Gene 519 (2013) 13–17

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Variants in endothelial nitric oxide synthase (*eNOS*) gene in idiopathic infertile Brazilian men☆,☆☆

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ARTICLE INFO

Article history:
Accepted 5 February 2013
Available online 16 February 2013

Keywords:
eNOS gene
Male infertility
Polymorphism
Sperm count

ABSTRACT

Purpose: In recent years, considerable concern has been expressed about the deleterious effects of reactive oxygen species (ROS) on sperm function, because ROS at high levels is potentially detrimental to sperm function and quality. Nitric oxide (NO) is a powerful anti-oxidant present in seminal plasma. The aim of the study was to analyze the distribution of the of endothelial nitric oxide synthase (*eNOS*) gene (T-786C, G894T, e 4a/b) polymorphisms in idiopathic infertile Brazilian men and evaluate the possible role of these polymorphisms in sperm count.

Methods: A case–control study was performed comprising 208 infertile men [n = 74 with non-obstructive azoospermia and n = 134 with severe oligozoospermia] and 201 fertile men as controls. Genotyping of *eNOS* polymorphisms was performed by real time (T-786C and G894T) and conventional PCR (4a/b). The results were analyzed statistically and a p-value < 0.05 was considered significant.

Results: According to the sperm count, relatively similar *eNOS* polymorphism genotypes and allele frequencies were found among the groups. Combined genotypes of the *eNOS* polymorphisms did not identify a haplotype associated with idiopathic infertility, even when the patients were separated in non-obstructive azoospermia or severe oligozoospermia.

Conclusion: In conclusion, the findings demonstrate that, in Brazilian population studied, genetic variations, T-786C, G894T, and e 4a/b, of the *eNOS* gene are not associated with male infertility.

1. Introduction

Male infertility constitutes a collection of different conditions exhibiting a variety of etiologies and a varying diagnosis. Defective sperm function is the most prevalent cause of male infertility and a difficult condition to treat. The etiology of impaired sperm production and function can be related to different congenital or acquired factors acting at pre-testicular, post-testicular or directly at the testicular level (Poongothai et al., 2009). In about 15% of the cases of male infertility genetic abnormalities could be present, including chromosomal aberrations and single gene mutations (Ferlin et al., 2006; O'Flynn O'Brien et al., 2010).

Oxidative stress and its role in the origins of male infertility were first appreciated in 1943, when the Scottish andrologist John MacLeod demonstrated that catalase could support the motility of human spermatozoa incubated under aerobic conditions (MacLeod, 1943). His explanation for these findings, that human spermatozoa are vulnerable to oxidative stress created by reactive oxygen species (ROS) such as H_2O_2, has been confirmed in a number of independent studies (Aitken, 1999; Baker and Aitken, 2005). In humans, spermatozoa generate reactive oxygen species (ROS) which are known to affect hyperactivation of spermatozoa, the acrosome reaction, and the attachment of spermatozoa to oocytes thereby contributing to the fertilization of oocytes (Abd-Elmoaty et al., 2010; de Lamirande and Gagnon, 1993; Makker et al., 2009).

Besides the beneficial effects of ROS, an excess of ROS is detrimental to spermatozoa and leads to damage of the DNA and plasma membrane through lipid peroxidation (Aitken et al., 1993). Because spermatozoa have discarded most of their cytoplasm during the final stages of spermatogenesis, the availability of cytoplasmic defensive enzymes is

Abbreviations: ABCD region, (Santo André, São Bernardo do Campo, São Caetano do Sul and Diadema County); Asp, aspartic acid; AZF, azoospermia factor; C, cytosine; CI, confidence interval; DNA, deoxyribonucleic; eNOS, endothelial nitric oxide synthase; FSH, follicle-stimulating hormone; Glu, glutamic acid; HWE, Hardy–Weinberg equilibrium; iNOS, inducible nitric oxide synthase; LH, luteinizing hormone; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOA, non-obstructive azoospermia; NOS, nitric oxide synthase; OR, odds ratio; PCR, polymerase chain reaction; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; SO, severe oligozoospermia; T, thymine; VNTR, variable number of tandem repeats; WHO, World Health Organization.

☆ Support: This work was supported by grants from FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) #2011/09900-9.

☆☆ Conflict of interest: None of the authors has any conflict of interest to disclose.

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0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.02.001