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# POTENTIAL ROLE OF NOS3 PROMOTER POLYMORPHISM (−786T>C / C786T) IN THE PATHOGENESIS OF CHRONIC MYELOPROLIFERATIVE NEOPLASMS

***Jakhonov Azizbek Kholmirzaevich*** *(Tashkent State Medical University, ALFRAGANUS UNIVERSITY)*

*Matkarimova Dilfuza Saburovna (Tashkent State Medical University)*

***Abstract:*** *Chronic myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders characterized by dysregulated proliferation of one or more myeloid cell lineages. While driver mutations (JAK2, CALR, MPL) are central, additional genetic and environmental modifiers likely influence disease initiation, progression, and complications. Endothelial nitric oxide synthase (NOS3), via its product nitric oxide (NO), modulates vascular tone, hematopoietic niche microenvironment, and regulation of oxidative stress and inflammation. The promoter polymorphism* ***−786T>C (aka C786T, rs2070744)*** *is known in other diseases to influence NOS3 expression and NO bioavailability. In this review and hypothesis article, we explore mechanistic rationales by which the −786T>C variant might contribute to the pathogenesis, vascular complications, and disease progression of MPNs. We survey what is known about NOS3 biology, the functional impact of −786T>C, and analogous disease associations, and propose testable models and research strategies. While direct empirical data are lacking at present, this article aims to stimulate investigation of NOS3 polymorphisms as potential modifiers in MPN pathophysiology.*

***Keywords:*** *NOS3, endothelial nitric oxide synthase, −786T>C, C786T, myeloproliferative neoplasms, nitric oxide, vascular niche, endothelial dysfunction*

## Introduction

### Chronic Myeloproliferative Neoplasms: Overview

Chronic myeloproliferative neoplasms (MPNs) represent a group of hematologic malignancies characterized by overproduction of one or more lineages

of myeloid cells in the bone marrow. Classic MPNs include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Википедия Driver mutations—most notably **JAK2 V617F**, **CALR**, **MPL**—explain a substantial portion of the disease biology. However, not all individuals with these driver mutations develop overt disease, and disease heterogeneity (e.g., rate of fibrosis progression, thrombosis risk, transformation to acute leukemia) suggests influence by additional genetic modifiers, epigenetic changes, and microenvironmental factors.

One key microenvironmental component is the **vascular and endothelial niche** within the bone marrow: endothelial cells, nitric oxide signaling, oxidative stress, and local hemodynamics can influence hematopoietic stem cell behavior, progenitor trafficking, and interactions with the extracellular matrix.

**NOS3 and Endothelial NO in Hematopoietic Environments**

Nitric oxide (NO) is a gaseous signaling molecule synthesized by endothelial nitric oxide synthase (**eNOS**, encoded by **NOS3**). NO regulates vascular tone, inhibits leukocyte adhesion, modulates angiogenesis, and participates in redox balance. Википедия In the bone marrow microenvironment, NO may influence hematopoietic progenitor cell quiescence, mobilization, and interaction with the endosteal or vascular niches, as well as modulate reactive oxygen species (ROS) and inflammatory signaling.

Given these roles, variability in NOS3 expression or activity could conceivably modulate the predisposition to clonal expansion, vascular events (e.g., thrombosis, vascular remodeling) or progression in MPNs.

**The −786T>C (C786T) Promoter Polymorphism**

The **−786T>C** (also referenced as **C786T**, rs2070744) polymorphism lies within the promoter region of NOS3. Studies in cardiovascular disease and other contexts suggest that the **C allele** is associated with reduced promoter activity, lower NOS3 mRNA expression, and diminished NO production, although results have been somewhat inconsistent. BioMed Central+3PubMed+3Википедия+3

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In cardiovascular meta-analyses, the T786C variant (i.e. presence of the C allele) was significantly associated with coronary artery disease risk (OR ~1.34–1.42) across ancestries. PubMed In prostate cancer, the −786T>C polymorphism was associated with progression and NOS3 transcript levels. PMC

Thus, although direct evidence in MPNs is lacking, the known functional and disease associations make −786T>C a candidate polymorphism for investigation as a disease modifier in MPNs.

**Aim of this article**: to review the biology of NOS3 and the known effects of −786T>C, explore theoretical mechanistic links to MPN pathogenesis, propose putative models, and suggest research strategies to test the hypothesis that NOS3 −786T>C influences MPN development, phenotype, or complications.

**NOS3 / eNOS: Structure, Regulation, and Function**

**Gene, Structure, and Isoforms**

- **NOS3** (endothelial nitric oxide synthase) is located on chromosome **7q35–q36**. BioMed Central+1
- eNOS is a constitutively expressed enzyme in endothelial cells; under basal conditions, it generates NO from the substrate L-arginine with cofactors (e.g. tetrahydrobiopterin, NADPH, flavins, calmodulin). Википедия
- Three NOS isoforms exist: endothelial (eNOS or NOS3), neuronal (nNOS or NOS1), and inducible (iNOS or NOS2). eNOS-derived NO is critical for vascular homeostasis. Википедия

**Regulation of NOS3 Expression and Activity**

NOS3 expression and activity are regulated at multiple levels:

- **Transcriptional regulation**: Promoter region elements, transcription factor binding (Sp1, AP-2, NF-κB, etc.), chromatin modifications, and promoter polymorphisms (e.g., −786T>C) affect gene transcription.
- **Post-transcriptional / mRNA stability**: 5′ and 3′ untranslated region elements, microRNAs, RNA-binding proteins.

- **Post-translational modifications & interactions**: Phosphorylation (e.g. at Ser1177), acylation (myristoylation, palmitoylation), interaction with caveolin-1, heat shock proteins, and subcellular localization.
- **Cofactor and substrate availability**: Tetrahydrobiopterin (BH4) deficiency, L-arginine limitation, oxidative degradation of BH4 lead to *eNOS uncoupling* (producing superoxide instead of NO).
- **Shear stress and mechanotransduction**: Hemodynamic shear stress upregulates eNOS expression and activity in vascular endothelium.

**Functional Roles of eNOS / NO**

NO produced by eNOS in endothelial cells diffuses to underlying vascular smooth muscle cells to activate soluble guanylate cyclase (sGC), increasing cyclic GMP (cGMP) and promoting vasorelaxation, thereby contributing to vascular tone regulation. Википедия

Beyond vasodilation, NO also:

- Inhibits platelet adhesion and aggregation
- Suppresses leukocyte adhesion and inflammation
- Exerts anti-proliferative effects on smooth muscle and endothelial cells (via cGMP or direct signaling)
- Acts in redox signaling, scavenging or generating reactive nitrogen species
- Modulates angiogenesis via cross-talk with VEGF and HIF pathways

In the bone marrow microenvironment, NO may influence vascular endothelial niche functions, barrier properties, hematopoietic stem cell (HSC) trafficking/migration, oxidative stress, and inflammatory tone.

**Functional Impact of the −786T>C (C786T) Polymorphism**

**Evidence from Genetic Association Studies**

- A large meta-analysis across 89 articles (69,235 individuals) found that the **T786C SNP** (C allele) is significantly associated with coronary artery disease (OR range ~1.34–1.42) in multiple genetic models and ancestries. PubMed

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- In a Tunisian population study of myocardial infarction, however, the −786T>C variant was not significantly associated with MI in that cohort. Ovid
- In preeclampsia (a vascular disorder of pregnancy), a Greek case-control study found **no significant association** between T-786C and disease status; haplotype analysis likewise failed to show strong association. BioMed Central
- In prostate cancer, −786T>C (C allele) was associated with higher NOS3 transcript levels and possibly with tumor progression, though interpretation is complex (e.g. bimodal behavior) and might reflect tumor-specific regulation. PMC
- In other contexts, the −786T>C polymorphism has been studied in relation to exercise response, blood pressure response to training, and endothelial responsiveness. (E.g., interactions in blood pressure lowering with exercise training have shown paradoxical responses in T786C carriers). Журналы физиологии

Thus, the literature suggests that the −786T>C polymorphism may have functional consequences under certain conditions, but its effect is context-dependent and may require environmental or stress triggers to manifest.

**Mechanistic Hypotheses about Functional Impact**

From molecular and cell-biological perspectives, the following mechanistic rationales can be proposed for how −786T>C influences NOS3 expression and NO production:

1. **Reduced promoter activity.** The C allele may disrupt binding of positive transcription factors (e.g. Sp1) or favor binding of repressor elements, decreasing basal transcription. Some reporter assays in endothelial cell lines have indeed shown lower promoter activity with the C allele (in some studies).
2. **Allele-specific chromatin differences.** Altered local chromatin structure (e.g. DNA methylation, histone modifications) around the polymorphic site may differentially affect transcription in C vs T alleles.
3. **Interaction with upstream regulators.** In conditions of oxidative stress, inflammatory signaling, or hemodynamic shear stress changes, the regulatory

impact of the −786T>C variant may be magnified (i.e. a "susceptibility allele" effect rather than a strong independent effect).

4. **"Uncoupling" under stress.** In individuals with the C allele and borderline NO synthesis, coexisting conditions (e.g. low BH4, L-arginine deficiency, oxidative stress) may exacerbate eNOS uncoupling, thereby shifting from protective NO to harmful ROS generation.

Taken together, these mechanisms suggest that carriers of the C allele may have **compromised endothelial NO production capacity**, particularly under stress or in disease states, which could predispose to endothelial dysfunction, vascular complications, or microenvironmental alterations.

**Hypothetical Links Between NOS3 −786T>C and MPN Pathogenesis**

Because direct empirical studies are not (to date) available, one must rely on mechanistic inference and analogy from other disease models. Below are several speculative pathways by which the −786T>C variant might influence MPN biology or its complications:

**1. Modulation of the Bone Marrow Vascular Niche**

- Hematopoietic stem and progenitor cells (HSPCs) reside in perivascular niches adjacent to sinusoidal endothelial cells. The local endothelial environment influences HSPC quiescence, proliferation, and egress.
- Reduced NO production from endothelial cells (due to −786T>C) may impair endothelial function, alter permeability, or dysregulate niche signals (e.g. through reactive oxygen species or altered cytokine gradients), which could favor clonal expansion of mutated progenitors or reduce suppression of aberrant growth.

**2. Promotion of Pro-thrombotic and Pro-adhesive Microenvironment**

- MPNs are known for increased risk of thrombosis. Endothelial dysfunction (reduced NO, increased adhesion molecules, procoagulant shift) is a major contributing factor to thrombogenesis.
- Carriers of the −786T>C C allele might have a baseline compromised endothelial NO capacity; in the context of MPN-driven inflammation and hypercoagulability, this could amplify thrombotic risk.

**3. Oxidative Stress, Inflammation, and Clonal Advantage**

- In MPNs, reactive oxygen species (ROS), chronic inflammation, and oxidative DNA damage contribute to genomic instability and disease progression.
- A lowered capacity for NO mitigation and redox balance in endothelial and perivascular cells (due to −786T>C) might exacerbate oxidative stress in the bone marrow microenvironment, favoring clonal evolution or fibrosis.

**4. Interaction with JAK/STAT or Other Oncogenic Signaling**

- Clonal signaling through JAK2, MPL, CALR, or downstream effectors may impose metabolic and oxidative stress on tissues, including endothelial compartments. A compromised NO system (via −786T>C) might render tissues less resilient to such stress, thereby facilitating tissue remodeling (e.g. microvascular rarefaction, fibrosis) or disease acceleration.

**5. Influence on Angiogenesis and Vascular Remodeling**

- As MPNs evolve, neovascularization, aberrant perfusion, and vascular remodeling in the marrow may occur. NO is a key regulator of angiogenesis; altered NO dynamics could influence pathological vessel formation, leading to abnormal niche architecture supportive of clonal cells.

**6. Epigenetic / Transcriptional Crosstalk**

- It is conceivable that NOS3 expression and activity might influence epigenetic regulators or redox-sensitive transcription factors (e.g. NF-κB, HIF, Nrf2) within endothelial or stromal compartments, thereby modulating gene expression milieus favorable or unfavorable to clonal expansion.

**Proposed Study Design to Test the Hypothesis**

To evaluate whether the −786T>C polymorphism contributes significantly to MPN pathobiology, the following experimental or clinical study designs might be undertaken:

**1. Case-Control Genetic Association Study**

- Enroll a cohort of patients diagnosed with MPNs (e.g. PV, ET, PMF) and matched healthy controls (matched for age, sex, ethnicity).

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- Genotype NOS3 −786T>C (rs2070744) and other known NOS3 SNPs (e.g. G894T, intron 4 VNTR).
- Compare allele frequencies and genotype distributions between MPN vs control groups.
- Stratify by driver mutation subtype (JAK2, CALR, MPL-negative) and by clinical phenotype (thrombosis history, fibrosis progression).
- Use logistic regression adjusting for confounders (age, sex, cardiovascular risk factors) to assess association (OR, CI).

**2. Cohort / Prospective Study of Disease Complications**

- Within an MPN patient cohort, genotype all participants for −786T>C.
- Prospectively track clinical events (thrombosis, disease progression to fibrosis or leukemic transformation, vascular complications).
- Perform survival analyses (Kaplan-Meier, Cox proportional hazards) to compare event-free survival between genotype groups (TT vs TC vs CC).
- Adjust for known prognostic factors (age, white cell count, driver mutation, JAK2 allele burden, treatment).

**3. Functional Ex Vivo and In Vitro Studies**

- Obtain bone marrow endothelial cells (or endothelial progenitor cells) from MPN patients (across genotypes).
- Assess NOS3 mRNA, eNOS protein levels, and NO production (e.g. by Griess assay, DAF-FM fluorescence) in relation to genotype.
- Under basal and stimulated conditions (e.g. shear stress, cytokine challenge, oxidative stress), compare functional differences in endothelial properties (e.g. adhesion molecule expression, barrier function, ROS generation).
- Co-culture assays: endothelial cells (with known genotype) co-cultured with hematopoietic progenitor cells (normal and mutated) to see how endothelial genotype influences proliferation, differentiation, adhesion, survival.

**4. Animal or Mouse Model Studies**

- Generate mice carrying humanized NOS3 promoter variants (T or C alleles) or knock-in −786C analogs.

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- Cross with murine MPN models (e.g. JAK2 V617F knock-in) to assess effects on disease onset, progression, thrombosis, microvascular changes, fibrosis.
- Examine bone marrow vascular architecture, NO bioavailability, oxidative stress markers, and clonal expansion dynamics.

**5. Integration with Multi-Omics and Biomarkers**

- In MPN patient cohorts, integrate genotype data with endothelial function biomarkers (e.g. flow-mediated dilation, circulating endothelial microparticles, NO metabolites, asymmetric dimethylarginine ADMA), inflammatory markers, ROS markers, and microvascular imaging.
- Use systems biology / network models to test whether NOS3 genotype is a latent modifier interacting with other molecular pathways in MPN.

**Potential Implications and Challenges**

**Implications**

- If a significant association is found, NOS3 −786T>C could serve as a *modifier gene* in MPN, helping explain inter-individual heterogeneity in disease phenotype or vascular complications.
- It could identify individuals at higher risk of thrombosis or disease progression, who may benefit from tailored preventive strategies (e.g. more aggressive aspirin or anticoagulation).
- It might point to therapeutic avenues — e.g. drugs that enhance NO bioavailability (e.g. statins, phosphodiesterase inhibitors, L-arginine/BH4 supplementation) as adjuncts in certain genotype subgroups.
- It would underscore the importance of the vascular niche and endothelial health in hematologic malignancies, promoting further niche-targeted research.

**Challenges and Limitations**

- **Lack of direct precedent**: No published study (as of my search) directly links −786T>C to MPNs, so initial results may be negative or weak.
- **Effect size**: The polymorphism may have only modest effect (low penetrance), requiring large sample sizes to detect.

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- **Population stratification / confounding**: Must carefully control for ethnicity, environmental exposures, cardiovascular risk factors.
- **Functional complexity and pleiotropy**: NOS3 is regulated by multiple interacting factors; genotype effect might be masked or modulated by other variants (e.g. in G894T) or environmental factors (e.g. oxidative stress).
- **Reverse causation**: In patients with advanced disease or therapy, endothelial dysfunction may already be altered, making it difficult to untangle cause vs effect.
- **Multiple hypothesis testing**: Correction for multiple comparisons (other SNPs, outcomes) is needed, which reduces power.

**Outline of a Full Manuscript**

Below is a suggested section-by-section scaffold. You can expand each section with more literature, tables, figures, and data.

1. **Introduction**
   - Background on MPNs, driver mutations and modifiers
   - Importance of bone marrow vascular niche
   - NOS3 / NO biology and rationale
   - Known associations of −786T>C in other diseases
   - Hypothesis and aims
2. **Materials and Methods**
   - Study populations, inclusion/exclusion criteria
   - Genotyping methods (PCR-RFLP, TaqMan, sequencing)
   - Endothelial / endothelial progenitor cell isolation and culture
   - NO / eNOS / ROS measurements
   - Co-culture assays, animal models (if applicable)
   - Statistical analyses
3. **Results** *(in a hypothetical or pilot dataset)*
   - Genotype and allele frequencies in MPN vs control
   - Clinical correlations (e.g. thrombosis rates, progression) by genotype
   - Endothelial functional assays by genotype

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- Co-culture outcomes
- Animal model phenotypes

4. **Discussion**
   - Interpretation of results
   - Comparison with other disease models (cardiovascular disease, cancer)
   - Biological plausibility, mechanistic insights
   - Limitations, alternative explanations
   - Clinical and translational implications
   - Future directions and recommendations
5. **Conclusion**
   - Summary of the hypothesis and key findings
   - Potential role of NOS3 −786T>C as modifier in MPNs
   - Call for further investigation
6. **Acknowledgments, Funding, Conflicts of Interest**
7. **References**
8. **Tables and Figures**
   - Table: Genotype distribution
   - Table: Clinical event rates by genotype
   - Figure: Model diagram of hypothesized pathways
   - Figure: NO production or endothelial assay differences
   - Supplementary data (if any)

**Sample (Hypothetical) Mini Results and Interpretation**

(*Note: purely illustrative, not real data*)

- Among 300 MPN patients and 300 matched controls, the C allele frequency at −786T>C is 0.25 in patients vs 0.18 in controls (p = 0.02).
- Adjusted OR for C allele carriers (TC + CC vs TT) is 1.45 (95% CI 1.08–1.94).
- Within MPN cohort, CC genotype is associated with higher incidence of thrombosis (hazard ratio 1.8, p = 0.03) after adjusting for age, JAK2 allele burden, and therapy.

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- In endothelial cell cultures, cells with CC genotype produce ~25% lower NO (Griess assay) under basal conditions and ~35% less under shear stress stimulation ($p < 0.01$).
- In co-culture, hematopoietic progenitor proliferation is modestly higher when cultured over CC-genotype endothelial monolayers (fold change +1.2, $p = 0.05$) compared to TT.
- In a murine model harboring knock-in of human −786C promoter variant plus JAK2 V617F, mice show faster onset of splenomegaly, greater vascular rarefaction in bone marrow sections, and more microthrombi in marrow sinusoids relative to −786T counterparts.

These hypothetical results, if replicated, would support the idea that the C allele acts as a modest modifier, lowering endothelial NO capacity, enhancing thrombosis, and subtly favoring clonal expansion or microenvironmental remodeling.

**Conclusion**

While canonical driver mutations dominate the pathogenesis of chronic myeloproliferative neoplasms, genetic modifiers and microenvironmental influences likely shape disease heterogeneity, complication risk, and progression. The NOS3 promoter polymorphism **−786T>C (C786T)** is an intriguing candidate variant because of its known functional associations in vascular disease, its plausible mechanistic links to endothelial function, and its potential to modulate the bone marrow vascular niche, oxidative balance, and thrombogenic potential. Although direct evidence in MPNs is lacking, the theoretical and indirect rationale is strong enough to justify systematic investigation via genetic association studies, functional assays, and integrative models. Discovering a role for NOS3 variants in MPNs could open new biomarker and therapeutic avenues centered on endothelial health and nitric oxide pathways.

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