RESEARCH ARTICLE

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Impact of Genetic Polymorphisms in *NF-κB2* and *TRAF3* Genes on Response to Bortezomib-Based Therapy in Multiple Myeloma Patients

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Abstract

Background: Multiple myeloma (MM), being the second most common hematological malignancy, has garnered significant attention. The ubiquitin proteasomal pathway (UPP), crucial for normal cell function, plays a pivotal role in myeloma pathophysiology, especially with the advent of bortezomib (BTZ). Dysregulation of the UPP has implications ranging from developmental abnormalities to cancer. **Objectives:** This study aimed to delineate the clinical characteristics of newly diagnosed multiple myeloma patients and investigate the influence of single nucleotide polymorphisms (SNPs) in *NF-κB2* and *TRAF3* genes on the risk and treatment response to bortezomib-based chemotherapy. **Materials and Methods:** Conducted at JIPMER, Pondicherry, this prospective study enrolled 184 participants, comprising cases and controls. DNA extraction from peripheral blood samples was followed by SNP analysis through Real-time Polymerase Chain Reaction. Patients were categorized into Good and Poor responders, and SNP associations with treatment response, response rates, and survival outcomes were assessed using chi-square and Kaplan-Meier analyses. **Results:** The median age of participants was 55 years, with backache being the most prevalent symptom (66.3%). Hypercalcemia (22%), renal failure (8.7%), and bone fractures (45.7%) were also observed, alongside high prevalence of anemia. Notably, the frequency of the *TRAF3* rs12147254 A allele was lower in cases compared to controls (31% vs. 49%, P-value=0.002). Poor responders exhibited higher frequencies of the GA+AA genotypes in *TRAF3* rs12147254 (OR-3.882(1.629-9.251), P-value-0.002) and NFKB2 rs1056890 (OR-3.308(1.366-8.012), P-value-0.008) when compared to good responders. The GA+AA genotype in *TRAF3* rs11160707 SNP correlated with improved progression-free survival. **Conclusion:** The study findings underscore a significant association between genetic polymorphisms and treatment response outcomes, suggesting their utility in prognostic determinations and clinical outcomes prediction in multiple myeloma patients.

Keywords: Bortezomib- multiple myeloma- response rate- overall survival- progression-free survival

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Introduction

Multiple myeloma (MM) stands as the second most common hematological malignancy, accounting for around 13% of all blood cancers, and unfortunately, it is usually regarded as incurable for the majority of those affected [1]. This disease is notably intricate, exhibiting various cytogenetic subtypes and characterized by an abnormal proliferation of monoclonal immunoglobulins. The overproduction of clonal plasma cells leads to damage in various organs, making it a challenging condition to manage effectively. Diagnosis of MM commonly hinges on the presence of myeloma-defining events during clinical evaluation, as indicated by the acronym CRAB - hypercalcemia, renal failure, anemia, and bone lytic lesions [2, 3].

The typical age for MM diagnosis falls within the range of 66 to 70 years, occurring at a frequency of roughly 0.02% to 0.03% [4]. Among newly diagnosed multiple myeloma (NDMM) patients, the most prevalent initial symptoms include anemia (73%), bone pain (58%), elevated creatinine levels (48%), fatigue (32%), hypercalcemia (28%), and weight loss (24%) [5]. In addition to MDE identification, diagnosis often demands the presence of certain criteria such as >60% clonal plasma cells observed in a bone marrow examination, findings from the involved/uninvolved serum free light chain (SFLC) assay, and the detection of more than one focal lesion through magnetic resonance imaging (MRI).

Furthermore, factors like disease stage, cytogenetic

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abnormalities, and the depth of response to therapy play crucial roles in influencing the prognosis and survival rates of MM patients [6, 7].

The standard treatment approach for NDMM typically involves high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) for patients aged 70 years or younger. For those not suitable candidates for ASCT, the administration of induction regimens is prolonged [8]. In both patient groups, the incorporation of immunomodulatory drugs (IMiDs), proteasome inhibitors (PIs), dexamethasone, and monoclonal antibodies (mAbs) has significantly altered their clinical outcomes, with previous clinical trials showcasing enhanced response rates and improved survival [9-12]. Notably, the introduction of bortezomib (BTZ) has resulted in a marked 50% enhancement in the overall survival rate of NDMM patients, establishing BTZ as the primary treatment choice for individuals battling MM [13, 14].

The ubiquitin-proteasome pathway (UPP) serves a crucial role in regulating protein stability and function. Targeting the UPP has emerged as a promising strategy for developing novel drugs and targeted therapies in combating various human cancers. BTZ specifically inhibits the activity of the 26S proteasome, a key player in intracellular protein degradation [15]. MM is characterized by extensive genetic diversity both between individuals and within the tumor itself, encompassing cytogenetic and molecular abnormalities. Apart from chromosomal alterations, recurring gene mutations have been identified in MM cases [16]. Among these mutations, one of significant importance affecting the efficacy of proteasome inhibitors is related to the nuclear factor kappa-B cell (NF-κB). NF-κB, a complex expressed in various cell types, acts as a pivotal regulator of genes implicated in immune function and inflammation. Alterations in the NF-κB pathway have been noted in approximately 17% of MM tumors and 40% of MM cell lines, underscoring its relevance in MM pathogenesis [17].

Another crucial player in modulating the NF-κB pathway is the Tumour receptor-associated factor (TRAF) gene, known for its role in dampening NF-κB function. TRAF genes are instrumental in overseeing immune responses and inflammatory processes [18].

It is theorized that the outcomes for distinct patient populations could potentially be enhanced through chemotherapy treatment. This study aimed to assess the impact of single nucleotide polymorphism (SNP) variants in MM patients undergoing Bortezomib-based chemotherapy.

Materials and Methods

This case–control genetic study was conducted at a tertiary care hospital in India. The study received ethical clearance from the Institute's ethics committee, and all participants provided written informed consent. Adhering strictly to the principles outlined in the Declaration of Helsinki and good clinical practice guidelines, the study enrolled participants based on specific eligibility criteria. A total of 92 patients diagnosed with NDMM and treated with a Bortezomib-based regimen between July 2017 and March 2021 were included, with follow-up extending until July 2022. Additionally, 92 healthy controls were recruited. Individuals with a history of comorbidities or cancer were excluded from the study.

The patient cohort was divided into two groups based on their response to treatment:

- Group I: Patients across all age groups diagnosed with MM exhibiting a positive response to BTZ treatment.
- Group II: Patients across all age groups diagnosed with MM showing a poor response to BTZ treatment.

Upon admission to the Department of Medicine and Medical Oncology, all NDMM patients underwent a comprehensive clinical assessment. The initial evaluation involved a detailed patient history, physical examination, and baseline blood studies. The biologic assessment encompassed a complete blood count (CBC), platelet counts, serum creatinine, serum electrolytes, serum calcium, serum albumin, lactate dehydrogenase (LDH), and beta2 microglobulin measurements. Subsequently, each patient underwent regular follow-ups spanning 4-6 cycles of Induction chemotherapy for response monitoring.

A 5-milliliter venous blood sample was obtained and transferred to a polypropylene centrifuge tube (Tarson-15 ml) containing 100µl of 10% Na2EDTA (Disodium Ethylenediamine-tetraacetic acid). The sample was then centrifuged at 1000 rpm (4ºC) for 10 minutes. Following centrifugation, the plasma supernatant was removed, and the pellet containing red blood cells (RBCs) and white blood cells (WBCs) was retained and stored for further analysis. Genomic DNA extraction from the WBCs was performed manually using the phenol-chloroform method. The isolated DNA was preserved at -20°C until the genotyping process was initiated. The quality and quantity of the extracted DNA were assessed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, DE 19810, USA). Subsequently, the DNA samples were appropriately diluted to a concentration of 50 ng/µl and incubated at 37ºC for 12 hours prior to further analysis.

Given the primary focus on investigating polymorphisms that might influence the progression of MM, the NF-κB gene pathways were of particular interest, considering their crucial role in MM pathogenesis. Genes linked to these molecular mechanisms were carefully chosen, with a specific emphasis on those with a minor allele frequency (MAF) exceeding 0.05, sourced from the 1000 Genomes database. The 1000 Genomes project was utilized for comparative purposes, enabling an exploration of population-level allele frequencies for the selected SNPs.

Genotyping Methodology

The *NF-κB2* and *TRAF3* gene variants were genotyped using Real-time Polymerase Chain Reaction (RT-PCR) in duplicate runs. Validated TaqMan SNP genotyping assays (*NF-κB2* G>A rs1056890, rs12769316 and *TRAF3* G>A rs11160707, rs12147254) kits obtained from Applied Biosystems were employed for this purpose. Sequence Detection Software (SDS) 7300, version 1.4, was utilized for absolute quantification and allelic discrimination analyses. Detailed information regarding the selected

SNPs can be found in Table 1. Standard RT-PCR protocols were followed for genotyping procedures.

Data Analysis and Response Assessment

Response evaluation adhered to the guidelines set by the International Myeloma Working Group (IMWG). Subsequent responses were categorized into good responders (sCR, CR, VGPR) and poor responders (SD, PD) based on defined criteria. Genotyping data and allele frequencies within the cases and controls were subjected to analysis through direct counting, with assessments for Hardy–Weinberg equilibrium conducted using the Chi-square test. Odds ratios and 95% confidence intervals were calculated using the same statistical method for further insights into the genetic associations observed.

Results

The study encompassed a total of 184 participants, with the healthy group comprising 62 males and 30 females, exhibiting an average age of 49.98 (±8.9) years. Among the 92 clinically NDMM patients undergoing Bortezomib-based therapy, 66 were male and 26 were female, with ages ranging from 33 to 75 years and a mean age of 55.24 ± 9.39 years. Notably, 49% of patients had comorbidities of hypertension and diabetes, while 10% were classified as overweight or obese. The median duration of symptoms was 3 months (ranging from 1 to 24). (Table 2)

The most prevalent symptoms reported were backache (66.3%), myalgia (53.3%), and bony swelling (12%) among the patients. Plasmacytoma was present in 21.7% of patients, while 19.6% exhibited paraparesis. IgGκ was identified as the most prevalent myeloma subtype, found in 42.4% of patients, with light chain myeloma observed in 11%. Beta 2 microglobulin and serum albumin mean values were recorded as 5.68±4.75 and 3.33±0.74, respectively. Furthermore, serum lactate dehydrogenase (LDH) levels were assessed in 50 patients.

NF-κB2 and *TRAF3* Gene Polymorphisms: Genotyping frequencies of *NF-κB2* (rs1056890, rs12769316) and *TRAF3* (rs11160707, rs12147254) gene polymorphisms were found to be in Hardy-Weinberg equilibrium. *NF-κB2* G>A rs12769316 demonstrated a G allele frequency of 64%, with an A allele frequency of 36%. The heterozygous genotype GA was detected in 41.3% of individuals, while homozygous genotypes GG and AA were observed in 43.5% and 15.2%, respectively. Allele frequencies were compared to the 1000 Genomes population and exhibited significant disparities from those documented among the population in Africa (AFR), as detailed in Table 3.

The allele frequencies of *NF-κB2* G>A rs1056890 were noted to exhibit a distribution of G allele at 58.7% and A allele at 41.3%, aligning closely with other subpopulations. Genotype frequencies for this variant were identified as GG (41.3%), GA (34.7%), and AA (24%). Correspondingly, for *TRAF3* G>A rs12147254, the genotype distributions were determined to be GG (51%), GA (36%), and AA (13%), with allele frequencies of G at 69% and A at 31%. Notably, these allele frequencies were statistically divergent from the South Asian subpopulation BEB.

The *TRAF3* gene variant rs12147254 unveiled a notable association with the treatment response in myeloma (Table 4). For the *TRAF3* G>A rs11160707 variant, the wild GG genotype was reported at 44.6%, while AA and GA genotypes accounted for 21.7% and 33.7%, respectively. The allele frequencies for this variant were 61.4% for G and 38.6% for A, aligning with findings from other populations.

Leveraging a genetic model analysis encompassing Co-dominant, dominant, and recessive models, the influence of *NF-κB2* and *TRAF3* genetic variants on the response to bortezomib-based therapy was rigorously evaluated. *NF-κB2* (rs1056890) Gene: Noteworthy associations were identified between the *NF-κB2* gene polymorphism (rs1056890) and the response to bortezomib-based treatment. Specifically, the heterozygous mutant genotype was linked with a higher prevalence of poor responders (73.8% versus 46%, with a p-value of 0.008). *TRAF3* (rs12147254) Gene: A similar trend was observed with the *TRAF3* gene variant (rs12147254), where the variant allele frequency surpassed that of the wild-type allele (66.7% versus 34%, with a p-value of 0.002), as detailed in Table 5. Linkage Disequilibrium Analysis: No significant findings were observed through the analysis of linkage disequilibrium in this context.

Utilizing Kaplan-Meier survival curves, we evaluated the overall survival (OS) and progression-free survival (PFS) across *NF-κB2* and *TRAF3* gene polymorphisms. The median OS was calculated to be 35 months with a 95% confidence interval of 52.18-60.27. Notably, the *TRAF3* rs11160707 SNP, specifically the GA/AA genotype, demonstrated a notable impact on PFS while not influencing OS significantly. In contrast, the GG+GA genotype of *NF-κB2* gene rs1056890 exhibited minimal effects on PFS. Conversely, the *TRAF3* rs12147254 GG+GA genotype emerged as a significant factor affecting OS (Figure 1).

Discussion

MM stands out as a genetically intricate and diverse neoplasm, contributing to tumor onset and advancement.

Table 1. Characteristic Features, rs IDs, and assay IDs of the Studied Polymorphisms

S. No.	Gene Name	rs Id	Gene Location	SNP Location	Assay Id
1	*NFKB2*	rs12769316	Chr 10	Upstream variant/ Downstream variant	C___1841559_10
	G>A	rs1056890			C___1118892_1_
2	*TRAF3*	rs11160707	Chr 14	Intron variant/ 3 Prime UTR variant	C__31068006_20
	G>A	12147254			C___3236637_10

Table 2. Baseline Demographic Characteristics and Laboratory Values among MM Patients

Table 2.1. Baseline Demographic characteristics among patients with Multiple Myeloma

S.No.	Variable (n=sample size)	Frequency(%)	
1	Co-Morbidity (n= 92)	Hypertension	12 (28)
		Diabetes Mellitus	9 (21)
		Heart Failure	3 (7)
		TB	3 (7)
		Renal Failure	1 (2.3)
		Chronic Obstructive Pulmonary Disease (COPD)	1 (2.3)
		More than one	14 (32.4)
2	Median duration of symptoms in months (n= 92)		3 (1-24)
3	Type of Symptoms (n= 92)	Backache	61 (66.3)
		Myalgia	49 (53.3)
		Fever	18 (19.6)
		Cough & Expectoration	17 (18.5)
		Difficulty in walking	11 (12)
		Bony swelling	11 (12)
		Headache	2 (2.2)
		Diplopia	1 (1.1)
4	Performance Status (n=92) ECOG	0	1 (1.3)
		1	42 (54.5)
		2	26 (33.8)
		3	6 (7.8)
		4	2 (2.6)

Table 2.2. Baseline Laboratory Values in Patients (Baseline Demographic characteristics and Laboratory values among MM patients)

S.No.	Variable	Value (M±S.D) or Median (range)
1	Hb g/dl (n= 92)	9.23±2.13
2	Total count WBC (103/µl) (n=92)	7.39±2.90
3	Differential count (%) (n=92)	
	Neutrophils	62.0±14.33
	Lymphocyte	29.54±12.82
	Monocyte	5.48±3.46
4	Total Platelet count (103/µl) (n=92)	217.87±93.48
5	NLR (n=92)	2 (0.5-13) / 2.87±2.18
5	S.Albumin g/dl (n=89)	3.33±0.74
6	S.Creatinine mg/dl (n=92)	1.31±1.22/ 1.09(0.15-8.96)
7	S.Calcium mg/dl (n= 89)	9.45±1.24
8	S.Lactate dehydrogenase mg/dl (n=50)	343.86±220.33
9	S.Alkaline Phosphatase g/dl (n= 88)	135.5 (45-579)
10	M-value g/dl (n= 70)	4.06±2.43/ 3.82 (0.10-10.60)
11	Serum Free Light Chain Assay (n= 92)	185.24±222.37/ 67.20 (0.58-779.0)
	Mean Kappa value (mg/l)	
	Mean Lambda value (mg/l)	88.48±167.32/ 12.00 (1.12-760.0)
	Mean kappa/lambda ratio	52.17±101.89/ 5.75 (0.01-495.4)
12	Beta 2 microglobulin(ng/ml) (n=92)	5.68±4.75/ 3.88 (1.04-20.70)

Table 3. Allele and Genotype Frequencies of Genes *NF-κB2* and *TRAF3* Compared to 1,000 Genome Population

SNP	SI	AFR	AMR	EAS	EUR	SAS				
						BEB	GIH	ITU	PJL	STU
N	92	661	347	504	503	86	103	102	96	102
NF-κB2 rs12769316										
G>A										
G	73.3	97.6	86.7	82.1	82	92.4	97.6	94.1	94.3	95.6
A	26.7	2.4*	13.3	17.9	18	7.6	2.4	5.9	5.7	4.4
NF-κB2 rs1056890										
G>A										
G	52	87.9	64.8	80.2	64.5	52.3	44.7	51.5	49	52
A	48	12.1	35.8	19.8	35.5	47.7	55.3	48.5	51	48
TRAF3 rs12147254										
G>A										
G	51	93.6	82.4	57.7	70.6	48.8	47.1	53.9	55.2	47.1
A	49	6.4	17.6	42.3	29.4	51.2*	52.9	46.1	44.8	52.9
TRAF3 rs11160707										
G>A										
G	76.6	99.9	83.7	86.7	99.4	97.1	93.7	95.6	97.9	96.6
A	23.4	0.1	16.3	13.3	0.6	2.9	6.3	4.4	2.1	3.4

N, number of subjects; SI, South Indian, AFR, African; AMR, American; EAS, East Asians; EUR, Europeans; SAS, South Asians, BEB, Bengali in Bangladesh GIH, Gujarat Indians in Houston, TX. ITU; Indian Telugu in UK PJL, Punjabi in Lahore; STU, Srilankan Tamils in the UK

Table 4. Association of the Genotypes in Cases and Controls

S.No.	Gene	rs id	Controls (n=92)		Cases (n=92)		p-value	OR (95%CI)
			GG	GA/AA	GG	GA/AA		
1	*NFKB2*	s12769316	52 (56.5)	40 (43.5)	40 (43.5)	52 (56.5)	0.59	1.253 (0.546-2.873)
		rs1056890	25 (27.2)	67 (72.8)	38 (41.3)	54 (58.7)	0.27	0.580 (0.220-1.529)
2	*TRAF3*	rs12147254	29 (31.5)	63 (68.5)	47 (51)	45 (49)	0.002	3.882 (1.629-9.251)
		rs11160707	52 (56.5)	40 (43.5)	41 (44.6)	51 (55.4)	0.35	0.677 (0.295-1.555)

Despite considerable advancements, there remains a gap in understanding the underlying biological mechanisms dictating the disease's progression. The pathogenesis of myeloma is intricately linked with genetic alterations impacting diverse cellular pathways. One such prominent pathway often disrupted in human cancers is the NF-κB signaling pathway, known for its role in evading apoptosis and promoting cell survival [19].

The development of MM is intricately associated with genomic instability leading to DNA damage. The hyperactivation of DNA repair mechanisms in MM cells grants them a survival advantage and confers resistance to therapeutic interventions, with the accumulation of new mutations over time further complicating treatment efficacy [20].

NF-κB2 and *TRAF3* genes play pivotal roles in governing the UPP. Defects at the molecular level can drive the unwarranted activation of both canonical and non-canonical NF-κB signaling pathways. Genetic variations within genes modulating the NF-κB pathway could elucidate the challenges encountered in achieving therapeutic efficacy with BTZ treatment, potentially shedding light on the nuances of treatment resistance mechanisms.

In this study, we explored the correlation between *NF-κB2* gene variants (rs12769316, rs1056890) and *TRAF3* gene variants (rs12147254, rs11160707) with the response to bortezomib-based therapy in MM patients. By scrutinizing genotypes and allele frequencies in MM cohorts, we sought to establish links between *NF-κB2*, *TRAF3* genes, and treatment outcomes, benchmarking them against the 1000 Genomes population database. Notably, there exists a scarcity of studies investigating the association between NF-κB pathway genes and clinical responses. Recent multicenter randomized trials have shed light on the safety and efficacy of bortezomib-based regimens for MM, yet comparative data across PAD, VCD, and VTD protocols in relapsed/refractory patients remains limited. This paucity of data has spurred further investigations into NF-κB family gene polymorphisms and their impact on patient outcomes under bortezomib-based treatments.

While BTZ primarily exerts its anti-myeloma effect through the inhibition of the transcription factor NF-κB, its broader implications on MM cell biology are manifold [21]. The *TRAF3* gene polymorphism rs11160707 emerges

Table 5. Genotyping Frequency data of *NF-κB2* and *TRAF3* Gene Polymorphism between Good Responders and Poor Responders.

NFKB2 rs12769316	GR (N=50)	PR (N=42)	Odds Ratio	
G > A			(95% CI)	P-value
GG	23 (46)	17 (40.5)	1	
GA	22 (44)	16 (38.1)		
AA	5 (10)	9 (21.4)		
Codominant model	22	16	0.984 (0.401-2.417)	0.971
GG vs GA				
GG vs AA	5	9	2.435 (0.691-8.587)	0.166
Dominant model	27	25	1.253 (0.546-2.873)	0.595
GG vs (GA+AA)				
Recessive model	45	33	0.500 (0.162-1.544)	0.228
(GG + GA) vs AA				
NFKB2 rs1056890				
GG	27 (54)	11 (26.2)	1	
GA	13 (26)	19 (45.2)		
AA	10 (20)	12 (28.6)		
GG vs GA	13	19	3.587 (1.327-9.699)	0.012
GG vs AA	10	12	2.945 (0.987-8.791)	0.053
GG vs GA+AA	23	31	3.308 (1.366-8.012)	0.008
GG + GA vs AA	40	30	0.625 (0.238-1.638)	0.339
TRAF3 rs12147254				
GG	33 (66)	14 (33.3)	1	
GA	12 (24)	21 (50)		
AA	5 (10)	7 (16.7)		
GG vs GA	12	21	4.125(1.603-10.617)	0.003
GG vs AA	5	7	3.300 (0.893-12.192)	0.07
GG vs GA+AA	17	28	3.882 (1.629-9.251)	0.002
GG + GA vs AA	45	35	0.556 (0.162-1.900)	0.34
TRAF3 rs11160707				
GG	24 (48)	17 (40.5)	1	
GA	15 (30)	16 (38.1)		
AA	11 (22)	9 (21.4)		
GG vs GA	15	16	1.506 (0.589-3.852)	0.393
GG vs AA	11	9	1.155 (0.393-3.395)	0.793
GG vs GA+AA	26	25	1.357 (0.592-3.110)	0.47
GG + GA vs AA	36	33	1.034 (0.382-2.799)	0.947

*1, used for reference; GR, good responder; PR, poor responder; OR, Odds ratio,; CI, confidence interval

as an independent and favorable prognostic factor for progression-free survival, suggesting that reduced *TRAF3* levels significantly correlate with prolonged progression-free survival in response to BTZ treatment, potentially indicating the activation of the non-canonical NF-κB pathway. This genetic association underscores the potential influence of rs11160707 on progression-free survival, likely mediated by *TRAF3* inactivation [22].

Gene polymorphisms within NF-κB2 and *TRAF3* possess the potential to exert a substantial impact on treatment outcomes in MM patients managed with bortezomib-based regimens. NP-κB family gene signatures have emerged as promising indicators for predicting myeloma risk and evaluating treatment responses to bortezomib-based therapies in preclinical settings. Such genetic insights hold immense promise for tailoring personalized treatment approaches and fostering a deeper understanding of therapeutic responses in MM management.

Our recent investigation did not reveal a significant association between the *NF-κB2* gene variant rs12769316 and treatment response outcomes or survival rates across patient genotypes. In contrast, Du et al. reported a higher frequency of the GA/AA genotype for *NF-κB2* rs12769316 compared to the GG genotype among patients (89.3% versus 69.1%, p-value 0.042). Notably, Du et al.'s findings

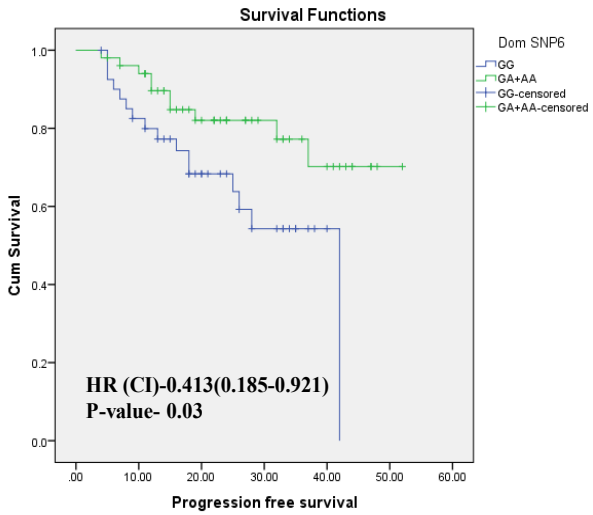

Fig. 1.1: TRAF3 rs11160707 PFS

GG vs GA+AA

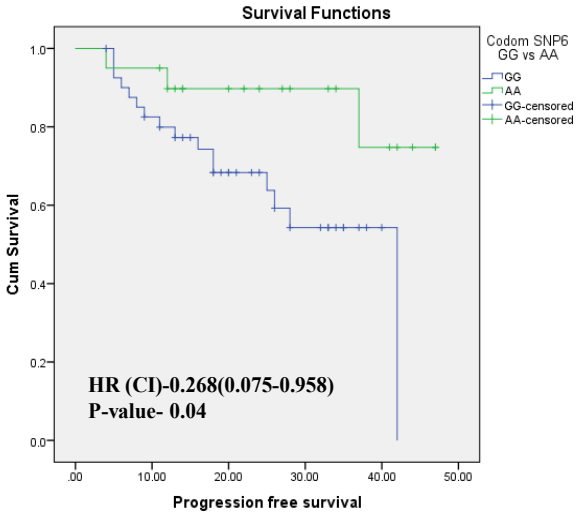

Fig. 1.2: TRAF3 rs11160707 PFS

GG vs AA

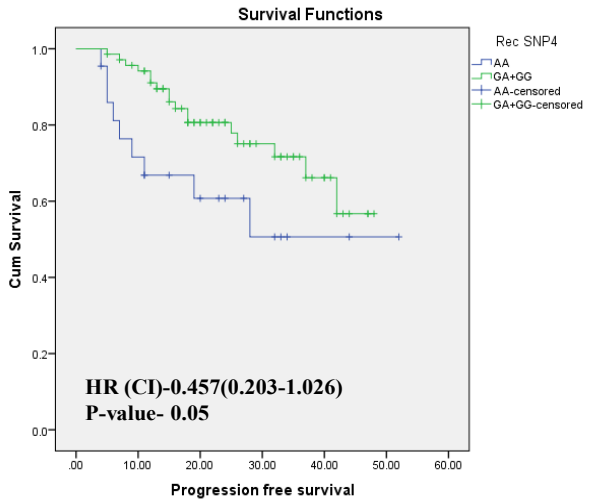

Fig. 1.3: NFKB2 rs1056890 PFS

AA vs GG+GA

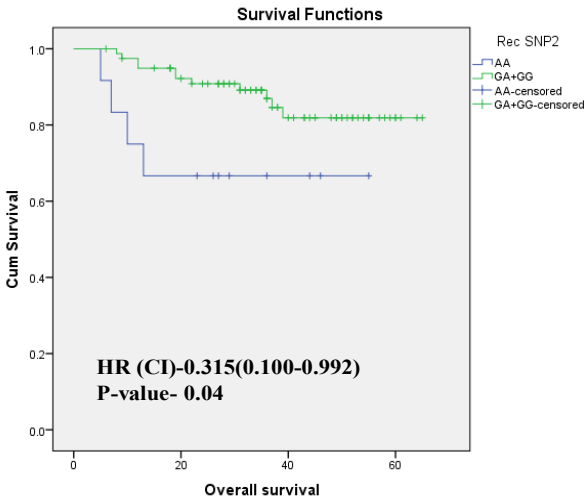

Fig. 1.4: TRAF3 rs12147254 OS

AA vs GG+GA

Figure 1. Kaplan-Meier Curves Showing Progression-Free Survival (PFS) and Overall Survival (OS) in *NFKB2* and *TRAF3* Genetic Variants by Using a Genetic Model of Genotyping Analysis. Figure 1.1, Association of rs11160707 genotyping (GG vs GA+AA) with PFS; Figure 1.2, Association of rs11160707 genotyping (GG vs AA) with PFS; Figure 1.3, Association of rs1056890 genotyping (AA vs GG+GA) with PFS. Figure 1.4 Association of rs12147254 genotyping ((AA vs GG+GA) with OS.

suggested that the variant alleles *TRAF3* rs11160707 and *NF-κB2* rs12769316 were linked to improved progression-free survival (PFS) and overall survival (OS), whereas the presence of rs1056890 was associated with poorer survival outcomes [23]. Conversely, an independent study noted that carriers of the GA & AA genotypes for *NF-κB2* gene variant rs12769316 exhibited reduced susceptibility to monoclonal gammopathies, particularly MM among male individuals (p-value=0.043), with no discernible impact on survival outcomes [24]. In contrast, the *TRAF3* rs12147254 polymorphism was reported to lack any association with MM risk [25].

Genetic aberrations observed in active MM play a pivotal role in the disease progression trajectory, underscoring the importance of targeting these early events for potential therapeutic benefits [26]. Bortezomib, a cornerstone in MM chemotherapy, has showcased notable response rates in both newly diagnosed and relapsed/refractory settings [27-29]. The antitumor activity of BTZ is attributed to the downregulation of numerous antiapoptotic genes and proteins, highlighting its efficacy in combating myeloma [30]. However, further comprehensive studies with expanded sample sizes are essential to validate and consolidate these findings. Moreover, given the limited literature available on the studied SNPs and their influence on MM treatment responses, additional research in this area is warranted to enhance our understanding of their clinical implications.

In conclusion, the examination of genotype and allelic frequencies of SNPs within *NF-κB2* and *TRAF3* genes yielded significant disparities compared to other subpopulations. Notably, the UPP emerged as a key player in regulating NF-κB2 signaling and appears to be intricately linked to the risk profile of MM. The outcomes of our study contribute valuable insights to the existing literature, pparticularly in the context of Indian data and may offer a promising genomic perspective for personalized therapy and prognostication.

Author Contribution Statement

All authors contributed equally in this study.

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Ethical approval

The study was approved by the Institutional Ethics Committee (JIP/IEC/2018/152).

Conflict of interest

None declared

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