ISSN 3060-4567

POTENTIAL ROLE OF THE BCL2 938 C>A PROMOTER POLYMORPHISM IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS: A HYPOTHESIS-DRIVEN REVIEW

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

Matkarimova Dilfuza Saburovna (Tashkent State Medical University)

Abstract: Background: *Chronic myeloproliferative neoplasms (CMPNs) are clonal hematopoietic stem cell disorders characterized by dysregulated proliferation, altered apoptosis, and inflammatory interactions. The* ***BCL2*** *gene encodes an anti-apoptotic protein that plays a key role in cell survival. A promoter single nucleotide polymorphism (SNP) at position –938 (C>A, known as rs2279115) in the BCL2 gene has been studied as a modulator of BCL2 expression, cancer risk, and prognosis in several malignancies. However, its significance in CMPNs is unknown.*

Objective: *This article reviews the biology of BCL2 and the known effects of the 938 C>A polymorphism, examines evidence in hematologic and other cancers, and proposes hypotheses and study designs for investigating the polymorphism's potential impact in CMPNs.*

Conclusions: *While direct evidence is lacking, the functional effect of the 938 C>A SNP on BCL2 promoter activity suggests it could modulate apoptosis stress responses in CMPN clones or the bone marrow niche. Well-designed genotypic and functional studies in CMPN patients are warranted to assess its role as a genetic modifier, prognostic marker, or therapeutic stratifier.*

Keywords: *chronic myeloproliferative neoplasm, BCL2, promoter polymorphism, –938 C>A, apoptosis regulation, genetic modifier*

Keywords

- *Chronic myeloproliferative neoplasms (CMPNs)*
- *BCL2 (B-cell lymphoma 2)*

ISSN 3060-4567

- *Promoter polymorphism –938 C>A (rs2279115)*
- *Apoptosis, cell survival*
- *Genetic susceptibility / modifier*
- *Hematologic neoplasms*

Introduction

Chronic myeloproliferative neoplasms (CMPNs) (also classically termed Philadelphia-negative myeloproliferative neoplasms, MPNs) include disorders such as polycythemia vera, essential thrombocythemia, and primary myelofibrosis. These conditions are defined by clonal expansion of hematopoietic progenitors, often driven by mutations in **JAK2**, **CALR**, or **MPL**, along with secondary or cooperating mutations in epigenetic regulators, splicing factors, and transcriptional regulators. Clinical heterogeneity in disease presentation, progression, thrombosis risk, fibrotic evolution, and leukemic transformation suggests the existence of genetic modifiers beyond the canonical driver mutations.

One possible class of genetic modifiers are polymorphisms in apoptosis and survival pathway genes. The **BCL2** gene encodes a prototypical anti-apoptotic protein of the BCL-2 family that regulates mitochondrial outer membrane permeabilization and thus the intrinsic apoptosis pathway. Variation in BCL2 regulation might influence how hematopoietic clones tolerate cellular stress, proliferate, or resist apoptosis.

Among BCL2 promoter variants, the **–938 C>A (rs2279115)** polymorphism has been studied across various cancers and hematologic diseases as a functional modulator of promoter activity and BCL2 expression. The C>A substitution occurs in the P2 (inhibitory) promoter region of BCL2, which can negatively regulate transcription from the main promoter. Several studies suggest that the –938 C allele and A allele differ in promoter binding and activity, with downstream effects on BCL2 expression, clinical features, and outcomes.

However, to date I am not aware of any published studies that examine the BCL2 –938 C>A polymorphism in CMPNs. This article aims to (1) review what is known about BCL2 and the –938 SNP, (2) explore how this SNP might influence

CMPN biology, and (3) propose experimental and clinical approaches to study its significance in CMPNs.

BCL2 Gene: Structure, Regulation, and Function

BCL2 gene structure and promoter context

The **BCL2** gene (located on chromosome 18q21.3) comprises multiple exons and is regulated by at least two promoter regions, commonly termed P1 (major) and P2 (inhibitory) promoters. The P2 promoter is upstream and acts to negatively regulate expression from the primary promoter (P1). The –938 C>A SNP lies within the P2 promoter region. In some reports, the –938C (wild-type) allele is associated with higher P2 promoter activity (i.e. more negative regulation) relative to the A allele. PubMed+4PubMed+4PubMed+4

Thus, the net effect of the SNP may be to modulate how strongly P2 suppresses P1, thereby influencing baseline BCL2 transcription levels under different cellular contexts.

BCL2 function in apoptosis and cell survival

BCL2 is one of the founding members of the BCL-2 family of proteins that regulate the intrinsic (mitochondrial) apoptosis pathway:

- Anti-apoptotic members (BCL2, BCL-XL, MCL1) act to inhibit pro-apoptotic effectors (e.g. BAX, BAK), preventing mitochondrial outer membrane permeabilization (MOMP) and cytochrome c release.
- Pro-apoptotic “BH3-only” proteins (BAD, BIM, PUMA, NOXA, etc.) sense cellular stress signals and can activate BAX/BAK or antagonize anti-apoptotic BCL2 family members.
- The balance of expression and interactions determines whether a cell undergoes apoptosis under stress (DNA damage, oxidative stress, cytokine deprivation) or survives.

In normal hematopoiesis, controlled apoptosis is essential to remove aberrant or excess progenitors. In neoplastic hematopoiesis, overexpression or dysregulation of BCL2 can confer survival advantage, increased resistance to apoptosis, and allow accumulation of aberrant clones.

BCL2 in hematologic neoplasms and cancer

- Overexpression of BCL2 is a common feature in certain lymphoid malignancies (e.g. follicular lymphoma, some forms of chronic lymphocytic leukemia) and is frequently targeted therapeutically (e.g. with venetoclax).
- In acute leukemias or myelodysplastic syndromes, BCL2 expression levels have been studied as biomarkers of resistance or prognosis.
- In non-hematologic cancers, BCL2 polymorphisms including –938 C>A have been associated with risk, tumor behavior, and patient outcomes in breast cancer, squamous cell carcinoma, prostate cancer, and others. PubMed+5BioMed Central+5PubMed+5

Given the central role of BCL2 in survival, variation in its regulatory control could theoretically influence the behavior of neoplastic clones in CMPNs, especially under stress or during clonal evolution.

The –938 C>A Polymorphism (rs2279115): Evidence, Function, and Implications

Epidemiologic associations: cancer risk and prognosis

A number of studies and meta-analyses have addressed the association between the BCL2 –938 C>A polymorphism and cancer risk or outcomes. Key findings include:

- A meta-analysis of 26 studies found that the **–938 C>A polymorphism** was modestly associated with **increased cancer risk** (dominant model OR ≈ 1.12, recessive model OR ≈ 1.38, allele model OR ≈ 1.15) overall; the association was stronger in Asian populations but not definitively observed in Caucasians. PubMed+1
- In oropharyngeal squamous cell carcinoma, the CC genotype was associated with poorer relapse-free and overall survival compared to other genotypes, and the –938 SNP was correlated with BCL2 expression. PubMed
- In transitional cell carcinoma of the bladder, –938 C>A was associated with relapse-free survival (allele dose effect) and was an independent predictor in multivariable analysis. PubMed

ISSN 3060-4567

- In chronic lymphocytic leukemia (CLL), an early study reported that the AA genotype was associated with shorter time to first treatment and overall survival, along with increased BCL2 expression. PubMed However, follow-up studies in independent CLL cohorts failed to replicate prognostic associations. PubMed
- In pediatric acute lymphoblastic leukemia (ALL), the –938 C>A genotype distribution was examined, but no significant correlation with clinical outcome, event-free survival or overall survival was found. BioMed Central
- In breast cancer, studies have found mixed results, with some reporting that the AA genotype is associated with risk or more aggressive tumor features, and others showing weaker or no associations. PubMed+2PMC+2

Overall, while many studies suggest that –938 C>A is functionally relevant, the magnitude and consistency of effect vary by cancer type, population, and study design.

Functional studies of the –938 C>A SNP

Functional investigations of the –938 C>A SNP, though limited, provide insight into how the polymorphism might influence BCL2 regulation:

- The –938 C allele is often reported to show higher promoter activity (in the context of the P2 regulatory region) and stronger binding of nuclear proteins, compared with the A allele. ui.adsabs.harvard.edu+4PubMed+4PubMed+4
- In the original CLL-related work, cells with the AA genotype exhibited higher BCL2 protein expression and correspondingly more apoptosis resistance. PubMed
- In oropharyngeal carcinoma, the –938 SNP correlated significantly with immunohistochemically assessed BCL2 protein levels. PubMed
- In breast cancer cell lines, the –938 polymorphism has been linked to differential BCL2 expression (though results and directionality can vary by cell line) in some studies. PMC+1

ISSN 3060-4567

- The precise molecular mechanism is hypothesized to involve differential binding of transcription factors (or repressor complexes) to the P2 region, influencing suppression strength over the main promoter.

Thus, it is plausible that in relevant cell types, the –938 C>A SNP exerts allele-dependent modulation of BCL2 transcription, which could influence how cells respond to apoptotic stimuli.

Limitations, inconsistencies, and caveats

- Some replication attempts have failed to confirm associations (especially in CLL). PubMed
- Many studies are retrospective and of modest sample size, increasing risk of bias, false positives, or population stratification.
- The functional context (cell type, chromatin environment, co-regulatory factors) likely modulates the real impact of the SNP, meaning that effects seen in one tissue may not generalize to others.
- Because BCL2 is part of a network of apoptosis regulators, compensatory mechanisms might mask or buffer the SNP's effect in vivo.
- The SNP effect likely is modest rather than strongly penetrant, meaning that a large sample size and careful controls are needed.

Hypothesized Significance of BCL2 –938 C>A in CMPNs

Although there is no published data specifically linking BCL2 –938 C>A to CMPNs, one can reason by analogy and biological plausibility to propose how the polymorphism could influence CMPN pathophysiology.

Potential mechanistic roles

1. **Modulation of clonal cell survival under stress**
   - CMPN clones (e.g. JAK2-mutated progenitors) endure oxidative stress, inflammatory cytokine signaling, and replicative stress. A genotype conferring slightly higher BCL2 expression may allow these clones to survive apoptotic pressures more robustly, aiding clonal persistence or expansion.

- Conversely, a genotype with less BCL2 expression might make clone viability more precarious, possibly curbing expansion or making cells more vulnerable to additional hits.

2. **Influence on subclone evolution and mutation acquisition**

- Clones that are more apoptosis-resistant might tolerate DNA damage or sublethal stress, increasing the likelihood of accumulating secondary mutations that drive disease progression or leukemic transformation.

3. **Interaction with inflammation and microenvironment signaling**

- CMPNs are associated with inflammatory cytokines (IL-6, TNF-α, etc.), reactive oxygen species (ROS), and bone marrow niche remodeling. These stresses may induce apoptotic signals; thus, BCL2 expression modulation could affect how hematopoietic progenitors respond to microenvironmental cues.

4. **Impact on treatment response or risk of transformation**

- If carriers of a certain genotype have clones that are more apoptosis-resistant, this might influence responsiveness to cytoreductive therapy, interferon-based regimens, or targeted agents.
- A genotype that supports survival may also predispose to fibrotic progression or leukemic transformation by enabling persistence of damaged or more aggressive subclones.

5. **Possible effect on normal hematopoietic competition**

- The BCL2 genotype may not only affect the malignant clone, but also normal progenitors. If the malignant clone has a survival advantage, it may outcompete normal hematopoiesis more effectively, influencing disease phenotype (e.g. higher counts, faster progression).

Hypothesis: genotype–phenotype associations in CMPNs

Based on the above, one might hypothesize:

- The **–938 C allele** (if associated with higher P2 suppressor activity and thus lower net BCL2 expression) might confer less apoptotic resistance, and thus **slower clonal expansion or lower risk** of aggressive phenotype.

ISSN 3060-4567

- The **–938 A allele** (if associated with weaker P2 suppression and thus higher net BCL2) might confer a modest survival advantage, possibly associated with a more proliferative or treatment-resistant CMPN phenotype.

Alternatively, depending on the actual direction of functional effect in hematopoietic progenitors (which may differ from other tissues), the allele–effect relationship could invert. Empirical testing is essential.

Clinically, one might expect that genotype may correlate with:

- Disease subtype (PV, ET, PMF)
- Hematologic parameters (leukocyte count, hematocrit, platelet count)
- Risk of thrombosis
- Rate of progression or time to fibrotic conversion
- Transformation to acute leukemia
- Survival or treatment response

Proposed Study Designs and Approaches

To test these hypotheses, the following research strategies are suggested:

Epidemiologic / association study in CMPN cohorts

1. **Study population**
   - A well-phenotyped cohort of CMPN patients (e.g. PV, ET, PMF), ideally with long-term follow-up and clinical annotation (thrombosis, transformation, treatments, survival).
   - Matched healthy controls from the same population for baseline allele frequency comparison.
2. **Genotyping the –938 C>A polymorphism**
   - Use PCR-RFLP, allele-specific PCR, TaqMan assays, or sequencing to determine genotypes (CC, CA, AA).
   - Confirm Hardy-Weinberg equilibrium in control cohort.
3. **Statistical association tests**
   - Compare genotype and allele frequencies between CMPN and controls (to test susceptibility).

ISSN 3060-4567

- Within CMPN patients, analyze associations of genotype with disease subtype, blood counts, molecular mutation burden (JAK2 V617F VAF, CALR, MPL, co-mutations).
- Time-to-event analyses (e.g. time to leukemic transformation, time to fibrotic progression, overall survival, thrombosis-free survival) using Kaplan-Meier and Cox regression, with genotype as a covariate (dominant, recessive, additive models).
- Multivariable adjustment for age, sex, known prognostic factors, co-mutations, and treatment variables.

4. **Subgroup analyses**

- Stratify by driver mutation (JAK2 vs CALR vs MPL).
- Stratify by allele burden (e.g. VAF) to see genotype effect at different mutation loads.
- Explore gene–environment interactions (e.g. with smoking, oxidative exposures).

5. **Power calculations and sample size**

- Given that the SNP effect is likely modest, large sample sizes (hundreds to thousands) may be necessary to detect statistically significant associations.
- Use preliminary effect size estimates (e.g. OR ~1.1–1.4) from meta-analyses in cancer as reference.

Functional / mechanistic studies

1. **Promoter reporter assays in hematopoietic cell lines**

- Clone BCL2 promoter fragments including P1 + P2 regions, containing either the –938 C or A allele, upstream of a luciferase reporter.
- Transfect into relevant hematopoietic progenitor cell lines (e.g. lineage-committed or stem-like models) and measure basal and induced promoter activity under baseline conditions and under stress (e.g. cytokines, oxidative stress, DNA damage).
- Compare activity of C vs A variant promoters.

ISSN 3060-4567

2. **Chromatin immunoprecipitation (ChIP) / transcription factor binding**

- Investigate whether nuclear proteins (transcription factors, repressors) differentially bind to the –938 region in C vs A allele context (e.g. via EMSA, ChIP assays).
- Examine histone modification marks or methylation state at the promoter in cells with different genotypes.

3. **Allele-specific expression (ASE) studies**

- In heterozygous patient samples, assess whether one allele is preferentially transcribed in bone marrow or peripheral blood mononuclear cells (e.g. by allele-specific quantitative RT-PCR or RNA-seq).
- Correlate allele expression bias with genotype and clinical features.

4. **In vitro survival / apoptosis assays**

- Establish cell lines or primary hematopoietic progenitors transduced to express BCL2 under the control of variant promoters or engineered genotype backgrounds, then challenge with apoptotic stressors (e.g. genotoxic agents, oxidative stress) and measure survival, caspase activation, mitochondrial membrane potential, etc.
- Compare responses across genotypes (C, CA, A).

5. **In vivo or ex vivo models**

- If possible, knockout/knock-in mouse models harboring the –938 variant (though this is technically challenging) could be used to investigate hematopoietic phenotypes, stress responses, and leukemogenesis in combination with other driver mutations.
- Alternatively, patient-derived xenograft or colony assays with BM cells of known genotype could be tested for differential expansion or resistance.

Integrative and translational analysis

- **Incorporation into prognostic models**: If genotype shows independent association with outcomes, it could be integrated into multivariable predictive models (together with clinical, molecular, cytogenetic factors).

- **Stratification for therapy**: If genotype correlates with treatment resistance or transformation risk, this could guide therapeutic intensification or monitoring.
- **Biomarker and decision support**: Ultimately, if validated, the –938 C>A polymorphism might serve as a genetic biomarker in CMPN risk stratification or progression prediction.

Challenges, Limitations, and Considerations

1. **Lack of prior data in CMPNs**
   - Because no published CMPN-specific investigations exist, the effect (if any) may be weak or confounded.
   - It will be critical to confirm that BCL2 is expressed and functionally relevant in CMPN clones or relevant progenitor subsets.
2. **Context specificity of SNP effect**
   - The functional consequence of the SNP may differ in hematopoietic progenitors vs epithelial or lymphoid cells, due to differences in transcription factor milieu, chromatin state, or epigenetic regulation.
   - The P2 promoter's suppressive function may vary by cell type or environmental condition.
3. **Redundancy in apoptosis regulation**
   - Many BCL2 family members and apoptosis regulators exist; the effect of BCL2 modulation alone may be buffered by compensatory pathways (e.g. MCL1, BCL-XL, BAX, BAK).
   - The net impact on cell survival may be modest.
4. **Population stratification and confounding**
   - Ethnic differences in allele frequency or linkage disequilibrium patterns could confound association results.
   - Large, well-matched controls and adjustment for population substructure (e.g. via principal component analysis) are essential.
5. **Multiple testing and false positives**

- Because many SNPs may be tested, correction for multiple comparisons is required.
- Replication in independent cohorts is critical.

6. **Effect size and required sample size**
   - The effect size is likely small; underpowered studies will yield inconclusive results or false negatives.
   - Meta-analyses or consortium-level collaboration may be necessary.
7. **Temporal dynamics and clonal heterogeneity**
   - CMPNs evolve over time with subclonal diversification; the genotype effect might vary in different disease phases.
   - Cross-sectional sampling may miss dynamic effects.

Proposed Manuscript Structure (Full 20-Page Version)

To expand this draft into a ~20-page manuscript, you might consider the following structure with expected content:

1. **Introduction** (2–3 pages)
   - Background on CMPNs, driver mutations, disease heterogeneity
   - Role of apoptosis and survival pathways in hematologic malignancies
   - Rationale for investigating BCL2 polymorphisms in CMPNs
2. **BCL2 Gene Biology** (2 pages)
   - Gene structure, promoter architecture (P1, P2)
   - Regulation of expression, transcriptional control
   - BCL2 protein function, interactions in apoptosis pathways
3. **The –938 C>A Polymorphism: Literature Review** (3 pages)
   - Genetic association studies in cancers and hematologic disorders
   - Meta-analysis results and caveats
   - Functional experimental evidence (reporter assays, binding studies)
   - Discordant findings and replication challenges
4. **CMPNs: Pathophysiology and Apoptosis Dynamics** (2 pages)
   - Clonal hematopoiesis, cell survival vs cell death balance
   - Role of inflammatory stress, oxidative stress, DNA damage

ISSN 3060-4567

- Evidence of apoptosis dysregulation in CMPNs (if any)

5. **Hypothesized Impact of 938 C>A in CMPNs** (2 pages)
   - Mechanistic models for genotype effect on clonal behavior
   - Possible clinical phenotype correlations
   - Examples of where variant might have relevance (thrombosis, progression)
6. **Proposed Experimental and Clinical Study Designs** (3 pages)
   - Case–control association studies, cohort design
   - Functional assays (promoter, ChIP, apoptosis)
   - Integration with molecular profiling, multivariable models
   - Power calculations, sample size estimation
7. **Potential Challenges and Mitigation Strategies** (1 page)
   - Context specificity, compensation, population effects
   - Strategies for robust design and replication
8. **Discussion and Future Directions** (2 pages)
   - What positive or negative results would mean
   - Translation to prognostic biomarker or therapeutic implications
   - Integration with multi-omics and large cohort studies
   - Consideration of related polymorphisms or additional BCL2 regulatory variants
9. **Conclusion** (½ page)
   - Summary of argument
   - Call for empirical CMPN-specific investigation
10. **References** (3–4 pages)
    - Include all primary research articles, review works, meta-analyses, functional studies

You may also include **Tables** and **Figures**, such as:

- Table of published association studies of –938 C>A in various cancers
- Table of hypothetical genotype–phenotype associations to test in CMPNs

- Schematic Figure: BCL2 promoter architecture and how –938 SNP may modulate regulation
- A flowchart of the experimental study plan

SAMPLE OF KEY REFERENCES (TO EXPAND)

1. Nückel H, Frey UH, Bau M, Sellmann L, Stanelle J, Dürig J, Jöckel KH, Dührsen U, Siffert W. Association of a novel regulatory polymorphism (–938C>A) in the BCL2 gene promoter with disease progression and survival in chronic lymphocytic leukemia. *Blood*. 2007;109(1):290-297. PubMed

2. Bachmann HS, Otterbach F, Callies R, Nückel H, Bau M, Schmid KW, Siffert W, Kimmig R. The AA genotype of the regulatory BCL2 promoter polymorphism (–938C>A) is associated with a favorable outcome in lymph node–negative invasive breast cancer patients. *Clin Cancer Res.* 2007 Oct 1;13(19):5790–5797. PubMed

3. Zhang N, Li X, Tao K, et al. BCL-2 (–938C>A) polymorphism is associated with breast cancer susceptibility. *BMC Med Genet.* 2011;12:48. PMC

4. Role of Bcl-2 (–938 C>A) polymorphism in susceptibility and prognosis of cancer: a meta-analysis. *PubMed*. 2014. PubMed

5. Hess J, Stelmach P, Eisenhardt A, et al. Impact of BCL2 polymorphisms on survival in transitional cell carcinoma of the bladder. *PubMed*. 2017. PubMed

6. Bachmann HS, Heukamp LC, Schmitz KJ, et al. Regulatory BCL2 promoter polymorphism (–938C>A) in prostate carcinoma. *Int J Cancer*. 2011. PubMed+1

7. Evaluation of Bax and BCL2 gene polymorphisms in Iraqi women with breast cancer (–938 C>A). *PubMed*. 2022. PubMed

8. Studies showing lack of significant prognostic effect in CLL replication cohorts. PubMed

9. Pediatric ALL cohort genotyping of –938 C>A: no significant correlation with outcomes.