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Endothelial nitric oxide synthase gene polymorphisms and erectile dysfunction in chronic pain

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ABSTRACT

Objectives: To investigate whether *endothelial nitric oxide synthase (eNOS)* T786C, 4VNTR and G894 T gene polymorphisms could mediate in andrological treatment response in Spaniards.
Subject patients/methods: The study participants were Spaniard males with erectile dysfunction (ED) and chronic pain (n = 105) recruited at the Pain Unit. *eNOS* polymorphisms were genotyped by quantitative polymerase chain reaction using Taqman specific probes. Statistical analyses were carried out using R-3.2.4 software.
Results: A total of 69 patients required andrological treatment and 76% of them improved ED upon iPED5 (20%), testosterone (35%) or iPDE5/testosterone treatment (45%); being significantly better in T786C-CC patients. Multivariate regression analysis indicated that age, opioid daily dose and carriage of T786C-C allele influenced the risk and ED severity in Spaniard chronic pain patients.
Conclusion: T786C polymorphism at *eNOS* locus appeared to be a major contributor in the variable erectile function iPDE5/testosterone response in Spaniards.

1. Introduction

Erectile function (EF) is regulated by relaxation and contraction of smooth muscle of penis that is mediated by nitric oxide (NO), the main vasoactive molecule mediator by induction of the production of 3′,5′-cyclic guanosine monophosphate (cGMP) and promotion of the relaxation of corpus cavernosum (Burnett, 2006; Thameem et al., 2008). Evidences show that functional polymorphisms within endothelial NO synthase (*eNOS*) gene interfere with normal EF (Thameem et al., 2008), down-regulating NO production, lowering cGMP levels (Wang and Wang, 2000; Yang et al., 2014) and leading to an increased erectile dysfunction (ED) predisposition (Erol et al., 2009; Jira et al., 2011).

Sexual problems are usually unrecognized and untreated in chronic illnesses like pain where other factors, as opioid induced androgen deficiency (Daniell, 2008; Ajo et al., 2017) and comorbidities, could worsen ED (Paice, 2003; Abs et al., 2000). In fact, approximately one half to two thirds of chronic non-cancer pain (CNP) patients reported reduced frequency in their sexual relationships (Buss and Leppert, 2014) and decreased desire/libido (Paice, 2003; Ambler et al., 2001); however, few of them received adequate treatment for these problems.

Knowledge gaps persist regarding ED prevalence and the influence of pharmacogenetics in andrological drug response (Chou et al., 2009). Genetic variants of *eNOS* gene (T786C, intron 4VNTR and G894T) have been widely studied and related to several vascular diseases. The *eNOS* G894T polymorphism in exon 7 was reported to cause an amino acid substitution (Glu to Asp at codon 298, Glu298Asp) resulting in disturbance of its activity and NO production (Lee et al., 2012), as well as T786C polymorphism that is located in the promoter region of the gene (Nakayama et al., 1999). *eNOS* intron 4 tandem repeats (4VNTR) polymorphism consists in a 27-bp variable number of tandem repeats

Abbreviations: eNOS, endothelial nitric oxide synthase; ED, erectile dysfunction; EF, Erectile function; NO, nitric oxide; cGMP, 3′,5′-cyclic guanosine monophosphate; CNP, chronic non-cancer pain; iPDE5, phosphodiesterase type 5 inhibitors; BMI, body mass index; VAS, Visual analogue scale; IIEF, International Index of Erectile Function; mSLQQ-QOL, modified Sexual Life Quality Questionnaire

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that influences protein synthesis and enzymatic activity (Sinici et al., 2010). All of these *eNOS* gene variants have been associated as independent risk factors in the pathogenesis of ED (Lee et al., 2012; Sinici et al., 2010; Erkan et al., 2006). However, these genetic association studies have been mostly conducted in other ethnic background populations (Casas et al., 2006) and to date, genetic studies examining these polymorphisms in chronic pain patients with ED in Spaniards are limited.

The present study aimed to explore a scarcely studied aspect of chronic pain: sexuality on patients chronically treated with opioids and whether *eNOS* polymorphisms, could be associated to a different EF response to andrological treatment.

2. Materials and methods

2.1. Study population

CNP patients receiving oral and/or transdermal opioid treatment for at least one year that spontaneously notified sexual dysfunction in their routine clinical controls were eligible for participation and derived to the Andrology Unit for diagnosis and treatment. All participants were informed about the study design and purpose by their clinician and gave written consent. Hospital Ethics Committee approved the study.

Prescreening was done in a total of 125 unrelated males of Spaniard origin, outpatients of the Pain Unit of Alicante Department of Health-General Hospital and 84 patients confirmed ED, from which 15 were excluded due to several reasons (did not meet inclusion criteria, were not suitable for andrological treatment, were lost in follow-up or other clinical base pathology as cancer). Thus finally, 69 patients were included according to the inclusion criteria. Patient's age ranged from 18 to 80 years old, all were free of alcohol or drug dependencies and none of them were taking hormone medication (supplementation or deprivation) or phosphodiesterase type 5 inhibitors (iPDE5) before the study. Patients who experienced any sexual dysfunction prior to the onset of chronic pain were excluded.

2.2. Outcomes

All patients received a thorough medical examination, including medical and drug history at baseline and upon 6 months of andrological treatment (final visit).

Demographic information was collected (patients' age, body mass index (BMI), marital status, ethnic background) at first visit. Validated scales and questionnaires completed at each visit were used to evaluate the clinical situation for each patient. Pain intensity and relief were measured using the Visual Analogue Scale (VAS) (McCormack et al., 1988). Both consist of a 100 mm horizontal line ranging from 0 (lowest) to 100 mm (highest), where the patient points on the line the intensity or relief of pain that feels, respectively. Quality of life of the patients was evaluated through the VAS-EuroQol Scale that consists in a vertical line from 0 (the worst imaginable health status) to 100 mm (the best imaginable) where the patient pointed his actual health status) (EuroQol, 1990).

The International Index of Erectile Function (IIEF) questionnaire was used to assess male sexual functioning, particularly the presence or absence of ED. The IIEF questionnaire consists of 15 items grouped into 5 sexual function domains: EF, orgasmic function, sexual desire, satisfaction with sexual intercourse and overall satisfaction. Higher scores in this questionnaire correspond to lower degrees of dysfunction. The domain assessing EF (IIEF-EF) includes 6 questions (maximum score of 30) and is a reliable measure for classifying the degree of ED as severe ($1-10$), moderate (11–16), mild (17–25) and normal/no dysfunction (≥ 26) (Rosen et al., 2002).

In addition, patients were asked to fulfill a questionnaire on sexual quality of life, the modified Sexual Life Quality Questionnaire (mSLQQ-QOL). This is a multidimensional tool on which patients and their partners were asked to compare their experiences prior to the onset of the ED with their experiences since treatment began. It presents 10 assessment items: frequency of sex; duration of sex; ease of insertion; ease of achieving orgasm; ease of initiating sex; pleasure of anticipation; carefree feelings during sex; pleasure of orgasm; pleasure overall; and partner pleasure (Fisher et al., 2005).

Furthermore, patients were encouraged to report their drug prescriptions and any noxious, unintended, or undesired effect associated to analgesic prescription using a self-completed questionnaire (World Health Organization, 1969). In order to translate the dose and route of each of the opioids that the patient received daily morphine equivalent daily dose (MEDD) was calculated using available references (Pergolizzi et al., 2008).

2.3. Andrological diagnosis and treatment

American and European Urological Associations Guidelines recommend an initial evaluation that includes sexual, medical, and psychosocial histories as well as laboratory tests thorough to identify comorbid conditions as cardiovascular disease (including hypertension, atherosclerosis, or hyperlipidemia), diabetes mellitus, depression, or alcoholism that may predispose patients to ED and that may contraindicate certain therapies (Hatzimouratidis et al., 2010; Montague et al., 2005). This assessment was performed at baseline visit at the Andrological Unit and after 6 months of follow-up at final visit as regular clinical controls.

Additional risk factors included smoking, pelvic, perineal, or penile trauma or surgery, neurologic disease, endocrinopathy, obesity, pelvic radiation therapy, Peyronie's disease, and prescription or recreational drug use. Finally, a history of the partner's sexual function was obtained. A focused physical examination evaluating the abdomen, penis, testicles, secondary sexual characteristics and lower extremity pulses was performed. Additional testing, as testosterone level measurement, was performed in selected patients.

Guidelines also recommend offering iPDE5 as first-line therapy for ED unless the patient has contraindications to its use. Thus, prescriptions were performed as usual for ED management and included iPDE5 and/or testosterone replacement. There were three main oral drugs categories for the ED treatment: vardenafil, sildenafil and tadalafil. All of these drugs belong to the group of iPDE5 and have similar mechanisms of action that consist in increasing the blood flow into the penis to facilitate erection when the patient is sexually stimulated. Patients with diminished libido and ED might present low serum testosterone levels. Testosterone levels were measured in blood samples. If testosterone levels were low, prescription of transdermal testosterone or testosterone gel was performed. The increase of male sexual functionning was calculated as the difference of IIEF (or IIEF-EF) scores at final visit from baseline.

Risk-factor modification, including lifestyle interventions as exercise and weight loss, were recommended to patients with ED. Patients who had serious cardiac disease, exertional angina or were taking several antihypertensive drugs were encouraged to seek for the advice of a cardiologist before beginning iPDE5 therapy.

2.4. eNOS genotyping

Approximately 2 mL of saliva were collected in PBS containing tubes. Genomic DNA was isolated with E.N.Z.A. Forensic DNA Kit (Omega bio-tek) according to manufacturer's instructions. Genotyping of T786C (rs2070744) and G894T (rs1799983) *eNOS* polymorphisms were performed by real time polymerase chain reaction (RT-PCR) using specific TaqMan probes MGB® (Applied Biosystems). All PCR amplifications were carried out in a RT- PCR Rotor Gene Q (Qiagen). The amplification parameters were as follows: 10 min initial denaturation at 95 °C, 40 cycles for 15 s at 92 °C, 90 s at 60 °C, and 1 min final extension at 60 °C. For the 27-bp intron 4VNTR *eNOS* polymorphism genotyping,

conventional PCR was performed with 200 ng of DNA followed by gel electrophoresis. There are three different alleles for this polymorphism: allele "a" of 453 bp, "b" of 480 bp, and "c" of 507 bp. The amplification parameters were as follows: 15 min initial denaturation at 96 °C, 35 cycles for 30 s at 95 °C, 20 s at 59 °C, and 45 s at 72 °C, and 10 min final extension at 72 °C. Primers used for PCR amplification were: forward 5′- TGGAAAGGTAGGGGGACTG-3′ and reverse 5′- GGTCACAGG CGTTCCAGTA-3′. PCR products were loaded in a 4% agarose gel and visualized under UV light.

2.5. Statistical analyses

Quantitative data is presented as mean ± standard deviation (SD). Shapiro Wilk test was used to test for normality. A *t*-test for independent samples or a Mann Whitney *U* test was used to assess group differences between two groups and ANOVA with Bonferroni's correction or Kruskal Wallis test was used for more than two groups. Relative frequencies of genotypes and alleles were calculated for each group. Subjects were grouped also as carriers and non-carriers, defined as participants who tested positive for the presence of the allelic variants (dominant model). The association between IIEF-EF and improvement of erectile functioning upon treatment and SNPs genotyped were examined using multiple logistic regressions adjusting for patients' age, body mass index, morphine equivalent daily dose, pain intensity, quality of sexual life and antidepressive use, at last dispensing. Analyses were carried out with R software package version 3.2.4 and p values < 0.05 were considered statistically significant.

3. Results

3.1. Study population

Demographic and clinical variables of the subject's pre- (baseline) and post-andrological treatment (final visit) are shown in Table 1. (See Fig. 1.)

All participants ($n = 125$, 57 ± 12 years, 100% Caucasian Spaniards) spontaneously reported decreased libido and/or ED, and were referred to the Andrology Unit where 84 (67%) patients confirmed the ED and 69 patients finished the. Thus, 15 (18%) patients were excluded due to several reasons (loss of follow-up, withdrawal of consent, ED associated to other pathologies, or they did not comply with the prescribed treatment).

Finally, 69 male CNP patients finished the study (58 ± 11 years-old, BMI 30 ± 5 kg/m^2). The majority were married (69%) followed by divorced (14%) and were retired (56%). The average duration of pain in chronic opioid therapy was 5.5 years (MEDD 115 ± 109 mg/day), with a baseline intensity of pain moderate (VAS 57 ± 25 mm) and moderate quality of life (VAS-EuroQol 52 ± 22 mm). Most of them received adjuvant drugs, mainly anticonvulsants and near 40% antidepressants. Comparison of demographic characteristics confirmed that the current sample was representative of patients who are typically seen at the Pain Unit of the Alicante General Hospital (data not shown).

Table 1
Demographic, clinical and biochemical variables.

	Baseline	Final visit	*p*-Value
Age (years)	57.9 ± 11.0	–	
BMI (kg/m^2)	30.4 ± 5.1	–	
Heart rate (bpm)	70.8 ± 12.4	68.6 ± 12.9	0.710
Systolic blood pressure (mm Hg)	133 ± 15.6	138 ± 15.3	0.455
Diastolic blood pressure (mm Hg)	76.8 ± 9.7	77.3 ± 14.8	0.901
Total testosterone (range 3–10 ng/mL)	3.2 ± 1.5	4.5 ± 3.4	**0.011**
Free testosterone (range 5.5–70.8 ng/mL)	26.7 ± 12.9	32.4 ± 23.8	0.168
Creatinine (mg/dL)	0.92 ± 0.25	1.30 ± 1.76	0.983
Total cholesterol (mg/dL)	179.8 ± 40.8	165.7 ± 70.5	0.952
LDL cholesterol (mg/dL)	110.5 ± 33.2	116.8 ± 32	0.497
HDL cholesterol (mg/dL)	46.3 ± 13.3	45.6 ± 15.2	0.989
Triglycerides (mg/dL)	145.5 ± 93.1	183.8 ± 158.6	0.629
MEDD (mg/day)	115 ± 109		
IIEF (5–75 scores)	26 ± 14.7	45.9 ± 20.1	< **0.001**
IIEF-EF (1–30 scores)	8.6 ± 6.9	18.6 ± 9.8	< **0.001**
mSLQQ-QOL (0–100 scores)	19.8 ± 16.2	54.3 ± 26.1	< **0.001**

BMI: body mass index; MEDD: Morphine equivalent daily dose; IIEF: International Index of Erectile Function; IIEF-EF: IIEF erectile function domain; msLQQ-QOL: modified Sexual Life Quality Questionnaire. Values are mean ± SD; Significant differences ($p < 0.05$) are written in bold.

3.2. Andrology unit treatment

Most of the patients presented at baseline visit a severe ED (73% IIEF-EF ≤ 10 scores; IIEF 26 ± 14.7 scores; IIEF-EF subdomain 8.6 ± 6.9 scores) and poor male sexual quality of life (mSLQQ-QOL 19.8 ± 16.2 scores). Multiple regression analysis showed that EF at baseline was positively influenced by a better sexual quality of life and negatively by age and BMI ($p < 0.05$, F-statistic = 9.94; $r^2 = 0.453$). Also, anxiolytic use, but not antidepressive, negatively influence EF severity ($p = 0.003$). At final visit, none of the variables analyzed (age, BMI, MEDD, sexual quality of life, drugs prescribed or genotype) significantly influenced ED response to the andrological treatment.

Andrological treatment of the patients included in the study is presented in Table 2. Patients were treated with iPDE5 (28%), testosterone (43%) or both (28%) with a significant improvement at the end of the study of male sexual functioning (IIEF 45.9 ± 20.1 scores), EF (IIEF-EF subdomain 18.6 ± 9.8 scores, $p = 0.000$) and sexual quality of life (mSLQQ-QOL 54.3 ± 26.1 scores, $p = 0.000$) (Table 1).

3.3. eNOS genotyping

Genotypes and allele frequencies for *eNOS* polymorphisms studied and their influence on IIEF-EF and mSLQ-QOL scores, are presented in Tables 3, 4 and 5.

Genotypes and alleles frequencies for T786C and G894 T polymorphisms were in agreement with the Hardy-Weinberg Equilibrium ($p \geq 0.05$). In our study, frequency of T786C-C allele of all patients genotyped was 57.1% and of G894 T-G allele 61.5% both similar to Caucasian prevalence. Variant "c" of intron 4VNTR polymorphism was not found in our sample. Allele "a" of intron 4VNTR "was present in only 2.2% in our study (Supplementary Fig. 1).

Upon 6 months of andrological treatment, IIEF-EF values tended to improve in all the patients independently of *eNOS* genotype. However, when analyzing by allele presence, patients homozygous for C allele of T786C exhibited the most significant improvement in EF response to andrological treatment.

All the patients except one were b/b for intron 4 VNTR polymorphism; thus the influence of 4VNTR genotype could not be analyzed.

Sexual quality of life was significantly improved in all the patients independently of their genotype. No differences were found also when the analysis was performed by allele (Table 5).

3.4. Interaction between clinical and eNOS gene polymorphisms

We further analyzed the interaction of these polymorphisms in relation to EF response (Table 4). Multiple regression analysis indicated that baseline EF was positively influenced by the carriage of T786C-C allele ($p = 0.029$); and negatively by age ($p = 0.015$) and MEDD ($p = 0.006$) (F-statistic = 5.259; $r^2 = 0.283$). Antidepressants prescription did not influence EF risk or response to andrological treatment, in this population.

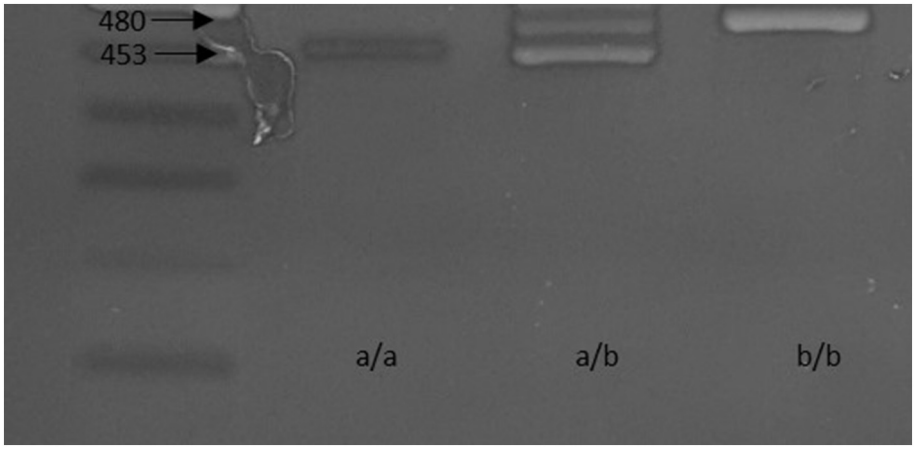

Fig. 1. IIEF-EF values for *eNOS* polymorphisms genotypes.

Table 2
Andrological treatment of patients included in the study.

	iPED5	Testosterone
Testosterone	28%	72%
iPED5	56%	28%
Vardenafil	25%	13%
Tadalafil	16%	9%
Sildenafil	16%	6%
Total	56%	72%

4. Discussion

In the present study, EF and sexual quality of life improved significantly after 6 months of andrological treatment in Spaniards CNP patients, even more in patients carrying T786C-CC genotype. To the best of our knowledge, this is the first association study of *eNOS* polymorphisms and EF in a population with CNP free of alcohol or drug dependencies.

NO has a crucial role in the vascular homeostasis, regulation of blood flow and blood pressure. Penile tumescence begins with the release of NO from either nonadrenergic noncholinergic nerve terminals or endothelial cells, and inadequate NO production during sexual stimulation can result from downregulation of *NOS* expression (Tsukada et al., 1998; Safarinejad et al., 2011). Several allelic variants of the *eNOS* gene can affect their expression levels. Most studied polymorphism is G894 T that leads to a decreased enzyme activity, decreased basal NO production and a 20% of lower activity (Lee et al., 2012). In a recent meta-analysis this low *eNOS* mRNA and serum NO levels, were observed in G894 T-GG genotype (Gao et al., 2017) with an increased risk for earlier onset of ED (OR = 3.572; $p < 0.020$). Further subgroup analysis based on ethnicity suggested that G894 T was significantly associated with risk of ED in Asians ($p = 0.003$), but not in Caucasians ($p = 0.166$) (Paradossi et al., 2004; Naber et al., 2001; Yang et al., 2017). In our study, no differences were observed for G894 T polymorphism, neither at baseline or final EF nor in response to andrological treatment.

In the same line, T786C polymorphism is tightly associated with an increased risk of ED. A reduced *eNOS* expression, *eNOS* mRNA and serum NO levels together with a decreased NO-induced vasomotor function were observed in individuals carrying the T786C-CC genotype (Gao et al., 2017). However, in our study this poor basal EF was the most improved after andrological therapy. In a population case-control

Table 4
Comparison of IIEF scores according to *eNOS* polymorphisms genotypes.

	IIEF-EF Baseline	IIEF-EF Final visit	EF Improvement (%)	#*p*-Value
T786C				
- TT	4.3 ± 4.5	13.5 ± 8.3	83	**0.024**
- TC	9.5 ± 7.2	15.3 ± 11.1	64	0.167
- CC	7.1 ± 5.1	23.9 ± 7	85	< **0.001**
**p* Value	0.168	0.079		
G894 T				
- GG	8.8 ± 6.7	21.0 ± 9.8	82	**0.004**
- GT	7.5 ± 6.5	17.3 ± 10.7	64	**0.006**
- TT	7.3 ± 6.3	21.4 ± 6.0	100	**0.014**
**p* Value	0.673	0.636		
Intron 4 VNTR				
- a/a	3	27	100	nd
- b/b	7.8 ± 6.5	20.5 ± 9.1	80	< **0.001**
**p* Value	nd	nd		

Data appears as mean ± SD. * *p* value: comparison between genotypes at baseline or final visit; #*p* value: comparison between baseline and final visit. Bold when $p < 0.05$.

Table 3
Frequencies of combined genotypes polymorphisms.

Polymorphism	Genotype ($n = 49$)			Allele 1 frequency		*p* Value
	1/1	1/2	2/2	Study	Population	
T786C (rs2070744)	30.6% (CC)	53.1% (CT)	16.3% (TT)	57.1% (C)	58%	ns
G894T (rs1799983)	35.4% (GG)	52.1% (GT)	122.5% (TT)	61.5% (G)	50–65%	ns
Intron 4VNTR	2.2% (a/a)	0% (a/b)	98.8% (b/b)	97.8% (b)	1–3%	ns

1: wild type allele; 2: mutant allele. Comparison between allele frequency in our study and data from 1000 genomes was performed by U-Man Whitney test. Intron 4VNTR has been obtained from references (Wang and Wang, 2000; Gao et al., 2017; Yang et al., 2017).

Table 5
Comparison of mSLQ-QOL scores according to *eNOS* polymorphisms genotypes.

	mSLQ-QOL Baseline	mSLQ-QOL Final visit	mSLQ-QOL Improvement	#*p*-Value
T786C				
- TT	22.3 ± 15.4	71.9 ± 11.8	52.5 ± 12.9	< **0.001**
- TC	16.5 ± 17.1	47 ± 31.4	36.4 ± 32.4	< **0.001**
- CC	20.1 ± 17.6	52.3 ± 23.9	35 ± 23.7	**0.003**
**p* Value	0.577	0.377	0.506	
G894 T				
- GG	19.1 ± 17.1	41.8 ± 26.6	30.5 ± 31.2	**0.028**
- GT	19.8 ± 17.1	57.4 ± 25.4	40.7 ± 25.1	< **0.001**
- TT	14.5 ± 14.4	56.6 ± 38.3	46.6 ± 33	0.056
**p* Value	0.819	0.482	0.624	
Intron 4 VNTR				
- a/a	26.3	nd	nd	nd
- b/b	19.7 ± 17.1	51.7 ± 28.7	36 ± 27.8	< **0.001**
**p* Value	nd	nd	nd	

Data appears as mean ± SD. **p* value: comparison between genotypes at baseline or final visit; #*p* value: comparison between baseline and final visit. Bold when $p < 0.05$.

study that enrolled 112 patients with ED and 156 age-matched healthy men, the risk genotype of T786C exhibited lower male sexual functioning than patients with the non-risk genotype (8.2 ± 4.5 vs 12.2 ± 5.0 IIEF-EF scores; $p < 0.015$). In agreement with this data, our study showed that Spaniard patients with T786C-CC genotype presented one of the worst basal male sexual functioning, although achieved the best ED response.

The influence of 4VNTR polymorphism could not be studied in our sample due to the low frequency of a/a genotype. Genotypic frequencies for this polymorphism are not well established and further studies in larger cohorts and in different ethnic backgrounds should be performed. In relation to the frequency of the *eNOS* gene intron 4VNTR polymorphism and its' influence on EF continue being controversial with scarce data supporting this (Erol et al., 2009; Liu et al., 2015; Wang et al., 2010; Yang et al., 2015). In a meta-analysis performed to estimate the association between *eNOS* polymorphisms and ED risk, the overall analysis showed a significant association to b/b variant (OR = 1.917, CI: 1.073–3.424) (Liu et al., 2015). However, these results are still preliminary and our study did not achieve enough power to analyze 4VNTR polymorphism influence in ED prevalence or response. Future studies with larger populations would be necessary.

It has been determined that *eNOS* polymorphisms are clinically relevant for other diseases. Decreased NO synthesis, *eNOS* gene polymorphisms, and/or arginine deficiency may cause increased levels of circulating NOS inhibitors, that contribute to vascular changes (as increase in arterial pressure or intraglomerular hypertension or progression of atherosclerosis) (Nehra, 2009) in different illnesses (Kojda and Harrison, 1999; Skovgaard et al., 2005) like chronic kidney disease (Hsu et al., 2012), osteoporosis (Gu et al., 2015) or coronary artery disease (Sung et al., 2015). Thus, ED might be a sign of cardiovascular o systemic problems as a sentinel symptom.

Our study presents some limitations that should be considered. First, the number of patients included in the study is very low, especially when polymorphisms with low frequency such as intron 4VNTR are studied. Second, sexuality is a complex process influenced by many factors (geographical location, lifestyle, dietary habits, personal and partner, for example) and may be influenced by many other circumstances. In our study, EF was influenced positively by better sexual quality of life; and coherently negatively by age, BMI, opioid daily dose and anxiolytic therapy. This points out the relevance to review patient's life-style and drug prescriptions, to improve ED as a first step intervention. Third, sexual dysfunction appears to be more commonly associated to chronic illnesses where other symptoms including fatigue, depression, muscular weakness and opioid drugs may affect sexual function apart of genotype (Schnatz et al., 2010; Uguz et al., 2011). Fourth, variants at *eNOS* locus may relate to the ethnic-specific predisposition (Tanus-Santos et al., 2001; Guo et al., 2005). Fifth, no control group was included (i.e. patients with chronic pain and opioid treatment without erectile dysfunction) that will allow to establish more robust conclusions. In addition, further studies using a large population size should be conducted. In addition, it would be interesting to analyze if response to andrological treatment was dosage dependent, however, in our study, most of the patients were receiving similar testosterone or iPED5 doses and no analysis could be made.

Our results support that T786C polymorphism of *eNOS* gene is associated with a different ED response in CNP Spaniards males that should be interpreted taking into consideration the genetic backgrounds, the multifactorial nature of ED and the fact that the results need to be replicated in other ethnic populations.

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Conflict of interest

Authors declare no conflict of interest.

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