AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Endothelial Nitric Oxide Synthase intron 4 VNTR Gene polymorphisms in European and African populations

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/130817> since 2016-01-04T09:52:07Z

Published version:

DOI:10.1007/s11033-012-1492-4

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

A. Santovito, P. Cervella, M. Delpero
Endothelial Nitric Oxide Synthase intron 4 VNTR Gene polymorphisms in
European and African populations
MOLECULAR BIOLOGY REPORTS (2012) 39
DOI: 10.1007/s11033-012-1492-4

The definitive version is available at:

<http://www.springerlink.com/index/pdf/10.1007/s11033-012-1492-4>

Endothelial Nitric Oxide Synthase intron 4 VNTR Gene polymorphisms in European and African populations

Alfredo SANTOVITO^{a,b}, Piero CERVELLA^a, and Massimiliano DELPERO^a

^a Department of Animal and Human Biology, University of Turin, Via Accademia Albertina n. 13, 10123 Torino (Italy)

*Corresponding Author:

Alfredo SANTOVITO

Dipartimento di Biologia Animale e dell'Uomo

Via Accademia Albertina n. 13

10123 – Torino (Italy)

Tel.: +39-0116704554

Fax: +39-0116704508

Abstract

Nitric oxide is an endogenous molecule that play an important role in the regulation of the systemic, cardiac and pulmonary circulations. We investigated the frequency of intron 4 VNTR *ecNOS* gene polymorphism in Ivorian and Italian populations. The frequencies of the bb, ab and aa genotypes were 0.422, 0.476, and 0.102, respectively, for the Ivorian sample, and 0.683, 0.300, and 0.017, respectively, for the Italian sample. The frequencies of 4b and 4a alleles were 0.660 and 0.340, respectively, for the Ivorian group, and 0.833 and 0.167, respectively, for the studied Italian population. The genotype frequencies were in Hardy-Weinberg equilibrium in both populations. No differences were found in *ecNOS* genotype and allele frequencies between sexes. The Ivorian population showed a significantly higher frequency of the 4a allele with respect to North African populations (Tunisia, Algeria and Morocco) and to the African-Brazilian sample. *Viceversa*, the Italian population did not show any significant differentiation with respect to other European

populations, with exception of the Holland and Turkey samples, confirming the high genetic homogeneity of Italy and Europe in the distribution of this polymorphism. In the Ivorian sample the 4a allele was prevalently found in a heterozygous status. A possible explanation could be that the heterozygote individuals produce a sufficient level of NO metabolites, although in lower amount with respect to the bb genotypes. As a consequence, the 4a allele is maintained prevalently in a heterozygous status and does not provoke an effective selective disadvantage for the carriers.

Key words: EcNOS; Nitric Oxide; genetic polymorphism; Italy; Ivory Coast

Introduction

Nitric oxide (NO) is an endogenous highly reactive molecule that play an important role in the regulation of vascular smooth muscle cell proliferation and migration, in the endothelial permeability, and in the endothelial-leucocyte interaction. Because of these properties, NO has been established as being a regulator of the systemic, cardiac and pulmonary circulations [1], with an important antithrombotic and antimicrobial role [2, 3]. An impairment of NO production has been showed to be related to many diseases, including coronary artery disease [4], ischemic stroke [5], hypertension [6], renal disease [7], and Alzheimer's disease [8]. At cellular level, an overproduction of NO has been described to be associated to DNA damage, cell cycle arrest, and cellular apoptosis [9], whereas reduction in basal NO release may predispose to hypertension, thrombosis, vasospasm and atherosclerosis [10].

NO is synthesized from the amino acid L-arginine by nitric oxide synthase (NOS) [11]. Three distinct isoforms of NOS, neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial constitutive NOS (ecNOS) have been identified in humans. ecNOS produces the majority of circulating NO [12] and, with nNOS, it is constitutively expressed in both airway and vascular endothelia. The continuously generated NO serve to maintain basal vascular NO production necessary for a good endothelial function and blood flow regulation, particularly coronary flow.

The ecNOS is encoded by a gene located on chromosome 7q35-q36 (13-15) and composed of 26 exons that span more than 21 kb of the genome [13]. Several polymorphisms were reported in the promotor region, exons, and introns of this gene, and an important one is represented by a 27-base pair core consensus VNTR (variable number of tandem repeats) polymorphism in the intron 4 [14]. The common wild-type 'b-allele' (4b allele) has five tandem 27-bp repeats and has been designated as *ecNOS4b*, while a rare 'a-allele' (4a allele) has been detected with only four repeats. Tsukada et al. (1998) [15] reported that *ecNOS* gene polymorphisms correlate with the circulating NO concentrations and individuals with bb and aa genotypes exhibit the highest and the lowest activity, respectively. The four repeats of VNTR, influencing the plasma NO levels and ecNOS protein

expression [12, 15], were reported to be involved in the pathogenesis of several diseases including coronary heart disease [4, 16,17], lung disease [18], hypertension [6, 19], atopic asthma [20], and early-onset colorectal cancer [21].

Published papers about this gene polymorphism refer mostly to case-control studies, and only few population genetic studies were published in order to assess the frequency of this polymorphism in populations worldwide. The aim of this study was to investigate the genetic variability at *ecNOS* locus in an African population from Ivory Coast and in an European population from Italy, for which no data has been published about *ecNOS* intron 4 VNTR polymorphism. We also compared our results with those reported from other control populations sampled worldwide, in particular from African and European groups.

Materials and Methods

Population studied

The African sampling was performed in Ouangolodougou, a rural village of 20.000 inhabitant located on the Northern Ivory Coast. One hundred twenty-eight unrelated individuals (59 males and 69 females, mean age 31.9 ± 14.5) were analyzed. The Italian sampling was performed in Piedmont, a region of Northern Italy. Two hundred and forty individuals (112 males and 128 females, mean age 58.36 ± 6.7) were analyzed. All the subjects were healthy volunteers and had received information about the study. The study has been approved by the local ethics committee and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

PCR Analysis

Peripheral blood samples (5-10 ml venipuncture) were collected in heparinized vacutainers and stored at -20°C . To extract DNA we used Chelex® solution protocol [22]. To determine the *ecNOS* genotype, a genomic DNA fragment of intron 4 of the *ecNOS* gene was amplified by PCR, using

the following primers: forward primer 5'-AGGCCCTATGGTAGTGCCTTT-3' and the reverse primer 5'-TCTCTTAGTGCTGTGGTCAC-3'. PCR reactions were carried out in a total volume of 25 µl containing 10 ng of DNA (template), with a final concentration of 1X Reaction Buffer, 1.5 mM of MgCl₂, 5% of DMSO, 250 µM of dNTPs, 0.5 µM of each primer, and 1U/sample of Taq DNA polymerase (Fischer, U.S.). The reaction profile was: 30 cycles of 94°C for 1 min, 56°C for 1 min., 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were separated by electrophoresis on a 4% Nusive high resolution agarose gel and visualized by ethidium bromide staining. Amplification yielded either a 420 bp (five repeats of the 27-bp sequence) fragment or a 393 bp (four repeats of the 27-bp sequence) fragment or both (heterozygous genotype). A 10% of the samples were amplified twice for checking the accuracy of the results. Moreover, in order to confirm that the amplified products represent genuine *ecNOS* intron 4 regions, selected PCR amplified segments, corresponding to a/a and b/b genotypes, were completely sequenced at both strands.

Statistical analysis

The allele and genotype frequencies were determined using GENEPOP program. Hardy-Weinberg equilibrium was evaluated by the Chi-square (χ^2) test using a 95% confidence interval. Allele frequencies obtained in the studied samples were compared with those reported for other European and African populations, through contingency tables analyzed with a χ^2 -test.

Results and Discussion

The frequency distribution of *ecNOS* 27-bp repeat polymorphism of intron 4 within the study groups was presented in Table 1. The frequencies of the bb, ab and aa genotypes were 0.422, 0.476, and 0.102, respectively, for the Ivorian sample, and 0.683, 0.300, and 0.017, respectively, for the Italian sample. The frequencies of 4b and 4a alleles were 0.660 and 0.340, respectively, for the Ivorian group, and 0.833 and 0.167, respectively, for the studied Italian population.

The genotype frequencies distribution of this polymorphism was in Hardy-Weinberg equilibrium in both populations. Because of existing gender differences in NO production [23, 24], allele and genotype frequencies of the *ecNOS* polymorphisms were analyzed separately in men and women. No differences were found in *ecNOS* genotype and allele frequencies distribution between sexes (Table 1).

The *ecNOS* intron 4 VNTR polymorphism has been analyzed in several human populations (Table 2), mostly in order to ascertain its association with several type of diseases. Results showed a significant ($P < 0.001$) decreasing gradient of the 4a allele frequency from Africa to Europe, America and Asia, probably ascribed to a founder effect due to ancient migrations of geographically distinct and isolated groups from Africa to other continents. In addition, being this polymorphism involved in the pathogenesis of several diseases, this different distribution pattern of the allele frequencies could also be the result of a selective pressure exerted on different populations.

Our Ivorian sample showed similar allele frequency values with respect to the other Ivorian sample genotyped for the same polymorphism, but a significantly ($p < 0.001$) higher frequency of the 4a allele with respect to North African populations (Tunisia, Algeria and Morocco) and to the African-Brazilian sample (Table 2).

This different distribution pattern among Africans could probably be attributed to the different evolutionary histories of the different ethnic groups, in particular of the Northern Africa populations with respect to West and South Africa groups. In Africa, the genetic structure of many otherwise genetically “neutral” systems, such as mitochondrial DNA variation, has already been demonstrated [25]. Therefore, it is not surprising to discover differences between west and north African populations.

Viceversa, the Italian population, did not show any significant level of differentiation with respect to other European populations previously analysed for the same genetic marker, with exception of the Holland and Turkey samples ($p < 0.001$), confirming the high genetic homogeneity of Italy and Europe in the distribution of this polymorphism.

According to many global surveys of genetic markers demonstrating that the African populations have consistently greater diversity than other populations [26, 27], our African sample showed an high frequency of heterozygous genotypes, indicating an high level of heterozygosity to the studied locus. It was found that the four repeats of VNTR influence the plasma NO levels. Indeed, the 4a allele of the *ecNOS* gene was related to low NO metabolite levels, and subjects homozygous for the 4a allele exhibit about 20% lower levels of NO metabolites than subjects with the b/b genotype [15]. Among Ivorians, the 4a allele was found prevalently in a heterozygous status. A possible explanation could be that the heterozygote individuals produce a sufficient level of NO metabolites, although in minor amount with respect to the bb genotypes. As a consequence, the 4a allele is maintained prevalently in a heterozygous status and does not provoke an effective selective disadvantage for the carriers.

In conclusion, we reported on novel frequency data as regards *ecNOS* intron 4 VNTR polymorphism in Ivorian and European populations, thereby extending previous observations obtained from other worldwide populations. Considering the implication of this polymorphism in many diseases and the scarcity of data about the prevalence of these diseases principally among African populations, further studies are needed to address the impact of selective constraints on the variability associated to this polymorphism in different populations and ethnic groups.

Acknowledgments

This work was supported by grants from the “Ministero Italiano dell’Università e della Ricerca Scientifica”

References

1. Rubino A, Loesch A, Burnstock G (1999) Nitric oxide and endothelin-1 in coronary and pulmonary circulation. *Int Rev Cytol* 189:59-93.

2. Draijer R, Astma DE, van der Laarse A (1995) c-GMP and nitric oxide modulate thrombin induced endothelial permeability: regulation via different pathways in human aortic and umbilical vein endothelial cells. *Circ Res* 76:199-208.
3. Gauthie TW, Scalia R, Murohara T (1995) Nitric oxide protects against leukocyte-endothelium interactions in the early stages of hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 15:1652-1659.
4. Park KW, You KH, Oh S, Chae IH, Kim HS, Oh BH, Lee MM, Park YB (2004) Association of endothelial constitutive nitric oxide synthase gene polymorphism with acute coronary syndrome in Koreans. *Heart* 90(3):282-285.
5. Munshi A, Rajeshwar K, Kaul S, Chandana E, Shafi G, Anilaa AN, Balakrishnac N, Alladi S, Jyothy A (2010) VNTR polymorphism in intron 4 of the eNOS gene and the risk of ischemic stroke in a South Indian population. *Brain Res Bull* 82:247-250.
6. Miyamoto Y, Saito Y, Kajiyama N et al., Yoshimura M, Shimasaki Y, Nakayama M, Kamitani S, Harada M, Ishikawa M, Kuwahara K, Ogawa E, Hamanaka I, Takahashi N, Kaneshige T, Teraoka H, Akamizu T, Azuma N, Yoshimasa Y, Yoshimasa T, Itoh H, Masuda I, Yasue H, Nakao K (1998) Endothelial nitric oxide synthase gene is positively associated with essential hypertension. *Hypertension* 32:3-8.
7. Schmidt RJ, Baylis C (2000) Total nitric oxide production is low in patients with chronic renal disease. *Kidney Int* 58:1261-1266.
8. Galbusera C, Facheris M, Magni F, Galimberti G, Sala G, Tremolada L, Isella V, Guerini FR, Appollonio I, Galli-Kienle M., Ferrarese C (2004) Increased susceptibility to plasma lipid peroxidation in Alzheimer disease patients. *Curr Alzheimer Res* 1:103-109.
9. Nguyen T, Brunson D, Crespi CL, Penman BW, Wishnock JS, Tannenbaum SR (1992) DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc Natl Acad USA* 89:3030-3034.

10. Candipan RC, Wang BY, Buitrago R, Tsao PS, Cooke JP (1996) Regression or progression. Dependency on vascular nitric oxide. *Arterioscler Thromb Vasc Biol* 16:44-50.
11. Bredt, DS, Snyder SH (1994) Nitric oxide: A physiologic messenger molecule. *Annu Rev Biochem* 63:175-195.
12. Wang XL, Mahaney MC, Sim AS, Wang J, Wang I, Blangero J, Almasy L, Badenhop RB, Wilcken DE (1997) Genetic contribution of the endothelial constitutive nitric oxide synthase gene to plasma nitric oxide levels. *Arterioscler Thromb Vasc Biol* 17:3147-3153.
13. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, Tsui LC, Schappert KT (1993) Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J Biol Chem* 268 (23):17478-17488.
14. Wang XL and Wang J (2000) Endothelial nitric oxide synthase gene sequence variations and vascular disease. *Mol Genet Metab* 70:241-251.
15. Tsukada T, Yokoyama K, Arai T, Takemoto F, Hara S, Yamada A, Kawaguchi Y, Hosoya T, Igari J (1998) Evidence of association of the eNOS gene polymorphism with plasma NO metabolite levels in humans. *Biochem Biophys Res Commun* 245:190-193.
16. Wang XL, Sim AS, Wang MX, Murrell GA, Trudinger B, Wang J (2000) Genotype dependent and cigarette specific effects on endothelial nitric oxide synthase gene expression and enzyme activity. *FEBS Lett* 471:45-50.
17. Ichihara S, Yamada Y, Fujimura T (1998) Association of a polymorphism of the endothelial constitutive nitric oxide synthase gene with myocardial infarction in the Japanese population. *Am J Cardiol* 81:83-86.
18. Barnes PJ & Belvisi MG (1993) Nitric oxide and lung disease. *Thorax* 48:1034-1043.
19. Jemaa R, Ben Ali S, Kallel A, Feki M, Elasmı M, Taieb SH, Sanhaji H, Omar S, Kaabachi N (2009) Association of a 27-bp repeat polymorphism in intron 4 of endothelial constitutive nitric oxide synthase gene with hypertension in a Tunisian population. *Clin Biochem* 42(9):852-856.

20. Lee YC, Cheon KT, Lee HB, Kim W, Rhee YK, Kim DS (2000) Gene polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in patient with asthma. *Allergy* 55(10):959-963.
21. Yeh CC, Santella RM, Hsieh LL, Sung FC, Tang R (2009) An intron 4 VNTR polymorphism of the endothelial nitric oxide synthase gene is associated with early-onset colorectal cancer. *Int J Cancer* 124(7):1565-1571.
22. Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a Medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* 10:506-513.
23. Forte P, Kneale BJ, Milne E, Chowienczyk PJ, Johnston A, Benjamin N, Ritter JM (1998) Evidence for a difference in nitric oxide biosynthesis between healthy women and men. *Hypertension* 32(4):730-734.
24. Tsang KW, Ip SK, Leung R, Tipoe GL, Chan SL, Shum IH, Ip MS, Yan C, Fung PC, Chan-Yeung M, Lam W (2001) Exhaled nitric oxide: the effects of age, gender and body size. *Lung* 179(2):83-91.
25. Salas A, Richards M, De la Fe T, Lareu MV, Sobrino B, Sánchez-Diz P, Macaulay V and Carracedo A (2002) The making of the African mtDNA landscape. *Am J Hum Genet* 71:1082-1111.
26. Goldstein DB, Lineares AR, Cavalli-Sforza LL, Feldman MW (1995). Genetic absolute dating based on microsatellites and the origin of modern humans. *Proc Natl Acad Sci* 92:6723-6727.
27. Garrigan D and Hammer MF. (2006) Reconstructing human origins in the genomic era. *Nature Reviews/Genetics* 7:669-680.
28. Jemaa R, Kallel A, Ben Ali S, Omar S, Chabrak S, Elasmı M, Haj Taieb S, Sanhaji H, Feki M, Mechmeche R, Kaabachi N (2007) Association of a 27-bp repeat polymorphism in intron

- 4 of endothelial constitutive nitric oxide synthase gene with myocardial infarction in Tunisian patients. *Clin Chem Lab Med* 45(11):1476-1480.
29. Djidjih R, Ghaffor M, Brun M, Gharnaout M, Salah SS, Boukouaci W, Djidjik H, Benyouness A, Koumaravelou K, Krishnamoorthy R, Abbadi MC, Charron D (2007) Constitutive nitric oxide synthase gene polymorphisms and house dust mite respiratory allergy in an Algerian patient group. *Tissue Antigens* 71:160-164.
30. Via M, González-Pérez E, Esteban E, López-Alomar A, Vacca L, Vona G, Dugoujon JM, Harich N, Moral P (2003) Molecular variation in endothelial nitric oxide synthase gene (eNOS) in western Mediterranean populations. *Coll Anthropol* 27:117-124
31. Rios DLS, D'Onofrio LO, Souza JK, QUEIROZ AM (2007) Smoking-dependent and haplotype-specific effects of endothelial nitric oxide synthase gene polymorphisms on angiographically assessed coronary artery disease in Caucasian- and African-Brazilians. *Atherosclerosis* 193:135-141.
32. Li R, Lyn D, Lapu-Bula R, Oduwole A, Igbo-Pemu P, Lankford B, Morgan J, Nkemdechi S, Liu G, Pack C, Silvestrov N, von Deutsch DA, Song Q, Abukhalaf IK, Ofili E (2004). Relation of endothelial nitric oxide synthase gene to plasma nitric oxide level, endothelial function, and blood pressure in African Americans. *Am J Hypertens* 17(7):560-567.
33. Mearin F, García-González MA, Strunk M, Zárate N, Malagelada JR, Lanas A (2006) Association between achalasia and nitric oxide synthase gene polymorphisms. *Am J Gastroenterol* 101(9):1979-1984.
34. Agema WRP, De Maat MPM, Zwinderman AH, Kastelein JJP, Rabelink TJ, Van Boven AJ, Feskens EJM, Boer JMA, Van Der Walle EE, Jukema JW (2004) An integrated evaluation of endothelial constitutive nitric oxide synthase polymorphisms and coronary artery disease in men. *Clin Sci* 107:255-261.

35. Akar N, Akar E, Cin S, Deda G, Avcu F, Yalçın A (1999) Endothelial nitric oxide synthase intron 4, 27 bp repeat polymorphism in Turkish patients with deep vein thrombosis and cerebrovascular accidents. *Thromb Res* 94(1):63-64.
36. Olcay A, Ekmekci CG, Ozbek U, Sezer M, Barcin C, Arslan E, Boztosun B, Nisanci Y (2006) Negative association of endothelial nitric oxide gene polymorphism with hypertension in Turkish patients: effect of ecNOS polymorphism on left ventricular hypertrophy. *Cardiovasc Ultrasound* 4:33.
37. Cine N, Hatemi AC, Erginel-Unaltuna N. (2002) Association of a polymorphism of the ecNOS gene with myocardial infarction in a subgroup of Turkish MI patients. *Clin Genet* 61(1):66-70.
38. Buraczynska M, Ksiazek P, Zaluska W, Nowicka T, Ksiazek A (2004) Endothelial nitric oxide synthase gene intron 4 polymorphism in patients with end-stage renal disease. *Nephrol Dial Transplant* 19:2302-2306.
39. Gardemann A, Lohre J, Cayci S, Katz N, Tillmanns H, Haberbosch W (2002) The T allele of the missense Glu(298)Asp endothelial nitric oxide synthase gene polymorphism is associated with coronary heart disease in younger individuals with high atherosclerotic risk profile. *Atherosclerosis* 160(1):167-175.
40. Sigusch HH, Surber R, Lehmann MH, Surber S, Weber J, Henke A, Reinhardt D, Hoffman A, Figulla HR (2000) Lack of association between 27-bp repeat polymorphism in intron 4 of the endothelial nitric oxide synthase gene and the risk of coronary artery disease. *Scand J Clin Lab Invest* 60:229-235.
41. Hefler LA, Ludwig E, Lampe D, Zeillinger R, Leodolter S, Gitsch G, Koelbl H, Tempfer CB (2002) Polymorphisms of the endothelial nitric oxide synthase gene in ovarian cancer. *Gynecol Oncol* 86(2):134-137.
42. Holla LI, Jurajda M, Pohunek P, Znojil V (2008) Haplotype analysis of the endothelial nitric oxide synthase gene in asthma. *Hum Immunol* 69(4-5):306-313.

43. Pulkkinen A, Viitanen L, Kareinen A, Lehto S, Vauhkonen I, Laakso M (2000) Intron 4 polymorphism of the endothelial nitric oxide synthase gene is associated with elevated blood pressure in type 2 diabetic patients with coronary heart disease. *J Mol Med* 78(7):372-379.
44. Fowkes FG, Lee AJ, Hau CM, Cooke A, Connor JM, Lowe GD (2000) Methylenetetrahydrofolate reductase (MTHFR) and nitric oxide synthase (eNOS) genes and risks of peripheral arterial disease and coronary heart disease: Edinburgh Artery Study. *Atherosclerosis* 150(1):179-185.
45. Lamnissou K, Ziogiannis P, Trygonis S, Demetriou K, Pierides A, Koptides M, Deltas CC (2004) Evidence for association of endothelial cell nitric oxide synthase gene polymorphism with earlier progression to end-stage renal disease in a cohort of Hellens from Greece and Cyprus. *Genet Test* 8(3):319-324.
46. Mamoulakis D, Bitsori M, Galanakis E, Vazgiourakis V, Panierakis C, Goulielmos GN (2009) Intron 4 polymorphism of the endothelial nitric oxide synthase *eNOS* gene and early microangiopathy in type 1 diabetes. *Int J Immunogenet* 36:153-157.
47. Luizon MR, Izidoro-Toledo TC, Simoes AL, Tanus-Santos JE (2009). Endothelial Nitric Oxide Synthase Polymorphisms and Haplotypes in Amerindians. *DNA Cell Biol* 28(7):329-334.
48. Serrano NC, Diaz LA, Casas JP, Hingorani AD, de Lucca DM, Páez MC (2010). Frequency of eNOS polymorphisms in the Colombian general population. *BMC Genetics* 11:54-61.
49. Cheon KT, Choi KH, Lee HB, Park SK, Rhee YK, Lee YC (2000) Gene polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in patients with lung cancer. *Lung* 178(6):351-360.
50. Koo KH, Lee JS, Lee YJ, Kim KJ, Yoo JJ, Kim HJ (2006) Endothelial nitric oxide synthase gene polymorphisms in patients with nontraumatic femoral head osteonecrosis. *J Orthop Res* 24(8):1722-1728.

51. Deng F, Hu Q, Tang B, He F, Guo S, Chen J, Li F, Wu X, Zhang J, Zhang H, Zhao J, Zhong H, He L, Li J, Zhang L, Wang S (2007) Endothelial nitric oxide synthase gene intron 4, 27 bp repeat polymorphism and essential hypertension in the Kazakh Chinese population. *Acta Biochim Biophys Sin* 39(5):311-316.
52. Lin NT, Lee MJ, Lee RP, Hong AI, Chen HI (2008) Analysis of endothelial nitric oxide synthase gene polymorphisms with cardiovascular diseases in eastern Taiwan. *Chin J Physiol* 51(1):42-47.
53. Tripathi G, Sharma RK, Baburaj VP, Sankhwar SN, Jafar T, Agrawal S (2008) Genetic risk factors for renal failure among north Indian ESRD patients. *Clin Biochem* 41(7-8):525-531.
54. Angeline T, Isabel W, Tsongalis GJ (2010). Endothelial nitric oxide gene polymorphisms, nitric oxide production and coronary artery disease risk in a South Indian population. *Exp Mol Pathol* 89:205-208.
55. Gururajan P, Gurumurthy P, Victor D, Srinivasa Nagaswara Rao G, Sai Babu R, Sarasa Bharati A (2011). Plasma Total Nitric Oxide and Endothelial Constitutive Nitric Oxide Synthase (ecNOS) Gene Polymorphism: A study in a South Indian Population. *Biochem Genet* 49:96-103.
56. Khajoe V, Kariyazono H, Ohno T, Ihara K, Mizuno Y, Kusuhara K, Hara T. (2003) Inducible and endothelial constitutive nitric oxide synthase gene polymorphisms in Kawasaki disease. *Pediatr Int* 45(2):130-134.
57. Nagase S, Suzuki H, Wang Y, Kikuchi S, Hirayama A, Ueda A, Takada K, Oteki T, Obara M, Aoyagi K, Koyama A (2003) Association of ecNOS gene polymorphisms with end stage renal diseases. *Mol Cell Biochem* 244(1-2):113-118.
58. Neugebauer S, Baba T, Watanabe T (2000) Association of the nitric oxide synthase gene polymorphism with an increased risk for progression to diabetic nephropathy in type 2 diabetes. *Diabetes* 49(3):500-503.

59. Asakimori Y, Yorioka N, Yamamoto I, Okumoto S, Doi S, Hirai T, Taniguchi Y (2001)

Endothelial nitric oxide synthase intron 4 polymorphism influences the progression of renal disease. *Nephron* 89(2):219-223.

Table 1 – Allele and Genotype Frequencies of intron 4 VNTR *ecNOS* gene polymorphism in the studied populations

Population	N	Genotype (frequency)			Allele (frequency)	
		bb	ba	aa	b	a
Ivory Coast	128	54 (0.422)	61 (0.476)	13 (0.102)	169 (0.660)	87 (0.340)
Male	58	28 (0.483)	22 (0.379)	8 (0.138)	78 (0.672)	38 (0.328)
Female	70	26 (0.372)	39 (0.557)	5 (0.071)	91 (0.650)	49 (0.350)
Italy	240	164 (0.683)	72 (0.300)	4 (0.017)	400 (0.833)	80 (0.167)
Male	112	78 (0.696)	34 (0.304)	0 (0.000)	190 (0.848)	34 (0.152)
Female	128	86 (0.672)	38 (0.297)	4 (0.031)	210 (0.820)	46 (0.180)

Table 2 – Allele and Genotype Frequencies of intron 4 VNTR *ecNOS* gene polymorphism in the studied and other populations worldwide distributed

Population	N	Allele (frequency)		References
		b	a	
<i>Africa</i>				
Ivory Coast	128	169 (0.660)	87 (0.340)*	Present Study
Tunisia	250	431 (0.862)	69 (0.138)*	[28]
Algeria	122	202 (0.828)	42 (0.172)*	[29]
Morocco (Berber)	117	190 (0.812)	44 (0.188)*	[30]
Ivory Coast	109	138 (0.633)	80 (0.367)	[30]
African-Brazilians	121	194 (0.802)	48 (0.198)*	[31]
African-Americans	58	81 (0.698)	35 (0.302)	[32]
<i>Pooled Africa</i>	905	1405 (0.776)	405 (0.224) ^{a,b,c}	
<i>Europe</i>				
Italy	240	400 (0.833)	80 (0.167) ^{&}	Present Study
Italy (Sardinia)	189	336 (0.889)	42 (0.111)	[30]
Spain	499	859 (0.861)	139 (0.139)	[30, 33]
Holland	466	839 (0.900)	93 (0.100) ^{&}	[34]
Turkey	488	873 (0.894)	103 (0.106) ^{&}	[35, 36, 47]
Polish	321	553 (0.861)	89 (0.139)	[38]
Germany	941	1572 (0.835)	310 (0.165)	[39, 40]
Austria	133	228 (0.857)	38 (0.143)	[41]
Czech Republic	316	517 (0.818)	115 (0.182)	[42]
Finland	110	184 (0.836)	36 (0.164)	[43]
Scotland	300	526 (0.877)	74 (0.123)	[44]
Greece	295	492 (0.834)	98 (0.166)	[45]
Greece, Crete	160	279 (0.872)	41 (0.128)	[46]
<i>Pooled Europe</i>	4458	7658 (0.859)	1258 (0.141) ^{a,d}	
<i>America</i>				
Amerindians	170	326 (0.959)	14 (0.041)	[47]
Colombia	859	1474 (0.858)	244 (0.142)	[48]
<i>Pooled America</i>	1029	1800 (0.875)	258 (0.125) ^b	
<i>Asia</i>				
South Korea	224	412 (0.920)	36 (0.080)	[49, 50]
China	216	392 (0.907)	40 (0.093)	[51, 52]
India	769	1274 (0.828)	264 (0.172)	[53, 54, 55]
Japan	1329	2409 (0.906)	249 (0.094)	[17, 56, 57, 58, 59]
<i>Pooled Asia</i>	2538	4487 (0.884)	589 (0.116) ^{c,d}	

* = significantly different (P<0.001) with respect to our studied Ivorian sample

& = significantly different (P<0.001) with respect to our studied Italian sample

^{a,b,c,d} = P<0.001