REVIEW PAPER

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Molecular characterization of multiple myeloma

B. Zeren Kiremitci*
Department of Genetics and Bioengineering, İzmir University of Economics, İzmir, Turkey

Elif Serap Gürler*
Department of Genetics and Bioinformatics, Kadir Has University, İstanbul, Turkey

Yağmur Kiraz
Department of Genetics and Bioengineering, İzmir University of Economics, İzmir, Turkey
https://orcid.org/0000-0003-3508-5617

Corresponding author: yagmur.kiraz@ieu.edu.tr

* equal contribution

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ABSTRACT

Multiple myeloma (MM) is a hematologic malignancy which occurs when plasma cells, a type of white blood cell, grow out of control and start to overproduce antibodies accumulating in the blood and bone marrow. Despite the recent advances, the survival rate for MM has not increased significantly which opens the need for identifying new molecular targets. This review article presents the most frequently observed gene mutations (KRAS (22.0%), NRAS (18.0%), DIS3 (9.3%), TTN (8.3%), ZNF717 (8.3%), TENT5C (7.3%), TP53 (7.3%) %), BRAF (6.3%), MUC16 (6.3%), RYR2 (5.4%), and LRP1B (5.4%)) in MM patients, with their rates, correlations, clinical significance, importance in the framework of MM, as well as potential novel targets collected from the literature. The genes and MM patients' dataset (211) were obtained from cBioportal. Summing up, in the study conducted in MM patients, 3 genes with the most frequent mutations were reported as KRAS, NRAS and DIS3. In addition, in the context of our literature reviews and the data obtained, it appears that the TZNF717, TTN, MUC16, RYR2 genes need further investigations within the framework of MM.

Introduction

Multiple myeloma (MM), or Kahler's Disease, is a disorder characterized by a malignant and uncontrolled division of antibody-secreting plasma cells (PCs) in the bone marrow. Even though the disease has a typical histologic diagnosis, it also presents a high level of genomic complexity, as well as significant differences in clinical features and patient survival [1]. Among all plasma cell neoplasms, MM is the second most common hematologic malignant disease with approximately 10% rate [2]. Due to its short survival time and high levels of fatal outcomes, it is frequently mislabeled as a rare disease. In fact, the proliferation of clonal PCs in the bone marrow is a defining feature of the disease and is marked by tumor cell secretion of monoclonal immunoglobulins (Igs) detectable in serum and/or urine. Accumulation of these abnormal PCs results in bone lesions and the destruction of bone tissue, bone marrow failure, and/or anemia [3].

Pathophysiology of MM comprises evolution in multiple stages that are monoclonal gammopathy of undetermined significance (MGUS), smoldering (asymptomatic) MM, symptomatic (intramedullary) MM, and extramedullary MM/plasma cell leukemia (PCL) [4]. The disease presents as the first step of monoclonal gammopathy of undetermined significance (MGUS). After the first step, the disease advanced to the subsequent ones, smoldering (asymptomatic) MM and symptomatic (intramedullary) MM. As the final stage of the disease, extramedullary or plasma cell leukemia is observed. MGUS evolves via branching evolution, as shown by many mutations and genomic aberrations at both the clonal and subclonal levels, leading to MM's genomic heterogeneity. The disease can be classified as hyperdiploid (having greater than the diploid number of chromosomes) or non-hyperdiploid (with fewer than the diploid number of chromosomes). In fact, hyperdiploid MM has usually been associated with a better prognosis [5].

Although there is still some ambiguity concerning the genomic nature of the disease, it has been established that the accumulation of abnormal PCs, chromosomal aberrations, hypermutated immunoglobulin genes, or dysregulation on MYC expression are significant causes in MM pathogenesis.

It was estimated that there would be about 34,920 new cases (1.8% of all cancer types) and about 12,410 deaths to occur due to MM in 2021. Additionally, the disease risk is higher for men than women [6]. According to the data obtained from different sources, over 50% of MM patients are reported to be over 60 ages, and nearly 3% of the patients are aged 40 and younger [7]. However, due to the developments in the understanding with regard to the genetic background of hematological neoplasm of PCs, the median survival time for patients has increased by three to four times in the last four decades [8].

The objective of this article is to analyze the genetic characterization of MM by using known genomic data and current knowledge in the literature. In view of such information, we aim to propose novel targets which could play a role in the disease diagnosis or/and prognosis, as well as in the development of new treatment methods.

Gene mutations in multiple myeloma

Samples from 211 patients using the cBioPortal for Cancer Genomics database [10] has allowed for the identification of gene mutations for MM. Parallel sequencing of paired tumor/normal sample of 203 MM patients has been performed [9–11]. According to the data obtained, significantly mutated genes have been listed. KRAS, NRAS, DIS3, ZNF717, TTN, TENT5C, TP53, BRAF, MUC16, RYR2, and LRP1B genes emerged as the eleven genes with the most frequent mutations. Results based on whole exome or whole genome sequencing of 203 MM matched tumor/normal sample pairs were retrieved from the cBioportal [10, 11]. As it is presented in the **Table 1**, some mutations of the genes are overlapping and seen in more than one sample. Even though the number of samples was greater for some mutations, the most common mutations are the following: KRAS (22.0%), NRAS (18.0%), DIS3 (9.3%), TTN (8.3%), ZNF717 (8.3%), TENT5C (7.3%), TP53 (7.3%), BRAF (6.3%), MUC16 (6.3%), RYR2 (5.4%), LRP1B (5.4%) (Figure 1A) which are mostly defined as missense mutations (putative driver and unknown significance). Gene altered in 67.32% of 205 cases, which equals 138 cases (**Figure 1A**).

Table 1. The analysis testing gene pairs across on OncoPrint. Gene mutations in pairs and separately in the study of MM patients

A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency
KRAS	DIS3	148	38	12	7	1.184	0.092	0.64	Co-occurrence
NRAS	ZNF717	156	32	12	5	1.022	0.17	0.64	Co-occurrence
KRAS	TENT5C	150	40	10	5	0.907	0.211	0.64	Co-occurrence
KRAS	RYR2	153	41	7	4	1.092	0.202	0.64	Co-occurrence
NRAS	TTN	155	33	13	4	0.531	0.368	0.64	Co-occurrence
NRAS	DIS3	153	33	15	4	0.306	0.46	0.64	Co-occurrence
KRAS	ZNF717	147	41	13	4	0.142	0.537	0.656	Co-occurrence

A

Mutated Genes (205 profiled samples)			
Gene	# Mut	#	Freq
KRAS	47	45	22.0%
NRAS	37	37	18.0%
DIS3	19	19	9.3%
ZNF717	18	17	8.3%
TTN	17	16	7.8%
TENT5C	15	15	7.3%
TP53	16	15	7.3%
BRAF	13	13	6.3%
MUC16	15	13	6.3%
RYR2	12	11	5.4%
LRP1B	13	11	5.4%

B

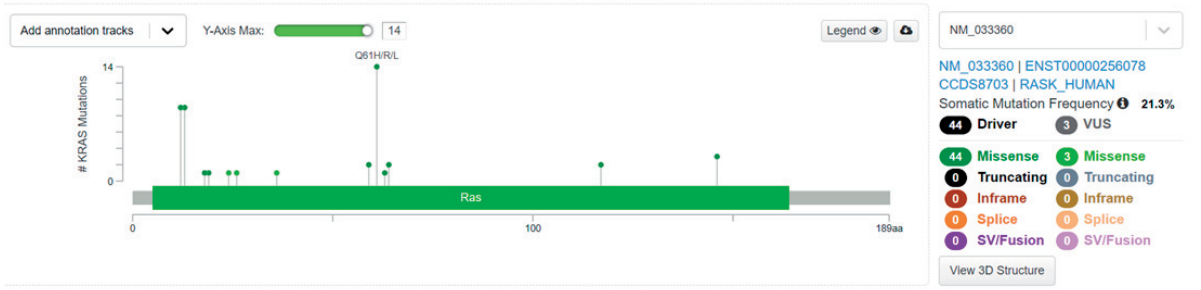
Figure 1. The most frequently mutated genes for 205 profiled samples from cBioPortal (*cBioPortal for Cancer Genomics*, n.d.-a). Figure 1A shows the mutated gene percentages. Figure 1B shows the genetic alterations of mutated genes for samples

KRAS mutations (mostly putative driver missense mutations) are seen in 22% of the samples whereas NRAS mutations (mostly putative driver missense mutations) are seen in 18% of the samples.

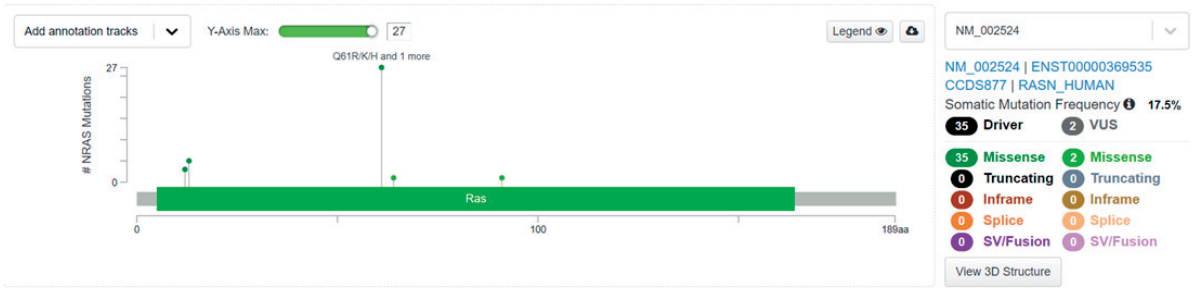
Although DIS3 mutations are found in 9%, ZNF717 mutation in 8%, and TTN mutation in 8% of the patients, they mostly contain unknown significance missense mutation (**Figure 1B**). Additionally, the locations of gene mutations are presented in **Figure 1B**. As seen in **Figure 1B**, there have been mutational overlaps. These are shown in **Table 1**, from the highest to the lowest numbers of co-occurrence mutations, according to the genes they overlap.

Figure 2 presents mutation types and locations of the KRAS, NRAS, DIS3 and ZNF717 genes with the highest mutation rates in MM. According to these data, KRAS mutations are missense mutations (47) and most of them are driver (44) mutations (**Figure 2A**). Similarly, the type which generates NRAS mutations also is a missense mutation (37) and most of them are defined as drivers (35) (**Figure 2B**). DIS3 mutation information consists of missense mutations and 7 of them are driver mutations (**Figure 2C**). Therefore, KRAS, NRAS and DIS3 genes can be considered as potential targets to inhibit the growth of cancer-causing cells. After emphasizing the importance of the mutations percentage in the abovementioned genes in MM patients, further studies should be conducted to address them. ZNF717 gene has been the most mutated gene, after KRAS, NRAS and DIS3 genes. In terms of the ZNF717 mutation, driver mutations are not defined, and there are missense mutations that have been indicated (17) (**Figure 2D**).

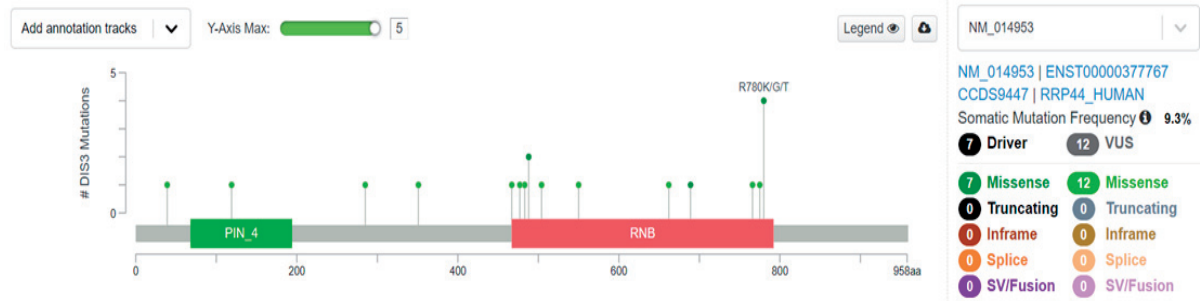
A. KRAS



B. NRAS



C. DIS3



D. ZNF717

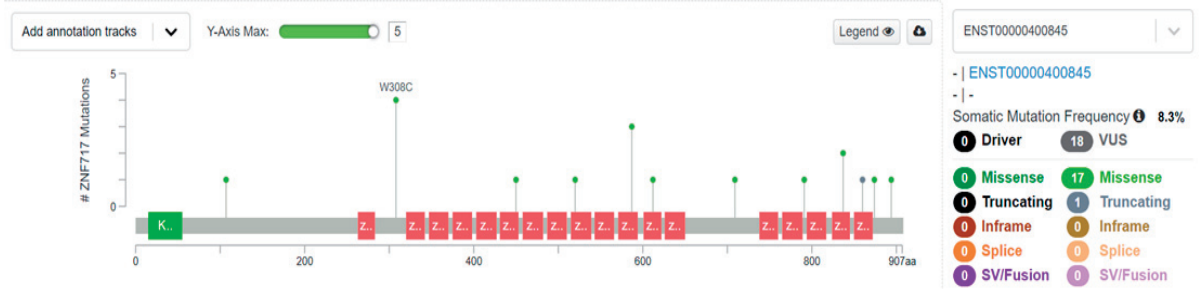


Figure 2. Representation of the most common mutations found on the most frequently mutated genes in MM dataset. A — KRAS, B – NRAS, C – DIS3, D — ZNF717 genes (*cBioPortal for Cancer Genomics*, n.d.-a)

Correlation Analysis between the Mutated Genes

As shown in **Figure 3** which has been obtained from the STRING database [12], KRAS, BRAF, and NRAS are the most related genes to each other from the curated databases. Even though the disease is seen as not fully curable, new advances in the treatment have shown that at least a small percentage of the patients may achieve the so-called operational cure [13]. Furthermore, Pasca et al. (2019) hypothesize that mutations in the KRAS/NRAS/BRAF genes are linked to a larger number of mutations per patient, and these genes are on the MAPK pathway checkpoint and/or MAPK inhibitors could be used as therapeutic

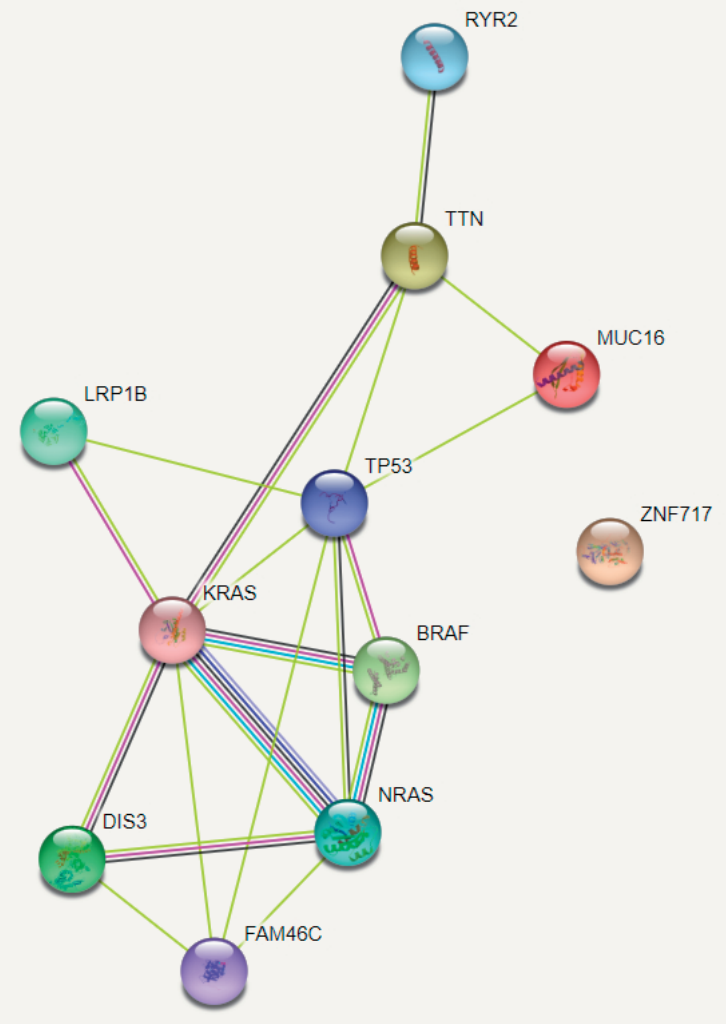


Figure 3. Physical network connection of the selected 11 genes from the STRING database. The purple and light blue edges show experimentally determined interactions. As a predicted interaction, black edges show the co-expression of genes (*11 Items (human) – the STRING Interaction Network*, n.d.)

agents for MM patients [14]. In addition, Kortüm et al. (2015) worked on fluorescence in-situ hybridization (FISH) and gene expression profiling to define the landscape of MM [15]. According to the mutation prevalence of the newly diagnosed patients; BRAF, NRAS, KRAS, TP53, DIS3, LRP1B are the genes that have multiple mutations and FAM46C has no mutation. Although, according to the cBioportal database, ZNF717 has an 8.3% mutation frequency [11], and there is text mining evidence for every 10 genes shown using yellow lines, ZNF717 has no physical network interaction with other mutated genes. Additionally, there is no protein homology evidence for the interested genes, although when the cluster tool of the database is used, BRAF, NRAS and KRAS genes are always clustered in the same group. As a physical network, an interaction is observed between only BRAF, NRAS, DIS3 genes which are referred to as 'driver' genes of MM from the curated and experimentally determined databases [16]. In addition, BRAF, NRAS, KRAS, and TP53 may be proto-oncogenes for a number of cancer types, such as thyroid, colorectal, or prostate cancer and have a role in the metabolic and signaling pathways [12].

CBio Gene Query Analysis

Data on which two genes were found together or separately in the study conducted with MM patients were obtained from cBioportal [11] (**Table 1**). This table contains information regarding gene

pairs in patients where mutations were found as co-occurring, separately or not observed at all. It is possible to access all possible gene pairs of interest from the cBioportal site [11]. In total, 7 patients with KRAS and DIS3 gene mutations were found, and these are the most common co-occurring gene mutations. Only KRAS mutation has been observed in 38 patients (without DIS3 mutation) and DIS3 mutation only in 12 individuals (without KRAS mutation). The number of individuals with the co-occurrence of the NRAS and ZNF717 genes has been recorded as 5. Moreover, 32 patients with only NRAS mutations (without ZNF717 mutations) and 12 patients with only ZNF717 mutations (without NRAS mutation) have been found. Likewise, mutations of KRAS and TENT5C have been observed in 5 patients. Those with only KRAS mutation and no TENT5C mutation have been recorded in 40 cases, and those with TENT5C mutation and no KRAS mutation have been recorded in 10 cases (**Table 1**).

Prognostic relevance of gene expressions

(See **Table 2**).

Significance of the identified gene signatures for MM

MM mutations have been identified by sequencing studies. KRAS, NRAS, TP53, FAM46C, DIS3, and BRAF mutations have a high risk of recurrence, as well as may play essential roles in the pathogenesis, progression, and prognosis of MM. Targeted sequencing analysis found the most common mutated genes to be the following: KRAS (36%), NRAS (20%), TP53 (16%), DIS3 (16%), FAM46C (12%). The induction of high-quality remission, including full response, is frequently the first treatment for MM. Treatment success necessitates the targeting of a diverse set of targets, including small subclones. In order to assess efficacy, it is essential to monitor gene changes in the tumor cell population under the pressure of developing treatments. Although mutation diversity constitutes an inherent feature of myeloma, it has been found that different genes in the same pathway cause multiple mutations (KRAS, NRAS, BRAF) in the same patient. Moreover, FAM46C and DIS3 have been found to be possible driver genes in MM. The finding of such driver gene mutations in MM has generated a considerable amount of expectations regarding the area of personalized treatment [20].

Table 2. Summary of gene and clinical significance. The summary of information regarding gene families, functions and clinical importance of KRAS, NRAS, DIS3, ZNF717, TTN, TENT5C, TP53, BRAF, MUC16, RYR2, and LRP1B genes, the most frequently mutations observed in MM patients

KRAS	The KRAS gene, a Kirsten Ras oncogene homolog from the mammalian Ras gene family, is responsible for producing a small GTPase superfamily protein. An activating mutation is caused by a single amino acid alteration. Lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, and colorectal cancer have all been linked to the transforming protein that results. Due to alternative splicing, two isoforms with different C-terminal regions are produced. Downstream signaling mechanisms of B Cell Receptor (BCR) and VEGF Signaling Pathway are two linked pathways. GTP binding is one of the Gene Ontology (GO) annotations for this gene, whereas HRAS is an essential paralog of this gene.
NRAS	This is an N-Ras oncogene that codes for a membrane protein which transports information between the Golgi apparatus and the plasma membrane. The ZDHHC9-GOLGA7 complex regulates this shuttling through palmitoylation and depalmitoylation. A guanine nucleotide-exchange factor activates the encoded protein, which possesses intrinsic GTPase activity, while a GTPase activating protein inactivates it. Diseases, such as somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, Noonan syndrome, and juvenile myelomonocytic leukemia, have been associated with NRAS mutation. Downstream signaling mechanisms of B Cell Receptor (BCR) and VEGF Signaling Pathway are the two linked pathways. GTP binding is one of the Gene Ontology (GO) annotations for this gene. HRAS is an essential paralog of this gene.
DIS3	The protein-coding gene DIS3 (DIS3 Homolog, Exosome Endoribonuclease, And 3'-5' Exoribonuclease) is found in exosomes. Plasma Cell Neoplasm and Perlman Syndrome are two diseases associated with DIS3. Unfolded Protein Response (UPR) and Gene Expression are the two pathways that are connected to it. RNA binding and endonuclease activity are two Gene Ontology (GO) annotations for this gene. DIS3L is a significant paralog of this gene.
ZNF717	Kruppel-associated box (KRAB) zinc-finger protein, which relates to a wide set of transcriptional regulators in mammals, is encoded by ZNF717. These proteins bind nucleic acids and are involved in a variety of physiological activities, such as cell proliferation, differentiation, and apoptosis, as well as viral replication and transcription regulation. On chromosome 1, a pseudogene of this gene was discovered. Gene Expression is one of its associated pathways. Nucleic acid-binding and DNA-binding transcription factor activity are two GO annotations connected to this gene. ZNF268 is a significant paralog of this gene.

Table 2 continued.

TTN	This gene produces a substantial amount of striated muscle protein. The product of this gene is split into two parts: an N-terminal I-band and a C-terminal A-band. On either side of a PEVK region rich in proline, glutamate, valine, and lysine, the I-band, which is the molecule's elastic component, comprises two areas of tandem immunoglobulin domains. The A-band comprises a combination of immunoglobulin and fibronectin repeats, as well as kinase activity, and is hypothesized to operate as a protein-ruler. A single titin molecule spans half the length of a sarcomere thanks to an N-terminal Z-disc area and a C-terminal M-line region which bind to the Z-line and M-line of the sarcomere, respectively. Titin also functions as an adhesion template for the formation of contractile machinery in muscle cells, since it comprises binding sites for muscle-associated proteins. It has been discovered as a chromosomal structural protein. In the I-band, M-line, and Z-disc sections of titin, there is a lot of variation. Variability in the I-band area contributes to variances in titin isoform elasticity and, as a result, to differences in elasticity of various muscle types. Autoantibodies to titin are generated in patients with the autoimmune diseases, such as scleroderma, and mutations in this gene are associated with the familial hypertrophic cardiomyopathy 9. Striated Muscle Contraction and Response to Elevated Platelet Cytosolic Ca2+ are two linked mechanisms. Nucleic acid binding and the same protein binding are two Gene Ontology (GO) annotations for this gene.
TENT5C	TENT5C is a Protein Coding gene (Terminal Nucleotidyltransferase 5C). Smoldering Myeloma and Monoclonal Gammopathy Of Uncertain Significance are two diseases linked to TENT5C. TENT5A is a significant paralog of this gene. Nucleotidyltransferase is a non-canonical poly(A) RNA polymerase that improves the stability and expression of mRNA. It primarily targets mRNAs that encode endoplasmic reticulum-targeted proteins and may be involved in the induction of cell death.
TP53	The transcriptional activation, DNA binding, and oligomerization domains of TP53 encode a tumor suppressor protein. The encoded protein reacts to a variety of cellular stressors by regulating target gene expression, resulting in cell cycle arrest, apoptosis, senescence, DNA repair, or metabolic alterations. TP53 mutations have been linked to several human malignancies, including hereditary tumors, such as Li-Fraumeni syndrome and Osteogenic Sarcoma. Multiple transcript variants and isoforms occur from alternative splicing of this gene and the utilization of various promoters. The usage of the various translation start codons from identical transcript variants has also been proven to result in additional isoforms. PI3K/AKT activation and Cell Cycle Mitotic are two linked pathways. DNA-binding transcription factor activity and protein heterodimerization activity are two Gene Ontology (GO) annotations for this gene. TP73 is a significant paralog of this gene.
BRAF	The BRAF protein is a member of the RAF family of serine/threonine protein kinases. This protein influences cell division, differentiation, and secretion via modulating the MAP kinase/ERK signaling pathway. Mutations in this gene, most notably the V600E mutant, are the most often detected cancer-causing mutations in melanoma, although they have also been found in non-Hodgkin lymphoma, colorectal cancer, thyroid carcinoma, non-small cell lung carcinoma, hairy cell leukemia, and lung adenocarcinoma. BRAF mutations are also linked to cardiofaciocutaneous, Noonan, and Costello syndromes, all of which have similar characteristics. Development Slit-Robo Signaling and CNTF Signaling are two pathways that are associated with it. Calcium ion binding and transferase activity, which transfers phosphorus-containing groups, are two Gene Ontology (GO) annotations for this gene. RAF1 is a significant paralog of this gene.
MUC16	The protein encoded by this gene belongs to the mucin family. Mucins are large molecular weight, O-glycosylated proteins located on the apical surfaces of epithelial and serve a vital role in establishing a protective mucous barrier. This protein is hypothesized to contribute to the formation of a barrier which protects epithelial cells from pathogens. This products of this gene have been used as markers for several malignancies, with higher expression levels linked to poorer outcomes. Clear Cell Adenocarcinoma and Ovarian Cyst are two diseases linked to MUC16. Defective C1GALT1C1 causes Tn polyagglutination syndrome (TNPS) and Diseases of Glycosylation are two of its linked pathways.
RYR2	This gene encodes a ryanodine receptor present in the sarcoplasmic reticulum of the heart muscle. The encoded protein is part of the calcium channel delivering calcium to the heart muscle and is made up of a tetramer of ryanodine receptor proteins and a tetramer of FK506 binding protein 1B proteins. Stress-induced polymorphic ventricular tachycardia and arrhythmogenic right ventricular dysplasia are linked to mutations in this gene. Activation of cAMP-Dependent PKA and CREB Pathway are two similar pathways. Calcium ion binding and protein kinase binding are two Gene Ontology (GO) annotations for this gene. RYR3 is a significant paralog of this gene.
LRP1B	A member of the low-density lipoprotein (LDL) receptor family is encoded by LRP1B. Due to their interactions with a range of ligands, these receptors perform a variety of functions in a proper cell function and development. This gene is disrupted in a variety of cancers. Lung cancer and Meier-Gorlin Syndrome 2 are two diseases associated with LRP1B. Calcium ion binding and low-density lipoprotein particle receptor activity are two Gene Ontology (GO) annotations for this gene. LRP1 is an essential paralog of this gene.
Ref	[17–19] Sources: https://www.genecards.org/ https://civicdb.org/home https://www.ncbi.nlm.nih.gov/

Copy-number changes and translocation studies performed on the newly diagnosed myeloma samples are examined in data sets with a long-term follow-up, and the effects of mutations are discussed. Although the most frequently mutated genes in the study were NRAS, KRAS, and BRAF, they constituted 44% of the cases. BRAF and DIS3 mutations showed an effect accompanying classical risk factors. 44% of patients with hypoactive/kinase-dead BRAF mutations presented concomitant changes in KRAS, NRAS, or activating BRAF mutations. They may have a role in the oncogenesis of MM by means of facilitating MAPK activation, which in turn may contribute to chemotherapy resistance. Thus, the findings show how important mutation screening is for better understanding newly diagnosed MM and may lead to patient-specific mutation-driven therapy methods [21].

Clonal evolution drives tumor progression, chemotherapy resistance, and relapse in cancer are known mechanisms; however, clonal selection induced by therapeutic pressure in MM has not been investigated to such a great extent. To investigate this problem, researchers used large-scale targeted sequencing of bone marrow PCs in MM patients at the diagnosis and recurrence following the same intense therapy. The most common mutations found at the diagnosis were KRAS (35%), NRAS (28%), DIS3 (16%), BRAF (12%) and LRP1B (12%). The mutational burden remained unaltered at recurrence. Chemotherapy resistance and recurrence may be caused by the newly acquired mutations in myeloma drivers, as well as (sub)clonal mutations which were present prior to the therapy [22].

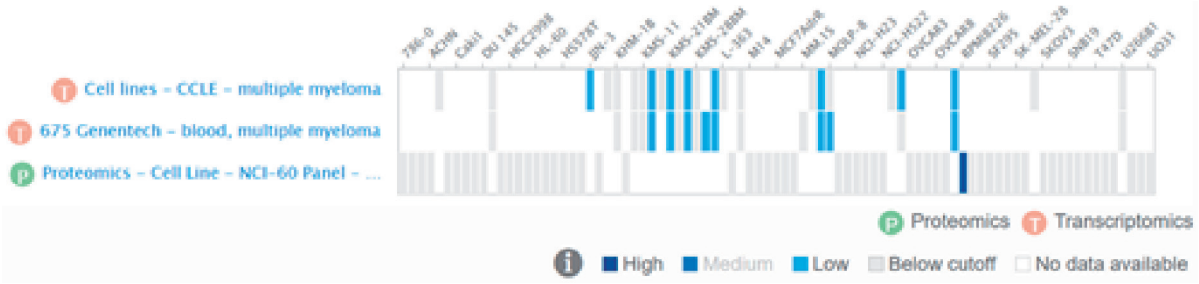
A Genome-wide association (GWAS) study found the 2q22 (rs61070260) variation affecting MM risk, and subsequently the association between rs61070260 in LRP1B and MM was evaluated. The results demonstrated that variation in LRP1B was highly correlated with MM susceptibility. In addition, a linkage disequilibrium (LD) study of this variation indicated an LD block containing exons 26-27-28 of the LRP1B gene, and the following sequencing analysis found three SNPs in exons 26 and 28 of LRP1B (rs762074421, rs756168629, rs113600691). A missense mutation leading in a transition from arginine to histidine at position 1661 of the LRP1B protein was not detected for the SNP rs756168629 in exon 26, and this mutation was estimated to be deleterious by SIFT and PolyPhen. These findings solidify the notion that LRP1B is a disease-associated gene involved in the development and progression of MM [23].

Although FAM46C is one of the most recurrently mutated genes in MM, its role in the pathogenesis of the disease has not yet been determined. According to one of the studies, wild-type (WT) FAM46C overexpression has induced cytotoxicity in MM cells. FAM46C mutations observed in MM patients eliminated this cytotoxicity, giving the mutant phenotype a survival benefit. In fact, FAM46C mutation is implicated in myeloma cell growth and survival, and this gene mutation is identified as a contributor to myeloma pathogenesis and disease development by means of the disruption of plasma cell differentiation [24].

Although the FAM46C gene is frequently mutated in MM, it has been found to encode a non-canonical poly(a) polymerase nc(PAP). Nevertheless, its target mRNAs and its role in the disease pathogenesis are much less known. A recent study using CRISPR-Cas9 technology and gene expression analysis has found that inactivation of FAM46C in MM down-regulated several mRNAs encoding Igs and ER-resident proteins. Surprisingly, poly(A) tail length determination tests have indicated that FAM46C expression is induced throughout plasma cell (PC) differentiation and that Ig mRNA encoding chains are substrates of ncPAP. In contrast, FAM46C loss increases the potential to migrate via upregulating the metastasis-associated lncRNA MALAT. The research has found that Ig mRNAs are targets of FAM46C, although they also revealed that this protein has a significant function in increasing antibody production during PC maturation, suggesting that its activity as a tumor suppressor is linked to the inhibition of myeloma cell migration [25].

Another factor associated with poor outcomes in MM is alterations of the tumor suppressor TP53. Once the oncogenic stress or DNA damage activates the p53 gene, it causes cell cycle arrest, or apoptosis depending on the biological environment, and its inactivation is linked to drug resistance in MM. The frequency of TP53 mutations increases with the development of the disease, from 5% at the diagnosis to 75% in late relapses [26].

A.



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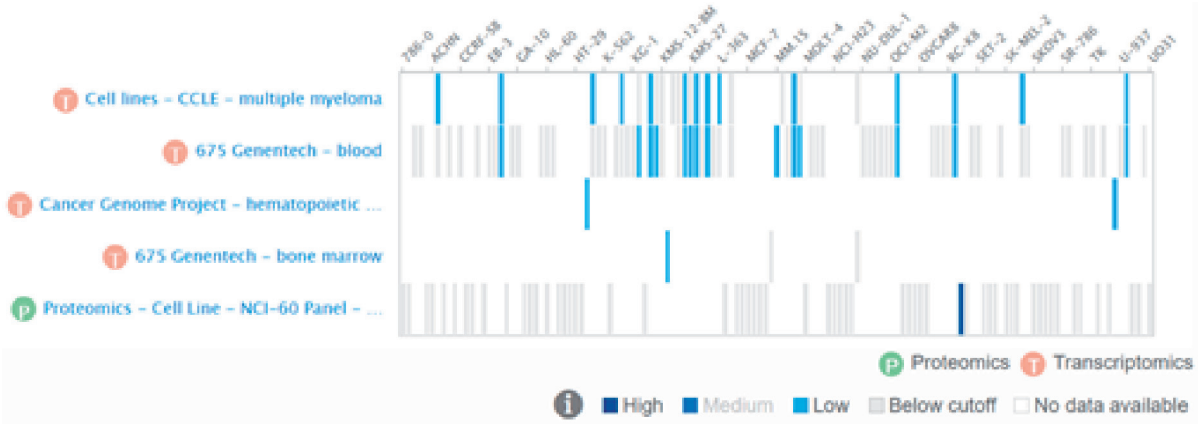

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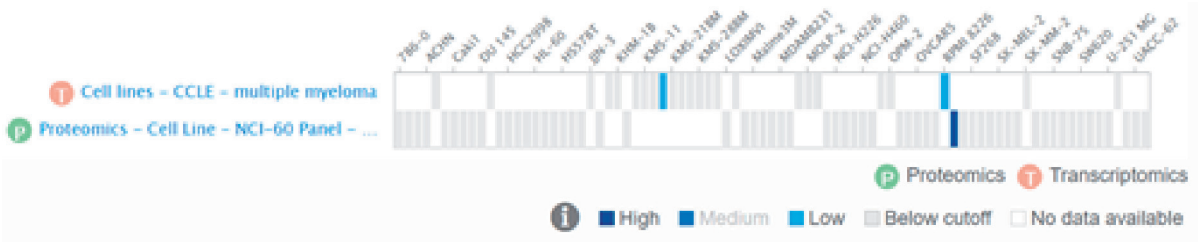

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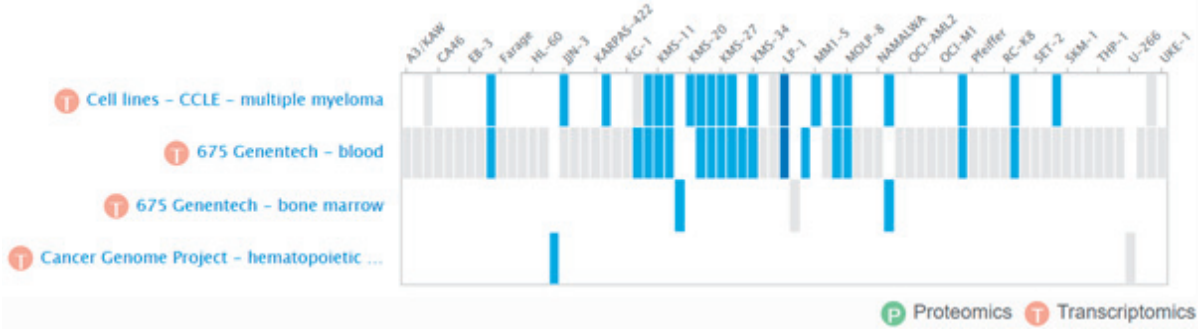

Figure 4. Figure 4. The expression pattern of RYR2, TTN, MUC16 and ZNF 717 genes in MM. A – RYR2, B – TTN, C – MUC16, D – ZNF717. 3 different experiments of RYR2 gene for MM. Two of them are transcriptomics experiments with CCLE – MM and 675 Genentech blood, MM, and the third is Proteomics – Cell Line – NCI 60 Panel – not applicable (Figure 4A). 5 different experiments have been performed showing the expression of TTN gene in MM (CCLE – MM, 675 Genentech – blood, Cancer Genome Project – Hematopoietic system – MM, 675 Genentech – Bone Marrow and Proteomics – Cell Line – NCI 60 Panel – not applicable) (Figure 4B). 2 experiments showing MM expressions of MUC16 gene were recorded (CCLE – MM and Proteomics – Cell Line – NCI 60 Panel – not applicable) (Figure 4C). Transcriptomics experiments have been performed in 4 different experiments (CCLE – MM, 675 Genentech – Blood, 675 Genentech – Bone Marrow, Cancer Genome Project – Hematopoietic system – MM) showing the expressions of the ZNF717 gene in MM (Figure 4D)

The expression levels of the RYR2, TTN, MUC16 and ZNF717 genes in MM homo sapiens are shown with the data obtained from the Expression Atlas (https://www.ebi.ac.uk/gxa/home) [27] (**Figure 4**). Expressions of these genes in cell lines were examined and tested. Generally, the expression levels of these genes were observed to be low (**Figure 4**). In fact, the low expression levels of these genes and their relationship with patient samples should be investigated at the molecular level. With these research results, a new approach can be obtained for the treatment of MM. Therefore, further studies are needed to clarify the mechanism and possible role of these genes in MM.

According to the graph shown in **Figure 5** obtained from cBioportal [11], the patients' cause of death (205 samples) changes depending on the mutated genes. Disease progression has been the cause of death for approximately 30% of the KRAS gene mutated patients, ~10% of DIS3, TTN, TENT5C or TP53 genes mutated patients, ~5% ZNF717 or LRP1B genes mutated patients. Additionally, disease progression has also been established to be the cause of death in the case of 20% of NRAS gene mutation patients. Although NRAS is the second most frequently mutated gene in MM, samples with NRAS gene mutations have a higher rate of cause of death defined as 'other' on the database (~ 40%). Moreover, a higher proportion of causes of death described as 'other' are found in patients with mutations on DIS3, TP53, BRAF, MUC16 and LRP1B. On the other hand, the frequency of RYR2 mutation ranks as 10th in Figure 1b, yet there is no available information regarding the cause of death of patients. According to the graph, death due to disease progression has the highest percentage only in patients with KRAS mutations. [11].

Novel targets (novel prediction markers)

Titin (TTN) is the largest known protein and the third most abundant filament in the cardiac and skeletal muscle, performing developmental, mechanical, and regulatory roles. With the widespread use of NGS, TTN has started to be reported as one of the main genes emerging in human hereditary diseases. Due to the essential role of TTN's, its size, modular structure, and numerous protein interactions, individual mutations may present greatly varied biological consequences and clinical symptoms. In the majority of cases, TTN mutations have been linked to a predominantly cardiac phenotype [28]. Thus, the impact on MM is of interest, given the potential for a diversity of its biological consequences.

According to the reports, TTN mutations may play an essential role in the immunotherapy of solid tumors and gastric cancer. However, no relationship has been shown between the TTN mutation and MM immunotherapy. Although research regarding the association between TTN mutation status and prognosis remains unclear, it is possible it may offer a new perspective to disease treatment [29].

Technical challenges and budgetary constraints make it difficult to achieve full Sanger Sequencing of TTN consisting of 364 exons. In addition, target enrichment strategies and sin-

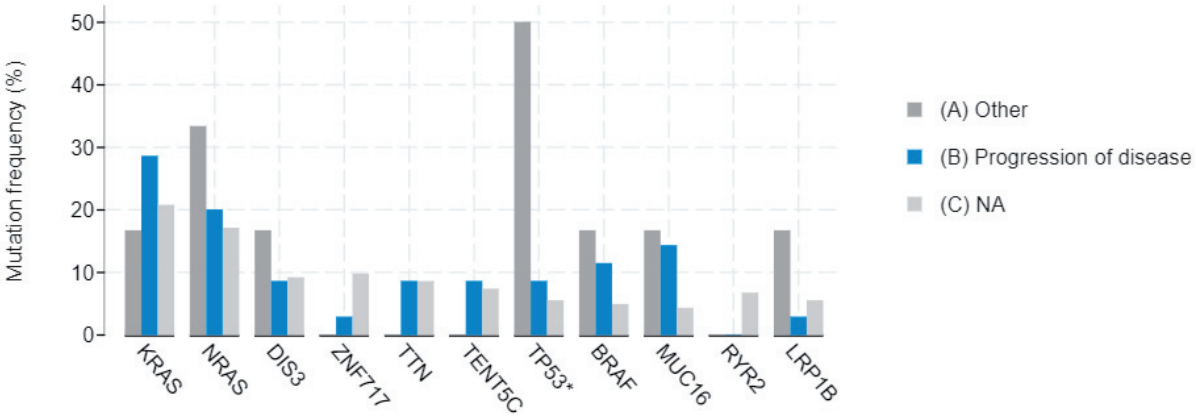

Figure 5. Patients' cause of death plot in correlation with mutation frequencies (*cBioPortal for Cancer Genomics*, n.d.-a)

gle-molecule sequencing studies used with second-generation sequencing may allow for diagnostic assays in this gene [28].

Recent NGS studies have shed light on cancer and its heterogeneity using bulk tumor samples. Tumor initiation and evolution are driven by sequential genetic changes in single cells, and single cell-sequencing has the potential of adding new insights into cancer studies in the bulk tumor genomic data [30]. In a study involving the use of these improvements, the clonal evolution of HBV-associated hepatocellular carcinoma has been approached by single-cell genome sequencing. It is widely recognized that the protein encoded by ZNF717 belongs to the zinc finger family, which plays an important role in the regulation of gene expression. Nevertheless, the precise role of ZNF717 in tumor pathogenesis has not been fully understood yet. In addition, although the effect of ZNF717 on MM remains unknown, its effect on hepatocellular carcinoma (HCC) has been investigated and the results have been shared in the past years. These results suggest that the tumor inhibitory effect of ZNF717 in HCC is likely mediated through the regulation of the IL-6/STAT3 pathway. Therefore, it can be assumed that ZNF717 is known to act as a tumor suppressor in HCC [30].

Furthermore, according to the studies from the STRING database, it has been observed that some genes, such as RAF1, UBE3A, BARD1, EXOS5C, AURKA, PIK3CA, RPA1, RPA3, SKIV2L2, are co-expressed with the BRAF, KRAS, and NRAS genes which, in turn, are the most frequently mutated and most related genes with MM. The conducted studies have demonstrated that microRNA-497 (miR-497) has a role as a tumor suppressor for several cancer types, and it has been proven that MiR497 overexpression inhibited MM cell proliferation, while promoting apoptosis by decreasing Raf-1 expression [31]. Additionally, SIRT1 is activated by a proteasome inhibitor, and it deacetylates GLI2 to improve hedgehog signaling (Hh) and treatment resistance in MM [32]. MDM2 gene is another proliferative gene for MM, since the overexpression of MDM2 enhances the survival and proliferation of MM cells.

Even though there co-expression evidence on the STRING database for UBE3A and BARD1 genes, these genes are only investigated in some cancers, such as cervical cancer (UBE3A) and leukemia (related with BARD1); however, there are no studies demonstrating their association with MM.

Methodology

The studies "Broad, Cancer Cell 2014" were selected from the cBioPortal database [10,11] and searched for "query by gene". In total, there were 205 samples containing mutation data out of 213 samples. Subsequently, the most frequent eleven genes (KRAS, NRAS, DIS3, TTN, ZNF717, TENT5C, TP53, BRAF, MUC16, RYR2, LRP1B) from 205 profiled samples were chosen for further research. Therefore, in order to understand the connection between the abovementioned eleven genes, a physical network analysis was conducted by the STRING database. Using the text-mining evidence data of the STRING database, literature research about the studied genes was completed. In cBioPortal Query Analysis [10, 11], mutation profiles (205 samples) of the chosen genes in 211 samples were found by the OncoPrint tool. Next, to understand the tendency of the genes, the Mutual Exclusivity tool was used and Table 1 was generated. 55 pairs of the genes were analyzed on the database and seven of the most paired genes were shown in Table 1. 'Mutations' tool of cBioPortal was used to understand mutation types and somatic mutation frequencies [11] (from Figure 4 to Figure 14). In the literature review, summary for each gene and clinical significance information was accessed through PubMed. Table 2, which contains the summary for all the investigated genes and their importance was created from the GeneCards, CivicDB and NCBI databases [17–19] Subsequently, the importance of the genes in MM was researched by means of literature survey. Then, expression results of the eleven genes were identified on the basis of the Expression Atlas database [27]. To analyze the patients' cause of death (disease progression / other causes) 'Plots' tool of cBioPortal [10, 11] was used and the 'cause of death table' was generated. In the final step of the analysis, to find and decide on the novel targets for the disease, the STRING database was used and the co-expressed genes were identified as the new targets.

In total, data comprising 211 MM samples was used from the cBioportal. These samples included 205 cases of patients where mutation data were found. Additionally, according to Lohr et al 203 samples had tumor-normal pairs which could be further analyzed [9]. Therefore, although the total number of 205 patients was provided on the website, the actual number of samples with tumor-norm pairs was determined to be 203. Furthermore, when selecting the abovementioned 11 genes for further analysis, it was observed that the number of samples should be 138. In fact, of the 203 samples studied, 138 had mutations in the 11 genes that were investigated and which were thought to be significant for MM (separately or together).

Conclusions

According to the study involving MM patients from the cBioPortal data set, ZNF717 mutation has been seen in 17 patients, TTN mutation in 17 patients, MUC16 mutation in 13 patients, and RYR2 mutation in 11 patients. Since the effect and mechanism of these mutations on MM have not been studied yet, more studies are necessary to investigate the roles of these genes in MM progression. Moreover, according to the STRING database studies, BRAF, NRAS, and KRAS have been found to be the most related genes with one another (co-expression, database, and experimental evidence) which affect MM. Additionally, samples have been studied in both of these databases, and the frequency of gene mutations has been found to vary, with some genes, such as DIS3, or TTN, proven to affect the disease.

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Conflict of interest statement
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