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Functional Variations in the *NOS3* Gene Are Associated With Erectile Dysfunction Susceptibility, Age of Onset and Severity in a Han Chinese Population

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ABSTRACT

Background: Impaired function of endothelial nitric oxide synthase (eNOS) is involved in the pathologic processes of erectile dysfunction (ED), and three functional polymorphisms (G894T, T-786C, and a tandem repeat of 27 bp in intron 4) in the *NOS3* gene, which encodes eNOS, are associated with the clinical characteristics of ED in several populations.

Aim: To investigate the effect of these variations of *NOS3* on ED phenotypes and the response to sildenafil in a Han Chinese population.

Methods: This case-control study enrolled 112 patients with ED and 156 age-matched healthy men. Their medical history and laboratory data were collected. ED severity and response to sildenafil were assessed using the five-item International Index of Erectile Function (IIEF-5) score. Routine polymerase chain reaction and Sanger sequencing were used to genotype the three polymorphisms of *NOS3*.

Outcomes: The frequencies of alleles, genotypes, and haplotypes of the loci in patients and controls; the IIEF-5 scores of patients carrying the risk and non-risk genotype; and the frequencies of risk and non-risk genotypes in patients with different ages at onset and responses to sildenafil were assessed.

Results: The frequencies of drinkers and diabetic and hyperlipidemic patients in the ED group were higher than those in the age-matched control group ($P < .05$). The distributions of alleles (G894T, $P < .005$; T-786C, $P < .015$), genotypes (G894T, $P < 0.015$; T-786C, $P < .010$), and haplotypes (G894T/T-786C, $P < .015$) of the *NOS3* polymorphisms were significantly different between patients with ED and controls. An increased risk for earlier onset of ED was observed in the G894T risk genotype carriers (odds ratio = 3.572; $P < .020$). Patients with the risk genotype of T-786C exhibited lower IIEF-5 scores than patients with the non-risk genotype (8.2 ± 4.5 vs 12.2 ± 5.0; $P < .015$). The influence of the T-786C or G894T genotype on the response to sildenafil was not observed.

Clinical Translation: The detectable effect of *NOS3* functional polymorphisms on ED suggests their application potential as a molecular biomarker in predicting ED susceptibility and severity in the Han Chinese population.

Strengths & Limitations: This study provides strong evidence that *NOS3* functional variation is an independent risk factor for ED in the Han Chinese population, which should be confirmed in larger cohorts considering the limited number of subjects in this study.

Conclusion: These results are the first to identify a clear association between *NOS3* functional variation and ED susceptibility, age at onset, and severity in the Han Chinese population. **Yang B, Liu L, Peng Z, et al. 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 2017;XX:XXX–XXX.**

Key Words: Erectile Dysfunction; International Index of Erectile Function; *NOS3*; Polymorphism; Sildenafil; Han Chinese

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INTRODUCTION

Erectile dysfunction (ED) is a multifactorial disorder that is defined as the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse.[1] The prevalence of ED is 18% in men older than 20 years and approximately 50% in men older than 40 years, and these men have varying degrees of ED.[2,3] Extensive research on the physiology and pathophysiology of ED has contributed to progress in understanding the pathomechanism of ED.[4,5] One notable finding is the important role of nitric oxide (NO) in the initiation and maintenance of an erection through a penile veno-occlusive mechanism.[6–8] Decreased expression of endothelial NO synthase (eNOS) has been observed in ED,[9,10] and eNOS-deficient mice have been found to exhibit decreased erectile function.[11] Gene transfer of eNOS has been found to partly restore erectile function in rats with streptozotocin-induced diabetes.[12] Taken together, these observations support an evident association between eNOS function and susceptibility to ED through the regulation of NO-mediated vascular dilation.

The *NOS3* gene, located on chromosome 7q36, contains 26 exons and encodes eNOS, an essential activator of endothelium-derived NO production. There are three polymorphic loci in the *NOS3* gene that have been associated with ED in previous studies,[13] including G894T (rs1799983) in exon 7,[14] T-786C (rs2070744) in the promoter region, and a variable number of tandem repeats (VNTR) of 27 bp in intron 4.[15,16] The eNOS that is encoded by the 894T allele exhibits decreased biological activity because of a defect in transport and affinity to caveolin-1.[17] The T→C mutation of the T-786C locus produces a binding site for replication protein A1, which downregulates eNOS expression by 50%.[18] The 27-bp motif of the VNTR in intron 4 encodes an siRNA-targeted *NOS3* mRNA, and the 4a allele, with four repeats of the motif, exhibits higher eNOS expression compared with the 4b allele, which has five repeats.[19] These findings suggest the functional effects of the polymorphic loci on eNOS expression and considerably explain their associations with ED.

However, the association of the *NOS3* polymorphisms with ED remains controversial because of the presence of significant heterogeneity across studies.[13] This heterogeneity can result from differences in the genetic composition of races, inclusion criteria, or the sample size of the population studied. The influence of *NOS3* on ED in the Han Chinese is largely unknown, but there is a strong possibility that the functional polymorphisms of the *NOS3* are linked to the phenotypic expression of ED in the largest ethnic population worldwide because of the deep involvement of eNOS function in the regulation of erection. To identify the hypothesis, we investigated *NOS3* polymorphisms in a Han Chinese population to explore the association of the *NOS3* variants that encode functionally decreased eNOS with the primary clinical features of ED, including susceptibility, age at onset, severity, and response to sildenafil.

METHODS

Study Population

A total of 268 Han Chinese men with ancestral homes in the Sichuan province were recruited from the Department of Urology, West China Hospital, Sichuan University (Chengdu, China) from 2013 to 2016, including 112 patients with ED and 156 age-matched healthy controls. The following inclusion criteria for patients with ED were used: (i) men younger than 60 years; (ii) the presence of regular sexual relationships with one partner in the past 6 months; (iii) a five-item International Index of Erectile Function (IIEF-5) score lower than 21; and (iv) normal testosterone levels (>3.5 ng/mL). The following exclusion criteria for patients with ED were used: (i) sexual hypoactivity and psychiatric disorders as confirmed by the Dissociative Experiences Scale, the Hamilton Rating Scale for Anxiety, and the Liebowitz Social Anxiety Scale; (ii) postoperative ED after pelvic, penile, or spinal surgery; or (iii) drug abuse. The ethical review board of West China Hospital, Sichuan University approved the study, and informed consent was obtained from all subjects.

Detailed family, medical, surgical, and psychosexual histories were recorded for patients with ED. Peripheral blood samples were collected and preserved at −20°C. Genomic DNA was extracted within 24 hours. Examinations including measurements of body mass index, blood pressure, blood lipid levels, plasma glucose levels, and testosterone levels were performed. Subjects smoking more than 10 cigarettes daily for at least 6 months were classified as smokers, and patients who consumed alcohol weekly for at least 6 months were classified as drinkers.[20] Subjects with triglyceride levels higher than 2.3 mmol/L or cholesterol levels higher than 6.2 mmol/L were diagnosed as having hyperlipidemia. Subjects exhibiting a systolic pressure higher than 140 mm Hg or a diastolic pressure higher than 90 mm Hg were considered to have hypertension. Subjects with fasting blood glucose levels higher than 7.1 mmol/L were diagnosed as having diabetes.[21]

All 112 patients with ED received erectile function assessments using IIEF-5 scores (S1) to classify the ED as mild (12–21), moderate (8–11), or severe (<7). Sildenafil was provided at a dose of 100 mg. IIEF-5 scores at least 3 months after dosing (S2) were recorded in 82 patients who attempted intercourse two to three times in 1 week. Differences between the values of S2 and S1 were calculated. The classification criterion for poor responders (PRs) to sildenafil was based on this difference: score lower than 2 for mild ED, score lower than 5 for moderate ED, and score lower than 7 for severe ED.[22] The patients who did not meet the criterion for PRs were classified as good responders (GRs) to sildenafil.

Genotyping of the Three Functional Polymorphisms of the *NOS3* Gene

Genomic DNA was extracted from peripheral blood samples using DNA isolation kits (Tiangen Inc, Beijing, China). The