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Comparison of Clinical Characteristics and Genetic Aberrations of Plasma Cell Disorders in Thailand Population

Chutirat Jirabanditsakul, MSc[1,2], Sumana Dakeng, PhD[2], Chutima Kunacheewa, MD[1], Yaowalak U-pratya, MSc[1], and Weerapat Owattanapanich, MD[1]

Abstract

Multiple myeloma is an incurable malignancy of plasma cells resulting from impaired terminal B cell development. Almost all patients with multiple myeloma eventually have a relapse. Many studies have demonstrated the importance of the various genomic mutations that characterize multiple myeloma as a complex heterogeneous disease. In recent years, next-generation sequencing has been used to identify the genomic mutation landscape and clonal heterogeneity of multiple myeloma. This is the first study, a prospective observational study, to identify somatic mutations in plasma cell disorders in the Thai population using targeted next-generation sequencing. Twenty-seven patients with plasma cell disorders were enrolled comprising 17 cases of newly diagnosed multiple myeloma, 5 cases of relapsed/refractory multiple myeloma, and 5 cases of other plasma cell disorders. The pathogenic mutations were found in 17 of 27 patients. Seventy percent of those who had a mutation (12/17 patients) habored a single mutation, whereas the others had more than one mutation. Fifteen pathogenic mutation genes were identified: *ATM, BRAF, CYLD, DIS3, DNMT3A, FBXW7, FLT3, GNA13, IRF4, KMT2A, NRAS, SAMHD1, TENT5C, TP53*, and *TRAF3*. Most have previously been reported to be involved in the RAS/MAPK pathway, the nuclear factor kappa B pathway, the DNA-repair pathway, the CRBN pathway, tumor suppressor gene mutation, or an epigenetic mutation. However, the current study also identified mutations that had not been reported to be related to myeloma: *GNA13* and *FBXW7*. Therefore, a deep understanding of molecular genomics would inevitably improve the clinical management of plasma cell disorder patients, and the increased knowledge would ultimately result in better outcomes for the patients.

Keywords

genetic, molecular, multiple myeloma, next-generation sequencing, Thailand

Abbreviations

AL, amyloid light-chain amyloidosis; *BM*, bone marrow; *CR*, complete remission; *gDNA*, genomic DNA; *M^{3}P*, a targeted MM-specific gene panel NGS sequencing; *MGUS*, monoclonal gammopathy of undetermined significance; *MM*, multiple myeloma; *NDMM*, newly diagnosed multiple myeloma; *NGS*, next-generation sequencing; *PCD*, plasma cell disorder; *PCR*, polymerase chain reaction; *RISS*, revised international staging system; *POEMS*, polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes; *RRMM*, relapsed/refractory multiple myeloma; sCR, stringent complete remission; *VAF*, variant allele frequency; *VGPR*, very good partial remission

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Introduction

Multiple myeloma (MM) is an incurable malignancy of plasma cells resulting from impaired terminal B cell development.[1,2] Plasma cells are immunoglobulin-secreting cells with a low proliferative capacity.[3,4] When plasma cells become myeloma cells, they secrete a monoclonal protein (M protein), which

[1] Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[2] Department of Clinical Microscopy, Faculty of Medical Technology, Mahidol University, Nakhon Pathom, Thailand

Corresponding Author:
Weerapat Owattanapanich, Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 10700 Bangkok, Thailand.
Email: weerapato36733@gmail.com

can be detected with serum protein electrophoresis and serum-free light chain assays.[5]

MM is the second most common hematologic malignancy with incidences that differ according to ethnicity.[6–8] Patients with MM in Asia account for approximately 35% of all cases worldwide. The incidence is high in many Asian countries, particularly China, Japan, South Korea, Singapore, Taiwan, and Thailand.[9] The incidence of MM in Thailand ranks third among the hematologic malignancies being surpassed by non-Hodgkin's lymphoma, and leukemia. MM is a heterogeneity disease, and the median age at diagnosis is 70 years.[7,8,10] Currently, the diagnosis of MM is based on the 2016 International Myeloma Working Group criteria and risk stratification using the revised international staging system (RISS).[11–13] However, recent studies have shown the importance of other genetic abnormalities that are not included in the RISS. As a result, several methods are being investigated to identify the most accurate strategy for stratifying the risk of patients, including fluorescence in situ hybridization and next-generation sequencing (NGS).[14] Although the use of genetically guided therapy is currently not standard practice, genetic mutational data would benefit guided treatment strategies, such as precision medicine.[15] Fifteen novel agents have also been approved for MM treatment.[16] Among them are proteasome inhibitors, immunomodulatory drugs, monoclonal antibodies, and chimeric antigen receptor T cells. The use of such agents has resulted in a dramatic improvement in the survival of patients with MM with a rise from 3 years to 10 years in the last decade.[16–18] Nevertheless, almost all patients with MM eventually have a relapse.[19]

MM is a complex heterogeneous disease resulting from various genomic mutations that lead to a variety of clinical characteristics and treatment outcomes.[20] The concept of clonal and subclonal evolution of MM has been the focus of research.[21–23] Molecular events, such as somatic mutations and epigenetic and chromosomal copy-number changes, have driven a multistep clonal evolution process. This process is important for the progression from the premalignant stage to MM, and then from MM to end-stage disease through lineage or branching patterns.[24,25] Moreover, the genetic mutations can guide therapeutic approaches.[20,26] In recent years, NGS technology, which involves whole-exome sequencing, has been used to identify the genomic mutation landscape and clonal heterogeneity in MM.[27–29] There is an MM-specific gene panel for targeting NGS sequencing (M^3P v3.0) that includes 88 frequently mutated genes and drug-related genes.[30] Although the genomic complexity of MM disease has been extensively studied in Western countries, data on Southeast Asian populations are still lacking. Therefore, this study set out to detect plasma cell disorders (PCDs) with genetic mutations in the Thai population by using targeted gene sequencing with the NGS technique.

Materials and Methods

This prospective observational study was conducted on Thai patients diagnosed with PCDs between May 2020 and March 2021. The inclusion criteria were as follows:

1. Patients aged 18 years or older.
2. Patients with PCDs. These included monoclonal gammopathy of undetermined significance (MGUS), smoldering MM, POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome, amyloid light-chain (AL) amyloidosis, newly diagnosed MM (NDMM), and relapsed/refractory MM (RRMM).
3. Patients requiring treatment and follow-up at Siriraj Hospital, Thailand.

All participants signed a consent form prior to enrollment. The baseline patient characteristics and initial laboratory results were recorded. This study was approved by the Siriraj Institutional Review Board (COA. No. SI 334/2020) and was registered in the Thai Clinical Trials Registry (number: TCTR20211020003).

Sample Preparation

As a routine practice of NGS testing in our institute, at least 12 mL of bone marrow (BM) aspiration was collected for mutational analysis. CD138 + plasma cells were then purified from those cells using the EasySep Human CD138 Positive Selection Kit (STEMCELL Technologies Inc).

Targeted Gene Sequencing by NGS

The initial process was DNA extraction, which was done using a QIAamp DNA Mini kit (Qiagen). A frozen pellet of CD138 + plasma cells was suspended in phosphate buffered saline, and the manufacturer's instructions were then followed. The concentration of extracted genomic DNA (gDNA) was measured using a Nanodrop System (Thermo Fisher Scientific) and a Qubit dsDNA HS assay (Qubit 3.0 Fluorometer; Life Technologies). The GeneRead QIAact Custom Panel (Qiagen), lymphoid neoplasms, and myeloma panel covering 59 targeted genes were used in the QIAGEN GeneReader NGS System.

An input of 40 ng of gDNA was used as the starting material for the construction of the DNA template library. Unique molecular index technology was integrated into a gene-specific primer-based target enrichment process.

The library preparation consisted of a single controlled multienzyme reaction for fragmentation, the end-repairing and A-tailing of the gDNA samples, adapter ligation, a target enrichment polymerase chain reaction (PCR), and a universal PCR that was complimentary with the adapter sequence. These processes generated the sequencing-ready DNA template. Next, the clonal amplification of the DNA template and emulsion PCR was performed to obtain the final library. This process was carried out using an automated GeneRead QIAcube instrument in accordance with the manufacturer's instructions. The sequencing of the DNA library template in the QIAGEN platform used sequencing-by-synthesis technology with fluorescently labeled, reversibly terminated deoxyribonucleotide triphosphates. The library templates were

sequenced for 150 cycles on a GeneReader instrument with an automated protocol.

Sequence Data Processing and Interpretation

Secondary analysis of the FASTQ reads, the output of the sequencing process by GeneReader, was performed by QIAGEN Clinical Insight Analyze (QCI-A) software. The read and call variants were aligned by the software using the hg19 reference. In addition, QCI-A automatically generated a quality control report and visualization of the sequencing results. The quality control criteria of the NGS in this study were qualified by the GeneRead QIAact Custom Panel certificate. In brief, each sample must consist of at least 95% of all regions of interest with a minimum UMI coverage of 30×, and at least 90% of all regions of interest with a minimum UMI coverage of 50×. The threshold to detect the variant allele frequency (VAF) was 3%. The final step with the QIAGEN GeneReader NGS System involved QIAGEN Clinical Insight Interpret (QCI-I), a web-based software. All mutations were manually reanalyzed by 2 investigators (C.J. and W.O.) using the COSMIC cancer database, VARSOME, and dbSNP. If different decisions were made on the conflicting results, the mutations in question were discussed until a consensus was reached. Mutations in *NRAS*, *BRAF, ATM,* and *TP53* genes by the NGS technique were confirmed by utilizing the Sanger sequencing technique.

Results

The 27 patients with PCDs comprised 17 with NDMM, 5 with RRMM, and 5 with other PCDs (3 patients with MGUS and 2 patients with AL amyloidosis). There were slightly more men than women, and the median age at the time of diagnosis was more than 60 years. Most of the patients had lytic bone lesions and anemia. In addition, the laboratory results showed a wide range of percentages for the plasma cells in the BM and for M protein. IgG monoclonal gammopathy was the most common M-protein subtype (63.0%), followed by IgA monoclonal gammopathy (18.5%).

For the 17 patients with NDMM, the staging was evaluated with the International Staging System (ISS). Two patients (11.8%) had ISS stage I, 6 (35.3%) had ISS stage II, and 9 (52.9%) had ISS stage III. Approximately three-fourths (76.4%) of the patients were treated with a bortezomib–cyclophosphamide–dexamethasone regimen. Two patients received a melphalan-based regimen, while the other 2 patients received a bortezomib–dexamethasone regimen and daratumumab–bortezomib–melphalan–prednisolone regimen, respectively. After the induction therapy, most of the patients (11/17; 64.7%) achieved remission and were evaluated to have had at least a very good partial response. However, the other 6 patients (35.3%) had stable disease (Table 1).

Ten of the 27 patients enrolled did not have mutations. Of those 10, 5 patients had NDMM, 2 had relapsed MM, and 3 had other PCDs (2 patients with AL amyloidosis and 1 with MGUS). The remaining 17 patients harbored at least one pathogenic mutation. Most (12/17; 70.6%) had only one pathogenic mutation, 4 had 2 pathogenic mutations, while 1 had 3 pathogenic mutations. The NDMM patients had a higher number of gene mutations than the patients in the other groups. The mean number of gene mutations was 1.1, 0.6, and 0.4 for the NDMM, RRMM, and PCD groups, respectively, but there were no statistically significant differences between the groups ($P = .239$; Figure 1). Of the mutations, 18 variants were classified as pathogenic or likely pathogenic in the following 15 genes: *ATM, BRAF, CYLD, DIS3, DNMT3A, FBXW7, FLT3, GNA13, IRF4, KMT2A, NRAS, SAMHD1, TENT5C, TP53,* and *TRAF3*. Two mutational variants were found in the *FBXW7*, *NRAS*, and *TENT5C* genes. Regarding pathogenic mutations found in more than 1 of the 27 patients, 4 patients had variants in the *GNA13* gene, whereas 3 patients had variants in the *NRAS* gene. However, 2 patients had variants in each of the following 3 genes: *DIS3*, *FBXW7*, and *TENT5C*. The oncoplots of the mutations in the 27 PCDs are presented in Figure 2.

Most pathogenic mutations were found in patients with NDMM. Mutations in those patients involved 13 genes. The gene reported the most frequently was *GNA13* (3/17; 17.6%). Each of the other genes (*NRAS*, *FBXW7*, and *TENT5C*) was found in 11.8% of the patients. In patients with MGUS, the mutated *DNMT3A* gene was commonly found. Mutations in the *DIS3* gene were found only in patients with relapsed MM. The *GNA13* mutated gene was present in both the NDMM and other PCDs groups, while the *NRAS* mutated gene was detected in the NDMM and relapse groups (Figure 3).

The VAF of the mutations ranged widely (3.6%-77.0% VAF). Most mutations (7/15; 46.7%) had a VAF less than 10% VAF, 40% of the mutated genes had a 15% to 50% VAF, and only 2 mutated genes had a VAF greater than 50%. The *FBXW7* mutated gene showed evidence of both 49% and 3.7% VAF. In contrast, the *NRAS* mutated gene only had clonal mutations, with 35%, 49%, and 77% VAF.

Discussion

To our knowledge, this was the first study to have identified somatic mutations in PCDs in the Thai population using targeted NGS. Due to the limited healthcare infrastructure, economic problems, and resource limitations in Thailand, several treatment modalities recommended by MM management guidelines were not available locally.[9] Targeted NGS is a comprehensive technology that enhances the understanding of genomic complexity in extremely heterogeneous PCDs. Moreover, the combination of fluorescence in situ hybridization and NGS data would likely be applied in the future to facilitate the decision-making on individual treatment protocols.

We identified 15 pathogenic mutation genes in 27 patients. Most of the mutations were previously reported. There was also clear evidence that mutations in the RAS/MAPK signaling pathway, such as mutations of the *KRAS, NRAS, BRAF*, and *NF1* genes play a role in the progression of myeloma,[31,32] which was comparable to our results. Two oncogenes (*NRAS*

Table 1. Data on Gene Mutations and Results of the Treatments of Individual Patients.

Patient	Group	Pathogenic variant detected				Induction dregimen	Treatment response
		Variant details			VAF		
M1	NDMM	*GNA13*	c.243_244delCG	p.E82fs*19	3.66%	VCD	CR
M2	NDMM	*TENT5C*	c.531dupT	p.E178*	6.87%	VCD	VGPR
M5	NDMM	*CYCLD*	c.1525G>T	p.E509*	5.56%	TCD	Nonresponse
M6	NDMM	-				VCD	CR
M8	NDMM	*FLT3*	c.2546G>A	p.R849H	52.0%	VCD	CR
M10	NDMM	*FBXW7*	c.187 °C > T	p.Q624*	49.0%	VCD	Nonresponse
		GNA13	c.243_244delCG	p.E82fs*19	3.57%		
		TRAF3	c.34 °C > T	p.Q114*	5.07%		
M12	NDMM	*BRAF*	c.1780G>A	p.D594N	15.0%	VCD	VGPR
		TENT5C	c.968_969delGG	p.G323fs*2	5.64%		
M13	NDMM	*ATM*	c.4235C>T	p.P1412L	46.0%	VCD	VGPR
		TP53	c.811G>A	p.E271K	7.39%		
M14	NDMM	*IRF4*	c.368A>G	p.K123R	38.0%	Vel/dex	Nonresponse
M15	NDMM	-				VCD	Nonresponse
M16	NDMM	*SAMHD1*	c.316C>T	p.R106*	44.0%	VCD	CR
M17	PCDs (MGUS)	*DNMT3A*	c.1453C>T	p.Q485*	19.0%	-	
M18	NDMM	-				MPT	VGPR
M19	NDMM	-				D-VMP	CR
M20	NDMM	*NRAS*	c.182A>G	p.Q61R	35.0%	VCD	Nonresponse
M21	NDMM	*GNA13*	c.243_244delCG	p.E82fs*19	3.57%	VCD	Nonresponse
		NRAS	c.182A>G	p.Q61R	49.0%		
M22	NDMM	-				VCD	CR
M25	PCDs (MGUS)	-				-	-
M26	PCDs (MGUS)	*GNA13*	c.243_244delCG	p.E82fs*19	6.06%	-	-
M28	PCDs (AL)	-				VCD	CR
M29	PCDs (AL)	-				VCD	CR
M30	RRMM	-				-	-
M34	RRMM	-				-	-
M45	RRMM	*NRAS*	c.181C>A	p.Q61K	77.0%	-	-
M51	RRMM	*DIS3*	c.1996delC	p.Q666fs*18	4.05%	-	-
M52	NDMM	*FBXW7*	c.349_351delGAG	p.E117del	3.72%	VCD	CR
		KMT2A	c.2629_2630delGA	p.D877fs*8	3.66%		
M54	RRMM	*DIS3*	c.1996delC	p.Q666fs*18	7.48%	-	-

Abbreviations: *AL,* amyloidosis; CR, complete remission; *D-VMP*, daratumumab-bortezomib-melphalan-prednisone; *MGUS*, monoclonal gammopathy of undetermined significance; *MPT*, melphalan/prednisolone/thalidomide; *NDMM*, newly diagnosed multiple myeloma; *PCD*, plasma cell disorder; *RRMM*, relapsed/refractory multiple myeloma; *TCD*, thalidomide/cyclophosphamide/dexamethasone; *VAF,* variant allele frequency; *VCD*, bortezomib/cyclophosphamide/dexamethasone; *Vel/dex*, bortezomib/dexamethasone; *VGPR*, very good partial remission.

and *BRAF*) were detected in the NDMM and RRMM groups, accounting for 18.2% (4/22). Our findings were comparable with a previous report that showed an *NRAS* mutation rate of approximately 20%.[33,34]

Our study also found 2 important mutations. They were the *CYLD* and *TRAF3* genes of the noncanonical NF-kB signaling pathway, which plays a key role in late-stage B-cell development.[35] These 2 mutations were found in 7.4% of our patients. However, this proportion was less than for the NDMM in general, which usually showed a rate of around 20%.[36,37] Moreover, we found that one patient with NDMM (7.4%) had both the *ATM* and *TP53* mutations. These mutations in the DNA repair pathway were important for the progression of the disease from its premalignant state to MM.[38] Their presence also indicated a poor prognosis for the disease.[27]

An E3 ligase is a target of cereblon modulators in the control of ubiquitinated proteins, particularly *IRF4*, a key transcription factor of plasma cell development.[39] Mutation of *IRF4* is rare. It was recently reported that this mutation led to the increased survival of myeloma cells and could affect the response to cereblon modulator treatment.[40] One of our patients harbored the *IRF4* mutation. He was treated with bortezomib and dexamethasone, but unfortunately, his response was sluggish.

The *DIS3* and *FAM46C* (*TENT5C*) mutated genes, which were putative tumor suppressor genes, were each identified in 7.4% of the patients. These genes have also been reported to be the most frequently found tumor-suppressor mutated genes in MM.[30] In our investigation, the *DIS3* gene was found only in relapsed patients. However, published reports have tended to minimize the potential of the *DIS3* mutation in familial MM.[41] Furthermore, this mutation was commonly found to be associated with deletion 13, which had a poor prognosis.[42]

We identified epigenetic mutations similar to previous reports on PCDs.[43,44] These involved the genes related to the

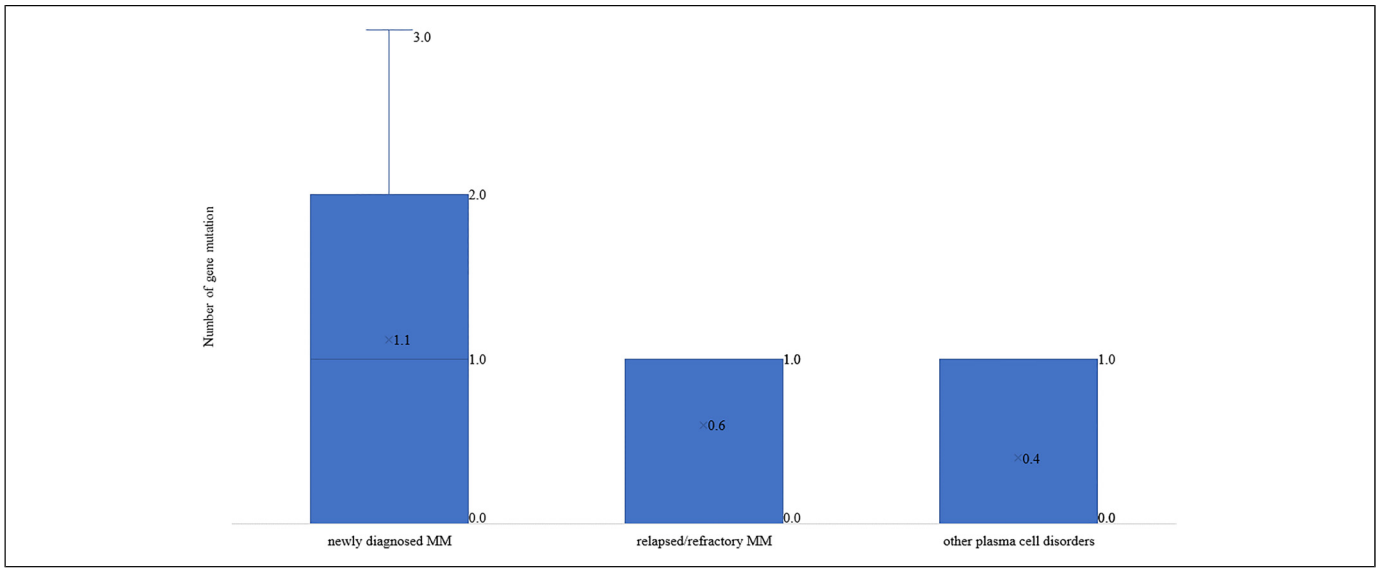

Figure 1. The number of gene mutations detected in a different group of patients.

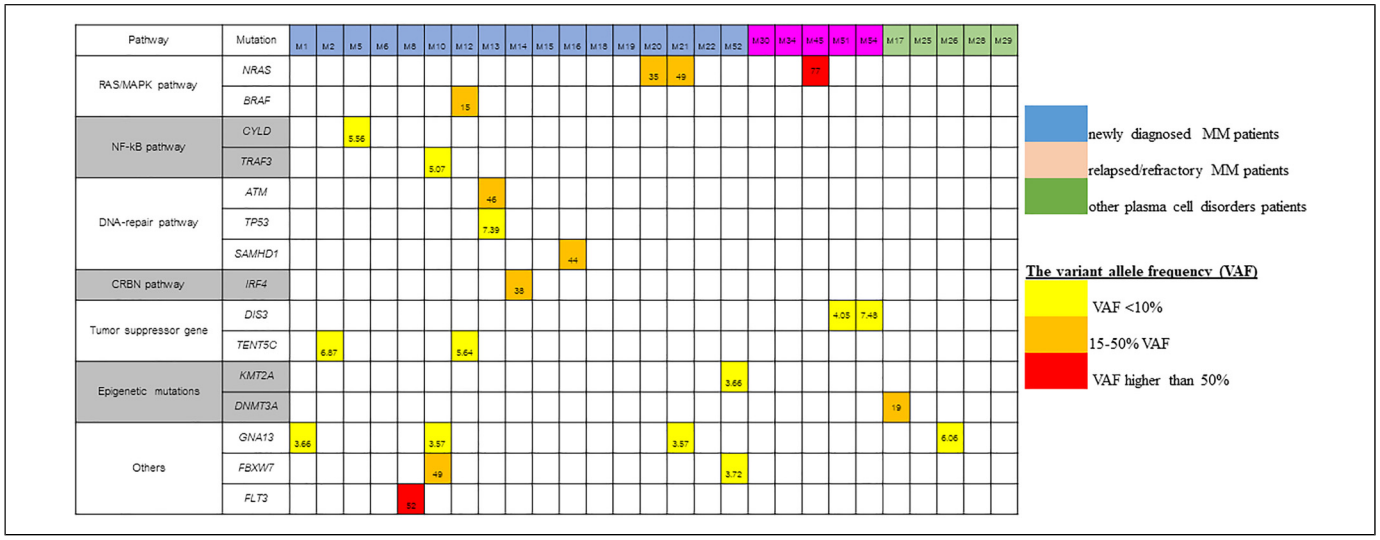

Figure 2. Oncoplots of mutations in 27 patients of plasma cell disorders.

DNA methylation regulators, histone methylation (*KMT2A* [3.7%]), and DNA methyltransferase (*DNMT3A* [3.7%]). The *KMT2A* mutated gene was found in MM, while the mutation of the *DNMT3A* gene was identified in patients with MGUS.[45]

Additionally, one of our NDMM patients had an *FLT3* mutation. The former study established that the FMS-like tyrosine kinase 3 ligand (FLT3L) level in the plasma and the *FLT3* overexpression correlated with the stage of the MM disease.[46,47] Moreover, the mutated *SAMHD1* gene, associated with the DNA repair in MM oncogenic pathways,[48] was also found in our study (Table 2).

Our study also identified new mutations that had not previously been reported to be related to myeloma; namely, *GNA13* and *FBXW7* (Figure 3). The *GNA13* mutated gene was reported in other B-cell lymphoid malignancies, which was related to the cell migration pathway.[52] In contrast, the *FBXW7* mutated gene was reported in T-cell leukemia and chronic lymphocytic leukemia, which was related to the NOTCH pathway.[53,54] Interestingly, the *FBXW7* mutation was found to be correlated with post-radiation induced thymic lymphomas from the mice model.[55] Nevertheless, further research is needed on the correlations of these gene mutations with PCDs.

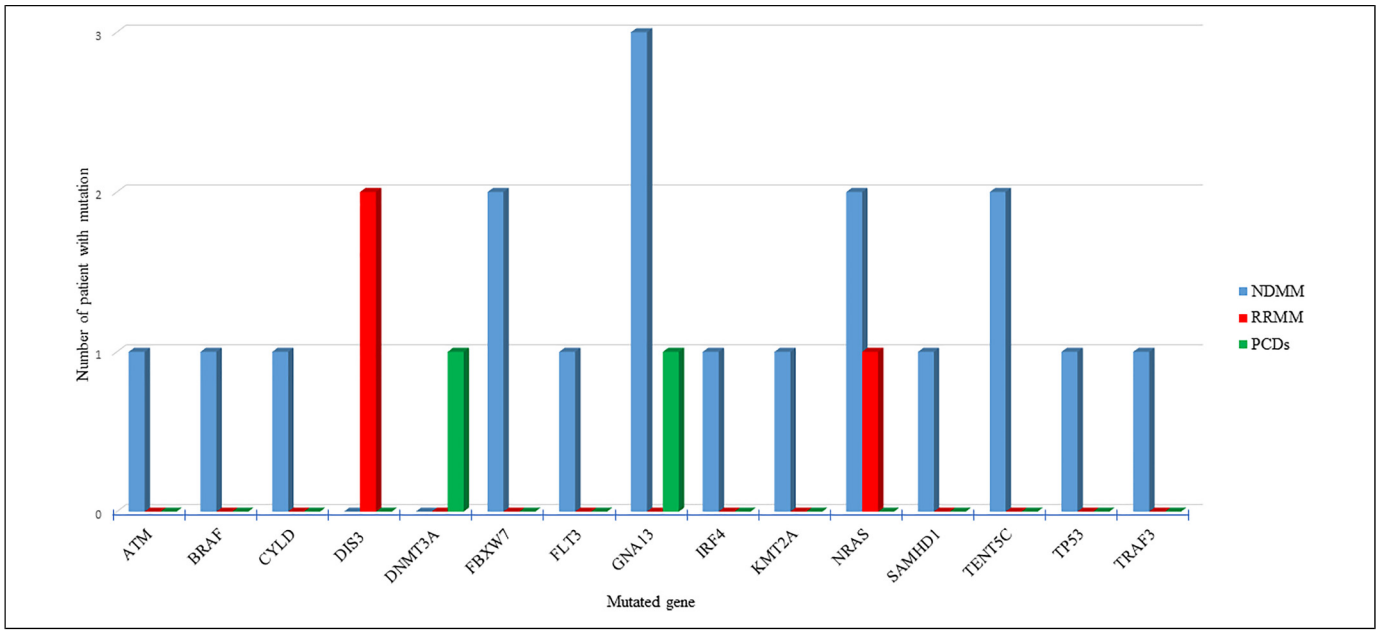

Figure 3. The mutational genes detected in 3 groups of patients.

Table 2. Proportions of Mutations in Multiple Myeloma Patients in This Study and Other Research.

	Kortuem *et al*[49]	Kortüm *et al*[50]	Kortüm *et al*[51]	This study
Patients' country	USA	Germany	USA	Thailand
Method	targeted NGS (M^3P v2.0)	targeted NGS(M^3P v3.0)	targeted NGS (M^3P)	custom targeted NGS
Patient numbers	142 NDMM	50 RRMM	A sequential sample post treatment 25MM patients	17 NDMM and 5 RRMM
Mutation incidence (range, average)	0-8, 1.5	0-13, 2.7	1.92, 2.12	0-3,1
ATM	NR	10	NR	3.7
BRAF	9	18	NR	3.7
CDKN2A	NR	NR	NR	7.41
CRBN	NR	12	NR	NR
CYLD	5	4	NR	3.7
DIS3	14	6	16,16	*7.41*
DNMT3A	NR	NR	NR	3.7
FBXW7	NR	NR	NR	7.41
FLT3	NR	NR	NR	3.7
GNA13	NR	NR	NR	14.81
IRF4	NR	4	NR	3.7
KMT2A	NR	NR	NR	3.7
KRAS	24	32	36,36	NR
MYD88	NR	2	NR	3.7
NRAS	17	26	20,16	11.11
SAMHD1	NR	NR	NR	3.7
SP140	NR	NR	12,12	NR
TENT5C (FAM46C)	8	12	12,16	7.41
TP53	9	26	16,16	3.7
TRAF3	11	8	NR	3.7

Abbreviation: *M3P*, a targeted MM-specific gene panel NGS sequencing; *MM,* multiple myeloma; *NDMM*, newly diagnosed multiple myeloma; *NGS*, next-generation sequencing; *NR*, not reported; *RRMM*, relapsed/refractory multiple myeloma.

Although the spectrum of the mutations and the treatment outcomes of the patients were broad, patients without pathogenic mutations appeared to have a good response to the induction therapy. The results showed that the *NRAS*, *CYLD*, *TRAF3*, and *IRF4* mutated genes were found in patients with stable disease. Moreover, the *NRAS* mutated gene mutation was found in both stable disease and relapse patients. Interestingly, these mutated genes may play a role in the progression of myeloma. On the other hand, patients with the same mutation had different response outcomes. For instance, patients who had 49% VAF of the *FBXW7* mutated gene did not respond to the induction therapy, whereas the patients who had 3.7% VAF of the mutation responded well to therapy. Of note, the burden of the mutation might affect the outcome of these patients.

There were some limitations of this study. This pilot study had a small sample size, which could not compare the number of mutations between NDMM and RRMM, the clinical outcomes between the mutated- and wild-type genes, and was difficult to derive an interpretation of the causative variants. However, the present investigation represented a solid starting point for more work on genomic complexities in Thai patients with PCDs. Our research is underway to expand the sample size in order to explore the exact incidence of the mutational landscape in the Thai population. Furthermore, the confirmation of the mutations by Sanger sequencing in this study was performed only in common gene mutations because of the limited funding. Therefore, we plan to confirm other uncommon mutations by the Sanger sequencing technique in the near future after collecting more samples.

Conclusions

The genetic landscape of Thai patients with plasma cell disorders demonstrates the heterogeneity of mutations. A deep understanding of molecular genomics would inevitably improve the clinical management of PCD patients, and the increased knowledge would ultimately result in better outcomes for the patients.

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Authors' Note

Ethical approval to report this case was obtained from the Ethics Committee for Research in Human Subjects at the Siriraj Institutional Review Board (SI 334/2020). And the written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORCID iD

Weerapat Owattanapanich https://orcid.org/0000-0002-1262-2005

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