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THE ROLE AND POTENTIAL SIGNIFICANCE OF THE G-84A POLYMORPHISM IN THE NOS1 GENE IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Abstract: Background: *Chronic myeloproliferative neoplasms (CMPNs) are clonal hematopoietic disorders characterized by overproduction of one or more myeloid lineages, driven by somatic mutations and modulated by the inflammatory microenvironment. Nitric oxide (NO), a key regulator of vascular tone, reactive nitrogen species, and signal transduction, is synthesized by nitric oxide synthases (NOS). Among them, neuronal NOS (NOS1) is classically expressed in neural tissues, but extra-neuronal roles in hematopoiesis and inflammation are plausible. A putative promoter polymorphism G-84A (i.e. at –84 base pairs upstream of the transcription start site) in NOS1 might alter gene expression and thereby influence disease risk or phenotype in CMPNs.*

Objective: *To review the biology of NOS1, nitric oxide pathways in hematopoiesis and inflammation, and to assess evidence and theoretical bases for involvement of a G-84A polymorphism in CMPNs. We propose mechanistic hypotheses, potential study designs, and clinical significance.*

Methods: *Literature search on NOS1, NOS polymorphisms, nitric oxide in hematopoiesis and myeloproliferative disorders, and general CMPN molecular pathogenesis.*

Results & Conclusions: *While direct evidence for G-84A in CMPNs is lacking, the role of NO in stem cell quiescence, oxidative stress, and inflammatory regulation suggests that functional variation in NOS1 may modulate disease initiation, progression, thrombosis risk, or transformation. Future case–control studies, functional assays, and integration into risk models are warranted.*

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Keywords: *Chronic myeloproliferative neoplasms, NOS1, nitric oxide synthase, promoter polymorphism, G-84A, hematopoiesis, inflammation, polymorphism, disease susceptibility.*

Keywords

- *Chronic myeloproliferative neoplasms (CMPNs)*
- *NOS1 (neuronal nitric oxide synthase)*
- *Promoter polymorphism, G-84A*
- *Nitric oxide (NO)*
- *Hematopoiesis*
- *Inflammation*
- *Genetic susceptibility*

Introduction

Chronic myeloproliferative neoplasms (CMPNs), or more broadly classical myeloproliferative neoplasms (MPNs), encompass disorders such as polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These disorders are characterized by clonal proliferation of hematopoietic stem/progenitor cells, often accompanied by morphological bone marrow changes, splenomegaly, and systemic symptoms. PMC+2ashpublications.org+2

Driver mutations in genes such as **JAK2**, **CALR**, and **MPL** are central to pathogenesis, leading to constitutive activation of JAK–STAT signaling pathways. ashpublications.org+2Wiley Online Library+2 Secondary or cooperating mutations in epigenetic regulators (e.g. TET2, ASXL1, EZH2) modulate disease evolution. Wiley Online Library+2ashpublications.org+2 Beyond genetic drivers, the inflammatory microenvironment is considered a key contributor to disease manifestations, symptom burden, progression, and thrombosis risk. Wiley Online Library+2PMC+2

Nitric oxide (NO) is a small gaseous signaling molecule produced by nitric oxide synthases (NOS). Three major isoforms exist: neuronal (nNOS, or NOS1), inducible (iNOS, or NOS2), and endothelial (eNOS, or NOS3). While NOS1 is classically associated with neuronal functions, increasing evidence supports roles

outside the nervous system, including regulation of vascular tone, redox signaling, and inflammatory modulation. Википедия Genetic polymorphisms in NOS genes have been studied in various disease contexts (e.g. cardiovascular disease, asthma, cancer), but their role in hematologic neoplasms is not well established.

In this article, we focus on a hypothetical or putative **G-84A** promoter polymorphism in the NOS1 gene (–84 G→A), exploring its potential functional significance, possible impact in CMPNs, and ways to investigate its relevance.

The Biology of NOS1 and Nitric Oxide in Hematopoietic and Inflammatory Systems

NOS1 structure, regulation, and expression

- The **NOS1** gene (neuronal nitric oxide synthase) encodes a protein that synthesizes NO from L-arginine, with cofactors such as NADPH, FAD, FMN, and tetrahydrobiopterin. Википедия
- In the nervous system, NOS1-derived NO acts as a neurotransmitter or neuromodulator, participating in synaptic plasticity, long-term potentiation, and neurovascular coupling. Википедия
- NOS1 contains regulatory domains including a PDZ domain and can interact with adaptor proteins (e.g. CAPON, PSD-95) for subcellular localization. Википедия
- Transcriptional regulation of NOS1 is complex and may involve promoter region elements, methylation, transcription factors, and epigenetic modifications.

Though classically associated with neurons, **extra-neuronal expression of NOS1** has been reported in non-neuronal tissues, including smooth muscle, macrophages, and possibly in vascular endothelium under certain conditions. The expression outside neural cells is lower and often tightly regulated.

Role of nitric oxide (NO) in hematopoiesis and the bone marrow microenvironment

While NOS2 (iNOS) and NOS3 (eNOS) have been more widely studied in immune or vascular contexts, NO derived from any NOS isoform can influence:

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1. **Stem/progenitor cell cycling and quiescence:** NO has been implicated in the regulation of hematopoietic stem cell (HSC) niche interactions, maintaining quiescence vs proliferation balance via redox signaling.

2. **Reactive nitrogen/oxygen species balance:** NO can combine with reactive oxygen species (ROS) to form peroxynitrite, affecting protein nitration, DNA damage, and signaling pathways.

3. **Modulation of cytokine signaling:** NO may influence inflammatory signaling (e.g. NF-κB, STAT, MAPK) by S-nitrosylation of key proteins or modulation of redox-sensitive transcription factors.

4. **Vascular and endothelial interactions:** In bone marrow vasculature, NO contributes to vasodilation, endothelial permeability, and reactive responses, which may influence trafficking of hematopoietic cells.

5. **Immune cell function:** NO influences macrophages, dendritic cells, and T cell signaling, potentially affecting the inflammatory milieu in CMPNs.

Given the importance of chronic inflammation in CMPN pathophysiology, functional variation in NOS genes could modulate disease features (e.g. thrombotic risk, fibrotic progression).

Evidence from NOS polymorphisms in disease

Although direct studies in CMPNs are sparse, polymorphisms in NOS genes (especially NOS2 or NOS3) have been linked to cardiovascular disease, asthma, cancer susceptibility, and other inflammatory diseases. PubMed For example, in asthma, NOS gene variants were meta-analyzed for associations with disease and nitric oxide levels. PubMed

The absence of strong data for NOS1 in hematologic neoplasms means one must rely on functional inference and related literatures.

Chronic Myeloproliferative Neoplasms: Pathogenesis, Inflammation, and Genetic Modulators

Pathogenetic overview

- CMPNs (or more specifically Philadelphia-negative MPNs) arise from acquisition of driver mutations in HSCs (JAK2 V617F, MPL, CALR). Wiley Online Library+2ashpublications.org+2
- These driver mutations activate JAK–STAT signaling, promoting proliferation and survival. ashpublications.org+1
- Secondary cooperating mutations in epigenetic regulators and splicing factors (e.g. TET2, ASXL1, SRSF2) shape disease heterogeneity, prognosis, and risk of leukemic transformation. Wiley Online Library+2ashpublications.org+2
- Clonal evolution over time, with accumulation of additional mutations, leads to disease progression, fibrotic transformation, or leukemic evolution. ashpublications.org+2PMC+2

Role of chronic inflammation and microenvironment

- CMPNs are increasingly viewed not only as proliferative hematologic diseases but also as **inflammatory neoplasms**. Wiley Online Library+2PMC+2
- Patients often display elevated cytokines (e.g. IL-1β, IL-6, TNF-α), chemokines, and evidence of oxidative stress. Wiley Online Library+1
- Inflammatory signaling contributes to symptoms (e.g. constitutional symptoms, cachexia), marrow fibrosis, and the prothrombotic state. Wiley Online Library+1
- The mutated clone may exert cell-extrinsic effects on the bone marrow niche, suppressing normal hematopoiesis and remodeling the microenvironment. PMC+1

Thus, genetic modifiers in oxidative/nitrosative stress or inflammation pathways could modulate the disease phenotype, progression, or complications.

Known genetic modifiers and SNP studies in CMPNs

- Some studies have explored polymorphisms beyond driver genes, e.g. in xenobiotic metabolism genes (GSTM1, GSTT1, NQO1) in MPNs, with mixed or modest associations. PubMed
- In one study, a panel of 50 genes in MPN patients revealed certain SNPs potentially correlated with susceptibility loci. spj.science.org

- In CMPN patients, SNPs in PTP1B were analyzed, but none directly implicated NOS1. tcr.amegroups.org
- No published studies specifically examine a G-84A polymorphism in NOS1 in CMPNs.

Given the dearth of direct empirical evidence, the following section explores plausible mechanistic hypotheses.

The Hypothetical G-84A Polymorphism in NOS1: Functional Hypotheses and Potential Impact in CMPNs

Rationale for considering G-84A in NOS1

- A promoter polymorphism at –84 (i.e. upstream from transcription start) lies within the proximal regulatory region. A G→A substitution could alter binding affinity for transcription factors, promoter methylation, or nucleosome positioning.
- If such a polymorphism results in altered NOS1 transcription (increase or decrease), it could modulate basal NO production in hematopoietic or stromal cells.
- In CMPNs, where the inflammatory and oxidative milieu is dysregulated, small shifts in NO homeostasis could have additive or modifying effects on disease behavior.

Possible functional consequences of G-84A variation

1. **Altered promoter activity**
   - The A allele might weaken or strengthen binding of specific transcription factors (e.g. Sp1, NF-κB, AP-1).
   - Reporter assays (promoter-luciferase) might reveal differential transcriptional activity.
2. **Differential epigenetic modification**
   - Polymorphisms can affect CpG context; G→A may abolish or create CpG sites, influencing DNA methylation or histone modification patterns.
3. **Allele-specific expression (ASE)**

- In heterozygotes, one allele might be preferentially expressed, detectable via RNA sequencing or allele-specific RT-PCR.

4. **Modulation of basal NO production and redox balance**

- A lower expression allele could reduce NO, possibly tipping redox balance toward ROS dominance, favoring DNA damage or clonal evolution.
- A higher expression allele might increase NO, which—while protective in some contexts—could generate reactive nitrogen species and nitrosative stress.

5. **Interaction with other pathways**

- The effect of G-84A might be modulated by inflammation, cytokines, or JAK–STAT signaling cross-talk.
- In the presence of JAK2 V617F (or other driver lesions), the influence of NOS1 variation might become more pronounced.

Potential influences on CMPN phenotypes

- **Disease susceptibility/risk**: Carriage of the A allele might confer modest increased or decreased risk of CMPN in susceptible populations.
- **Phenotypic subtype modulation**: The effect of G-84A might influence whether a patient develops PV vs ET vs PMF, based on differential NO-mediated signals.
- **Thrombotic risk**: NO is a vasodilator and inhibitor of platelet aggregation; lower NO (if A allele reduces expression) might predispose to thrombosis.
- **Fibrosis progression**: NO-mediated signaling may influence fibroblast activation, collagen deposition, or angiogenesis in marrow stroma.
- **Transformation to leukemia**: Increased oxidative/nitrosative stress or DNA damage may accelerate additional mutational events and leukemic conversion.

A hypothetical model

1. In healthy hematopoiesis, NOS1 expression is low but contributes to basal NO signaling regulation.
2. In a predisposed HSC bearing a driver mutation (e.g. JAK2), the presence of a lower-expression NOS1 allele (G-84A variant) reduces NO-mediated

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suppression of ROS and inflammatory feedback, thereby facilitating further clonal expansion or genomic instability.

3. Conversely, a higher-expression allele might mitigate or delay progression by better redox control.

4. The effect is likely modest and may only be evident in conjunction with other genetic modifiers, environmental exposures (e.g. smoking, oxidative stress), or inflammatory stimuli.

Proposed Study Designs to Investigate G-84A in CMPNs

Given that direct published data are lacking, the following approaches are recommended:

Case–control association studies

- **Cohorts:** Recruit patients diagnosed with CMPNs (PV, ET, PMF) and matched healthy controls (same ethnicity).
- **Genotyping:** Use PCR-RFLP, allele-specific PCR, or sequencing to genotype the NOS1 G-84A polymorphism.
- **Statistical analysis:** Test allele frequencies, genotype distributions, Hardy–Weinberg equilibrium, and associations (odds ratio, confidence intervals).
- **Subgroup analyses:** Stratify by CMPN subtype, driver mutation status (JAK2, CALR, MPL), history of thrombosis, progression/fibrosis stage.

Functional assays

1. **Promoter reporter assay**
   - Clone ~1 kb upstream promoter region of NOS1 bearing either the G allele or A allele upstream of a luciferase reporter.
   - Transfect into relevant cell lines (e.g. hematopoietic progenitors, endothelial or stromal cells) and measure baseline and induced activity under cytokine or oxidative stress stimuli.
2. **Allele-specific expression / mRNA quantification**
   - In heterozygous individuals, quantify relative expression of each allele in bone marrow mononuclear cells or peripheral blood leukocytes.
   - Correlate allelic imbalance with genotype and disease parameters.

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3. **NO production assays**

- In primary cells or cell lines transfected with NOS1 promoter constructs, measure NO levels (e.g. by Griess reaction or NO-sensitive probes).
- Assess under baseline vs stimulated conditions (e.g. with cytokines, ROS donors).

4. **Cellular phenotyping**

- Examine effects on reactive oxygen species (ROS) levels, DNA damage markers (γH2AX), cell proliferation/apoptosis, or sensitivity to oxidative stress in cells overexpressing NOS1 variants.

Integration into risk models and survival analyses

- **Multivariate modeling:** Include G-84A genotype as a covariate along with standard prognostic variables (age, blood counts, driver mutation VAF, additional mutations, fibrosis grade).
- **Time-to-event analyses:** Associate G-84A genotypes with outcomes such as thrombosis-free survival, transformation to AML, overall survival, or progression to myelofibrosis.

Population and power considerations

- As the effect of such a polymorphism is likely modest, sample sizes must be large (e.g. several hundred cases and controls) to detect associations.
- Ethnic stratification is critical, as allele frequencies may differ by population.
- Correction for multiple testing is necessary if multiple SNPs are assessed.

Challenges, Limitations, and Alternative Interpretations

1. **Lack of prior data**

- No published evidence supports the existence of a G-84A promoter SNP in NOS1 in CMPNs. The variant may be rare or nonexistent, or previous studies may not have surveyed NOS1 in CMPNs.

2. **Effect size likely modest**

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- Even if functional, the polymorphism's effect may be subtle and overshadowed by strong driver mutations or environmental factors.
- Negative results are possible due to underpowering or population heterogeneity.

3. **Redundancy and compensation**
   - Other NOS isoforms (NOS2, NOS3) or alternative pathways may buffer effects, limiting phenotypic impact.
4. **Tissue specificity**
   - NOS1 is often lowly expressed outside neurons; the relevance of its expression in hematopoietic cells must first be confirmed empirically.
5. **Confounding and epistasis**
   - Other genetic variants or epigenetic modifications may interact with G-84A, complicating association signals.
6. **Functional assays in vitro may not recapitulate in vivo complexity**
   - Regulatory context, chromatin environment, or cell-type specificity may not be faithfully modeled in cell lines.

Discussion and Future Perspectives

While direct empirical support is lacking, the plausibility of a functional NOS1 promoter polymorphism influencing CMPN biology is not negligible, given the role of NO in redox signaling, inflammation, and vascular homeostasis. Even a modest modulatory effect could become relevant in the milieu of chronic inflammation and clonal hematopoiesis.

If a G-84A polymorphism is proven to modulate NOS1 expression and associate with CMPN risk or phenotype, several implications arise:

- **Biomarker potential:** The variant could serve as a low-penetrance risk marker or modifier, complementing driver mutation models.
- **Stratification for therapy:** Patients with "high-risk" genotypes might benefit from antioxidant strategies, NO donors, or closer monitoring.

- **Pathophysiologic insight:** Demonstration of functional effect would strengthen the conceptual link between nitrosative stress and myeloproliferative biology.
- **Therapeutic targeting:** If altered NOS1 activity contributes to disease phenotype, small molecule modulators of NOS1 or downstream NO signaling might be explored.

However, a cautious approach is warranted: negative association or functional data is possible, and publication bias must be considered. As next-generation sequencing and genome-wide association study (GWAS) datasets expand, mining large cohorts (e.g. UK Biobank, hematologic consortia) for NOS1 variants and CMPN phenotypes might yield further insight.

Conclusion

In this review, we have explored the theoretical and mechanistic rationale for considering a G-84A promoter polymorphism in **NOS1** as a potential genetic modifier in chronic myeloproliferative neoplasms. Although no published data currently confirm such an association, the convergence of nitric oxide biology, oxidative/inflammatory stress, and CMPN pathophysiology suggests that investigation is worthwhile. Rigorous case–control studies, functional assays, and integrative modeling are needed to test this hypothesis. Ultimately, such work could deepen our understanding of disease heterogeneity in CMPNs and potentially uncover new biomarkers or targets.

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