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Research article

Gene mutations in newly diagnosed multiple myeloma patients detected by next-generation sequencing technology☆

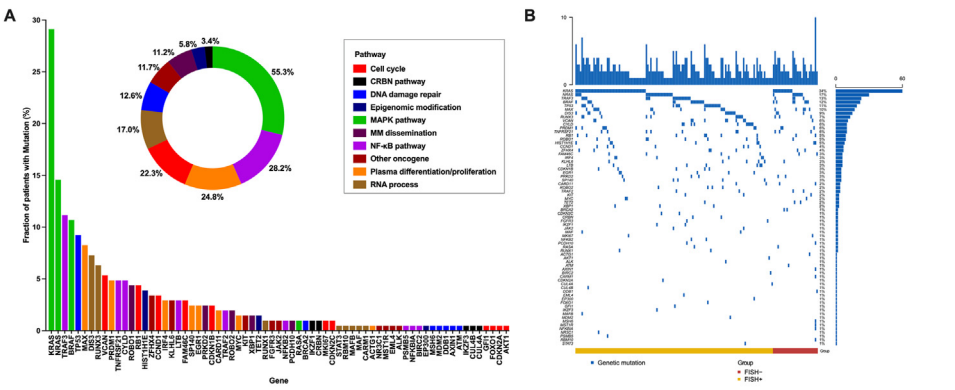
Yutong Wang, Mengzhen Wang, Bin Chu, Minqiu Lu, Lei Shi, Shan Gao, Yuan Chen, Qin Yan, Na Ji, Li Bao*

Department of Hematology, Beijing Jishuitan Hospital, Capital Medical University, Beijing 100035, China

HIGHLIGHTS

- The correlation between clinical characteristics and genetic mutations detected by 92 gene next-generation sequencing panels authorized by invention patent grants was analyzed in newly diagnosed multiple myeloma of the Chinese population.
- The distribution of genetic mutation is similar to data reported in Western countries. The gene mutation affects the pathway of the RNA process is more frequently occurring in males and age less than 70 years patients.
- The International Staging System (ISS) Stage III correlated with gene mutations in the NK-κB pathway while Revised ISS (R-ISS) Stage III correlated with the DNA damage repair pathway. Cytogenetic abnormalities detected by fluorescence *in situ* hybridization also have a certain correlation with gene mutations.

GRAPHICAL ABSTRACT



Genetic distribution of mutations in 206 patients with newly diagnosed multiple myeloma. (A) Gene mutation frequencies are depicted with varied colors denoting diverse gene pathways and the constituent diagram illustrating the genetic profile. (B) The distribution of genetic mutation in patients with or without cytogenetic abnormalities detected by FISH. FISH: Fluorescence *in situ* hybridization; MM: Multiple myeloma

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ABSTRACT

Background: Multiple myeloma (MM) is a heterogeneous plasma-derived hematopoietic malignancy with complex genetic mutation contributing to the pathogenesis. Though gene sequencing has been applied in MM, genetic features from Chinese MM patients are reported less. We investigated the genetic mutation of newly diagnosed multiple myeloma (NDMM) patients and explore its correlation with cytogenetic abnormalities detected by fluorescence *in situ* hybridization (FISH).

Methods: A total of 206 patients with NDMM were enrolled. After enriching plasma cells with CD138 magnetic beads, 92 MM-related target gene mutations were detected by the Illumina sequencing platform, and six common genetic abnormalities were detected by FISH.

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* Corresponding author: Hematology Department, Beijing Jishuitan Hospital, Capital Medical University, No. 31 East Xinjiekou Street, Xicheng District, Beijing 100035, China.

E-mail address: baoli@jst-hosp.com.cn (L. Bao).

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Results: 162 cases (78.6%) had at least one gene mutation detected by NDMM. The top 5 mutated genes were *KRAS, NRAS, TRAF3, BRAF,* and *TP53*. Cytogenetic abnormalities detected by FISH have a certain correlation with gene mutations, t(11;14) translocations are often accompanied by *CCND1* and *TP53* mutations, *KLHL6* in t(4;14), *SP140, CDKN1B* and *PRKD2* in t(14;16) and t(14;20) translocations. The mutation ratio was higher for *EGR1*, while lower of *CCND1* in patients with gain 1q21. The *TP53* mutation was more likely in patients with 17p deletion. The gene mutation affects the pathway of the RNA process is more frequently occurring in males and age less than 70 years patients. The International Staging System (ISS) Stage III correlated with gene mutations in the NK-κB pathway while Revised ISS (R-ISS) Stage III correlated with the DNA damage repair pathway.
Conclusions: There are various gene mutations in NDMM patients, mainly *RAS/MAPK* and *NF-κB* pathway gene pathways.

Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by abnormal clonal plasma cells, which is the second most common hematologic malignancy afflicts around 18,000 people in China per year[1] and 155,000 worldwide.[2] Despite the novel treatment including proteasome inhibitors (PIs), immunomodulatory drugs (IMiD), and CD38 monoclonal antibody, MM remains incurable with a median progression-free survival (PFS) of 41 months[2] due to the refractory and inevitable drug-resistant relapse. A previous study has reported a lot of oncogenes and tumor suppression genes taking part in the tumorigenesis and progression of MM, including *Bcl-2, C-myc, RAS, TP53* et al.[3] MM has vast heterogeneity which was discovered by modern sequencing methods applied to large clinical trial cohorts in the post-genomic era.[4] Next-generation sequencing (NGS) has been widely used in Western countries to establish the MM gene mutation landscape, which found some gene mutations like *PRDM1, TGDS, TRAF3*, and *ZFHX4* were related to survival.[5–7] Therefore, the gene mutation of Chinese MM patients remains to be reported and provide data to identify pathogenesis and prognosis as the key to the modern management of MM.

Here, we designed an NGS targeted to capture 92 myeloma-specific genes that are authorized patent for an invention to identify the mutations of newly diagnosed MM (NDMM) patients. Using this approach, we were able to identify gene mutation markers as well as their interaction with characteristics and cytogenetic abnormalities detected by fluorescence *in situ* hybridization (FISH) and both confirm previous findings. We evaluated this panel on 206 patients with NDMM to report the gene mutations and correlations with cytogenetic abnormalities, with the further aim of exploring the impact of mutations in patients without cytogenetic abnormalities.

Methods

Clinical data of patients

From October 2019 to July 2023, patients who were newly diagnosed with MM received FISH and NGS detection of myeloma cells, were enrolled in this retrospective study. The diagnosis, International Staging System (ISS) staging, and Revised ISS (RISS) staging MM patients were performed according to the International Myeloma Working Group consensus.[8] The baseline clinical data of patients were obtained through our electronic medical record system.

Interphase FISH analysis and high-risk chromosome abnormality concept

Before receiving induction therapy, all patients accepted bone biopsy to collect 4 ml of bone marrow fluid in heparin anticoagulant tubes. The monoclonal plasma cells were identified in 196 patients by flow cytometry. The purified bone marrow myeloma cells were obtained through CD138 whole blood sorting magnetic beads using autoMACSTM Pro Separator (Miltenyi Biotec, Germany). The purified CD138+ cells were harvested and microscope slides were performed according to standard protocols of manufactory instruction[9] of DNA denaturation, hybridization, slide washing, slide preparation, and counterstaining. The other 10 patients without BM monoclonal plasma cells received FISH detection of slices from biopsy samples in extramedullary disease (EMD). All slices were roasted, dewaxed, boiled, and digested with gastric enzymes beforehand. Two hundred nuclei were analyzed for each probe with a 100× objective fluorescence microscope (BX51, Olympus, Japan) by single and triple emission filters. The probes used in this study including GSP P53/CSP17(17p13), GSP 1q21, GSP CCND1/IGH (11q22/14q32), GSP FGFR3/IGH (4p16/14q32), GSP MAF/IGH (14q32/16q23) and GSP MAFB/IGH (14q32/20q21) (LBP medicine science and technology, Guangzhou, China) to detect del(17p), gain/-amplification 1q21, t(11;14), t(4;14), t(14;16), and t(14;20), respectively. Interphase FISH results were described according to the standards of the International System for Human Cytogenetic Nomenclature (ISCN) 2016.[9] We considered patients who have three signals more than the threshold of 1q21 as Gain 1q21, while four or more signals as Amplification 1q21. The cutoff points were 8.0% for P53 deletion and 5.0 % for remaining positive values (established from our laboratory by mean of 15 normal controls plus ± 3 times of standard deviations).

Next-generation DNA sequencing (NGS)

NGS detection was conducted in CD138 purified BM aspirate samples from 196 patients, while in biopsy samples of EMD from 10 patients without monoclonal plasma cells. Genomic DNA was extracted from $CD138^+$ cells using the QIAamp Blood DNA Mini Kit and biopsy samples using the GeneReadTM FFPE Kit (QIAGEN, Germany). The panel sized 344 kb including 92 MM-related genes was designed through AmpliSeq for the Illumina Gene Assay with the Illumina Design Studio platform (https://designstudio.illumina.com/). The library was constructed with an AmpliSeqTM Library PLUS for the Illumina kit (Illumina, USA). For each sample dataset, the mean sequence depth was above 1000x, and the 0.2x uniformity was not less than 0.85; otherwise, the library was reconstructed and sequenced. After library construction, sequencing was performed on an Illumina MiSeq Reagent Kit v3 (150 cycles) (Illumina, USA) DX system. The quality requirements for the DNA sequencing process include DNA/library and sequencing quality assessment. The DNA extraction concentration needs to be higher than 10 ng/μL and the absorbance ratio (260/280) should be between 1.8 and 2.0. The library concentration needs to be higher than 0.3 ng/μL. The sequencing quality assessment including the Ontarget reply ratio, Proportion of Q30 data volume when offline, and Uniformity all should be higher than 80%. Meanwhile, the average sequencing depth should be greater than 1000x. Only when the above conditions are all met can the sequencing be judged to have reached quality control.

Mutation analysis

The original data were pre-processed by the DNA Amplicon Analysis Module in Illumina Local Run Manager to transform to FastQ files and call single-nucleotide variation (SNV) and insertion/deletion. Then the sequencing reads were aligned based on the human reference genome hg19 (GRCh37) using Burrows–Wheeler alignment (BWA, version.

0.7.17). Local duplication alignment, base quality correction, and mutation analysis were performed on bam files using the analysis system's Pisces software (Pisces 5.2.11.163). The InDel realignment and base quality recalibration were performed to control quality using BaseRecalibrator and IndelRealigner, respectively, from the Genome Analysis Toolkit (GATK; version 3.8; Broad Institute, Cambridge, MA, USA). The ANNOVAR software performed the annotation for the Variant Call Format (VCF).[10] Databases of ExAC_ALL, gnomAD_ALL, and 1000G were used to identify single nucleotide polymorphisms (SNPs) that were excluded from further analysis. The variant allele frequency (VAF) was calculated as the ratio of mutation reads to coverage reads, which might help eliminate germline mutation and evaluate the content of tumor cells. The identified variants were filtered by the following criteria: the VAF exceeding 5%, the maximum gene mutation frequency reported less than 0.01% in ExAC_ALL and gnomAD_ALL databases, the genetic mutation recorded in COSMIC database hematology disease with more than two non-synonymous mutations.

Statistical analysis

The clinical variables were presented as median values and ranges for continuous data, and proportions for categorical data. Pearson correlation analysis was used to examine the relationship between gene mutation, cytogenetic abnormalities, and baseline characteristics performed by R software v. 4.1.1, with a P value less than 0.05 regarded as significant.

Results

Patient characteristics

In nearly four years, a total of 206 newly diagnosed multiple myeloma (NDMM) patients were included in this study, of which 109 (52.9%) were males and 97 (47.1%) were females. The median age was 63.5 (30–88) years. The high-risk patients classified as ISS stage III and R-ISS stage III were 76 (36.9%) and 35 (17.0%), respectively. The major induction treatment regimens were proteasome inhibitors (PIs) + immunomodulators (IMiDs) based (128 patients, 62.1%) and PIs based (60 patients, 29.1%). The other patients received a CD38 antibody-based regimen and others (one for IMiDs and one refused treatment). Among the patients, 87 (79.1%) achieved complete response or very good partial response, while 64 (31.1%) achieved at least partial response and received autogenetic stem cell transplantation subsequently. Twenty patients (18.2%) had diabetes mellitus. The cytogenetic abnormalities detected by FISH including gain 1q21 (105 patients, 51.0%), amplification 1q21 (88 patients, 42.7%), del 17p (28 patients, 13.6%), t(4;14) (34 patients, 16.5%), t(11;14) (46 patients, 22.3%), t(14;16) (six patients, 2.9%) and t(14;20) (one patient, 0.5%). These baseline characteristics of NDMM patients are summarized in Table 1.

Table 1
Baseline characteristics of newly diagnosed multiple myeloma patients.

Characteristics	NDMM (n = 206)
Sex, *n* (%)	
Male	109 (52.9)
Female	97 (47.1)
Age (years), median (range)	63.5 (30–88)
M protein subtype,*n*	
IgG/IgA/IgD	93/52/1
κ/λ/non-secreting	24/27/9
ISS stage[a], I/II/III (*n*)	75/52/76
R-ISS stage[a], I/II/III (*n*)	51/117/35
Induction treatment regimen, *n* (%)	
PIs + IMiDs based	128 (62.1)
PIs based	60 (29.1)
CD38 antibody-based	16 (7.8)
Others	2 (1.0)
ASCT, *n* (%)	64 (31.1)
FISH, *n* (%)	
Gain 1q21	105 (51.0)
Amplification 1q21	88 (42.7)
del 17p	28 (13.6)
t(4;14)	34 (16.5)
t(11;14)	46 (22.3)
t(14;16)	6 (2.9)
t(14;20)	1 (0.5)

[a] Three patients failed to detect the level of β2-microglobulin who could not stage in ISS and R-ISS. ASCT: Autogenetic stem cell transplantation; FISH: Fluorescence *in situ* hybridization; IMiDs, Immunomodulators; ISS: International staging system; NDMM: Newly diagnosed multiple myeloma; PIs: Proteasome inhibitors; R-ISS: Revised ISS.

The gene mutation of NDMM patients

Information on gene mutation at diagnosis is presented as a waterfall plot in Figure 1. A total of 162 patients (78.6%) detected at least one gene mutation in this study, of which one patient (0.5%) had ten gene mutation, four patients (1.9%) with six gene mutation, six patients (2.9%) with five gene mutation, 22 patients (10.7%) with four gene mutation, 31 patients (15.0%) with three gene mutation, 53 patients (25.7%) with two gene mutation and 45 (21.8%) with one gene mutation. There were 44 patients (21.4%) without cytogenetic abnormalities detected by FISH, and only 14 (6.8%) of them also detected no gene mutation by NGS.

The top five mutation genes were *KRAS* (60 patients, 29.1%), *NRAS* (30 patients, 14.6%), *TRAF3* (23 patients, 11.2%), *BRAF* (22 patients, 10.7%) and *TP53* (19 patients, 9.2%). The details of the gene mutation ratio are presented in Figure 2.

The relationship between gene mutation and characteristics of baseline

The gene mutation affects the pathway of the RNA process and is more frequently occurring in males (22/104 vs. 10/102, $P = 0.025$) and those less than 70-year-old patients (24/85 vs. 8/89, $P = 0.018$) [Table 2]. The *RUNX3* that participated in the pathway of the RNA process is also mutated higher in male (11/104 vs. 1/102, $P = 0.003$) NDMM patients. The ISS Stage III correlated with gene mutations in the NK-κB pathway (24/127 vs. 22/76, $P = 0.003$), *TRAF3* (10/127 vs. 12/76, $P = 0.043$), and *CARD11*(4/127 vs. 0/76, $P = 0.031$) mutation, while R-ISS Stage III correlated with the DNA damage repair pathway (18/168 vs. 7/35, $P = 0.019$).

The relationship between gene mutation and cytogenetic abnormalities

The results of relevance analysis between gene mutation and cytogenetic abnormalities detected by FISH are displayed in Figure 3. There was a significantly higher ratio of *EGR1* mutation (5/105 vs. 0/101, $P = 0.026$), while a lower ratio of *CCND1* mutation (1/105 vs. 6/101, $P = 0.048$) in patients with gain 1q21. The del 17p was only positively correlated with *TP53* mutation (6/28 vs. 17/178, $P = 0.016$). The t(11;14) was more likely coexisted with *CCND1* (6/46 vs. 1/160, $P < 0.001$) and *TP53* (9/46 vs. 10/160, $P = 0.006$) mutation. In patients with t(4;14), *KLHL6* was more frequently accompanied (3/34 vs. 3/172, $P = 0.006$). Furthermore, gene mutation of *SP140, CDKN1B* and *PRKD2* were more common in patients with t(14;16)/t(14;20) (1/7 vs. 4/199, $P = 0.038$ for all).

The analysis of cases paired with NGS detection

In this study, three patients received an examination of these 92 myeloma-related gene mutations before treatment and after relapse. One patient was diagnosed as MM, a type of kappa light, with gain 1q21, amp 1q21, and t(4;14). As shown in Figure 4A, the VAF of gene mutation in BM was lower than biopsy from EMD around the thoracic vertebra (4.2% vs. 90.9%). The lower content of myeloma cells in BM liquid (Flow cytometry indicated 0.1%) was the main cause. The EMD data suggested

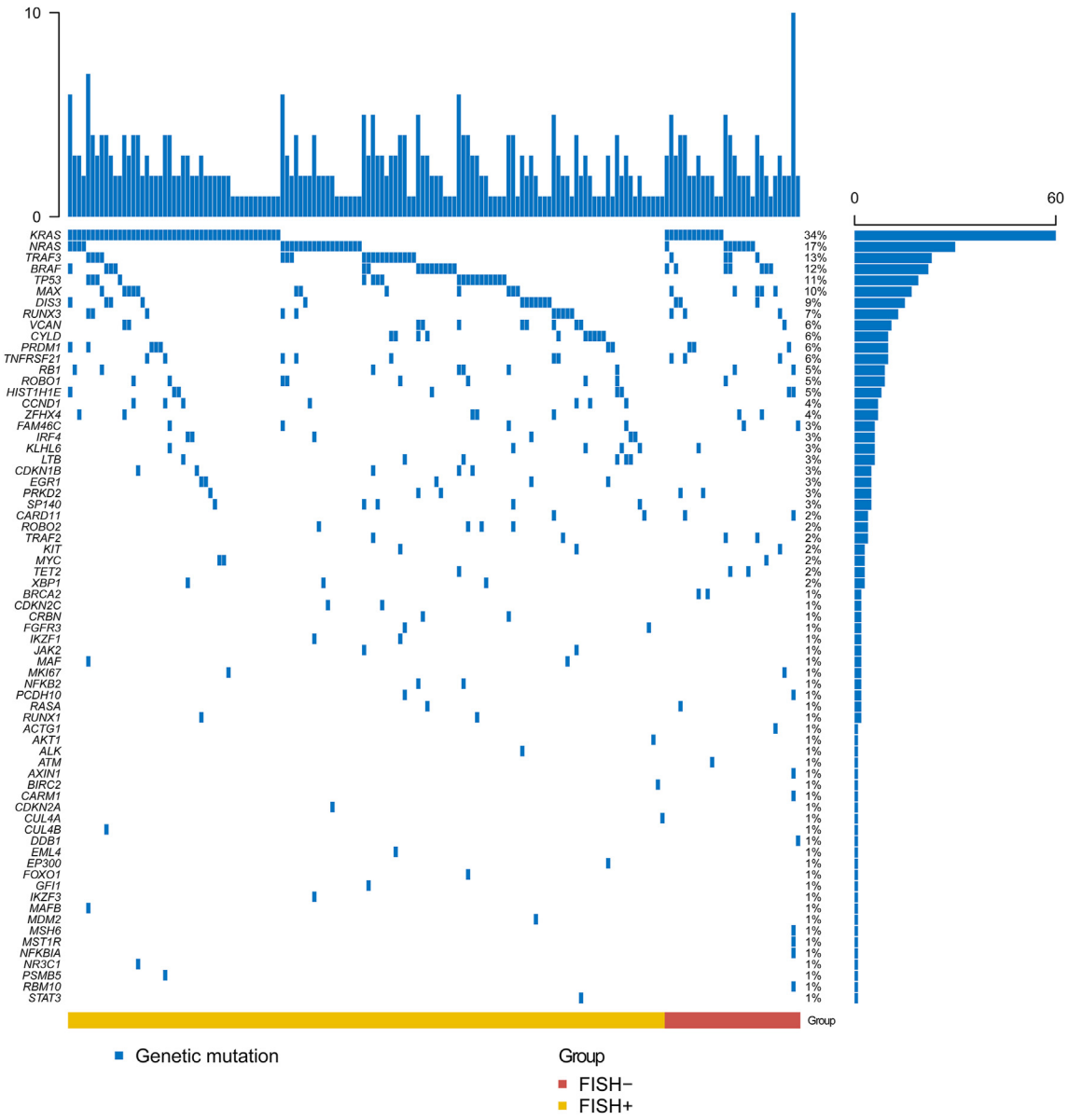

Figure 1. The distribution of genetic mutation in 206 newly diagnosed multiple myeloma patients with or without cytogenetic abnormalities detected by FISH. FISH: Fluorescence *in situ* hybridization.

the *PRDM1* mutation seemed to be a biallelic mutation in this patient, which was hard to infer from BM data. As shown in Figure 4B, the *ERN1* mutation was only detected when relapsed of this patient. The other patient had no myeloma cells in BM when relapsed, but a lot of EMD including skin, tonsil, and thoracic vertebra. The gene mutations were altered in EMD tissue when relapsed, from *TET2* and *NRAS* to *TET2, KRAS*, and *ZFHX4* [Figure 4C].

Discussion

Multiple myeloma had complex heterogeneous recurrent genomic aberrations including immunoglobulin gene translocations, copy number abnormalities, complex chromosomal events, transcriptomic and epigenomic deregulation, and mutations that define various molecular subgroups with distinct outcomes.[11] The gene mutation is one of the genetic events that results in chromosomal instability, in which the driver gene mutation may interfere with the cycle and prompt accelerated proliferation.[12] The genomic landscape of MM had been revealed by NGS detection. However, the gene mutations reported by different studies are various due to differences in sequencing methods, detection of gene regions (coding regions or hotspot regions), and data processing. Here we reported the somatic mutations in NDMM patients in the Chinese population by targeted NGS panel authorized patent for invention. The data suggested the distribution of genetic mutation is similar to data reported in Western countries. The gene mutation affects the pathway of the RNA process is more frequently occurring in males and age less than 70 years patients. The ISS Stage III correlated with gene mutations in the NK-κB pathway while R-ISS Stage III correlated with the DNA damage repair pathway. Cytogenetic abnormalities detected by FISH also have a certain correlation with gene mutations. The target NGS detection is a reliable technology to understand the genomic complexity and combined with cytogenetic detection might be applied to facilitate the decision-making on individual treatment protocols.

Brian A Walke et al. demonstrated the top ten mutation genes of *KRAS, NRAS, DIS3, FAM46C, BRAF, TP53, HUWE1, TRAF3, ATM,* and *EGR1* in 1273 patients with NDMM by whole exome sequencing (WES).[3] The other study involved 418 NDMM patients which conducted 246 target gene sequencing reported the top ten mutation genes were *KRAS,*

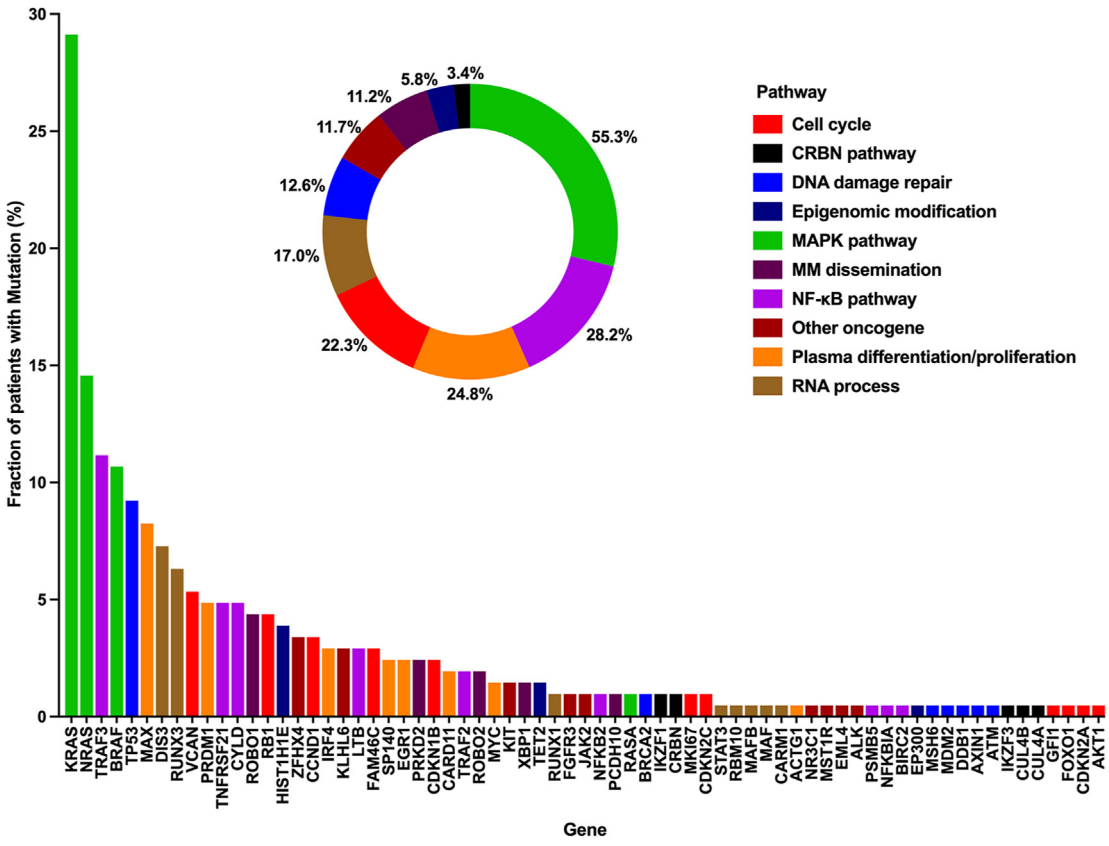

Figure 2. Gene mutation frequencies are depicted with varied colors denoting diverse gene pathways and the constituent diagram illustrating the genetic profile.

Table 2
The correlation between baseline characteristics and genetic mutation.

Variables	Gene mutation frequency	*P* value
Age, <70 years vs ≥ 70 years,*n/N*		
RNA process	24/85 vs. 8/89	0.018
Sex, Male vs. Female (*n/N*)		
RNA process	22/104 vs. 10/102	0.025
RUNX3	11/104 vs. 1/102	0.003
ISS stage[a], I-II vs. III (*n/N*)		
NK-κB pathway	24/127 vs. 22/76	0.003
TRAF3	10/127 vs. 12/76	0.043
CARD11	4/127 vs. 0/76	0.031
R-ISS stage[a], I-II vs. III (*n/N*)		
DNA damage repair	18/168 vs. 7/35	0.019

[a] Three patients failed to detect the level of β2-microglobulin who could not stage in ISS and R-ISS. ISS: International stage system; R-ISS: Revised international stage system.

NRAS, FAT4, FAM46C, TP53, FAT3, DNAH9, PCLO, DIS3, and *BRAF.*[5] In our study, 206 NDMM patients received 92 target gene sequencing of the CDS region, in which 162 patients (78.6%) had at least one gene mutation. The top ten mutation genes were comparable to previous studies, in clouding *KRAS, NRAS, TRAF3, BRAF, TP53, MAX, DIS3, RUNX3, VCAN,* and *CYLD*. The recent research on target NGS detection from Thailand reported different ranks to the above-mentioned study.[13] One study conducted in 40 NDMM patients with 30 target gene sequencing reported the common mutations in Chinese were *ATM, CUL4B, IRF4, KRAS,* and *NRAS.*[14] The other study from China examined the unpurified BM samples of 28 target genes and reported the most common gene mutations were *NRAS, PRDM1, FAM46C, MYC, CCND1, LTB, DIS3, KRAS, CRBN, DNMT3A, IRF4,* and *BRAF.*[15] Our positive rate of gene mutation was improved by CD138 purified and expanded detection region to CDS compared to these two Chinese studies, while still lower than WES detection. In addition, there were 99 patients (48.1%) had the gene mutation of the *RAS/MAPK* pathway (*KRAS, NRAS,* and *BRAF*), and 45 (21.8%) patients with *NF-κB* pathway-related gene mutation (*TRAF3, CYLD, TRAF2, IRF4, IFKB2* and *TNFRSF21*), which is comparable with WES data in 463 NDMM patients (43% and 17% with *RAS/MAPK* and *NF-κB* pathway-related gene mutation).[6]

The genomic complexity of MM is also reflected in the correlation of gene mutations and cytogenetic abnormalities. The distinct IgH translocation subgroups have been reported enriched for some mutations. For example, *CCND1, IRF4, LTB* and *HUWE1* are almost exclusively mutated in t(11;14), while *FGFR3, PRKD2, ACTG1, DIS3* in t(4;14), *ATM, BRAF, MAF, TRAF2, EP300* and *DIS3* in t(14;16).[11,12] We also found a significant correlation between t(14;16) and t(14;20) translocations and *SP140, CDKN1B,* and *PRKD2* mutation, while *CCND1* and *TP53* mutations on one hand, and *KLHL6* on the other hand are almost exclusively mutated in t(11;14) and t(4;14), respectively. The variation of results may be due to differences in the population, number of cases, and detection methods of diverse studies. All these researches indicated the abnormal expression of fusion genes caused by IgH translocation may increase the mutation rate of some genes.[12] Moreover, data in our study also suggested patients with del 17p had a significantly higher ratio of *TP53* mutation compared with NDMM patients without del 17p (28.6% vs. 6.8%, $P = 0.016$). Laurence Lodédeng et al. found all *TP53* mutations in 20 patients presenting in del 17p group.[16] The other research involved 1766 tests and reported 27 of 28 patients with *TP53* mutation coexisted with del 17p.[17] A mutated *TP53* may further destabilize the genome, which promotes additional chromosome segregation errors of del 17p.[18] However, no research has confirmed the chronological order of del 17p and *TP53* mutation in NDMM patients, which remains further study to elucidate.

Based on the MyeloTama Genome Project (MGP) of WES detection in 1273 NDMM patients, Walker et al. established a high-risk, double-hit group by bi-allelic *TP53* inactivation or amplification 1q21 with ISS stage III.[7] In our research, six NDMM patients (2.9%) had del 17p coexisted

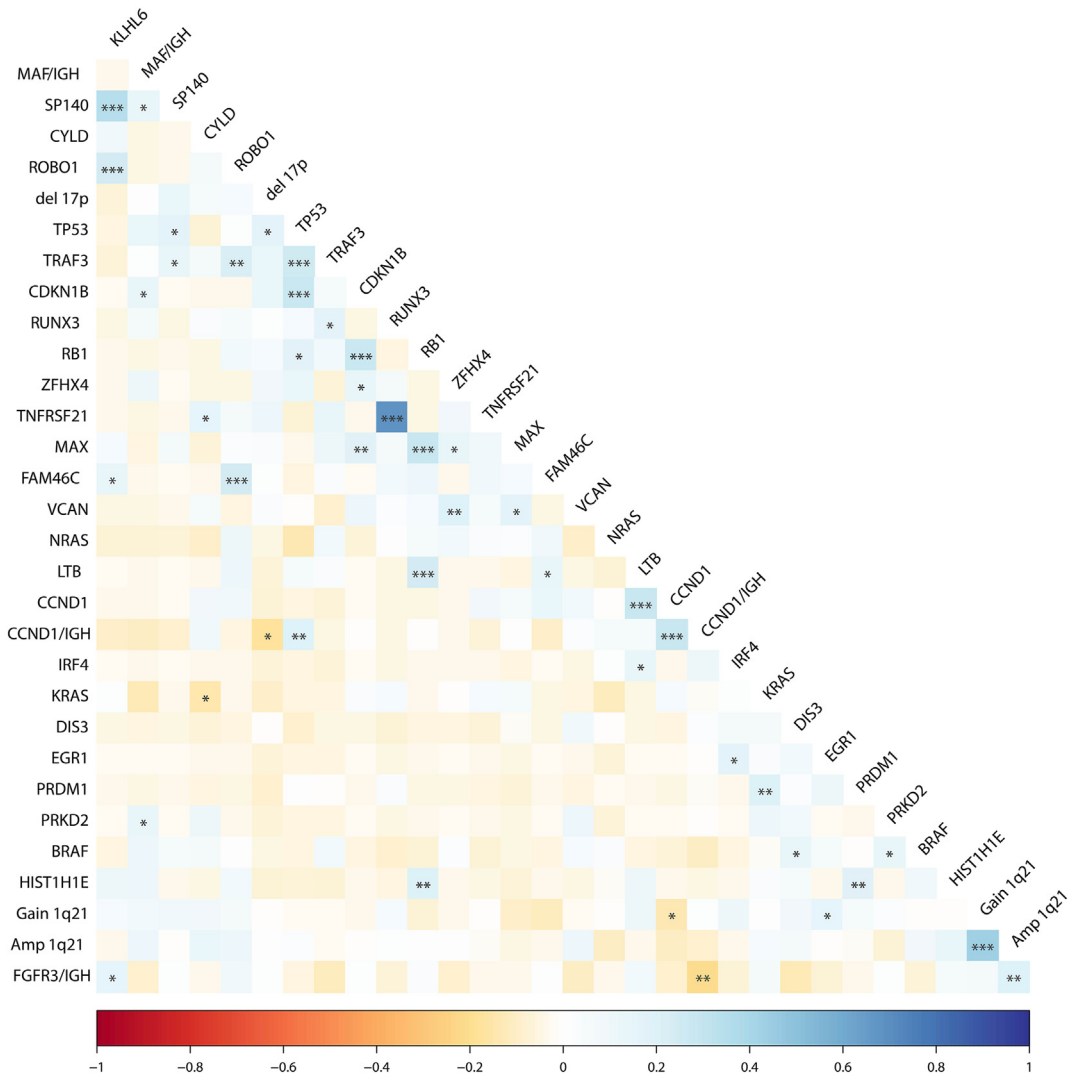

Figure 3. Pearson correlation analysis of gene mutation and cytogenetic abnormalities concurrence. *P value < 0.05, **P value < 0.001, **P value < 0.0001. The bar below presents the Pearson correlation coefficient for measuring linear dependence between two various.

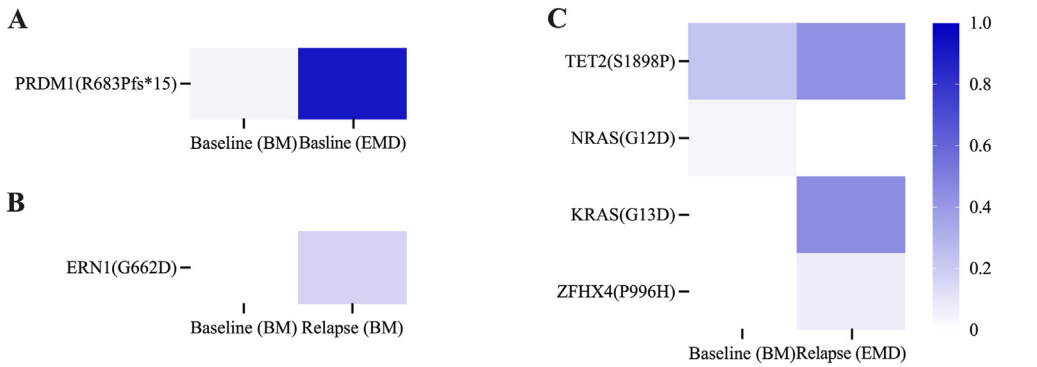

Figure 4. The alteration of genetic mutation in three patients with case-paired detection before treatment and after the first relapse. (A)the alteration of gene mutation between bone marrow in baseline and extramodular disease after relapse; (B and C) the alteration of gene mutation in bone marrow from baseline to relapse.

with *TP53* mutation in the other allele, while 9 with bi-allelic *TP53* mutation (4.4%). The target gene mutation detection by NGS including *TP53* can further identify ultra-high-risk patients, especially in patients with FISH detection negative. Taking the third patient in Figure 4 as an example, she had no high-risk cytogenetic abnormalities at diagnosis and no other high-risk factors displayed in NCCN guidline.[19] But NGS detection indicated she had *TET2* and *NRAS* (G12D) mutation. The *NRAS* (G12D) mutation has been identified as significantly reducing myeloma sensitivity to single-agent bortezomib therapy.[20] Meanwhile, Pavia et al. reported that *TET2* and *NRAS* were the most frequently mutated in NDMM patients with myelodysplastic syndrome-associated phenotypic alterations, indicating shorter PFS and overall survival (OS).[21] The

medicine targeting *NRAS* might overcome the poor prognosis of MM patients.[22] In addition, new mutations of *KRAS* and *ZFHX4* were detected in EMD tissue, which may be due to the spatial genomic heterogeneity[23] or tumor evolution.[24] Although these gene mutations were categorized as tier II with potential clinical significance,[25] they provide an important basis for prognosis in MM.

There were some limitations of this study. The relatively small samples to multiple multiparameters of baseline and short time of follow-up limited to analysis of the prognostic value of genetic mutations in this pilot. However, the present results provided a reliable method to examine the genetic mutation for myeloma patients which is authorized by invention patent grants. Our research is underway to conduct the multi-center cohort and expand the sample size to explore the prognosis value of genetic mutation further to help select the appropriate treatment option.

In conclusion, the genetic mutation of Chinese patients with MM demonstrates heterogeneity and is closely related to characteristics and cytogenetic abnormalities. The proposed mutated genes with prognostic value still need large cohort and prospective clinical trials to clarify.

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Authors contribution

Li Bao and Yutong Wang contributed to the study design and manuscript preparation. Bin Chu, Minqiu Lu, Lei Shi, Shan Gao, and Yuan Chen provided the patients and collected data. Na Ji conducted the FISH detection, while Qi Yan conducted the NGS detection. Yutong Wang and Mengzhen Wang analyzed the data which presented as the figures. All authors approved the final manuscript.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Beijing Jishuitan Hospital (No.202104-46). The pan-informed consent was written and obtained from each patient before data collection, detection, and analysis.

Data availability statement

The data supporting this study's findings are not publicly available due to containing information that could compromise the privacy of research participants; nevertheless, some data are available from Li Bao. Email: baoli@jst-hosp.com.cn.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpt.2023.12.004.

References

1. Liu J, Liu W, Mi L, et al. Burden of multiple myeloma in China: an analysis of the Global Burden of Disease, Injuries, and Risk Factors Study 2019. *Chin Med J*. 2023; 136:2834–2838. https://doi.org/10.1097/CM9.0000000000002600.
2. Cowan AJ, Green DJ, Kwok M, et al. Diagnosis and management of multiple myeloma: a review. *JAMA*. 2022;327:464–477. https://doi.org/10.1001/jama.2022.0003.
3. Walker BA, Mavrommatis K, Wardell CP, et al. Identification of novel mutational drivers reveals oncogene dependencies in multiple myeloma. *Blood*. 2018;132: 587–597. https://doi.org/10.1182/blood-2018-03-840132.
4. Harding T, Baughn L, Kumar S, Van Ness B. The future of myeloma precision medicine: integrating the compendium of known drug resistance mechanisms with emerging tumor profiling technologies. *Leukemia*. 2019;33:863–883. https://doi.org/10.1038/s41375-018-0362-z.
5. Bolli N, Biancon G, Moarii M, et al. Analysis of the genomic landscape of multiple myeloma highlights novel prognostic markers and disease subgroups. *Leukemia*. 2018;32:2604–2616. https://doi.org/10.1038/s41375-018-0037-9.
6. Walker BA, Boyle EM, Wardell CP, et al. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. *J Clin Oncol*. 2015;33:3911–3920. https://doi.org/10.1200/JCO.2014.59.1503.
7. Walker BA, Mavrommatis K, Wardell CP, et al. A high-risk, Double-Hit, group of newly diagnosed myeloma identified by genomic analysis. *Leukemia*. 2019;33: 159–170. https://doi.org/10.1038/s41375-018-0196-8.
8. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15: e538–e548. https://doi.org/10.1016/S1470-2045(14)70442-5.
9. Daudignon A, Quilichini B, Ameye G, Poirel H, Bastard C, Terre C. Cytogenetics in the management of multiple myeloma: an update by the Groupe francophone de cytogénétique hématologique (GFCH). *Ann Biol Clin (Paris)*. 2016;74:588–595. https://doi.org/10.1684/abc.2016.1178.
10. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38:e164. https://doi.org/10.1093/nar/gkq603.
11. Hassan H, Szalat R. Genetic predictors of mortality in patients with multiple myeloma. *Appl Clin Genet*. 2021;14:241–254. https://doi.org/10.2147/TACG.S262866.
12. Neuse CJ, Lomas OC, Schliemann C, et al. Genome instability in multiple myeloma. *Leukemia*. 2020;34:2887–2897. https://doi.org/10.1038/s41375-020-0921-y.
13. Jirabanditsakul C, Dakeng S, Kunacheewa C, Up Y, Owattanapanich W. Comparison of clinical characteristics and genetic aberrations of plasma cell disorders in Thailand population. *Technol Cancer Res Treat*. 2022;21:15330338221111228. https://doi.org/10.1177/15330338221111228.
14. Hu Y, Chen W, Wang J. Mutations in thirty hotspot genes in newly diagnosed Chinese multiple myeloma patients. *OncoTargets Ther*. 2019;12:9999–10010. https://doi.org/10.2147/OTT.S216289.
15. Fan Y, Wang SJ, Liu YF, et al. Gene mutation and overexpression of newly diagnosed multiple myeloma patients. *J Exp Hematol*. 2022;30:166–169. https://doi.org/10.19746/j.cnki.issn.1009-2137.2022.01.027.
16. Lode L, Eveillard M, Trichet V, et al. Mutations in TP53 are exclusively associated with del(17p) in multiple myeloma. *Haematologica*. 2010;95:1973–1976. https://doi.org/10.3324/haematol.2010.023697.
17. Thakurta A, Ortiz M, Blecua P, et al. High subclonal fraction of 17p deletion is associated with poor prognosis in multiple myeloma. *Blood*. 2019;133:1217–1221. https://doi.org/10.1182/blood-2018-10-880831.
18. Quintyne NJ, Reing JE, Hoffelder DR, Gollin SM, Saunders WS. Spindle multipolarity is prevented by centrosomal clustering. *Science*. 2005;307:127–129. https://doi.org/10.1126/science.1104905.
19. Callander NS, Baljevic M, Adekola K, et al. NCCN Guidelines® Insights: Multiple Myeloma, Version 3.2022. *J Natl Compr Canc Netw*. 2022;20:8–19. https://doi.org/10.6004/jnccn.2022.0002.
20. Mulligan G, Lichter DI, Di Bacco A, et al. Mutation of NRAS but not KRAS significantly reduces myeloma sensitivity to single-agent bortezomib therapy. *Blood*. 2014;123:632–639. https://doi.org/10.1182/blood-2013-05-504340.
21. Maia C, Puig N, Cedena MT, et al. Biological and clinical significance of dysplastic hematopoiesis in patients with newly diagnosed multiple myeloma. *Blood*. 2020;135: 2375–2387. https://doi.org/10.1182/blood.2019003382.
22. Balaratnam S, Torrey ZR, Calabrese DR, et al. Investigating the NRAS 5' UTR as a target for small molecules. *Cell Chem Biol*. 2023;30:643–657. https://doi.org/10.1016/j.chembiol.2023.05.004.
23. Rasche L, Chavan SS, Stephens OW, et al. Spatial genomic heterogeneity in multiple myeloma revealed by multi-region sequencing. *Nat Commun*. 2017;8:268. https://doi.org/10.1038/s41467-017-00296-y.
24. Dutta AK, Alberge JB, Sklavenitis-Pistofidis R, Lightbody ED, Getz G, Ghobrial IM. Single-cell profiling of tumour evolution in multiple myeloma - opportunities for precision medicine. *Nat Rev Clin Oncol*. 2022;19:223–236. https://doi.org/10.1038/s41571-021-00593-y.
25. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, American society of clinical oncology, and college of American pathologists. *J Mol Diagn*. 2017;19:4–23. https://doi.org/10.1016/j.jmoldx.2016.10.002.