Genetic Analysis of Triple-Negative Myeloproliferative Neoplasms Using Whole-Exome Sequencing

Muhammad Arif[1], Yasar Mehmood Yousafzai[2], Munira Borhany[3]
[1] Khyber Medical University, Peshawar
[2] Khyber Medical University, Peshawar
[3] Ziauddin Medical University Hospital, Karachi

Triple-negative Polycythemia Vera · Triple-negative Essential Thrombocythemia · Triple-negative Primary Myelofibrosis

Introduction

- Triple-negative Myeloproliferative neoplasms lack canonical JAK2, MPL and CALR mutations and are diagnostically challenging.
- Nevertheless, these disorders may harbor non-canonical driver mutations and additional alterations involving epigenetic regulators and tumor-suppressor pathways.
- Defining this hidden molecular landscape may improve biological classification and identify variants requiring functional validation.
- This study aimed to characterize germline and somatic genetic variants in triple-negative myeloproliferative neoplasms using whole-exome sequencing.

Methods

- This first study from Pakistan, conducted at Khyber Medical University, Peshawar, included 16 patients with JAK2-, MPL- and CALR-negative Polycythemia Vera, Essential Thrombocythemia or Primary Myelofibrosis.
- Whole-exome sequencing of high-quality DNA was performed at 100× depth on the Illumina platform.
- Matched blood and buccal controls were available for 7 patients; 9 patients lacked matched controls.
- Somatic variants were identified using Genome Analysis Toolkit Mutect2.
- Annotated variant call format files were reviewed sequentially for driver, myeloid-related and non-myeloid genes.
- Variants were assessed using Mutation Taster, Polymorphism Phenotyping version 2 and Sorting Intolerant From Tolerant, and checked against the Catalogue of Somatic Mutations in Cancer, the Database of Single Nucleotide Polymorphisms, ClinVar and published literature.

Conclusion

- These preliminary findings highlight substantial genetic heterogeneity in triple-negative myeloproliferative neoplasms, with recurrent mutations in epigenetic regulators, tumor-suppressor genes and non-canonical drivers.
- Non-classical JAK2, CALR and MPL variants, together with frequent ASXL1, ARID1B, TET2, TP53 and RNF145 alterations, warrant further molecular investigation and functional validation in larger matched cohorts.

STUDY DESIGN | 16 Patients | 100× Illumina Whole-Exome Sequencing | 7 matched blood–buccal pairs | 9 unmatched cases | First study of its kind in Pakistan

Results

Results Description

A total of 16 triple-negative myeloproliferative neoplasm patients were studied, including 11 males (68.8%) and 5 females (31.2%), with a mean age of 37.3 years. Diagnoses comprised Polycythemia Vera in 7 patients (43.8%), Essential Thrombocythemia in 6 (37.5%), and Primary Myelofibrosis in 3 (18.8%).

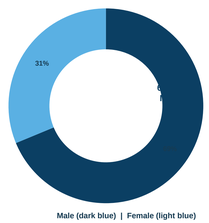
Primary driver gene variants were detected in 7 events. JAK2 and CALR variants were each identified in 3 of 16 patients (18.8%), while an MPL mutation was detected in 1 of 16 patients (6.3%). Of these, 4 of 7 variants (57.1%) were novel, 3 of 7 (42.9%) were previously reported, 5 of 7 (71.4%) were somatic, and 2 of 7 (28.6%) were germline.

Additional myeloid-related variants were detected in TP53, ASXL1, TET2, and ARID1B, with 20 variants overall. ARID1B was most frequently affected, with 8 of 20 variants (40.0%), followed by TP53, ASXL1, and TET2, each with 4 of 20 (20.0%). Most variants were somatic, 19 of 20 (95.0%), and 6 of 20 (30.0%) were novel.

Less frequent myeloid-related variants involved DNMT3A, IKZF1, NF1, TET3, and CREBBP, comprising 12 events overall: CREBBP, 4; TET3, 3; IKZF1, 2; NF1, 2; and DNMT3A, 1.

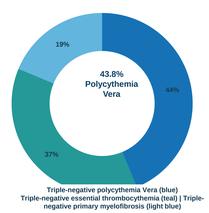
Non-myeloid variants were mainly seen in RNF145, accounting for 10 of 16 patient-events (62.5%), followed by ZNF236, SREBF2, and DIAPH1, each with 2 of 16 (12.5%). Overall, 45 distinct variants across 16 genes represented 55 patient-level variant events.

Figure 1A. Sex distribution



Male (dark blue) | Female (light blue)

Figure 1B. Disease distribution



Triple-negative polycythemia Vera (blue)
Triple-negative essential thrombocythemia (teal) | Triple-negative primary myelofibrosis (light blue)

Figure 2. Primary-driver gene events

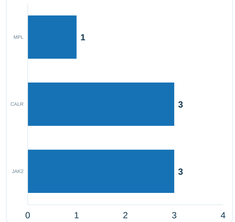
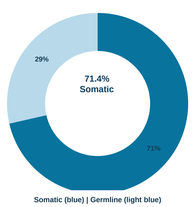


Figure 5B. Somatic vs germline drivers



Somatic (blue) | Germline (light blue)

Figure 3A. Frequent myeloid genes

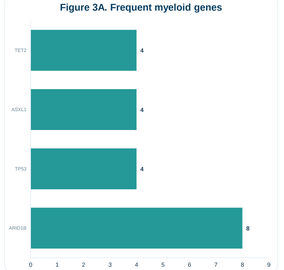


Figure 3B. Other myeloid genes

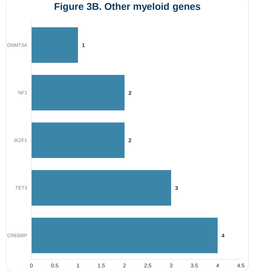


Figure 4. Non-myeloid genes

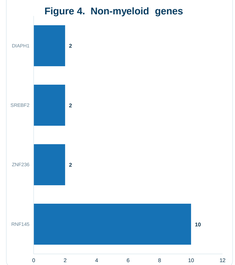
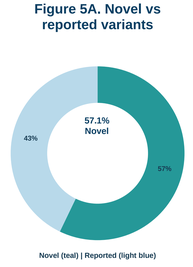


Figure 5A. Novel vs reported variants



Novel (teal) | Reported (light blue)

Complete Gene-Variant Profile: 45 Distinct Variants Across 16 Genes

Gene	Variants
JAK2	c.2429C>T → p.T810I; c.1077delA → p.R360Gfs*9; c.2698G>T → p.E900X
MPL	c.1639G>A → p.A547T
CALR	c.184A>G → p.K62E; c.242T>C → p.F81S; c.151C>A → p.L51I
TP53	c.662A>G → p.E221G; c.1A>G → p.M1?; c.931_932insTCCACCTGTGTCTCCCTACCTTTCCTTGCCTCTTTCCTAGCACTGCCCAACA → p.Asn311Ilefs*43; c.1087A>G → p.R363G
ASXL1	c.1927delG → p.G645Vfs*58; c.2257delC → p.T754Lfs*18; c.3022C>A → p.H1008N; c.2167_2168del → p.L724Efs*49
TET2	c.172delA → p.S59Vfs*8; c.5090G>T → p.G1697V; c.4615_4617del → p.Q1542del; c.5792A>T → p.E1931V
ARID1B	c.519_521del → p.H179del; c.848A>T → p.D283V; c.588_589insCAGCAGCAGCAG → p.Q214_H215insQQQQ; c.352_354del → p.S124del; c.516_517del → p.A173Pfs*141; c.518_519insCA → p.H174Tfs*21; c.4626delT → p.Y1543Ifs*21
DNMT3A	c.918G>A → p.W306X

Gene	Variants
IKZF1	c.1558T>C → p.X520Q; c.1491C>A → p.Y497X
NF1	c.8023del → p.P2675Hfs*43; c.2062G>T → p.E688X
TET3	c.4158C>A → p.C1386X; c.2905_2906insAGTCAATCGCTATTT → p.P969delinsQSIAIS; c.4298C>T → p.A1433V
CREBBP	c.6895C>T → p.Q2299X; c.1687A>G → p.N563D; c.319C>T → p.P107S; c.80G>A → p.S27N
RNF145	c.68delA → p.K23Rfs*17; c.80delA → p.N27Tfs*13; c.80dupA → p.N27Kfs*44; c.68dupA → p.K24Efs*47
ZNF236	c.1379dupA → p.M463Nfs*104
SREBF2	c.203_205del → p.S74del
DIAPH1	c.3700_3702del → p.E1234del

Acknowledgements

Funded by NIH–HRI Pakistan (NHCG-22/R2-69). We thank the participating centres, collaborators, laboratory staff and research assistant Umar.

Authors & affiliations

Muhammad Arif[1]; Yasar Mehmood Yousafzai[2]; Munira Borhany[3]
[1,2] Khyber Medical University, Peshawar
[3] Ziauddin Medical University Hospital, Karachi

References

1. Arber et al. Blood. 2022. 2. Khoury et al. Leukemia. 2022.
3. Grinfeld et al. N Engl J Med. 2018.