	0.580	
Vitality (%)					
Control	86.91	87.03	87.13	0.960	0.240
Patient	81.43	81.18	81.44	0.970	
Motility (% progressive)					
Control	47.88	47.09	46.58	0.690	0.200
Patient	28.75	30.77	30.63	0.470	
Morphology (% normal)					
Control	12.19	11.47	11.09	0.440	0.500
Patient	7.04	7.79	7.44	0.700	
***SOD2* c.47T>C(rs4880)**	**TT**	**TC**	**CC**		
Concentration (10^6/ml)					
Control	105.02	92.77	80.00	0.580	**0.044**
Patient	56.97	53.34	36.13	0.450	
Total sperm count (10^6)					
Control	286.83	249.58	190.25	0.590	0.760
Patient	146.81	159.80	130.42	0.270	
Vitality (%)					
Control	87.15	86.81	86.00	0.760	**0.000**
Patient	81.06	82.46	75.63	**0.002**	
Motility (% progressive)					
Control	46.79	47.83	49.90	0.550	0.160
Patient	29.17	32.02	27.28	**0.048**	
Morphology (% normal)					
Control	11.57	11.56	12.50	0.920	0.180
Patient	7.53	7.88	6.00	0.920	
***CAT* -262 C > T (rs1001179)**	**CC**	**CT**	**TT**		
Concentration (10^6/ml)					
Control	100.11	103.74	ND	0.850	0.780
Patient	51.83	60.11	112.00	0.300	
Total sperm count (10^6)					
Control	273.89	264.01	ND	0.880	0.430
Patient	144.35	170.73	425.60	0.068	
Vitality (%)					
Control	87.10	86.40	ND	0.450	0.610
Patient	81.14	82.17	ND	0.600	
Motility (% progressive)					
Control	47.41	45.59	ND	0.310	0.610
Patient	30.15	30.62	38.50	0.470	

(Continues)

TABLE 3 (Continued)

SNP Semen parameters	Genotype			*p*-Value*	*p*-Value**
Morphology (% normal)					
Control	11.55	11.90	ND	0.740	0.420
Patient	7.38	8.56	7.00	0.750	
***NOS3*–786C>T (rs2070744)**	**CC**	**CT**	**TT**		
Concentration (10^6/ml)					
Control	100.11	103.74	ND	0.078	0.960
Patient	51.83	60.11	112.00	0.480	
Total sperm count (10^6)					
Control	273.89	264.01	ND	0.330	0.960
Patient	144.35	170.73	425.60	0.420	
Vitality (%)					
Control	87.10	86.40	ND	0.220	**0.024**
Patient	81.14	82.17	78.00	0.130	
Motility (% progressive)					
Control	47.41	45.59	ND	0.890	**0.017**
Patient	30.15	30.62	38.50	0.740	
Morphology (% normal)					
Control	11.55	11.90	ND	0.960	0.110
Patient	7.38	8.56	7.00	0.880	

Note: One-way ANOVA was used to compare the mean of the semen parameters and genotypes.

Abbreviations: *CAT*, Catalase; ND, not detected; *NOS3*, endothelial nitric oxide synthase; *SOD1*, Superoxide dismutase 1; *SOD2*, Manganese superoxide dismutase.

**P-value* based on a comparison between genotypes of patients and controls.; ***P-value* based on a comparison between genotypes of whole samples of the study. Bold formats represent statistical significance ($p < 0.05$).

11.76% of the control. Only one patient carrying homozygous TT genotype was found in the study samples (Table 2).

Our data showed that there was no significant difference in genotype ($p = 0.401$) and allele frequency ($p = 0.209$) between the group of infertile patients and the fertile men.

4.6 | NOS3–786 C>T (rs2070744)

In the case of the *NOS3*–786 C>T variant, we identified 62 patients (57.94%) and 69 healthy individuals (81.18%) carrying TT genotype, while 43 patients (40.19%) and 15 healthy subjects (17.65%) having CT genotype. Only three individuals had wild-type CC genotype, including two patients and one fertile man.

We detected a difference in the distribution of genotypes and allele frequencies between the patient and control groups with $p = 0.003$ and $p = 0.002$, respectively. At the genotype level, no statistically significant difference was found between the disease group and the healthy group. However, at the allele level, T carriers had a lower risk of disease (OR = 0.397, 95% CI: 0.213–0.711, $p = 0.002$) and vice versa (Table 2).

4.7 | Association between the semen parameters and SNP genotypes

To evaluate the influence of SNP genotypes on sperm quality, we compared the semen parameters (% sperm morphology, % sperm motility, sperm vitality, sperm concentration, and total sperm count) between patients and fertile men for each genotypes (Table 3). The results showed that there were a significant difference for *SOD2* c.47T>C variant with respect to the concentration ($p = 0.044$) and sperm vitality ($p = 0.000$). Similarly, for the *NOS3* –786C>T variant, we also found a difference between genotypes and the vitality ($p = 0.024$) and the motility ($p = 0.017$). Within the infertile men, we identified a significant difference between the *SOD2* c.47T>C variant and the sperm vitality ($p = 0.002$) and the sperm motility ($p = 0.048$, Table 3).

Comparing the mean of these parameters with each SNP genotypes, we detected that only the sperm vitality rate of TC and TT genotypes of SOD2 c.47T>C (rs4880) variant were higher than CC genotype carriers for whole samples (Figure 1A) and for the infertile patients (Figure 1B).

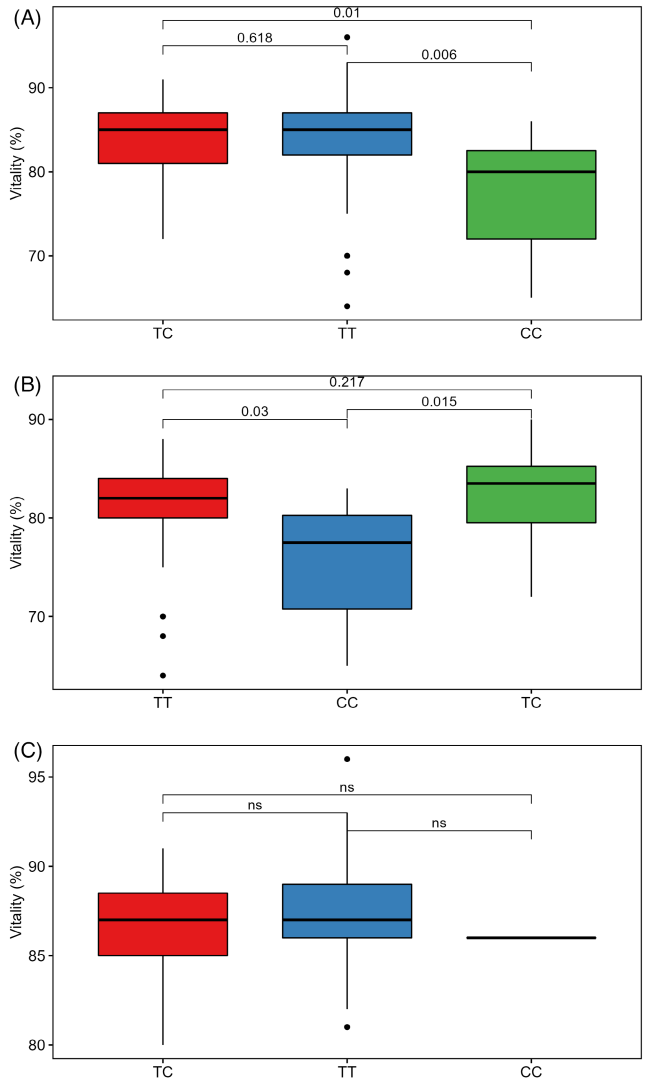

FIGURE 1 The association between sperm vitality and Manganese superoxide dismutase (*SOD2*) genotype. Wilcoxon test was used to evaluate the association between sperm vitality and *SOD2* genotypes. *P-value* was adjusted with Bonferroni correction. The association between sperm vitality (% vital) and genotype of *SOD2* for whole samples (A), in infertile cases (B), and healthy men (C). Sperm vitality rate in infertile cases with TT and TC genotype was higher than in patients with CC genotype. Meanwhile, no statistically significant differences were found between genotypes in the healthy group.

4.8 | Impact of gene–gene interaction on male infertility

The enzymes SODs and CATs protect sperm from destruction by superoxide (O_2^-) and hydrogen peroxide (H_2O_2). We hypothesized that a given patient carrying a combination of unfavorable alleles of the above SNPs might have a higher risk of male infertility than an individual carrying wild-type alleles. Therefore, multivariate logistic regression method was used to test the interaction between *SOD1* 7958G>A and *SOD2* c.47T>C, *SOD1* 7958G>A and *CAT* -262C>T, *SOD2* c.47T>C and *CAT* -262C>T, and all three variants. Our results are presented in Figure 2. The patients who carried both *SOD1* 7958GA and *SOD2* c.47TC genotypes had a 4.343-fold higher infertility risk than wild-type carriers (95% CI: 1.467–13.799, $p = 0.005$). The patients with the *SOD1*:7958GA×*CAT*: -262CT genotype pattern had a higher risk of the disease than the wildtype genotypes with OR = 4.101 (95% CI: 1.103–20.93, $p = 0.039$). Similarly, patients with the genotype pattern of *SOD2*: TC+CC×*CAT*: TC+TT also had a higher risk of male infertility (OR = 3.166, 95%CI: 1.116–10.588, $p = 0.027$) than the wildtype genotype carriers. In particular, the risk of male infertility in the carriers of the combination of *SOD1*:GA×*SOD2*:TC×*CAT*: CT and *SOD1*: 7958GA+AA×*SOD2*: c.47TC+CC×*CAT* -262: CT+TT genotypes increased more than 8 times (95% CI: 1.264–248.69, $p = 0.04$) and 7 times (95% CI: 1.587–62.709, $p = 0.007$) compared with the wild-type carriers, respectively.

5 | DISCUSSION

Nowadays, male infertility, which makes up approximately 50% of general infertility, is considered a serious health problem. Among multifactors have been reported, oxidative stress is one factor attributed to male infertility.[27–29] For this reason, genetic polymorphisms of antioxidant genes might contribute to the risk of male infertility. In this study, we reported the association of four polymorphisms of antioxidant genes (*SOD1*: 7958G>A, *SOD2*: c.47T>C, *CAT*: -262C>T, and *NOS3*: -786C>T) with observed infertility in our patients.

SOD1 and SOD2 play an important role in eliminating ROS and these antioxidant enzymes protect cells from free radicals and damage by oxidative stress. The polymorphism 7958G>A (rs4998557) of *SOD1* was reported to be possibly associated with colorectal cancer,[30] sexually dimorphic manner,[31] sudden sensorineural hearing loss in the Japanese population,[32] or Alzheimer's disease.[33] To date, although there has been no study on the influence of *SOD1* gene polymorphisms on infertility in humans, studies on mice have shown that there was impaired fertility in SOD1 deficient mouse sperm[34] or a decreased of spermatogenic cells in sod1-knockout mice under heat stress.[35] Our disease–control study has identified the heterozygous genotype 7958GA of *SOD1* in the infertile men was significantly higher than in control men (Table 2), suggesting that the GA genotype might be a risk for male infertility.

For *SOD2*, previous studies have shown that the *SOD2* c.47T>C (rs4880) polymorphism was associated with male infertility.[6,10,36–39] Most studies show that the SOD2 rs4880 CC genotype has an association with a low level of SOD activity, except for the study of Garcia-Rodriguez et al.[39] The results of this study indicated that the frequency of wild-type TT genotype was significantly lower in the male infertility group than in the control group, suggesting a protective role for the T allele. In contrast, C allele carriers had a higher risk of disease, consistent with the results of other authors.[6,10,36–38]

For variant *CAT* -262C>T (rs1001179), this study showed that there was no significant difference in genotype and allele frequency between the group of infertile patients and the fertile men

Gene x gene interaction	Patients	Controls	OR (95% CI)	*p-value*
SOD1 (rs4998557) x SOD2 (rs4880)				
GGxTT	10	15	Ref	
GGxTC+CC	21	25	1.252 (0.463 - 3.474)	0.646
GA+AAxTC+CC	78	58	1.999 (0.839 - 4.951)	0.109
GAxTC	**27**	**9**	**4.343 (1.467 - 13.799)**	**0.005**
GA+AAxTT	41	41	1.488 (0.599 - 3.83)	0.380
SOD1 (rs4998557) x CAT (rs1001179)				
GGxCC	21	21	Ref	
GGxCT+TT	2	5	0.422 (0.049 - 2.311)	0.423
GAxCT	**13**	**3**	**4.101 (1.103 - 20.93)**	**0.039**
GA+AAxCT+TT	16	5	3.093 (0.992 - 11.166)	0.060
SOD2 (rs4880) x CAT (rs1001179)				
TTxCC	47	51	Ref	
TCxTC	11	5	2.334 (0.774 - 8.075)	0.122
TCxTC+TT	12	5	2.542 (0.859 - 8.706)	0.084
TC+CCxTC+TT	**15**	**5**	**3.166 (1.116 - 10.588)**	**0.027**
SOD1 (rs4998557) x SOD2 (rs4880) x CAT (rs1001179)				
GGxTTxCC	10	13	Ref	
GGxTC+CCxCT+TT	2	3	0.886 (0.889 - 6.937)	0.240
GAxTCxCT	**8**	**1**	**8.819 (1.264 - 248.69)**	**0.044**
GA+AAxTTxCT+TT	4	3	1.685 (0.287 - 11.308)	0.525
GA+AAxTC+CCxCT+TT	**13**	**2**	**7.614 (1.582 - 62.709)**	**0.007**

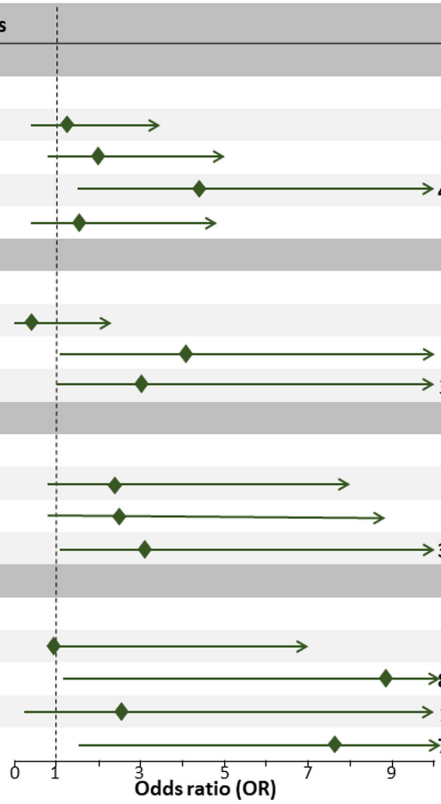

FIGURE 2 The gene–gene interaction on male infertility. The patients, who carried both Superoxide dismutase 1 (*SOD1*) 7958GA and Manganese superoxide dismutase (*SOD2*) c.47TC genotypes had a 4.3-fold; the patients with the *SOD1*:7958GA×*CAT*: -262CT genotype pattern had a 4.1-fold. The patients, who had a combination of *SOD1* 7958GA+AA, *SOD2* c.47TC+CC, and *CAT* -262CT+TT genotypes increased nearly 8 times the risk of infertility compared with the wild-type carriers. Bold formats represent statistical significance ($p < 0.05$).

(Table 2). Our result is consistent with those of Bousnane et al[40] on the Algerian group of infertile patients and Faure et al[36] but different from the result of other studies have reported that there was a significant difference in CT heterozygous genotype between the patient group and the control group.[14,39]

Genetic variants of *NOS3* might be associated with sperm defects.[8,21–23,41] Previous studies indicated that the *NOS3*–786C allele was associated with the risk for poor semen parameters in Iranians[23] or associated with higher levels of sperm DNA fragmentation and increased a risk for Chinese male infertility.[20,24] Although the frequency of the *NOS3*–786C allele in this study is very low (only three individuals), the results obtained in this study are consistent with those of other studies.[36,42] In particular, the frequency of *NOS3*–786 CT genotype of infertile men was higher than in the healthy men, suggesting that this genotype may have a higher risk of male infertility. Meanwhile, genotype TT carriers had a reduced risk (Table 2).

SODs and CAT are important enzymes regulated by the nuclear factor erythroid 2-related factor 2/ antioxidant respond element (NRF2/ARE) signaling pathway,[43] involved in cell protection by scavenging the superoxide anion. Furthermore, gene–gene interactions between polymorphisms of antioxidant genes might affect male infertility.[10,38,44] Regarding the polymorphisms of three genes *SOD1*, *SOD2*, and *CAT*, which were genotyped in this study, we found that the patients carried both *SOD1*: 7958GA and *SOD2*: c.47TC genotypes, the patients with the *SOD1*: 7958GA×*CAT*: -262CT, and the patients with the *SOD1*: 7958GA+AA×*CAT*: -262CT + TT genotype pattern had an increased risk of male infertility. Especially, infertile men with genotype *SOD1*:7958GA+AA, *SOD2*: c.47TC+CC, and *CAT*: -262CT+TT had a nearly 8-fold higher infertility risk than wild-type carriers (Figure 2). These data suggest that the gene–gene interaction between SOD1 7958G>A and SOD2 c.47T>C, SOD1 7958G>A and CAT -262C>T, SOD2 c.47T>C, and CAT -262C>T, and all three variants might increase risk of male infertility in Vietnamese men.

The weakness of this study is that it was performed on a relatively small sample size. In the future, it would be necessary to conduct further investigation on a larger sample size for better statistical analysis.

In conclusion, our study determined the association between antioxidant genes polymorphism and male infertility in Vietnamese men. We also identified that gene–gene interaction between *SOD1* 7958G>A and *SOD2* c.47T>C, *SOD1* 7958G>A and *CAT* -262C>T, *SOD2* c.47T>C, and *CAT* -262C>T, and all three variants might increase risk of male infertility in Vietnamese patients. To the best of author's knowledge, this is the first study to explore the link between *SOD1* 7958G>A polymorphism and male infertility. Further research with large sample size and more antioxidant gene polymorphisms

should be considered to understand better the association between polymorphisms and male infertility in the Vietnamese population.

AUTHOR CONTRIBUTIONS

Conceptualization: NDT, BHA, TDP; Funding acquisition: NDT, BHA, BMD; Data curation, Formal analysis, and Investigation: MTHT, VPN, NHH; Roles/Writing - original draft: BHA, NDT; Writing - review & editing: BHA, TDP, BMD, NDT, NVH.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study may be requested from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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