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Landscapes of immunoglobulin heavy-chain gene repertoire and its cytogenetic abnormalities in Chinese patients with multiple myeloma

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Multiple myeloma (MM) is a heterogeneous disease, the full understanding of whose pathogenesis remains elusive. While B-cell receptors are known to play a pivotal role in myeloma pathogenesis, the characterization of immunoglobulin heavy-chain (*IGH*) gene repertoire and their clinical significance in Chinese patients has not been fully explored. In this study, we analyzed the profiling of clonal *IGH* gene rearrangements via NGS assay, and its cytogenetic abnormalities by FISH in a cohort of 301 Chinese patients with newly diagnosed MM. We identified a particular subgroup, which was characterized by a marked overrepresentation of *IGHV4-39*. Additionally, *IGHV4-39* was correlated with a higher somatic hypermutation rate and shorter HCDR3 length. Notably, *IGHV4-39* was significantly more prevalent in patients with high-risk cytogenetic abnormalities, particularly the recurrent *IGH* translocations involving t(4;14). Our findings, represented the largest *IGH* data in MM series from Asia, and investigated the association between specific *IGHV* and cytogenetic alterations in Chinese MM patients for the first time.

Keywords Multiple myeloma, Immunoglobulin heavy-chain gene rearrangement, Cytogenetic abnormalities, Next-generation sequencing, *IGHV4-39*

Multiple myeloma (MM) is a lymphoid neoplasm characterized by the abnormal proliferation of a pathological clone of B-cells at a specific stage of differentiation[1]. Like other types of B-lymphoid malignancies, immunoglobulin heavy-chain (*IGH*) gene rearrangement begins at the earliest stages of B-cell development and involves a series of recombination events between *V*, *D*, and *J* genes, as well as somatic hypermutation (SHM), resulting in the formation of unique *V(D)J* sequences[2,3]. This process confers an extremely high diversity to B-cell receptors repertoires, enabling them to recognize different antigens effectively[4]. The detection of *IGH* gene rearrangements via next-generation sequencing (NGS) has further facilitated the study and identification of clonal B-cell populations in the bone marrow. This technology has helped shed light on the biology of myeloma cells, indicating that they are post-switch B-cells that have undergone antigen selection after traversing the germinal center and that there is a bias in the usage of certain *IGHV* genes.

Over the past decade, significant efforts have been made to improve the clinical outcomes in MM. However, MM remains largely incurable, with most patients experiencing multiple relapses and eventually becoming refractory to treatment. As a heterogeneous disease, previous studies support MM development through a multistep process via the acquisition of sequential genetic hits. A significant proportion of myelomas (50–70%) carry translocations targeting the switch regions of the *IGH* genes located at chromosome 14q32[5]. These aberrant rearrangements juxtapose oncogenes into the proximity of the powerful *IGH* enhancers, driving abnormal expression of the translocated oncogenes. Several studies have hypothesized that these characteristics, together

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with the biased usage of certain *VH* gene segments, establish a molecular archaeology for myeloma and shape the IG repertoire prior to the acquisition of the malignant phenotype[6]. It is noteworthy that there is disparity in disease incidence and clinical behavior among patients from different ethnic and geographical backgrounds[7,8]. For instance, the median age of onset of MM in China is 57.9–59 years old[8,9], which is notably lower than that observed in Western countries[10–12]. While the *IGH* repertoire might be ethnicity-specific, neither this repertoire nor the applicability of NGS-based *IGH* clonality testing has ever been reported in Chinese populations with MM, except for our previous study[13,14].

Another notable point is that antigen stimulation is considered a key environmental driver of malignant transformation. In this regard, *IGH* gene segment usage in myeloma appears to reflect some degree of both positive and negative selection by environmentally encountered antigens[15]. Molecular studies of *IGH* gene rearrangements have provided valuable insights into the pathogenesis of MM in the context of epidemiology.

In this study, we aimed to better understand the repertoire of IG gene usage and molecular archaeology among Chinese patients with MM. We conducted an analysis of the *IGH* repertoire in 301 patients with newly diagnosed MM (NDMM). Our goal was to investigate the distribution and usage of clonal *IGH* gene rearrangements in the Chinese population with NDMM. We identified a unique subgroup with a biased usage of certain *IGHV* genes. We then investigated the usage of *D* and *J* genes, HCDR3 length, the status of SHM among this subgroup. Lastly, we explored the correlation between a specific *VH* gene and recurrent *IGH* translocations, aiming to uncover the relationship between antigen selection and chromosomal abnormalities in MM.

Materials and methods

Sample and clinical data

A total of 301 bone marrow samples were obtained from the MM specialty biobank of the National Clinical Research Center for Hematologic Diseases, sourced from NDMM patients treated at the First Affiliated Hospital of Soochow University between February 2019 and July 2024. The diagnostic and treatment response criteria for MM were assessed according to the criteria of the International Myeloma Working Group (IMWG) consensus[16].

Cytogenetics analysis by fluorescence in situ hybridization

The FISH (fluorescence in situ hybridization) panel included *IGH* translocations such as t(4;14) (p16.3; q32), t(11;14) (q13; q32), t(14;16) (q32; q23), and t(14;20) (q32; q21), as well as other aberrations such as Gain(1q21), deletion (Del) (13q14) and Del(17p13). Purified CD138 + plasma cells were isolated as previously reported[17]. High-risk cytogenetic abnormality (HRCA) was defined by the presence of any of t(4;14), t(14;16), t(14;20), or Del(17p13) at diagnosis[18]. Standard risk cytogenetic abnormality (SRCA) was defined by the absence of these abnormalities[18].

Clonality determination via NGS

IGH-based NGS of the *V(D)J* rearrangements was performed according to Chinese expert consensus on laboratory standardized technical specifications using NGS in MM[19]. Briefly, genomic DNA was isolated from fresh bone marrow aspirates at diagnosis, and purified DNA samples were quantified using the Qubit DNA Assay Kit (Qubit 4.0, Thermo Fisher Scientific, Inc.). Library preparation and clonality testing were conducted using the commercially available LymphoTrack assays kit-PGM (Invivoscribe, Inc. San Diego, CA, USA), targeting the *IGH* FR1 fragment. Sequencing results were analyzed using the LymphoTrackS5-PGM Software version 2.4.5 (Invivoscribe, Inc.). Sequence information was analyzed using IgBLAST (https://www.ncbi.nlm.nih.gov/igblast) databases and the international ImMunoGeneTics information system (IMGT) (http://www.imgt.org). The criteria for *IGH* clonality were determined as previously described[13]. The SHM status of *IGHV* was assessed using the closest germline gene[20].

Statistical analysis

R Studio (Version 4.2.3, https://www.r-project.org/) and GraphPad Prism 8.3.0 were utilized for all statistical analyses and plotting. The comparison of categorical variables was performed by the Chi-square test or Fisher's exact test. Median and interquartile ranges were calculated for quantitative variables. Mann–Whitney U test and Student t-test were employed to analyze intergroup differences in SHM rates and heavy-chain complementarity-determining region 3 (HCDR3) length. All statistical tests were two-tailed, and P-values < 0.05 were considered statistically significant.

Results

Baseline characteristics of the study population

A total of 301 patients were enrolled in the study, comprising 156 males and 145 females, with a median age of 61 years across the study population. Approximately 66.7% of patients were classified as Revised International Staging System (R-ISS) stage II. The predominant immunoglobulin isotype was IgG (58.2%), followed by IgA (27.1%), IgD (3.0%) and IgM (0.3%). 31 patients were light chain only MM and 3 patients were Non-secretory. FISH detected 78 patients (26.4%) were t(4;14), and 45 patients (15.2%) were t(11;14), 6 patients were t(14;16), and only 1 patient was t(14;20). There were 105 patients (35.5%) detected with HRCA. The other detailed characteristics of the 301 NDMM patients were presented in Table 1.

Distribution of clonal rearrangements

A total of 322 clonal *IGH* rearrangements from 301 patients were identified, comprising 282 productive and 40 unproductive rearrangements. The analysis flowchart of this study was summarized in Fig. 1. Among these patients, 262 cases (87.0%) presented only one productive rearrangement, 16 cases (5.3%) presented

Characteristic	N = 301
Gender—no. (%)	
Male	156 (51.8)
Female	145 (48.2)
Age, years—median [range]	61 [29–80]
Hemoglobin, g/L—median [range]	94 [39–169]
LDH, U/L- median [range]	157.8 [67.1–523.0]
Serum β2-microglobulin, mg/L—median [range]	3.6 [1.0–78.1]
ISS stage—no. (%)	
I	62 (21.3)
II	135 (46.4)
III	94 (32.3)
Missing	10
R-ISS stage—no. (%)	
I	43 (14.8)
II	194 (66.7)
III	54 (18.5)
Missing	10
Immunoglobulin isotype—no. (%)	
IgG	174 (58.2)
IgA	81 (27.1)
IgD	9 (3.0)
IgM	1 (0.3)
Light-chain only—no. (%)	31 (10.4)
Non-secretory—no. (%)	3 (1.0)
Missing	2
FISH—no. (%)	
t (4; 14)	78 (26.4)
t (11; 14)	45 (15.2)
t (14; 16)	6 (2.0)
t (14;20)	1 (0.3)
Del (13q14)	129 (43.6)
Del (17p13)	35 (11.8)
Gain (1q21)	171 (57.8)
Cytogenetic abnormality—no. (%)	
Standard risk	191 (64.5)
High-risk	105 (35.5)
Missing	5

Table 1. Clinical characteristics of 301 newly diagnosed multiple myeloma patients. *LDH,* Lactate dehydrogenase; *ISS,* International Staging System; *R-ISS,* Revised International Staging System.

one productive and one unproductive rearrangement, 18 cases (6.0%) presented only one unproductive rearrangement, 2 cases (0.7%) presented two productive rearrangements, and 3 cases (1.0%) presented two unproductive (see Supplementary Fig. S1 online).

IGH gene repertoire

To profile the *IGH* rearrangement repertoire, we analyzed the *V*, *D*, and *J* genes rearrangements. We identified 43 functional *IGHV* genes in 282 productive rearrangements. Among these, *IGHV3* was the most frequent subgroup (53.5%), followed by *IGHV4* (23.8%), *IGHV1* (8.9%), *IGHV2* (8.9%), *IGHV5* (3.5%), *IGHV7* (1.1%), and *IGHV6* (0.4%). According to the *IGHV* analyses, *IGHV3-30* was the most expressed (12.4%), followed by *IGHV3*-23 (8.2%), *IGHV4-39* (8.2%), *IGHV4-59* (6.7%), and *IGHV3-21* (6.0%), accounted for almost half of the series (41.5%) (Fig. 2a). Comparison between our series and other two studies previously reported[21,22], we found a significantly higher usage of *IGHV3-21* and *IGHV4-39* (6.0% vs. 3.1% and 8.2% vs. 3.4%, respectively; $P < 0.05$). Particularly, *IGHV4-39* usage was significantly more frequent in our study than in Western MM patients from previously reported by Gkoliou et al[21]. (8.2% vs. 3.4%, $P = 0.0037$) (Fig. 2b). And *IGHV3-9* and *IGHV4-31* was significantly lower than Korean MM patients [22] (2.8% vs. 11.6% and 2.1% vs. 9.3%, respectively; $P < 0.05$). *IGHV4-39* was not observed in 43 MM patients from Korea (see Supplementary Table S1 online).

Among the *IGHD* family, we defined 25 *IGHD* genes in our series. *IGHD3* and *IGHD2* were the two most abundant subgroups, comprising 36.4% and 16.0%, respectively. *IGHD3-10* (10.9%), *IGHD6-13* (9.5%), *IGHD3-*

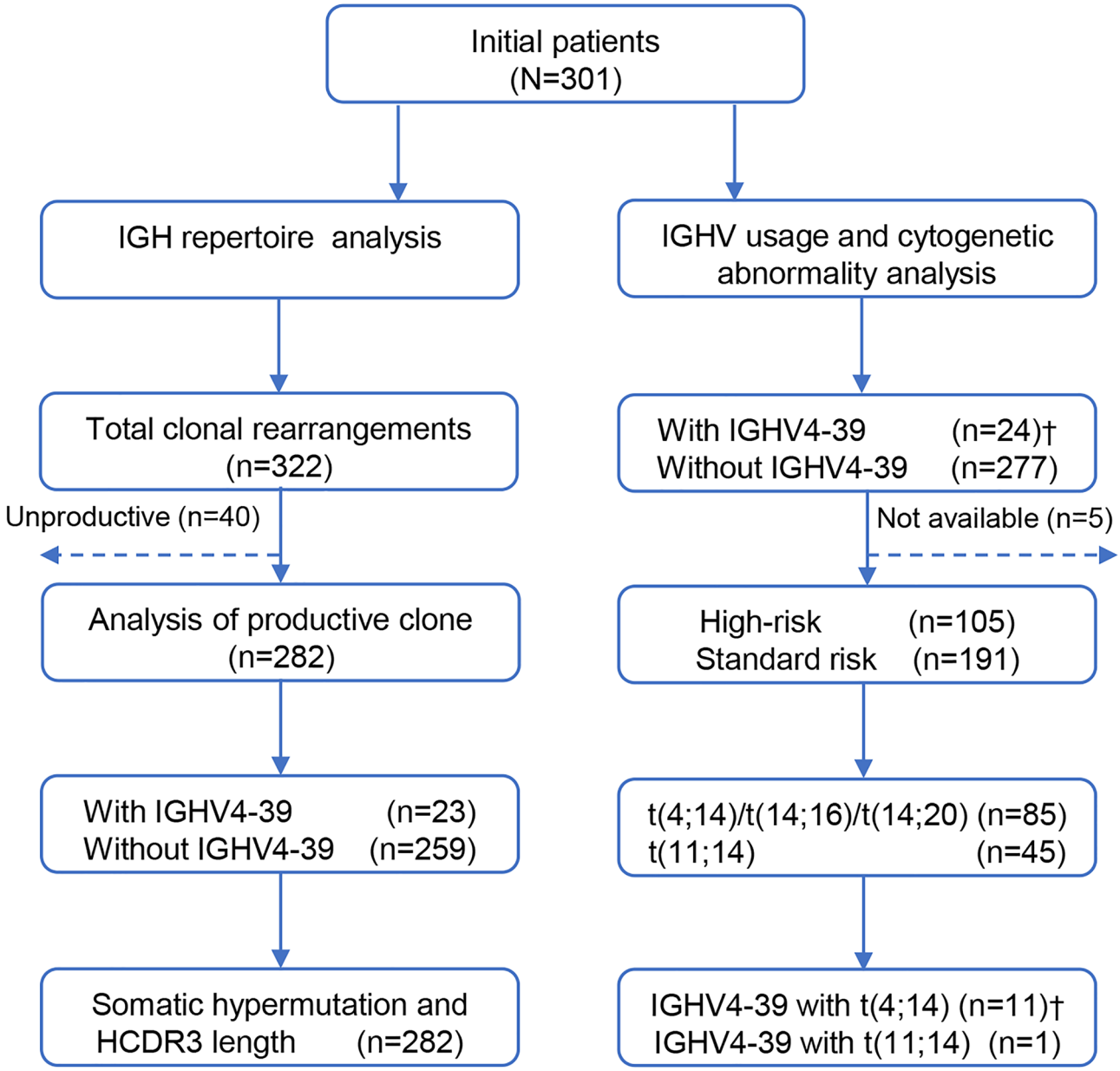


Fig. 1. Analysis flowchart. † Including one case with an unproductive rearrangement.

16 (8.4%), *IGHD1-26* (6.9%), *IGHD5-12* (6.9%) were the five predominant genes (Fig. 2c). The predominant *IGHD* gene didn't show any significant differences compared to other two groups (Fig. 2d). The detailed information on the comparison of *IGHD* gene among the three groups was summarized in Supplementary Table S2.

According to *IGHJ* gene usage, we noticed that, *IGHJ4* (45.6%) and *IGHJ6* (26.3%) were the most frequent segments in our cohort. In addition, the frequency of *IGHJ3* usage in our cohort was significantly lower than that in Western MM (7.8% vs. 13.6%, $P = 0.015$). Meanwhile, *IGHJ6* usage was significantly higher than that in Western series. (26.3% vs. 19.7%, $P = 0.030$) (Fig. 2e). There was no significant difference in other *IGHJ* genes among the three groups (see Supplementary Table S3 online).

Antigen selection imprint on IGHV4-39

Considering the remarkable bias of *IGHV4-39* usage in our series, we subsequently investigated the antigen selection imprint on *IGHV4-39*. In 23 MM patients with productive *IGHV4-39* rearrangements, no common antigen epitopes were found (see Supplementary Fig. S2 online). Each patient had their own unique HCDR3 sequence (see Supplementary Table S4 online). A biased pairing of certain functional *IGHD* genes and *IGHJ* genes was identified, with 7 out of 23 cases (30.4%) recombining with *IGHD3* and *IGHJ4* in addition to *IGHV4-39*. Further subgroup analysis revealed 3 cases with *IGHD3-10* and *IGHJ4* (Fig. 3a). The median rate of *IGHV* SHM with *IGHV4-39* was significantly higher compared to those without *IGHV4-39* (11.1% vs. 9.0%, $P = 0.0056$).

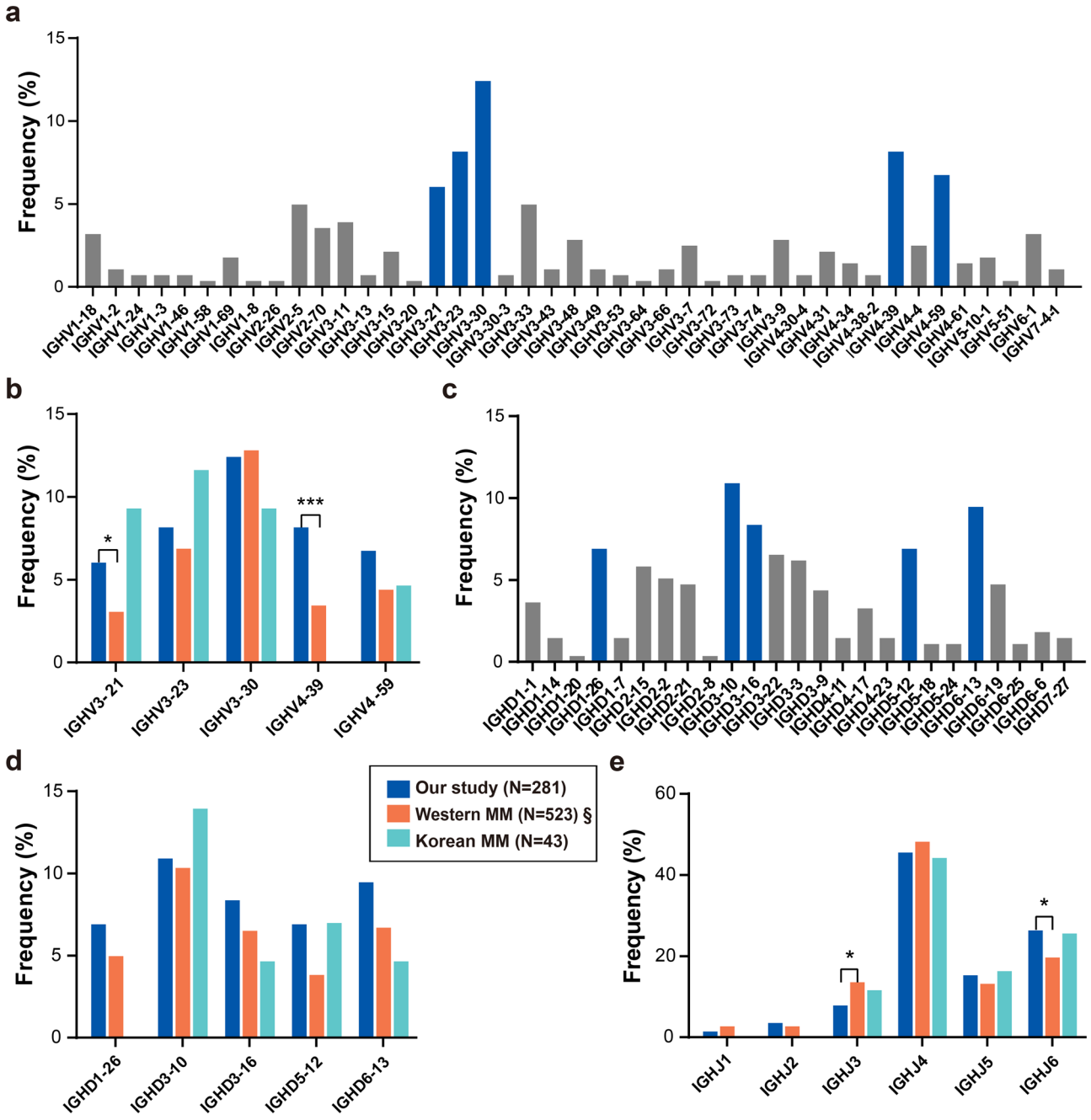


Fig. 2. *IGH* gene repertoire by NGS-based assay in multiple myeloma. (**a**) Bar plot showing the frequency of *IGHV* gene usage in our MM series: 43 *IGHV* genes were shown on the X-axis, with the five most abundant *IGHV* genes depicted in blue. (**b**) Comparison of the five most abundant *IGHV* genes between our series and two other previously reported studies. *P*-values were calculated using Chi-square test. (**c**) Bar plot showing *IGHD* gene usage in our cohort: 25 *IGHD* genes were shown on the X-axis, with the five most abundant *IGHD* genes depicted in blue. (**d**) Comparison of the five most frequent *IGHD* genes in three groups. *P*-values were calculated using Chi-square test. (**e**) Bar plot showing the *IGHJ* gene usage in our group and the comparison with two other groups. *P*-values were calculated using Chi-square test. * $P<0.05$, *** $P<0.005$. § 523 patients from collaborating institutions in Greece (n = 176), Italy (n = 72), Spain (n = 201), and the IMGT/LIGM-DB public database (n = 74). The sequence datasets from the Italian and Spanish groups have been reported previously[23,24].

Comparing the five most abundantly used *IGHV* subgroups, SHM rate of *IGHV4-39* was significantly higher than that of *IGHV3-21* (median: 11.1% vs. 7.8%; $P=0.0005$) (Fig. 3b). The length of the HCDR3 region was significantly shorter in patients with *IGHV4-39* than without *IGHV4-39* group (median: 14 vs. 16 amino acids, $P=0.0321$; Fig. 3c). Comparing between the *IGHV* subgroups, *IGHV4-39* was significantly shorter than *IGHV3-*

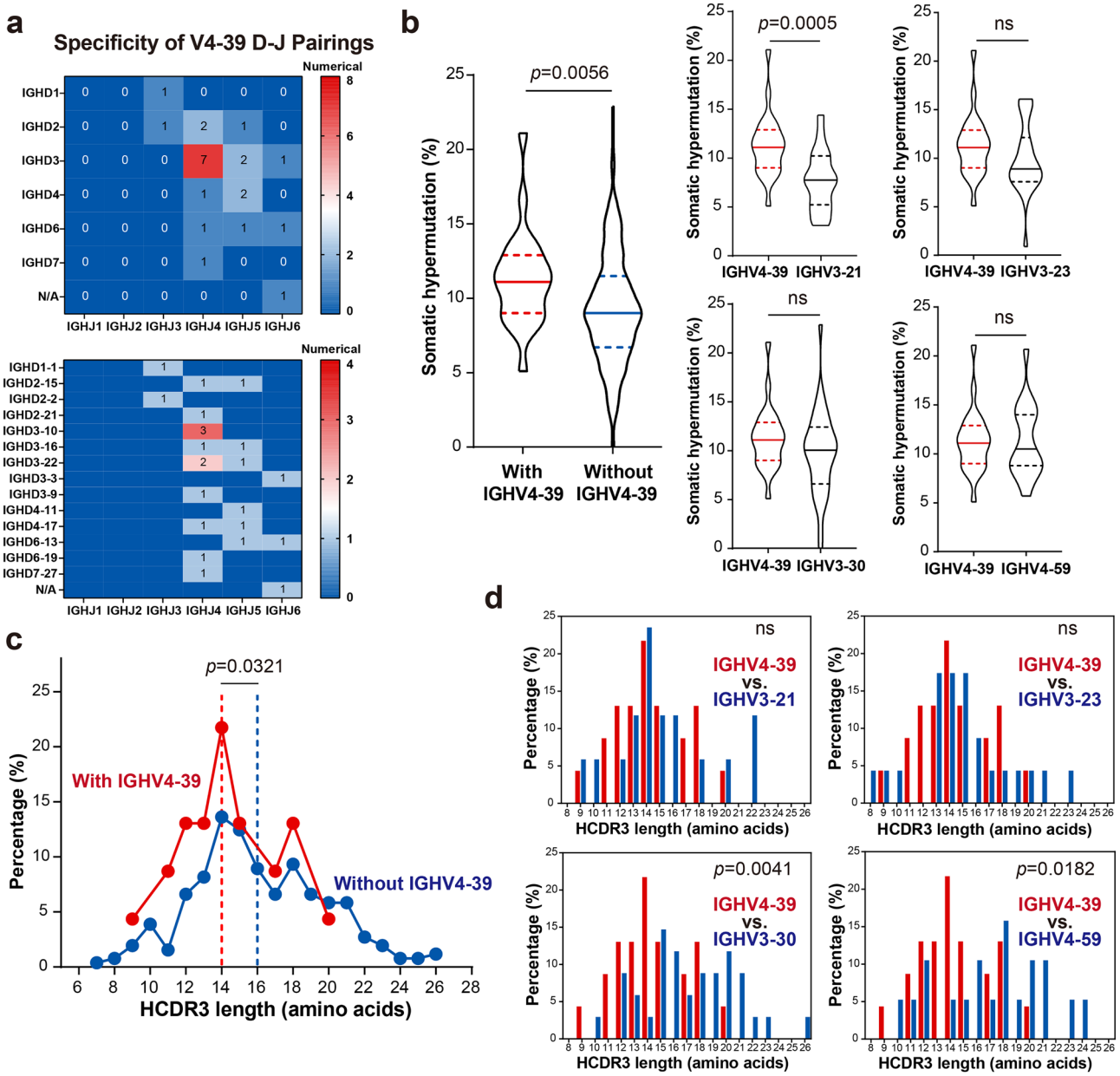
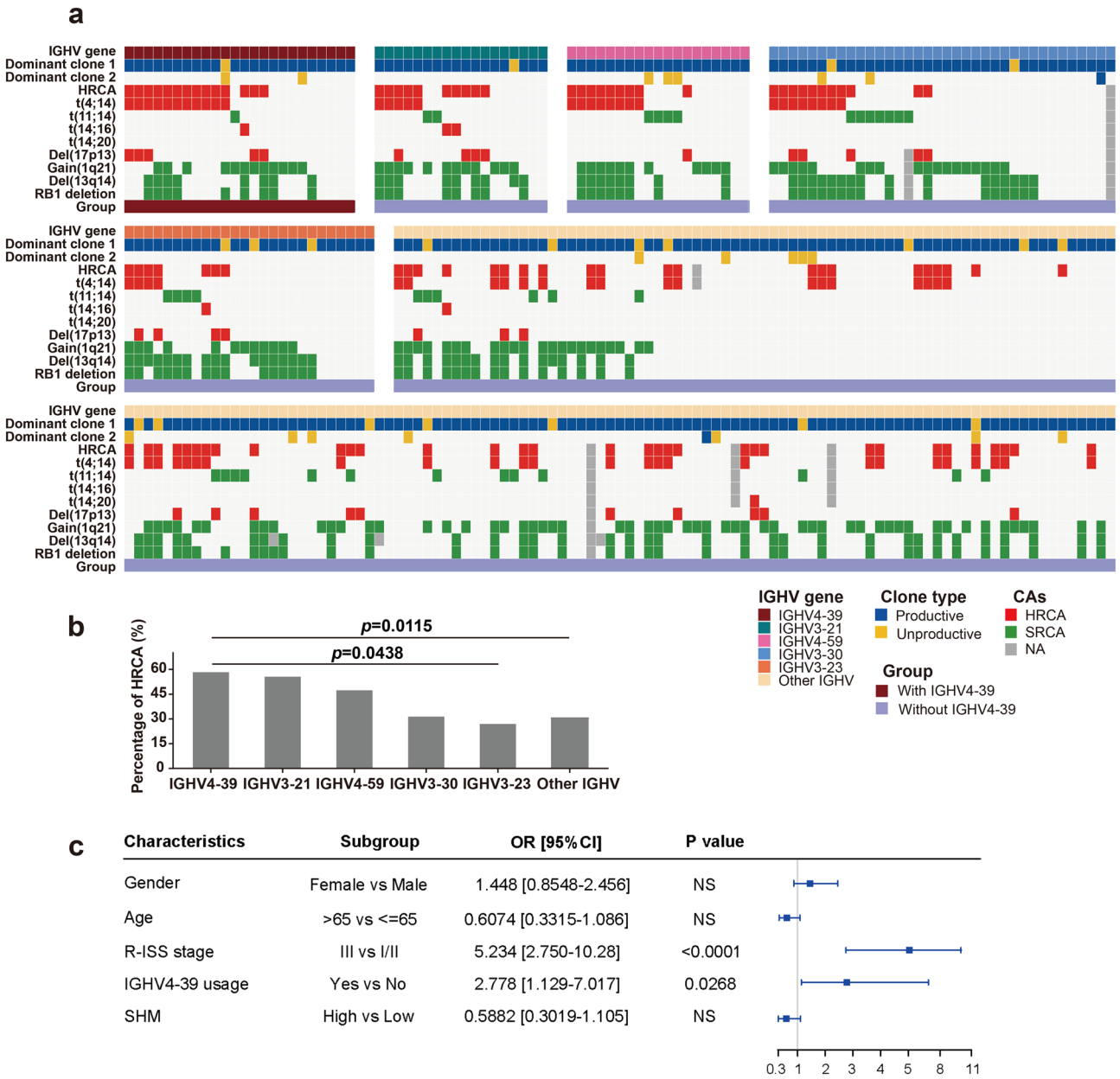


Fig. 3. Specificity characteristics of biased pairings, somatic hypermutation rates and HCDR3 lengths in multiple myeloma patients with *IGHV4-39* clonal rearrangement. (**a**) Heatmap showing the number of biased pairings of productive *IGHD* and *IGHJ* genes in *IGHV4-39* rearrangement (n = 23). (**b**) Violin plot showing the distinct somatic hypermutation rates in patients with *IGHV4-39* compared to other *IGHV*. *P*-values for each *IGHV* subgroup were calculated using Mann Whitney test, $P < 0.05$. (**c**) Line chart showing the percentage of distinct HCDR3 lengths in patients with and without *IGHV4-39*. Statistical differences were calculated by Mann Whitney test, $P < 0.05$. (**d**) Comparison of the HCDR3 lengths between *IGHV4-39* and four other *IGHV* gene subgroups. *IGHV3-21* group, n = 17; *IGHV3-23* group, n = 23; *IGHV3-30* group, n = 34; *IGHV4-59* group; n = 19. *P*-values for each comparison group were calculated using Student t-test, $P < 0.05$.

30 (mean: 14.5 vs. 17.2 amino acids; $P = 0.0041$) and *IGHV4-59* (mean: 14.5 vs. 17.1 amino acids; $P = 0.0182$) (Fig. 3d).

Cytogenetic abnormalities and IGHV4-39

FISH analysis was performed in 296 out of 301 patients (98.3%) at the time of diagnosis. The relationship between *IGHV* gene and cytogenetic abnormalities were illustrated in Fig. 4a. The proportion of *IGHV4-39* accompany with HRCA was significantly higher than that of *IGHV3-23* (58.3 vs. 26.9%; $P = 0.0438$) and other *IGHV* group (58.3% vs. 31.0%, $P = 0.0115$) (Fig. 4b). When comparing the baseline characteristic between patients with and without *IGHV4-39*, we observed that cytogenetic abnormality was the only statistically differential factor (High-risk: 58.3% vs. 33.8%; $P = 0.0294$), especially high-risk t(4;14) alteration (45.8% vs. 24.6%; $P = 0.0435$) (Table 2).



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Characteristics	Subgroup	OR [95% CI]	P value
Gender	Female vs Male	1.448 [0.8548-2.456]	NS
Age	>65 vs <=65	0.6074 [0.3315-1.086]	NS
R-ISS stage	III vs I/II	5.234 [2.750-10.28]	<0.0001
IGHV4-39 usage	Yes vs No	2.778 [1.129-7.017]	0.0268
SHM	High vs Low	0.5882 [0.3019-1.105]	NS

Fig. 4. Cytogenetic abnormalities and multivariate analysis in 301 multiple myeloma patients. (**a**) Landscape of *IGHV* gene subgroups and cytogenetic abnormalities. (**b**) Bar plot displaying the comparison of HRCA proportion between cases with *IGHV4-39* clonal rearrangement and five other *IGHV* subgroups. Statistical differences were calculated by Fisher's exact test, $P<0.05$. (**c**) The forest plots display the odds ratios (ORs) (95% CIs) and *P* values of clinical features derived multivariate logistic regression analyses for HRCA in the overall cohort. $P<0.05$. NS, not significant.

Furthermore, a multivariate analysis was performed to adjust for potential confounders such as age, sex, R-ISS stage and so on. We found that R-ISS stage (III vs. I/II: OR 5.234, 95% CI 2.75–10.28, $P<0.0001$) and *IGHV4-39* (Yes vs. No: OR 2.778, 95% CI 1.129–7.017, $P=0.0268$) contributed to HRCA (Fig. 4c). These results further confirmed an independent association between *IGHV4-39* and HRCA in our MM cohort.

We subsequently analyzed the correlation between the unique *IGHV* usage and *IGH*/14q32-ralated chromosomal translocations. We identified that *IGHV4-39* had the highest frequency (14.1%, 12/85) in patients involving *IGH*/14q32-related HRCA. *IGHV4-39* was the most frequently used (14.1%; 11/78) in t(4;14) group. Conversely, only 1 case with *IGHV4-39* (2.2%; 1/45) was detected in t(11;14) group (Fig. 5). But the statistical difference was not reached between two groups.

To explore the impact of SHM rate and HCDR3 length between t(4;14) and *IGHV4-39*. We divided all patients into four groups according to the cytogenetic abnormalities. The results showed t(4;14) with *IGHV4-39* had a significantly higher mutation rate than that of non-t(4;14) without *IGHV4-39* (median SHM: 12.0% vs. 8.9%,

Characteristic	With IGHV4-39 (n = 24)		Without IGHV4-39 (n = 277)		
	no	%	no	%	*P*-value
Age					0.4960[a]
< 65	17	70.8	177	63.9	
> = 65	7	29.2	100	36.1	
Gender					0.2991[a]
Male	10	41.7	146	52.7	
Female	14	58.3	131	47.3	
ISS stage					0.6786[a]
I	5	23.8	57	21.3	
II	13	52.4	122	45.7	
III	6	23.8	88	33.0	
Missing	0		10		
R-ISS stage					0.7320[b]
I	2	8.3	41	15.4	
II	17	70.9	177	66.3	
III	5	20.8	49	18.3	
Missing	0		10		
Cytogenetic abnormality					**0.0294**[a]
Standard risk	10	41.7	180	66.2	
High-risk	14	58.3	92	33.8	
Missing	0		5		
FISH					
t (4; 14)	11	45.8	67	24.6	**0.0435**[a]
t (11; 14)	1	4.2	44	16.2	0.1447[b]
t (14; 16)	1	4.2	5	1.8	0.4006[b]
t (14;20)	0	0	1	0.4	NS[b]
Del (17p13)	5	20.8	30	11.0	0.1801[b]
Gain (1q21)	12	50.0	159	58.5	0.5563[a]
Missing	0		5		

Table 2. Baseline characteristics in MM patients with and without clonal *IGHV4-39* gene rearrangements. Hypothesis testing: a, Pearson chi-square test; b, Fisher's exact test; *P* value < 0.05; NS, not significant.

P = 0.0278). There was no statistical difference among the other groups (see Supplementary Fig. S3a online). Furthermore, we analyzed the distribution of HCDR3 lengths and found no significant correlation between *IGHV* mutation rate or t(4;14) and HCDR3 length (see Supplementary Fig. S3b and S3c online).

Clinical relevance (an exploratory study)

To further investigate the potential clinical implications of *IGHV4-39*, we conducted an exploratory analysis of the association between *IGHV4-39* and clinical outcome. 274 patients were available for follow-up, including 24 cases with *IGHV4-39*. The median of follow-up time was 28 months (range: 1–75 months). No statistically significant differences were found between patients with or without *IGHV4-39* in both progression-free survival (PFS) and overall survival (OS) ($P > 0.05$, see Supplementary Fig. S4a and S4b online). However, these results should be interpreted with caution due to the overall patient population is highly heterogeneous, with all stages and different treatments.

Discussion

We investigated the *IGH* gene repertoire of a cohort of 301 NDMM with Chinese patients via NGS assay at diagnosis for the first time. Our results represented the largest *IGH* data in MM series from Asia. Consistent with previous studies[25], the vast majority of patients (93.0%) displayed at least one productive clone. Gkoliou et al.[21] studied *IGH* gene repertoire from Western MM patients in a largest population, including Greece (n = 176), Italy (n = 72), Spain (n = 201), and the IMGT/LIGM-DB public database (n = 74). Medina et al.[24] presented the characterization of *IGH* gene rearrangements in 413 myeloma patients treated in Spanish trials, including 113 patients characterized by NGS. Only 43 Korean patients were reported from Asia[22]. The repertoire of *IGH* gene usage in Chinese patients with MM has not been reported to date. We compared the *VDJ* gene usage among Chinese, Western, and Korean patients with MM. MM patients displayed a skewed *IGH* gene repertoire in our cohort. Consistent with Western population, *IGHV3-30*, *IGHD*3-10, and *IGHJ*4 was the most abundant *IGHV*, *IGHD* and *IGHJ* gene, respectively. However, variations were noted in the *VDJ* gene usage. Notably, *IGHJ6*

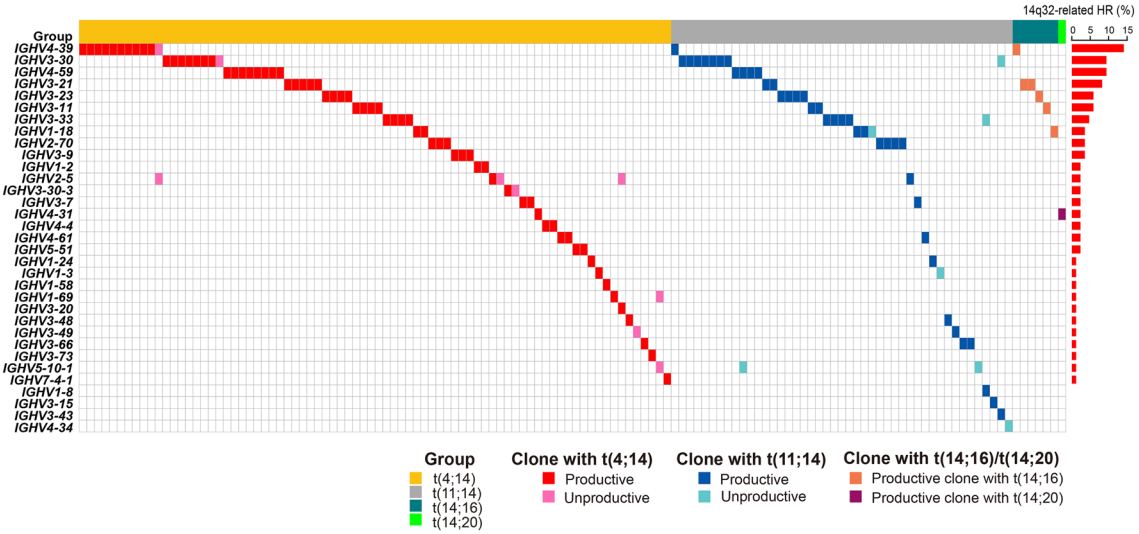

Fig. 5. Analysis of *IGHV* gene and *IGH*/14q32-ralated chromosomal translocations. Waterfall plots showing the association between *IGHV* gene and *IGH*/14q32-related cytogenetic abnormalities, including t(4;14), t(11;14), t(14;16) and t(14;20). The red bar plot on the right side showing the frequency of 14q32-related HRCA. NA, not available.

was more prevalent, whereas *IGHJ3* was relatively rare in Chinese patients compared to Western counterparts. Remarkably, *IGHV4-39* (8.2%) was prominently used in our patients, but relatively lower in Western MM cohorts with an incidence of 3.4%[21] and no in Korean patients[22]. This gene segment appears to be specifically favored in Chinese myeloma cases. Intriguingly, both our study[26] and previous research[27] indicated a higher incidence of *IGHV4-39* usage in Chinese chronic lymphocytic leukemia (CLL) compared to Western CLL. Medina et al.[24] reported that gene selection was biased in MM, with a significant overrepresentation of *IGHV3*, *IGHD2*, and *IGHD3*, as well as of the *IGHJ4* gene group, compared to the normal B-cell repertoire. Kim et al.[22] found *IGHV3-9*, *IGHV4-31*, and *IGHD3-3* were common in Korean patients with MM but no in Western patients. Furthermore, our and other previous studies have demonstrated that Chinese patients exhibit distinct *IGHV* gene usage and gene mutation profiles in CLL and Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia compared to Western cohorts[28,29]. These findings suggested the ethnicity and geography play roles in shaping *IGHV* usage. Therefore, the slightly different *IGH* gene repertoire, particularly the elevated frequency of *IGHV4-39* gene usage in Chinese MM patients, may be attributed to their distinct ethnic backgrounds and antigenic stimulation within the same disease context.

We further investigated the characteristics of *IGHV4-39* gene rearrangement, including the HCDR3 sequence, *D-J* gene pairing, SHM rate, and HCDR3 length. Notably, we observed that 30.4% of patients exhibited biased pairing of *IGHD3* and *IGHJ4* genes. *IGHV* genes play a crucial role in epitope binding affinity determination and B cell differentiation in B cell neoplasms[15]. Extensive studies have examined *IGHV* sequences in various human B-cell tumors. Previous research has confirmed that *IGHV4-39* was characterized by exhibits the highest affinity for non-muscle myosin II, an autoantigen potentially implicated in CLL promotion[30]. Furthermore, the usage of *IGHV4-39* and along with stereotyped HCDR3 has been identified as an independent risk factor for Richter syndrome transformation[31]. However, MM cases with stereotyped HCDR3 have not been identified so far[21]. Interestingly, a higher SHM rate and shorter HCDR3 length were observed in the patients with *IGHV4-39* compared to those without *IGHV4-39* in our cohort. The SHM status serves as evidence of the origin of tumor B cells and reflects the status of tumor clones before and after transformation[32]. It's established that the SHM status of *IGHV* is a prognostic factor in CLL[33]. Myeloma cases with mutated *IGHV* genes suggest derivation from B cells undergoing a germinal center reaction in response to antigen stimulation. The relatively higher mutation rate observed in *IGHV4-39* among MM patients prompts discussion on whether it reflects developmental antigen selection induced by a specific exogenous antigen.

To the best of our knowledge, this study is the first to elucidate the association between clonal *IGH* rearrangement and cytogenetic alterations in MM. We investigated the correlation between *IGHV* and cytogenetic abnormalities in MM patients using cytogenetic data obtained at diagnosis. The t(4;14) translocation was the most common *IGH* translocation in our cohort, consistent with a report by Fan H et al. in Chinese MM patients[34], which is higher than that reported in Western cohorts (~ 15–20%), as previously described[24]. We first observed that the *IGHV4-39* gene exhibited higher expression in MM patients with HRCA compared to other *IGHV* subgroups. Importantly, the *IGHV4-39* gene was more frequently detected in cases with t(4;14) than those without. The presence of specific cytogenetic abnormalities confers heterogeneity in prognosis. The t(4;14) defines a unique subtype associated with poor clinical outcomes, as previously reported[35]. Previous studies have identified certain genetic lesions, such as recurrent *IGH* translocations and hyperdiploidy, as oncogenic drivers in MM[36]. These translocations typically result in overexpression of partner genes. Among them, only the t(4;14) generates a fusion protein involving *MMSET* and *FGFR3* genes, which significantly influences disease behavior,

including transforming ability, impact on MM cell growth and survival, and clinical outcome[37]. However, it has been reported that the t(4;14) alone is insufficient to directly lead to myeloma. Recent research identified true high-risk t(4;14) patients by analyzing the coordinates of translocation breakpoints in the *NSD2* gene[38]. Similarly, in our study, we observed that cases with t(4;14) were more likely to be accompanied by the *IGHV4-39* gene segment, suggesting that distinct *IGHV* repertoires in MM patients may contribute to secondary genetic changes.

Our study also tries to explore the potential clinical implications of *IGHV4-39* gene rearrangement in an exploratory fashion. No statistically significant differences were found between patients with or without *IGHV4-39* for survival. Spanish's previous study presented the use of *IGHD2* and *IGHD3* groups were associated with improved prognostic features and prolonged progression-free survival rates for MM patients[24]. These findings unveiled *IGH* gene rearrangements could be considered as new molecular markers for PFS and OS in MM. Whether *IGHV4-39* could serve as a prognostic marker still need to further research. Additional studies are needed to better understand the nature and mechanism of *IGHV4-39*.

Several limitations should be considered in our present study. Although the overall cohort is large, only 24 cases were *IGHV4-39* rearrangement, some analyses (e.g., SHM rate and HCDR3 length) may be underpowered. Additionally, it might be insufficient for assessing the impact of *IGHV4-39* on clinical outcomes with a relatively small representative sample size and short follow-up period. Lastly, as a single-center retrospective study, the results may not be fully representative of the Chinese population. Further studies involving a larger number of Chinese patients with MM, especially those with *IGHV4-39*, would be helpful for confirming our observations. Corroboration from a larger cohort with more comprehensive data is necessary to ensure the validity of our study.

In conclusion, we have presented the distinct clonal profiles of *IGH* gene rearrangements in Chinese NDMM patients. The clonal *IGHV4-39* rearrangement, as a particular subgroup, was characterized by predominant usage, high SHM status and short HCDR3 length, as well as association with high-risk cytogenetic abnormalities involving t(4;14) in our cohort. Our findings, for the first time, profiled the *IGH* repertoire and investigated the correlation between specific *IGHV* and cytogenetic lesions in Chinese MM patients.

Data availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA010104) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human.

Received: 28 December 2024; Accepted: 26 May 2025
Published online: 02 July 2025

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Acknowledgements
All the samples were from Hematologic Biobank, National Clinical Research Center for Hematologic Diseases, Jiangsu Provincial Science and Technology Resources (Clinical Resources). Coordination Service Platform, The First Affiliated Hospital of Soochow University.

Author contributions
L.Y. and C.F. designed the study and edited manuscript. Y.G and L.Y. wrote the initial manuscript and analyzed the data. J.C.,Y.C., Y.C. and M.Z. performed Ig-NGS clonality assay. C.X. and T.T. collected patient's samples and performed DNA extraction. J.P., L.Y., J.S., S.J., X.S., W.Y. and S.Y. collected the data. Z.C., D.W. and S.C. gave comments and revised it for important content. All authors contributed to the final version of the manuscript.

Funding
This study was supported by grant from the National Key R&D Program of China (2019YFA0111000, 2022YFC2502700), the National Natural Science Foundation of China (82020108003, 82330008, 81400114, 81970136, 81970142), Jiangsu Hematology Medical Innovation Center (CXZX202201), the Open Project of Jiangsu Biobank of Clinical Resources (SBK202003001, SBK202003003).

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
The study was approved by the First Affiliated Hospital of Soochow University Medical Ethics Committee.

Informed consent
All enrolled patients provided written informed consent, in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, principles originating from the Declaration of Helsinki, and study site-specific regulations.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-04158-2.

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