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Genetic polymorphism of glial cell-derived neurotrophic factor (GDNF) in male infertility

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Infertility is defined as the failure to establish a pregnancy after a year of regular and unprotected sexual intercourse [1]. Males are solely responsible for 20–30% of infertility cases but contribute to 50% of cases overall. In 30–40% of the cases, the etiology remains unknown, and so is described as idiopathic male infertility [2]. Genetic anomalies such as chromosomal disorders, mitochondrial DNA mutations, deletions in Y chromosome and endocrine abnormalities have been described in infertile men [3]. Some 10–15% of cases of azoospermia and severe oligozoospermia are secondary to genetic causes, the microdeletion of Y chromosome and a wide range of structural autosomal anomalies and mutations in single genes are associated with male infertility [4].

Glial cell-derived neurotrophic factor (GDNF), a member of transforming growth factor-beta superfamily, plays an important role in spermatogenesis, is produced and secreted by Sertoli cells from birth through adulthood [5]. Spermatogonial stem cells (SSCs) express GDNF receptors including GFRα1 and c-RET tyrosine receptor [6]. The GDNF signalling pathway is regulated by follicle-stimulating hormone and plays a key role in SSC renewal and spermatogonial differentiation. Disruption of GDNF/c-RET signalling results in abnormal spermatogenesis due to deficit in SSC renewal [7]. Overall, SSC interactions with interstitial and peritubular cells are critical for SSC function and are an important underlying factor promoting male fertility [5]. These data suggest that GDNF and its signalling plays important role in human male infertility. We hypothesised a link between *GDNF* single nucleotide polymorphism (SNP) rs2075680 and the risk of male infertility.

We recruited 185 men with infertility and 200 fertile men as controls. At least three seminal fluid analyses, carried out after 3–5 days of sexual abstinence, were performed to ascertain their infertility status. Semen analysis has been performed according to World Health Organization recommendations [8]. We excluded patients with a positive history of epididymo-orchitis, prostatitis, genital trauma, cryptorchidism, chromosomal abnormalities, testicular torsion, bilateral absence of the vas deferens, varicocele, hypogonadotropic hypogonadism, seminal infections, drug and alcohol user; and also chronic diseases such as diabetes. All the patients provided a medical history and underwent physical examinations. Controls were healthy men with normal semen parameters who had at least one child without benefiting from assisted reproductive technologies. The study has been performed in compliance with the 1964 Helsinki declaration.

Genomic DNA was extracted from peripheral blood cells using the GPP Solution (Gen Pajoohan, Iran) according to manufacturer's instruction. A ratio of A260/A280 absorbance was used to qualify extracted DNA. Primers were designed using oligo primer analysis software (version 7.54, Molecular Biology Insights Inc., Cascade, CO, USA). These were GDNFF (5′-AACCTCCCCTAACCCGTTC-3′) and GDNFR (5′-TTTCCTCGCGCCTGTCGAAG-3′). Polymerase chain reaction (PCR) was performed in a total reaction volume of 25 µl containing 50 ng of genomic DNA, 1 U DNA polymerase, 1 × reaction buffer (750 mM Tris-HCl (pH 9.0), 500 mM KCl, 200 mM $(NH_4)_2SO_4$), 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.25 µM of each primer. Amplification was performed using a thermal cycler (Bio-RAD, USA) according to the following protocol; the initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 60 s at 60 °C, extension for 45 s at 72 °C with a final extension time of 2 min at 72 °C. The resulting 508 bp DNA fragment was digested with *Alu1* restriction enzyme (Thermo Scientific) generating two fragments of 416 and 92 bp only in the presence of the A allele.

Allele frequencies of the *GDNF* polymorphism were estimated by gene counting. Hardy–Weinberg equilibrium (HWE) of the genotypes was analysed by the chi-square (χ^2) test. Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated. Associations

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were regarded as significant at $P < 0.05$. Data was analysed on SPSS v 20.0 (Chicago, IL, USA).

The 185 idiopathic infertile men were aged mean [SD] 38.6 [16.7] years – the 200 controls 36 [18] years ($P > 0.05$). In the control subjects and cases, the genetic distributions of *GDNF* SNP rs2075680 did not deviate from the HWE (controls $\chi^2 = 2.29$; $P = 0.12$, cases $\chi^2 = 0.11$, $P = 0.73$). The frequencies of *GDNF* homozygous major (CC), heterozygous (CA) and homozygous minor (AA) genotypes in controls were 54%, 36% and 10%, and 40.5%, 44.3% and 15.1 % in infertile men, respectively ($p = 0.025$). In the codominant model, compared with genotype CC, the CA or AA genotypes were linked to infertility risk. In the dominant model, individuals with the AA and CA genotypes were at higher risk of infertility when compared with the CC genotype. A higher prevalence of the A allele was present in infertile cases (37.3%) than in controls (28%) (Table 1). The patients were classified as azoospermia (30.3%), oligozoospermia (44.3%) and asthenospermia (25.4%). The genotype analyses revealed strong associations of AA genotype and A allele with azoospermia, but no links with oligozoospermia. However, the A allele carriers seemed to confer protective effects on male infertility risk in asthenospermic men (Table 2).

We hypothesised an association between a SNP (rs2075680) located in *GDNF* and the risk of male infertility by a case-control approach. Our results showed that A allele of rs2075680 could confer a genetic predisposition male infertility. Moreover, AA genotype carriers have an increased risk of infertility of about two times higher than those with the CC genotype. We also found the AA genotype to be associated with azoospermia. In the literature, few studies have been conducted regarding association between *GDNF* SNPs and different diseases. A case-control study conducted by Fernandez et al. did not find any association between gene variants of *GDNF* (rs2075680, rs2910797 and rs11111) and Hirschsprung disease [9]. A study of 930 young adults reported that *GDNF* rs3096140 might be involved in the genetic background of smoking, independent of anxiety characteristics [10]. In the adult mouse, GDNF is a pivotal paracrine regulator of the numbers, replication and differentiation of SSCs and progenitor spermatogonia. Inhibition of GDNF signalling reduces the replication of SSCs, and promotes their differentiation [7]. An *in vitro* and *in vivo* study has elucidated the expression of GDNF in normal and Sertoli cell-only (SCO) testes. It has been shown that the Sertoli cells are the primary source of GDNF in the human testis and in the SCO testis these cells produce substantially less GDNF compare to normal testis [6].

Table 1. Genotype and allele frequencies of *GDNF* polymorphic site and risk of male infertility.

Model	Genotype	Controls *N* (%)	Cases *N* (%)	OR (95%CI)	*P*-value
Codominant	CC	108 (54)	75 (40.5)	Reference	
	CA	72 (36)	82 (44.3)	1.64 (1.06–2.52)	0.02
	AA	20 (10)	28 (15.1)	2.01 (1.05–3.84)	0.03
Dominant	CC	108 (54)	75 (40.5)	Reference	
	AA + CA	92 (46)	110 (59.5)	1.72 (1.14–2.57)	0.008
Recessive	CA + CC	180 (90)	157 (84.9)	Reference	
	AA	20 (10)	28 (15.1)	1.60 (0.87–2.96)	0.12
Overdominant	CC + AA	128 (64)	103 (55.7)	Reference	
	CA	72 (36)	82 (44.3)	1.41 (0.93–2.13)	0.09
Alleles	C	288 (72)	232 (62.7)	Reference	
	A	112 (28)	138 (37.3)	1.53 (1.12–2.07)	0.006

OR: odds ratio; CI: confidence interval; GDNF: glial cell-derived neurotrophic factor.

Table 2. The *GDNF* rs2075680 genotype and allele distributions in three groups of infertile men.

Cases	Genotype/Allele	*N* (%)	OR (95%CI)	*P*-value
Azoospermia	CC	18 (32.1)	Reference	–
N = 56	CA	22 (39.3)	1.11 (0.55–2.24)	0.75
	AA	16 (28.6)	2.38 (1.06–5.30)	0.03
	AA + CA	38 (67.8)	1.43 (0.76–2.39)	0.25
	C	58 (51.8)	Reference	–
	A	54 (48.2)	1.56 (1.02–2.39)	0.03
Oligozoospermia	CC	25 (30.5)	Reference	–
N = 82	CA	48 (58.5)	1.75 (0.98–3.12)	0.05
	AA	9 (11)	0.96 (0.40–2.31)	0.93
	AA + CA	57 (69.5)	1.55 (0.89–2.70)	0.11
	C	98 (59.8)	Reference	–
	A	66 (40.2)	1.13 (0.77–1.64)	0.51
Asthenospermia	CC	32 (68)	Reference	–
N = 47	CA	12 (25.5)	0.34 (0.16–0.71)	0.004
	AA	3 (6.4)	0.25 (0.07–0.88)	0.03
	AA + CA	15 (32)	0.31 (0.16–0.63)	0.001
	C	76 (80.8)	Reference	–
	A	18 (19.1)	0.39 (0.22–0.69)	0.001

OR: odds ratio; CI: confidence interval; GDNF: glial cell-derived neurotrophic factor.

We focused on SNP in the 5′upstream region of *GDNF* gene, rs2075680, consisting of a nucleotide substitution C to A with chromosomal location 37840540. Many genetic variations located in the upstream regions of genes are likely to be regulatory. One mechanism is that the genetic variants within upstream regions may influence gene transcription by modulating the binding affinity of a transcription factor to the DNA and leading to unusual protein expression [11]. Several groups investigated the potential association between gene polymorphisms and male infertility [12–14]. A recent study determined that the AG genotype of *VDAC3* (rs16891278) showed a significantly lower sperm concentration compared with the AA genotype [13]. Trang et al., in a Vietnamese population, revealed that the polymorphisms of *NAT2* (rs1799929, rs1799930) and GSTP1 (rs1138272, rs1695) are associated with idiopathic male infertility [14]. We have previously shown that individuals with the variant *ApE1* (–656T > G) TG genotype had a significantly increased risk of female infertility (P = 0.035, OR = 1.98, 95% CI = 1.04–3.74) [15].

We recognize certain limitations to our results. Firstly, our sample size is relatively small; the result should be interpreted with caution until confirmed in larger studies. Secondly, this study only considers a local population that may limit the application of these findings to other populations. Thirdly, since only one SNP of *GDNF* has been investigated in the present study, we cannot exclude the possibility that other genetic variants could have a role in idiopathic male infertility susceptibility. Finally, numerous factors act individually and together to influence risk of male infertility. This work represents an advance in biomedical science because it demonstrates a link between the GDNF (rs2075680) polymorphism and male infertility, suggesting the SNP may help in diagnosis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. International committee for monitoring assisted reproductive technology (ICMART) and the WHO revised glossary of ART terminology, 2009. Fertil Steril. 2009;92(5):1520–1524.
[2] Ray A, Shah A, Gudi A, et al. Unexplained infertility: an update and review of practice. Reprod Biomed Online. 2012;24(6):591–602.
[3] Lee JY, Dada R, Sabanegh E, et al. Role of genetics in azoospermia. Urology. 2011;77(3):598–601.
[4] Miyamoto T, Minase G, Shin T, et al. Human male infertility and its genetic causes. Reprod Med Biol. 2017;16(2):81–88.
[5] Potter SJ, DeFalco T. Role of the testis interstitial compartment in spermatogonial stem cell function. Reproduction. 2017;153(4):R151–R162.
[6] Spinnler K, Köhn FM, Schwarzer U, et al. Glial cell line-derived neurotrophic factor is constitutively produced by human testicular peritubular cells and may contribute to the spermatogonial stem cell niche in man. Hum Reprod. 2010;25(9):2181–2187.
[7] Parker N, Falk H, Singh D, et al. Responses to glial cell line-derived neurotrophic factor change in mice as spermatogonial stem cells form progenitor spermatogonia which replicate and give rise to more differentiated progeny. Biol Reprod. 2014;91(4):92.
[8] WHO. Laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization; 2010. p. 7–113.
[9] Fernandez RM, Ruiz-Ferrer M, Lopez-Alonso M, et al. Polymorphisms in the genes encoding the 4 RET ligands, GDNF, NTN, ARTN, PSPN, and susceptibility to Hirschsprung disease. J Pediatr Surg. 2008;43(11):2042–2047.
[10] Kotyuk E, Nemeth N, Ronai Z, et al. Association between smoking behaviour and genetic variants of glial cell line-derived neurotrophic factor. J Genet. 2016;95(4):811–818.
[11] Benson CC, Zhou Q, Long X, et al. Identifying functional single nucleotide polymorphisms in the human CArGome. Physiol Genomics. 2011;43(18):1038–1048.
[12] Mazjin MA, Salehi Z, Mashayekhi F, et al. Evaluation of GPx1 Pro198Leu polymorphism in idiopathic male infertility. Mol Biol (Mosk). 2016;50(1):89–93.
[13] Pan L, Liu Q, Li J, et al. Association of the VDAC3 gene polymorphism with sperm count in Han-Chinese population with idiopathic male infertility. Oncotarget. 2017;8(28):45242–45248.
[14] Trang NT, Huyen VT, Tuan NT, et al. Association of N-acetyltransferase-2 and glutathione S-transferase polymorphisms with idiopathic male infertility in Vietnam male subjects. Chem Biol Interact. 2018;286:11–16.
[15] Yousefi M, Salehi Z, Mashayekhi F, et al. The association of ApE1-656T>G and 1349T>G polymorphisms and idiopathic male infertility risk. Int Urol Nephrol. 2015;47(6):921–926.