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# Association of NOS3, VEGF, ACE and ACE2 polymorphisms with erectile dysfunction severity and tadalafil response in diabetic men

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## Abstract

This study investigated whether common polymorphisms in the NOS3, VEGF, ACE and ACE2 genes are associated with erectile dysfunction (ED) severity and response to tadalafil treatment in men with diabetes mellitus (DM). Seventy-eight men with DM and ED received tadalafil 5 mg once daily for 3 months. Erectile function was assessed using the 5-item International Index of Erectile Function (IIEF-5) at baseline and post-treatment. Genotyping was performed using competitive allele-specific PCR and conventional PCR for the ACE insertion/deletion polymorphism. Tadalafil significantly improved median IIEF-5 scores from 14 (IQR 11–17) to 19.5 (IQR 16–22) ($p < 0.001$), with 47 patients (60.3%) classified as responders (≥ 4-point increase). A nominal association with baseline ED severity was observed for ACE2 rs2285666, as T allele carriers had lower odds of moderate-to-severe ED compared with C allele carriers (OR 0.20, 95% CI 0.04–0.94; $p = 0.041$). However, no associations remained significant after correction for multiple testing. These findings suggest that common polymorphisms in these genes do not significantly predict tadalafil response in men with DM and ED.

## 1. INTRODUCTION

Erectile dysfunction (ED) is a common male sexual disorder with a substantial impact on quality of life and overall health. Its prevalence increases with age and is particularly high among men with metabolic and cardiovascular diseases. Diabetes mellitus (DM) represents one of the strongest risk factors for ED, with diabetic men developing erectile impairment earlier and experiencing more severe forms of the condition compared with the general population. The mechanisms underlying diabetic ED are complex and involve endothelial dysfunction, autonomic neuropathy, cavernosal smooth muscle alterations and impaired penile vascular hemodynamics (1). Penile erection is a hemodynamic process critically dependent on intact endothelial and smooth muscle function. The central molecular pathway mediating erection is nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) signaling (2–4). During sexual stimulation, endothelial nitric oxide synthase (eNOS), encoded by the *NOS3* gene, catalyzes the production of NO, which activates soluble guanylate cyclase in cavernosal smooth muscle cells, leading to increased intracellular cGMP levels. cGMP induces smooth muscle relaxation through protein kinase G–dependent mechanisms, allowing arterial dilation, sinusoidal expansion, and veno-occlusive trapping of blood within the corpora cavernosa. Disruption of this signaling pathway represents a key mechanism in the development of ED (3, 4). Chronic hyperglycemia promotes oxidative stress, endothelial dysfunction, autonomic neuropathy, and structural remodeling of cavernosal tissue (2, 5). Reduced NO bioavailability due to impaired eNOS activity and increased reactive oxygen species (ROS) results in diminished cGMP generation and compromised erectile function (4, 5). Functional polymorphisms in *NOS3* have been associated with altered NO production and increased risk of ED (6), suggesting that genetic variability in this pathway may influence both baseline erectile capacity and pharmacological response.

Vascular endothelial growth factor (VEGF) plays a pivotal role in endothelial survival, angiogenesis and maintenance of cavernosal smooth muscle architecture. Experimental studies have demonstrated that VEGF restoration improves erectile function in diabetic models (7). Polymorphisms in *VEGF* promoter regions may influence gene expression and thereby modulate endothelial repair capacity in the diabetic corpus cavernosum.

The renin-angiotensin-aldosterone system (RAAS) further regulates penile vascular tone and tissue remodeling. Angiotensin II promotes vasoconstriction, oxidative stress, inflammation, and fibrosis within the corpus cavernosum (2). Angiotensin-converting enzyme (ACE) facilitates angiotensin II production, while angiotensin-converting enzyme 2 (ACE2) counterbalances this pathway by converting angiotensin II into angiotensin-(1–7), a peptide with vasodilatory and anti-fibrotic properties. Dysregulation of the ACE/ACE2 axis may therefore shift the penile microenvironment toward vasoconstriction and structural deterioration, exacerbating ED severity. Importantly, *ACE2* is located on the X chromosome, and its functional polymorphisms may exert a direct phenotypic impact in men.

Phosphodiesterase type 5 inhibitors (PDE5I), including tadalafil, enhance erectile function by inhibiting cGMP degradation and amplifying residual NO-mediated signaling (4). However, their efficacy depends on sufficient upstream NO production and preserved cavernosal structure. Treatment response varies considerably among individuals, and men with diabetes frequently demonstrate reduced responsiveness to PDE5 inhibitors (1). While clinical factors, such as duration of diabetes and glycemic control are known to contribute to this variability, genetic determinants of treatment response remain insufficiently characterized (6, 8).

The aim of our study was to investigate whether selected *NOS3*, *VEGF*, *ACE* and *ACE2* polymorphisms are associated with baseline ED severity and response to tadalafil treatment in men with DM.

## 2. MATERIAL AND METHODS

# 2.1. Study design

This prospective genetic association study included patients with a diagnosis of type 1 DM, type 2 DM and concomitant ED. All participants were assessed using standardized protocols for clinical evaluation, data collection, genotyping and statistical analysis. The study was conducted in accordance with the Declaration of Helsinki and the Slovenian Code of Medical Ethics and received ethical approval from the National Medical Ethics Committee of the Republic of Slovenia at the Ministry of Health (KME 0120–337/2023/3).

## 2.2. Study participants

A total of 78 participants with a clinical diagnosis of DM and ED were enrolled in the study. Participants were recruited from the University Medical Centre Ljubljana and the Health Centre Kočevje between May 2024 and March 2025. All participants have completed International Index of Erectile Function (IIEF)-5 questionnaire before treatment.

ED severity was classified into five categories: absence of ED (IIEF-5 score 22–25), mild (17–21), mild to moderate (12–16), moderate (8–11), and severe (1–7) (9).

All patients received tadalafil 5 mg once daily for a duration of three months. Post-treatment assessment using the IIEF-5 questionnaire was performed at a follow-up visit three months after initiation of tadalafil therapy. Participants demonstrating an increase of ≥ 4 points in the IIEF-5 score were considered responders to treatment (8).

Inclusion criteria were confirmed diagnosis of DM and ED (participants who scored 21 points or less on the International Index of Erectile Function (IIEF)-5 questionnaire), age between 40 and 65 years and signed informed consent. Exclusion criteria included treatment with organic nitrates, a history of cardiovascular events, primary hypogonadism, prostate malignancy, psychogenic ED, premalignant or malignant genital lesions, Peyronie's disease, congenital penile disorders, previous pelvic injury, surgery or radiotherapy, and prostate-specific antigen (PSA) level ≥ 3.0 µg/L.

## 2.3. DNA isolation and genotyping

Genomic DNA was extracted from peripheral venous blood collected in EDTA-containing tubes (BD Vacutainer Blood Collection Tube, Becton Dickinson, NJ, USA). DNA isolation was performed using the FlexiGene DNA Kit (Qiagen, Germany) according to the manufacturer's instructions. *NOS3*, *VEGF, ACE*, and *ACE2* polymorphisms were selected based on their minor allele frequencies (MAFs) in Caucasian population and previously reported functional relevance. Only SNPs with MAF > 0.05 were included in the analysis.

The selected polymorphisms were genotyped using competitive allele-specific PCR (KASP) assays according to the manufacturer's protocol (LGC Biosearch Technologies, UK).

*ACE* rs1799752 insertion/deletion polymorphism was determined using modified classic PCR method described by Knoell et al. with the addition of 5% DMSO (10). Using the established primers (F: 5-GCCCTGCAGGTGTCTGCAGCATGT-3 and R: 5-GGATGGCTCTCCCCGCCTTGTCTC-3), we could distinguish between the D and I alleles, resulting in 319-bp and 597-bp amplicons to differentiate between homozygotes for insertion and individuals without insertions or heterozygotes. To ensure genotyping accuracy, control samples with known genotypes were included on each PCR plate, and approximately 20% of samples were randomly selected and re-genotyped.

## 2.4. Statistical analysis

Continuous variables were presented as medians with interquartile ranges (IQR; 25–75%), and categorical variables as frequencies. Minor allele frequencies (MAFs) were calculated for all polymorphisms, and deviation from Hardy–Weinberg equilibrium (HWE) was assessed using the $\chi^2$ test. Both additive and dominant genetic models were evaluated. SNPs with fewer than 3 carriers of the polymorphic allele were excluded from analysis. For *ACE2*, which is located on the X chromosome, only a single allele per individual was considered. For the ACE insertion polymorphism, only homozygotes for the deletion could be reliably determined.

The normality of continuous variables was assessed using the Shapiro–Wilk test. As normality assumptions were not met, non-parametric methods were used for further analyses: Mann–Whitney test for comparisons between two independent groups. Kruskal–Wallis test for comparisons among more than two independent groups and Wilcoxon signed-rank test for comparison of related samples.

Associations between polymorphisms and categorical variables were assessed using logistic regression, with results expressed as odds ratios (ORs) and 95% confidence intervals (CIs). All statistical tests were two-tailed. To account for multiple testing due to the inclusion of nine SNPs, Bonferroni correction was applied; $p < 0.006$ was considered statistically significant, whereas $p$ values between 0.006 and 0.050 were regarded as nominally significant. Statistical analyses were performed using IBM SPSS Statistics version 29.0 (IBM Corporation, Armonk, NY, USA).

Power analysis indicated that for a polymorphism with MAF of 30%, the study had 80% power to detect ORs ≥ 4.0 for classification of ED severity and OR ≥ 3.8 for ≥ 4-point improvement in IIEF-5 score. Power calculation was performed using the PS Power and sample size program, version a57e8c3 (11).

# 3. RESULTS

## 3.1. Patients' characteristics

A total of 78 male patients with DM and ED were included. Median age was 58 (IQR 53–62.3) years and the median duration of DM was 11 (IQR 6–17) years. Majority of participants had type 2 DM (88.5%). Median HbA1c was 7.2% (IQR 6.4–8.3). Baseline hormonal and biochemical

characteristics are summarized in Table 1.

Table 1
Clinical characteristics of the included patients with DM and ED (N = 78).

| Variable | Category/unit | Number / median (25%-75%) |
|---|---|---|
| Age | Years, median (25%-75%) | 58 (53-62.3) |
| Duration of DM | Years, median (25%-75%) | 11 (6–17) |
| HbA1c | %, median (25%-75%) | 7.2 (6.4–8.3) |
| DM type | 1, N (%) | 9 (11.5) |
| | 2, N (%) | 69 (88.5) |
| Total testosterone [1] | nmol/L, median (25%-75%) | 11.7 (8.9–17.5) |
| Free testosterone [1] | pmol/L, median (25%-75%) | 27.9 (19.8–38.3) |
| SHBG [1] | nmol/L, median (25%-75%) | 31 (22–47) |
| LH [1] | IU/L, median (25%-75%) | 4.3 (3.4–5.7) |
| FSH [1] | IU/L, median (25%-75%) | 7 (5-11.2) |
| Prolactin [1] | µg/L, median (25%-75%) | 5.9 (4.3–7.8) |
| TSH [1] | mIU/L, median (25%-75%) | 2 (1.4–2.5) |
| T4 [1] | pmol/L, median (25%-75%) | 15.3 (14.2–16.5) |
| T3 [1] | pmol/L, median (25%-75%) | 5.2 (4.9–5.6) |
| PSA [1] | µg/L, median (25%-75%) | 0.68 (0.39–1.25) |

The number of missing data is given in [] brackets. DM – diabetes mellitus, SHBG – Sex hormone-binding globulin, LH- Luteinizing hormone, FSH – Follicle-stimulating hormone, TSH – Thyroid-stimulating hormone, T4 – Thyroxine, T3 – Triiodothyronine, PSA – Prostate-specific antigen.

IIEF-5 results are presented in Table 2. At baseline, the median IIEF-5 score was 14 (IQR 11–17). A total of 16 patients (20.5%) had moderate ED, while 8 patients (10.3%) had severe ED before treatment. After three months of tadalafil 5 mg daily, the median IIEF-5 score significantly increased to 19.5 (IQR 16–22) points ($p < 0.001$), corresponding to a median improvement of 4 points (IQR 2–7). Overall, 63 patients (80.8%) demonstrated any improvement in IIEF-5 score after treatment, while 47 patients (60.3%) met the predefined responder criterion of ≥ 4-point increase in IIEF-5 score.

Table 2
IIEF-5 questionnaire results.

| Variable | Category/unit | |
|---|---|---|
| ED severity before treatment | Mild, N (%) | 22 (28.2) |
| | Mild to moderate, N (%) | 32 (41.0) |
| | Moderate, N (%) | 16 (20.5) |
| | Severe, N (%) | 8 (10.3) |
| IIEF-5 before treatment | Points, median (25%-75%); [min-max] | 14 (11–17); [5–21] |
| IIEF-5 after treatment | Points, median (25%-75%); [min-max] | 19.5 (16–22); [6–25] |
| IIEF-5 change | Points, median (25%-75%); [min-max] | 4 (2–7); [0–15] |
| IIEF-5 improvement of at least four points after treatment | No, N (%) | 31 (39.7) |
| | Yes, N (%) | 47 (60.3) |

IIEF-5: International Index of Erectile Function, ED – Erectile dysfunction.

## 3.2. Genotype frequencies

Genotype distributions for *NOS3*, *VEGF*, *ACE* and *ACE2* polymorphisms are presented in Table 3. Minor allele frequencies were consistent with previous European population reports and no significant deviations from HWE were observed for autosomal polymorphisms (all $p > 0.05$). For *ACE2*

(X-linked), allele frequencies are reported for the hemizygous state in men and HWE was not determined. *VEGF* rs699947 and rs833061 were in complete linkage disequilibrium, therefore only rs699947 was included in further analyses.

Table 3
Genotype frequencies for the studied polymorphisms, minor allele frequency (MAF) and agreement with Hardy-Weinberg equilibrium (HWE).

| Gene | Polymorphism | Description | Genotype | N (%) | MAF (%) | $P_{HWE}$ |
|---|---|---|---|---|---|---|
| *NOS3* | rs2070744 | NC_000007.14:g.150992991C > T | TT | 27 (34.6) | 37.2 | 0.067 |
| | | | TC | 44 (56.4) | | |
| | | | CC | 7 (9.0) | | |
| | rs1799983 | NC_000007.14:g.150999023T > G | GG | 28 (35.9) | 37.8 | 0.299 |
| | | | GT | 41 (52.6) | | |
| | | | TT | 9 (11.5) | | |
| *VEGF* | rs699947 | NC_000006.12:g.43768652A > C | AA | 21 (26.9) | 49.4 | 0.651 |
| | | | CA | 37 (47.4) | | |
| | | | CC | 20 (25.6) | | |
| | rs833061 | NC_000006.12:g.43769749C > T | CC | 21 (26.9) | 49.4 | 0.651 |
| | | | CT | 37 (47.4) | | |
| | | | TT | 20 (25.6) | | |
| | rs1570360 | NC_000006.12:g.43770093A > G | GG | 41 (52.6) | 28.8 | 0.405 |
| | | | GA | 29 (37.2) | | |
| | | | AA | 8 (10.3) | | |
| *ACE* | rs1800764 | NC_000017.11:g.63473168C > T | TT | 25 (32.1) | 41.7 | 0.473 |
| | | | TC | 41 (52.6) | | |
| | | | CC | 12 (15.4) | | |
| | rs1799752 [1] | NC_000017.11:63488529:ATACAGTCACTTTT:ATACAGTCACTT TTTTTTTTTTTTTGAGACGGAGTCTCGCT CTGTCGCCCATACAGTCACTTTT | No insertion/heterozygote | 56 (71.8) | / | / |
| | | | Homozygous for insertion | 21 (26.9) | | |
| | rs4343 | NC_000017.11:g.63488670G > A | GG | 16 (20.5) | 53.2 | 0.623 |
| | | | GA | 41 (52.6) | | |
| | | | AA | 21 (26.9) | | |

The number of missing data is given in [] brackets.

A- adenine, G – guanine, C – cytosine, T – thymine, MAF – minor allele frequency, $P_{HWE}$ – p-value of the $\chi 2$ test for HWE.

*gene is located on the X chromosome – only alleles given, not genotypes (hemizygous state)

| Gene | Polymorphism | Description | Genotype | N (%) | MAF (%) | $P_{HWE}$ |
|---|---|---|---|---|---|---|
| *ACE2** | rs2285666 | NC_000023.11:g.15592225C > T | C | 59 (75.6) | 24.4 | / |
| | | | T | 19 (24.4) | | |
| | rs1978124 | NC_000023.11:g.15599940T > C | T | 40 (51.3) | 48.7 | / |
| | | | C | 38 (48.7) | | |
| The number of missing data is given in [] brackets. | | | | | | |
| A- adenine, G – guanine, C – cytosine, T – thymine, MAF – minor allele frequency, $P_{HWE}$ – p-value of the $\chi 2$ test for HWE. | | | | | | |
| *gene is located on the X chromosome – only alleles given, not genotypes (hemizygous state) | | | | | | |

## 3.3. Association of polymorphisms with erectile dysfunction severity

No significant associations were observed between *NOS3*, *VEGF* or *ACE* polymorphisms and baseline IIEF-5 score under additive or dominant genetic models (Table 4). Logistic regression analyses did not identify associations with moderate or severe ED (Table 5). Nominal association was observed only for *ACE2* rs2285666. Carriers of the T allele had lower odds of moderate or severe ED compared with C allele carriers (OR 0.20, 95% CI 0.04–0.94; p = 0.041). Although this did not remain significant after Bonferroni correction, the direction and magnitude of effect suggested a potential protective role. Similarly, *ACE2* rs1978124 demonstrated a non-significant trend toward lower odds of severe ED (OR 0.40, 95% CI 0.15–1.09; p = 0.074).

Table 4
Association of the studied polymorphisms with IIEF-5 score before treatment and with change after treatment.

| Gene | Polymorphism | Genotype | IIEF-5 before treatment<br>Median (25%-75%) | P value | Change in IIEF-5 after treatment Median (25%-75%) | |
|---|---|---|---|---|---|---|
| *NOS3* | rs2070744 | TT | 15 (12–18) | $P_{add}$=0.185 | 4 (2–7) | $P_{add}$=0.824 |
| | | TC | 13 (11–16) | | 4 (2–7) | |
| | | CC | 16 (10–18) | | 4 (0–12) | |
| | | TC + CC | 13 (11–17) | $P_{dom}$=0.099 | 4 (2–7) | $P_{dom}$=0.711 |
| | rs1799983 | GG | 15 (10-17.8) | $P_{add}$=0.926 | 4.5 (0-6.8) | $P_{add}$=0.826 |
| | | GT | 13 (11–17) | | 4 (2.5-7) | |
| | | TT | 14 (10-17.5) | | 4 (1.5–8.5) | |
| | | GT + TT | 13.5 (11–17) | $P_{dom}$=0.750 | 4 (2–7) | $P_{dom}$=0.585 |
| *VEGF* | rs699947 | AA | 14 (11–16) | $P_{add}$=0.890 | 5 (2-7.5) | $P_{add}$=0.122 |
| | | CA | 13 (10.5–18) | | 4 (0-5.5) | |
| | | CC | 14 (8.8–17) | | 5.5 (3.3–8.8) | |
| | | CA + AA | 14 (10.5–17.5) | $P_{dom}$=0.852 | 4 (2–7) | $P_{dom}$=0.650 |
| | rs1570360 | GG | 14 (11-16.5) | $P_{add}$=0.854 | 4 (2.5-7) | $P_{add}$=0.189 |
| | | GA | 14 (10–18) | | 3 (1.5–5.5) | |
| | | AA | 13.5 (11.5–15.5) | | 7 (3.3–8.8) | |
| | | GA + AA | 14 (11–18) | $P_{dom}$=0.786 | 4 (2–7) | $P_{dom}$=0.529 |
| *ACE* | rs1800764 | TT | 14 (11-16.5) | $P_{add}$=0.668 | 4 (1.5-9) | $P_{add}$=0.704 |
| | | TC | 14 (10–17) | | 4 (2–7) | |
| | | CC | 15 (11.5–17.8) | | 4 (0-6.8) | |
| | | TC + CC | 14 (11–17) | $P_{dom}$=0.743 | 4 (2–7) | $P_{dom}$=0.560 |
| | rs1799752 | No insertion/heterozygote | 14 (11–17) | P = 0.787 | 4 (2–7) | P = 0.254 |
| | | Homozygous for insertion | 14 (11-16.5) | | 6 (2-10.5) | |
| | rs4343 | GG | 16.5 (12.3–18.8) | $P_{add}$=0.175 | 4 (0.5–5.8) | $P_{add}$=0.427 |
| | | GA | 13 (10–16) | | 4 (2–7) | |
| | | AA | 14 (11-16.5) | | 6 (2-10.5) | |
| | | GA + AA | 13.5 (10–16) | $P_{dom}$=0.069 | 4 (2-7.3) | $P_{dom}$=0.422 |
| *ACE2* | rs2285666 | C | 13 (11–17) | P = 0.110 | 4 (2–7) | P = 0.669 |
| | | T | 15 (13–18) | | 4 (0–8) | |
| | rs1978124 | T | 13 (10-16.8) | P = 0.093 | 4 (2-8.5) | P = 0.249 |
| | | C | 15 (12–17) | | 4 (0–7) | |

$P_{add}$: P-values for the additive model determined by the Kruskal-Wallis test; $P_{dom}$: P-values for the dominant model determined by the Mann-Whitney test

Table 5
Association of the studied polymorphisms with moderate or severe erectile dysfunction (ED) before treatment.

| Gene | SNP | Genotype | Mild/mild to moderate ED | Moderate/severe ED | OR (95% CI) | P value |
|---|---|---|---|---|---|---|
| | | | N (%) | N (%) | | |
| *NOS3* | rs2070744 | TT | 22 (81.5) | 5 (18.5) | Ref. | |
| | | TC | 27 (61.4) | 17 (38.6) | 2.77 (0.88–8.71) | 0.081 |
| | | CC | 5 (71.4) | 2 (28.6) | 1.76 (0.26–11.84) | 0.561 |
| | | TC + CC | 32 (62.7) | 19 (37.3) | 2.61 (0.85–8.05) | 0.094 |
| | rs1799983 | GG | 19 (67.9) | 9 (32.1) | Ref. | |
| | | GT | 29 (70.7) | 12 (29.3) | 0.87 (0.31–2.47) | 0.799 |
| | | TT | 6 (66.7) | 3 (33.3) | 1.06 (0.21–5.21) | 0.947 |
| | | GT + TT | 35 (70) | 15 (30) | 0.90 (0.33–2.45) | 0.844 |
| *VEGF* | rs699947 | AA | 15 (71.4) | 6 (28.6) | Ref. | |
| | | CA | 26 (70.3) | 11 (29.7) | 1.06 (0.32–3.44) | 0.926 |
| | | CC | 13 (65) | 7 (35) | 1.35 (0.36–5.04) | 0.659 |
| | | CA + AA | 39 (68.4) | 18 (31.6) | 1.15 (0.38–3.46) | 0.799 |
| | rs1570360 | GG | 29 (70.7) | 12 (29.3) | Ref. | |
| | | GA | 19 (65.5) | 10 (34.5) | 1.27 (0.46–3.52) | 0.644 |
| | | AA | 6 (75) | 2 (25) | 0.81 (0.14–4.57) | 0.807 |
| | | GA + AA | 25 (67.6) | 12 (32.4) | 1.16 (0.44–3.04) | 0.762 |
| *ACE* | rs1800764 | TT | 17 (68) | 8 (32) | Ref. | |
| | | TC | 28 (68.3) | 13 (31.7) | 0.99 (0.34–2.87) | 0.980 |
| | | CC | 9 (75) | 3 (25) | 0.71 (0.15–3.35) | 0.664 |
| | | TC + CC | 37 (69.8) | 16 (30.2) | 0.92 (0.33–2.56) | 0.872 |
| | rs1799752 | No insertion/ heterozygote | 40 (71.4) | 16 (28.6) | Ref. | |
| | | Homozygous for insertion | 14 (66.7) | 7 (33.3) | 1.25 (0.43–3.67) | 0.685 |
| | rs4343 | GG | 13 (81.3) | 3 (18.8) | Ref. | |
| | | GA | 27 (65.9) | 14 (34.1) | 2.25 (0.55–9.22) | 0.261 |
| | | AA | 14 (66.7) | 7 (33.3) | 2.17 (0.46–10.20) | 0.328 |
| | | GA + AA | 41 (66.1) | 21 (33.9) | 2.22 (0.57–8.66) | 0.251 |
| *ACE2* | rs2285666 | C | 37 (62.7) | 22 (37.3) | Ref. | |
| | | T | 17 (89.5) | 2 (10.5) | 0.20 (0.04–0.94) | **0.041** |
| | rs1978124 | T | 24 (60) | 16 (40) | Ref. | |
| | | C | 30 (78.9) | 8 (21.1) | 0.40 (0.15–1.09) | 0.074 |

## 3.4. Association of polymorphisms with response to tadalafil

We also evaluated whether genetic variability in the NO pathway, angiogenic signaling or RAAS influenced tadalafil response. None of the investigated polymorphisms were associated with change in IIEF-5 after treatment (Table 4) or good response to tadalafil (overall IIEF-5 improvement of ≥ 4-points) (Table 6).

Table 6
Association of the studied polymorphisms with an improvement in erectile dysfunction (ED) of at least 4 points after treatment.

| Gene | SNP | Genotype | Improvement ≥ 4 points (IIEF-5) No N (%) | Improvement ≥ 4 points (IIEF-5) Yes N (%) | OR (95% CI) | P value |
|---|---|---|---|---|---|---|
| *NOS3* | rs2070744 | TT | 10 (37) | 17 (63) | Ref. | |
| | | TC | 18 (40.9) | 26 (59.1) | 0.85 (0.32–2.28) | 0.746 |
| | | CC | 3 (42.9) | 4 (57.1) | 0.78 (0.14–4.24) | 0.778 |
| | | TC + CC | 21 (41.2) | 30 (58.8) | 0.84 (0.32–2.19) | 0.722 |
| | rs1799983 | GG | 12 (42.9) | 16 (57.1) | Ref. | |
| | | GT | 15 (36.6) | 26 (63.4) | 1.30 (0.49–3.47) | 0.600 |
| | | TT | 4 (44.4) | 5 (55.6) | 0.94 (0.21–4.26) | 0.933 |
| | | GT + TT | 19 (38) | 31 (62) | 1.22 (0.48–3.14) | 0.674 |
| *VEGF* | rs699947 | AA | 8 (38.1) | 13 (61.9) | Ref. | |
| | | CA | 18 (48.6) | 19 (51.4) | 0.65 (0.22–1.93) | 0.438 |
| | | CC | 5 (25) | 15 (75) | 1.85 (0.48–7.06) | 0.370 |
| | | CA + AA | 23 (40.4) | 34 (59.6) | 0.91 (0.33–2.54) | 0.857 |
| | rs1570360 | GG | 14 (34.1) | 27 (65.9) | Ref. | |
| | | GA | 15 (51.7) | 14 (48.3) | 0.48 (0.18–1.28) | 0.144 |
| | | AA | 2 (25) | 6 (75) | 1.56 (0.28–8.74) | 0.616 |
| | | GA + AA | 17 (45.9) | 20 (54.1) | 0.61 (0.24–1.52) | 0.289 |
| *ACE* | rs1800764 | TT | 10 (40) | 15 (60) | Ref. | |
| | | TC | 16 (39) | 25 (61) | 1.04 (0.38–2.88) | 0.937 |
| | | CC | 5 (41.7) | 7 (58.3) | 0.93 (0.23–3.78) | 0.923 |
| | | TC + CC | 21 (39.6) | 32 (60.4) | 1.02 (0.38–2.68) | 0.975 |
| | rs1799752 | No insertion/ heterozygote | 22 (39.3) | 34 (60.7) | Ref. | |
| | | Homozygous for insertion | 8 (38.1) | 13 (61.9) | 1.05 (0.37–2.95) | 0.924 |
| | rs4343 | GG | 6 (37.5) | 10 (62.5) | Ref. | |
| | | GA | 17 (41.5) | 24 (58.5) | 0.85 (0.26–2.78) | 0.784 |
| | | AA | 8 (38.1) | 13 (61.9) | 0.98 (0.25–3.73) | 0.970 |
| | | GA + AA | 25 (40.3) | 37 (59.7) | 0.89 (0.29–2.75) | 0.837 |
| *ACE2* | rs2285666 | C | 22 (37.3) | 37 (62.7) | Ref. | |
| | | T | 9 (47.4) | 10 (52.6) | 0.66 (0.23–1.88) | 0.436 |
| | rs1978124 | T | 14 (35) | 26 (65) | Ref. | |
| | | C | 17 (44.7) | 21 (55.3) | 0.67 (0.27–1.66) | 0.381 |

## 4. DISCUSSION

In this prospective study of men with DM and ED, tadalafil significantly improved erectile function, but the investigated *NOS3* (rs2070744, rs1799983), *VEGF* (rs699947, rs1570360, rs833061) and *ACE* (rs1800764, rs1799752, rs4343) polymorphisms were not associated with baseline ED severity or tadalafil response after correction for multiple testing. Only *ACE2* rs2285666 showed a nominal association with lower odds of moderate or severe ED before treatment.

Several previous studies investigated the association of *NOS3* polymorphisms with ED, mostly focusing on rs2070744 and rs1799983. In Iranian men with vasculogenic ED, Safarinejad et al found that rs2070744 and rs1799983 were strong genetic predictors of vasculogenic ED and that the combination of the rs2070744 C allele with the rs1799983 T allele further increased the risk (12). In a Han Chinese population, Yang et al likewise reported that functional *NOS3* polymorphisms were associated with ED susceptibility, earlier onset, and greater severity, although they did not influence sildenafil response (13). Segura et al observed in men with chronic pain that rs2070744 influenced both baseline ED severity and therapeutic response, with the CC genotype showing worse baseline erectile function but greater improvement during treatment, whereas rs1799983 was not significant in that cohort (14). Muniz et al also found that *NOS3* variation modified sildenafil response, with rs2070744 and related haplotypes influencing pharmacologic responsiveness according to ED subtype (15). However, not all studies support these associations. Andersen et al reported no association between *NOS3* rs1799983 and ED complaints in a population-based Brazilian sample (16) and Eisenhardt et al found that *NOS3* rs1799983 did not influence ED risk or age at onset in a German ED population with substantial cardiovascular comorbidity (17). Our negative findings for rs2070744 and rs1799983 are therefore not isolated; rather, they fit the pattern of heterogeneity already seen in the literature. One plausible explanation is that, in diabetic ED, the contribution of individual *NOS3* polymorphisms is likely attenuated by the profound endothelial dysfunction, oxidative stress, autonomic neuropathy and structural cavernosal remodeling that characterize the diabetic phenotype, making single-locus effects more difficult to detect.

For *VEGF*, prior ED studies are more limited, but relevant to the exact polymorphisms assessed here. Lee et al examined rs699947, rs1570360, and rs833061 in Taiwanese men and found that carriers of the rs699947 A allele had a higher prevalence of ED and worse erectile function, supporting a role for altered angiogenic signaling in ED susceptibility (18). In contrast, the main pharmacogenetic evidence comes from Lacchini et al, who showed that *VEGF* promoter polymorphisms influenced sildenafil responsiveness in both clinical and postoperative ED, with rs1570360 and rs699947 associated with poorer treatment response, presumably through lower *VEGF* expression and impaired coupling between angiogenesis and the nitric oxide–cGMP pathway (19). Our findings did not replicate those associations for either baseline severity or tadalafil response. This difference may reflect several factors. First, previous positive studies were conducted largely in non-diabetic or mixed ED populations, whereas our cohort consisted exclusively of men with DM. Second, the angiogenic and endothelial environment in diabetic ED is chronically impaired, which may reduce the relative effect size of common *VEGF* promoter polymorphisms. Third, prior pharmacogenetic signals were generated mainly for sildenafil, whereas our study used daily tadalafil, and drug-specific differences in pharmacodynamics cannot be excluded.

The available evidence for the role of *ACE* in ED is limited and less consistent. The most studied ED polymorphism is the *ACE* insertion/deletion polymorphism, rs1799752. In men with metabolic syndrome, Mazo et al reported that the deletion genotype was associated with increased ED risk and greater severity, suggesting that RAAS activation may aggravate vascular and microvascular impairment in erectile tissue (20). Andersen et al, however, found no overall association between *ACE* I/D and ED complaints in the Brazilian population, although they suggested a possible age-dependent effect in men aged 40 to 55 years (21). Eisenhardt et al additionally reported that the *ACE* I/D polymorphism influenced sildenafil response, with better response among men carrying the II genotype, particularly in those with cardiovascular disease (22). By contrast, our study found no significant association between *ACE* rs1799752 and either ED severity or tadalafil response. Importantly, we did not identify prior ED-specific studies evaluating *ACE* rs1800764 or *ACE* rs4343, which means that our negative findings for these 2 polymorphisms appear to address a gap rather than contradict an established ED literature. This point matters because it suggests that, for *ACE*, the literature remains centered on rs1799752, and that extension to additional common polymorphisms has not yet yielded reproducible disease-specific signals.

The most novel aspect of our study is the inclusion of *ACE2* polymorphisms rs2285666 and rs1978124. We did not identify prior ED-focused genetic association studies specifically examining these *ACE2* polymorphisms, so direct comparison with earlier ED cohorts is not possible. Nevertheless, the broader cardiovascular and diabetes literature makes our nominal signal for rs2285666 biologically plausible. Huang et al evaluated *ACE2* polymorphisms, including rs1978124 and rs2285666, in a Chinese hypertension cohort, supporting the idea that common *ACE2* polymorphisms can influence vascular phenotypes (23). Patel et al studied Caucasian subjects with DM and found that *ACE2* rs1978124 was associated with reduced ejection fraction in men and greater left ventricular mass in women, showing that *ACE2* variation can modify cardiovascular structure and function in diabetic populations (24). Additional diabetes-related data also link rs2285666 to DM complicated by stroke, again implicating this locus in vascular vulnerability (25). Against that background, our observation that carriers of the rs2285666 allele had lower odds of moderate or severe ED is mechanistically coherent, even though it did not remain significant after Bonferroni correction. Because *ACE2* counterbalances angiotensin II by generating angiotensin-(1–7), a vasodilatory and anti-fibrotic peptide, altered *ACE2* activity could reasonably affect penile vascular tone, endothelial homeostasis, and tissue remodeling. The fact that *ACE2* is X-linked further increases its relevance in men, who are hemizygous at this locus. Our results therefore do not establish *ACE2* as a confirmed determinant of diabetic ED, but they do support it as an underexplored pathway deserving targeted replication.

Another important finding is the absence of clear pharmacogenetic predictors of tadalafil response across all investigated loci. Earlier studies suggested that *NOS3*, *VEGF* and *ACE* variation may influence response to sildenafil (15, 19, 22), yet even within that literature the direction and magnitude of effect vary by population and ED subtype.

This study has several strengths, including its prospective design, standardized treatment protocol and use of a validated instrument (IIEF-5) for assessment of erectile function. The cohort was well characterized and genotyping was performed using reliable methods with appropriate quality control. However, several limitations should be acknowledged. The relatively small sample size limits statistical power, particularly for detecting

modest genetic effects typical of complex traits. Therefore, smaller but clinically relevant associations cannot be excluded. The candidate gene approach restricts analysis to predefined polymorphisms and does not capture the broader genetic architecture of ED. Additionally, residual confounding by unmeasured factors such as obesity, smoking status, diabetic complications and concomitant medications cannot be excluded. Finally, the ethnically homogeneous population may limit generalizability to other populations.

## 5. CONCLUSIONS

In this prospective study, daily tadalafil significantly improved erectile function in men with DM, yet common polymorphisms in *NOS3*, *VEGF* and *ACE* do not appear to predict baseline ED severity or therapeutic response, while *ACE2* polymorphisms show only nominal protective effects. These findings underscore the multifactorial nature of diabetic ED and the challenges of identifying single-gene pharmacogenetic predictors. Future research should employ larger, multi-ethnic cohorts, genome-wide or polygenic analyses and standardized phenotyping to identify clinically relevant biomarkers, ultimately facilitating personalized treatment strategies for this high-burden condition.

## Declarations

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**CRediT authorship contribution statement**

**Boštjan Hostnik:** Writing – review & editing, Writing – original draft, Investigation. **Andrej Janež:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Katja Goričar:** Writing – review & editing, Formal analysis, Data curation. **Tanja Blagus:** Writing – review & editing, Investigation, Data curation. **Vita Dolžan:** Writing – review & editing, Supervision. **Jasna Klen:** Writing – review & editing, Supervision, Methodology, Conceptualization.

**Declaration of competing interest**

The authors have no conflict of interest to disclose.

**Data Availability Statement**

Additional data are available from the corresponding author on reasonable request.

## References

1. Defeudis G, Mazzilli R, Tenuta M, Rossini G, Zamponi V, Olana S, idr. Erectile dysfunction and diabetes: A melting pot of circumstances and treatments. Diabetes Metab Res Rev. februar 2022;38(2). doi:10.1002/dmrr.3494
2. Gratzke C, Angulo J, Chitaley K, Dai Y tian, Kim NN, Paick JS, idr. Anatomy, Physiology, and Pathophysiology of Erectile Dysfunction. J Sex Med. januar 2010;7(1):445–75. doi:10.1111/j.1743-6109.2009.01624.x
3. De Tejada I nigo S aenz, Angulo J, Cellek S, Gonz\' alez-Cadavid N, Heaton J, Pickard R, idr. Pathophysiology of Erectile Dysfunction. J Sex Med. 1. januar 2005;2(1):26–39. doi:10.1111/j.1743-6109.2005.20103.x
4. Andersson KE. Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. Pharmacol Rev. december 2011;63(4):811–59. doi:10.1124/pr.111.004515 PubMed PMID: 21880989.
5. Moore CR, Wang R. Pathophysiology and treatment of diabetic erectile dysfunction. Asian J Androl. november 2006;8(6):675–84. doi:10.1111/j.1745-7262.2006.00223.x
6. Mostafa T, Hassan A, Alghobary MF, Abdelrahman SH. Effect of Genetic Polymorphism on the Response to PDE5 Inhibitors in Patients With Erectile Dysfunction: A Systematic Review and a Critical Appraisal. Sex Med Rev. oktober 2020;8(4):573–85. doi:10.1016/j.sxmr.2020.05.005
7. Dall'Era JE, Meacham RB, Mills JN, Koul S, Carlsen SN, Myers JB, idr. Vascular endothelial growth factor (VEGF) gene therapy using a nonviral gene delivery system improves erectile function in a diabetic rat model. Int J Impot Res. maj 2008;20(3):307–14. doi:10.1038/ijir.2008.1
8. Marchal-Escalona C, Herrera-Imbroda B, Clemente-Postigo M, Alcaide-Torres J, Quiñonero A, Marchal M, idr. PDE5A Polymorphisms Influence on Sildenafil Treatment Success. J Sex Med. 1. julij 2016;13(7):1104–10. doi:10.1016/j.jsxm.2016.04.075
9. Rosen RC, Cappelleri JC, Smith MD, Lipsky J, Pe~ na BM. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. Int J Impot Res. december 1999;11(6):319–26. doi:10.1038/sj.ijir.3900472 PubMed PMID: 10637462.

10. Knoell DL, Johnston JS, Bao S, Kelley KA. A Genotyping Exercise for Pharmacogenetics in Pharmacy Practice. Am J Pharm Educ. september 2009;73(3):43. doi:10.5688/aj730343
11. Dupont WD, Plummer WD. Power and sample size calculations. Control Clin Trials. april 1990;11(2):116–28. doi:10.1016/0197-2456(90)90005-M
12. Safarinejad MR, Khoshdel A, Shekarchi B, Taghva A, Safarinejad S. Association of the T-786C, G894T and 4a/4b polymorphisms of the endothelial nitric oxide synthase gene with vasculogenic erectile dysfunction in Iranian subjects. BJU Int. junij 2011;107(12):1994–2001. doi:10.1111/j.1464-410X.2010.09755.x
13. Yang B, Liu L, Peng Z, Lu D, Ren Z, Liu S, idr. Functional Variations in the *NOS3* Gene Are Associated with Erectile Dysfunction Susceptibility, Age of Onset and Severity in a Han Chinese Population. J Sex Med. 1. april 2017;14(4):551–7. doi:10.1016/j.jsxm.2017.02.003
14. Segura A, Ballester P, Ajo R, Inda M del M, Urbano A, Muriel J, idr. Endothelial nitric oxide synthase gene polymorphisms and erectile dysfunction in chronic pain. Gene. 2019;721:100005. doi:10.1016/j.gene.2019.100005
15. Muniz JJ, Lacchini R, Rinaldi TO, Nobre YTDA, Cologna AJ, Martins ACP, idr. Endothelial nitric oxide synthase genotypes and haplotypes modify the responses to sildenafil in patients with erectile dysfunction. Pharmacogenomics J. april 2013;13(2):189–96. doi:10.1038/tpj.2011.49
16. Andersen ML, Guindalini C, Santos-Silva R, Bittencourt LRA, Tufik S. Association Analysis of Endothelial Nitric Oxide Synthase G894T Gene Polymorphism and Erectile Dysfunction Complaints in a Population-Based Survey. J Sex Med. 1. marec 2010;7(3):1229–36. doi:10.1111/j.1743-6109.2009.01606.x
17. Eisenhardt A, Stief C, Porst H, Wetterauer U, Weidner W, R\" ubben H, idr. Genetic association study of the GNB3 C825T, the ACE I/D and the eNOS G894T polymorphisms and the risk to develop erectile dysfunction in a German ED population. Andrologia. avgust 2010;42(4):218–24. doi:10.1111/j.1439-0272.2009.00975.x PubMed PMID: 20629643.
18. Lee YC, Huang SP, Tsai CC, Cheng KH, Juan YS, Wu WJ, idr. Associations of VEGF Gene Polymorphisms with Erectile Dysfunction and Related Risk Factors. J Sex Med. 1. april 2017;14(4):510–7. doi:10.1016/j.jsxm.2017.02.009
19. Lacchini R, Muniz JJ, Nobre YTDA, Cologna AJ, Martins ACP, Tanus-Santos JE. VEGF genetic polymorphisms affect the responsiveness to sildenafil in clinical and postoperative erectile dysfunction. Pharmacogenomics J. oktober 2013;13(5):437–42. doi:10.1038/tpj.2012.39
20. Mazo EB, Gamidov SI, Mamedov MN, Iremashvili VV. Association between the insertion/deletion polymorphism of the angiotensin-converting enzyme gene and erectile dysfunction in patients with metabolic syndrome. Int J Impot Res. januar 2008;20(1):68–72. doi:10.1038/sj.ijir.3901598
21. Andersen ML, Guindalini C, Santos-Silva R, Bittencourt LRA, Tufik S. Angiotensin-Converting Enzyme Polymorphism and Erectile Dysfunction Complaints in the Brazilian Population. J Sex Med. 1. avgust 2010;7(8):2791–7. doi:10.1111/j.1743-6109.2010.01796.x
22. Eisenhardt A, Sperling H, Hauck E, Porst H, Stief C, Rübben H, idr. ACE gene I/D and NOS3 G894T polymorphisms and response to sildenafil in men with erectile dysfunction. Urology. julij 2003;62(1):152–7. doi:10.1016/S0090-4295(03)00137-7
23. Huang W, Yang W, Wang Y, Zhao Q, Gu D, Chen R. Association study of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms and essential hypertension in northern Han Chinese. J Hum Hypertens. 1. december 2006;20(12):968–71. doi:10.1038/sj.jhh.1002090
24. Patel SK, Wai B, Ord M, MacIsaac RJ, Grant S, Velkoska E, idr. Association of ACE2 Genetic Variants With Blood Pressure, Left Ventricular Mass, and Cardiac Function in Caucasians With Type 2 Diabetes. Am J Hypertens. 1. februar 2012;25(2):216–22. doi:10.1038/ajh.2011.188
25. Wu YH, Li JY, Wang C, Zhang LM, Qiao H. The *ACE2* G8790A Polymorphism: Involvement in Type 2 Diabetes Mellitus Combined with Cerebral Stroke. J Clin Lab Anal. marec 2017;31(2):e22033. doi:10.1002/jcla.22033