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Cytogenetic evolution in myeloproliferative neoplasms with different molecular abnormalities

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ABSTRACT

We investigated the changes in chromosomal abnormalities in myeloproliferative neoplasm (MPN) patients during long-term follow-up. In total, 28 MPN patients (22 with primary myelofibrosis and 6 with polycythemia vera) were included. Among them, 25 patients underwent serial bone marrow (BM) biopsies during disease progression, and 3 patients had cytogenetic abnormalities at initial diagnosis but lacked follow-up BM biopsies. *JAK2*, *CALR*, and *MPL* mutation analyses were performed. Targeted sequencing analysis was conducted in 11 patients. Among the 28 patients, 21 (75.0%) had cytogenetic abnormalities either at diagnosis (8/26) or during follow-up. The median time from the initial analysis to the appearance of additional cytogenetic abnormalities was 8.4 years. Among the chromosomal abnormalities at initial diagnosis, trisomy 8 (3/26, 11.5%) was the most frequent, followed by gain of 1q, del(20q), and del(9q) (each in 2/26). Among all chromosomal abnormalities, including those that occurred during follow-up, the most frequent was del(20q) and +1q (8/28, 28.6%), followed by del(6p) (14.3%) and trisomy 8 (10.7%). Del(20q) was more frequent in *CALR*-mutated patients (4/6, 66.7%) than in *JAK2*-mutated patients (3/19, 15.8%, $P = 0.016$). The presence of cytogenetic abnormalities at initial diagnosis was associated with poor prognosis. Cytogenetic evolution may provide interesting insights into the disease course.

1. Introduction

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders that are characterized by the proliferation of myeloid lineages. MPNs comprise the following three major disease entities: polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocythemia (ET) [1]. MPNs are insidious diseases at early stages; however, with progression of the disease, the disease ends in bone marrow failure due to myelofibrosis or transformation to the blast phase [1]. The importance of key driver mutations in *JAK2*, *CALR* or *MPL* genes is well known in the pathogenesis of MPNs [2]. There are an increasing number of mutations under extensive research for their impact on the pathogenesis and prognosis of MPNs, including genes involved in epigenetic regulation, splicing, and signaling [3]. The ongoing studies on genetic abnormalities of MPNs provide insights into the emergence and progression of disease [2].

Meanwhile, cytogenetic abnormalities are detected in approximately 14–20% of PV patients [4–6], 30% to 50% of PMF patients [7–11], and 5%–10% of ET patients [12,13]. There is no specific MPN-defining cytogenetic abnormality; however, there are several recurrently found chromosomal abnormalities, including del(20q), +8, +9, +1q, and del(13q) [6,11]. These cytogenetic abnormalities have been revealed to be associated with the prognosis of MPN patients and incorporated into prognostic scoring systems of PMF. The previous scoring systems of MPN, the international prognostic system (IPSS) [7] and dynamic IPSS (DIPSS) [14], were modified to generate the DIPSS-plus [15], which includes karyotype information for the stratification of risk groups. The original DIPSS-plus system stratified cytogenetic risk groups into two tiers, “favorable” and “unfavorable” [15]. The unfavorable karyotypes included complex karyotypes, such as +8, −7/7q-, i(17q), −5/5q-, 12p-, inv.(3) or 11q23 rearrangements. The prognostic impact of cytogenetic abnormalities has been actively studied, and a recent study from a large cohort of PMF patients suggested a three-tiered cytogenetic risk model [11]. In addition to prognostic

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impact, data about cytogenetic abnormalities and their phenotypic and molecular correlations have been accumulated. Studies on the association of cytogenetic abnormalities and several recurrent somatic mutations of MPNs, such as *JAK2*, *CALR*, *MPL*, *ASXL1*, *SRSF2*, *U2AF1*, and *IDH1* and *IDH2*, have been performed [10,11].

Because the disease course of MPN is generally chronic, the temporal changes and clonal architecture of somatic mutations and their correlation with the disease course may provide valuable insights into the pathogenesis of the disease. Several studies were performed on the clonal evolution of somatic mutations and copy number variations [3,8,16–18]. However, long-term follow-up data on cytogenetic changes and their correlations with the molecular, hematologic, and clinical characteristics of disease are limited, especially since the discovery of novel mutations. In this study, we studied the changes in chromosomal abnormalities in MPN patients during long-term follow-up and investigated the correlation of the molecular and hematologic characteristics of the disease.

2. Materials and methods

2.1. Patients

In total, 28 MPN patients, including 22 PMF patients and 6 PV patients, were included in this study. Among them, 25 patients were selected for this study because they underwent serial bone marrow biopsies and conventional karyotyping during disease progression over a median of 12.3 years (range, 2.6–25.3 years). Three patients who did not have follow-up bone marrow biopsy results were also included in this study because they presented cytogenetic abnormalities at initial diagnosis. The intervals of follow-up bone marrow biopsies were variable among the patients, and the reasons for bone marrow biopsies were also variable, such as suspicion of disease progression due to aggrevation of anemia and transfusion dependency, appearance of blasts in peripheral blood or revisit of the patients after long-term follow-up loss. Bone marrow biopsies were performed twice in 12 patients, 3 times in 9 patients, and 4 times in 4 patients. Among 12 patients in whom bone marrow biopsies were performed twice, 2 patients underwent follow-up bone marrow biopsies within 2 to 3 years of the initial diagnosis, 2 patients within 7 years of initial diagnosis, and 8 patients within 10 years of initial diagnosis. The information about the patients is presented in Supplemental Table S1. The MPNs were diagnosed according to the 2016 revised World Health Organization (WHO) classification criteria [1]. All the bone marrow specimens were reviewed by two hematopathologists (S.Y.K. and M.K.) and the diagnosis and subclassification of MPN were reassessed according to the WHO 2016 criteria. Bone marrow fibrosis (MF) was assessed according to the European consensus grading system on a scale of MF-0 to MF-3 [19]. At the initial diagnosis, among 22 PMF patients, 4 patients had reticulin fibrosis grade 2 or 3 and were classified as overt PMF, and the remaining 18 patients had grade 0 or 1 fibrosis and satisfied other criteria for the diagnosis of PMF including megakaryocytic proliferation and atypia; these patients were diagnosed as prefibrotic/early PMF. Fibrosis was sequentially assessed in the follow-up bone marrow samples. The following laboratory and clinical information was obtained for each patient: dates of diagnosis and therapy initiation, age, sex, hemoglobin levels, leukocyte count, platelet count, and conventional G-banding cytogenetic analyses of bone marrow cells. Six patients whose mutational analysis data were reported in a previous study were included in this cohort [20]. Cytogenetic studies were performed as part of the routine diagnostic work-up, and karyotypes were recorded according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016 [21]. This study complied with the Declaration of Helsinki. This study was reviewed and approved by the Chungnam National University Hospital Institutional Review Board (Daejeon, Republic of Korea).

2.2. Molecular analysis

Genomic DNA was extracted from either bone marrow aspirate samples or peripheral blood of 21 patients. For 7 patients, *JAK2* V617F mutation-positive results were available from medical records. DNA was extracted using the ExiPrep kit (Bioneer, Daejeon, Korea) according to the manufacturer's instructions. For those patients who had gDNA available, allele-specific PCR and pyrosequencing were performed for *JAK2* V617F analysis according to previously described methods [20,22,23]. For 7 patients, *JAK2* V617F mutation-positive results were available from medical records; *JAK2* V617F mutation analysis was performed by allele-specific PCR and Sanger sequencing. Sanger sequencing, pyrosequencing, and fragment length analysis were performed to detect the *CALR* exon 9 frame-shift mutation as described in a previous study [20,24]. The results of the *CALR* mutational analysis of 6 patients for whom the *CALR* results were positive were reported in a previous study [20]. For mutation analysis of the *MPL* gene, Sanger sequencing of *MPL* exon 10 was performed as previously described [24]. The PCR products were purified and sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA) and an AB 3500 automatic sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the primers described. For eleven samples, next-generation sequencing (NGS) was performed as a part of a targeted gene panel that included 138 genes for myeloid malignancies, including *JAK2*, *MPL*, *CALR*, *SRSF2*, *SF3B1*, *U2AF1*, *CSF3R*, *IDH1*, *IDH2*, *TP53*, *ABL1*, and *BCR*. The gDNA shearing, standard library production and hybridization were performed by Celemics (Celemics Inc., Seoul, Korea), followed by paired-end 150-bp rapid-run sequencing on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA). The raw data were mapped to the reference genome (hg19). Variant analysis pipelines, annotation, and filtering processes were performed as described in previous studies [20,25]. For eleven samples with NGS data, additional copy number analyses were performed with the CopywriteR software package [26] using data from a normal peripheral blood sample as a control. CopywriteR is a tool for detecting copy number aberrations using targeted sequencing data by exploiting the off-target sequencing reads. The copy number analyses by CopywriteR were performed using R 3.5.1 (www.r-project.org).

2.3. Statistical analysis

Fisher's exact test and the χ^2 test were used to compare categorical variables, and the Mann-Whitney *U* test was used for continuous variables. The overall survival (OS) and the time from the initial diagnosis to when MF-2 or MF-3 bone marrow fibrosis was detected were estimated using the Kaplan-Meier method, and differences between the survival curves were analyzed using the log-rank test. Statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Chromosomal abnormalities and other characteristics

The clinical and laboratory characteristics of the 28 patients (22 PMF patients and 6 PV patients) are presented in Table 1. Bone marrow cellularity and megakaryocyte counts were not significantly different between PMF and PV patients. Among the 26 patients for whom the initial karyotyping results were available, excluding 2 patients for whom no metaphase data were available, 8 patients (30.8%) had cytogenetic abnormalities at diagnosis. Ultimately, chromosomal abnormalities were found in 21/28 patients (75.0%) either at diagnosis or during follow-up. The median time from diagnosis to the detection of the first cytogenetic abnormalities was 7.2 years (range, 0.0–24.4 years). Fifteen patients presented with the emergence of novel

Table 1
Clinical and laboratory characteristics of the 28 patients with myeloproliferative neoplasms (MPNs).

Variable	Total MPN (n = 28)	PMF (n = 22)	PV (n = 6)	*P*-value‡
Male/female (% male)	12/16 (42.9)	10/12 (45.5)	2/4 (33.3)	0.595
Age at diagnosis (years)	59.0 (34.8–80.5)	59.0 (36.6–80.5)	60.0 (34.8–65.4)	0.867
Hemoglobin (g/dL)	13.5 (7.1–20.6)	11.8 (7.1–20.6)	18.6 (15.1–19.4)	0.002
Leukocytes ($\times 10^9$/L)	11.68 (3.44–47.27)	11.27 (3.44–47.27)	13.43 (8.46–31.50)	0.401
Absolute neutrophils ($\times 10^9$/L)	7.85 (0.74–37.82)	5.13 (0.74–37.82)	8.85 (4.81–21.42)	0.314
Platelets ($\times 10^9$/L)	669 (16–1786)	776 (16–1786)	356 (134–756)	0.038
Circulating blasts (%)	0 (0–1)	0 (0–1)	0 (0–1)	0.316
BM cellularity (%)	83 (50–100)	83 (50–100)	78 (55–95)	0.754
Megakaryocytes per HPF in initial BM sample[a]	10.3 (1.2–25.0)	10.8 (1.2–25.0)	6.2 (4.0–16.0)	0.168
Megakaryocytes per HPF in follow-up BM sample[a]	8.0 (1.2–30.4)	8.9 (1.2–30.4)	5.5 (4.0–10.0)	0.218
Chromosomal abnormalities at initial diagnosis, n (%)	8/26 (30.8)	7/21 (33.3)	1/5 (20.0)	0.562
Chromosomal abnormalities at initial diagnosis or during follow-up, n (%)	21 (75.0)	16 (72.7)	5 (83.3)	0.595
Time from diagnosis to detection of chromosomal abnormalities (years)	7.2 (0.0–24.4)	6.0 (0.0–18.4)	11.2 (0.1–24.4)	0.137
No. of cases with additional chromosomal abnormalities were occurred during follow-up, n (%)	15 (53.6)	11 (50.0)	4 (66.7)	0.468
Time to detection of additional abnormal clones (years)	8.4 (3.6–24.4)	8.3 (3.6–18.4)	13.8 (3.7–24.4)	0.240
Time from initial diagnosis to chromosomal testing with no clonal change (years)	7.8 (2.6–19.5)	7.7 (2.6–19.5)	10.7 (10.7–10.7)	0.439
Mutations				0.306
JAK2 V617F mutation	19 (67.9)	13 (59.1)	6 (100)	
CALR mutations	6 (21.4)	6 (27.3)	0 (0)	
MPL mutation	2 (7.1)	2 (9.1)	0 (0)	
Negative for *JAK2*, *CALR*, and *MPL* mutations	1 (3.6)	1 (3.6)	0 (0)	
Reticulin fibrosis (MF 2-3) at initial diagnosis, n (%)	4 (14.3)	4 (18.2)	0 (0)	0.259
Progression of fibrosis in subsequence disease course, n (%)[b]	24 (85.7)	21 (95.5)	3 (50.0)	0.005
Progression to accelerated or blast phase, n (%)	4 (14.3)	3 (13.6)	1 (16.7)	0.851
Deceased, n (%)	7 (25.0)	7 (31.8)	0 (0)	0.111
Follow-up (years)	11.1 (0.2–25.5)	10.2 (0.2–20.9)	11.8 (1.1–25.5)	0.823

Data are presented as the median (range) for continuous variables and the number of cases (percentage) for categorical variables unless otherwise indicated.
Abbreviations: BM, bone marrow; HPF, high power field; MF, myelofibrosis; n, number; PMF, primary myelofibrosis; PV, polycythemia vera.
[a] Average megakaryocytes counted in 10 high power fields (400×) from bone marrow biopsies.
[b] The number of patients who presented with bone marrow fibrosis (MF 2-3 at the final follow-up test).
‡ *P* values were calculated using the χ^2 test for categorical variables and the Mann–Whitney U test for continuous variables between the PMF and PV patient groups.

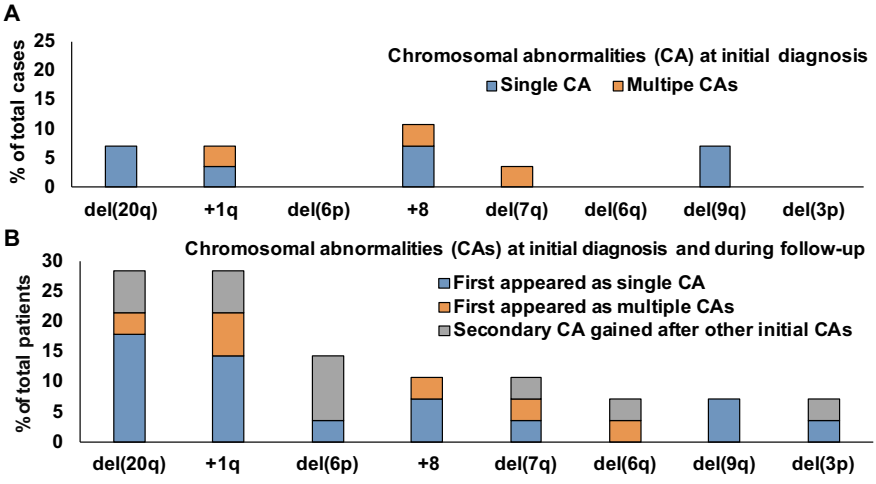

Fig. 1. The frequency distributions of common chromosomal abnormalities (CAs) in 28 myeloproliferative neoplasm (MPN) patients. (A) The chromosomal abnormalities at initial diagnosis were classified as single abnormalities or multiple abnormalities. (B) The total chromosomal abnormalities at initial diagnosis and during follow-up are presented, and the frequencies of abnormalities that first appeared as single abnormalities, abnormalities that appeared with other abnormalities and secondary abnormalities that were gained after other abnormalities.

cytogenetic abnormalities either from normal karyotypes (13 patients) or from abnormal karyotypes with single chromosomal abnormalities (2 patients), and the median time to the detection of additional karyotypes was 8.4 years (range, 3.6–24.4 years). Among 9 patients for whom the follow-up cytogenetic data were available and who exhibited either stable normal karyotypes (7 patients) or single chromosomal abnormalities (2 patients) at diagnosis, the median duration of follow-up with no clonal cytogenetic changes was 7.8 years (range, 2.6–19.5 years). There were 19 patients (67.9%) with *JAK2* V617F mutation, 6 patients (21.4%) with *CALR* mutations, 2 patients (7.1%) with *MPL* mutations, and 1 patient (3.6%) without mutations. In total, 6 PV patients had *JAK2* V617F mutations, 13 PMF patients (59.1%) had *JAK2* V617F mutations, and 6 PMF patients (27.3%) had *CALR* mutations. At initial diagnosis, 4 PMF patients presented with MF-2 or MF-3 bone marrow fibrosis. Ultimately, 24 patients (85.7%) had MF-2 or MF-3 bone marrow fibrosis during follow-up.

3.2. Chromosomal abnormalities and molecular abnormalities

Among the chromosomal abnormalities found in 8 patients who presented abnormalities at initial diagnosis, the most frequent chromosomal abnormality was trisomy 8 (3/26, 11.5%), and the gain of the long arm of chromosome 1 (+1q), del(20q), and del(9q) were each detected in 2/26 patients (%) (Table 2 and Fig. 1A). Seven patients (87.5%) presented single chromosomal abnormalities, and 1 patient presented multiple chromosomal abnormalities (47,XY,+1,der(1;7)

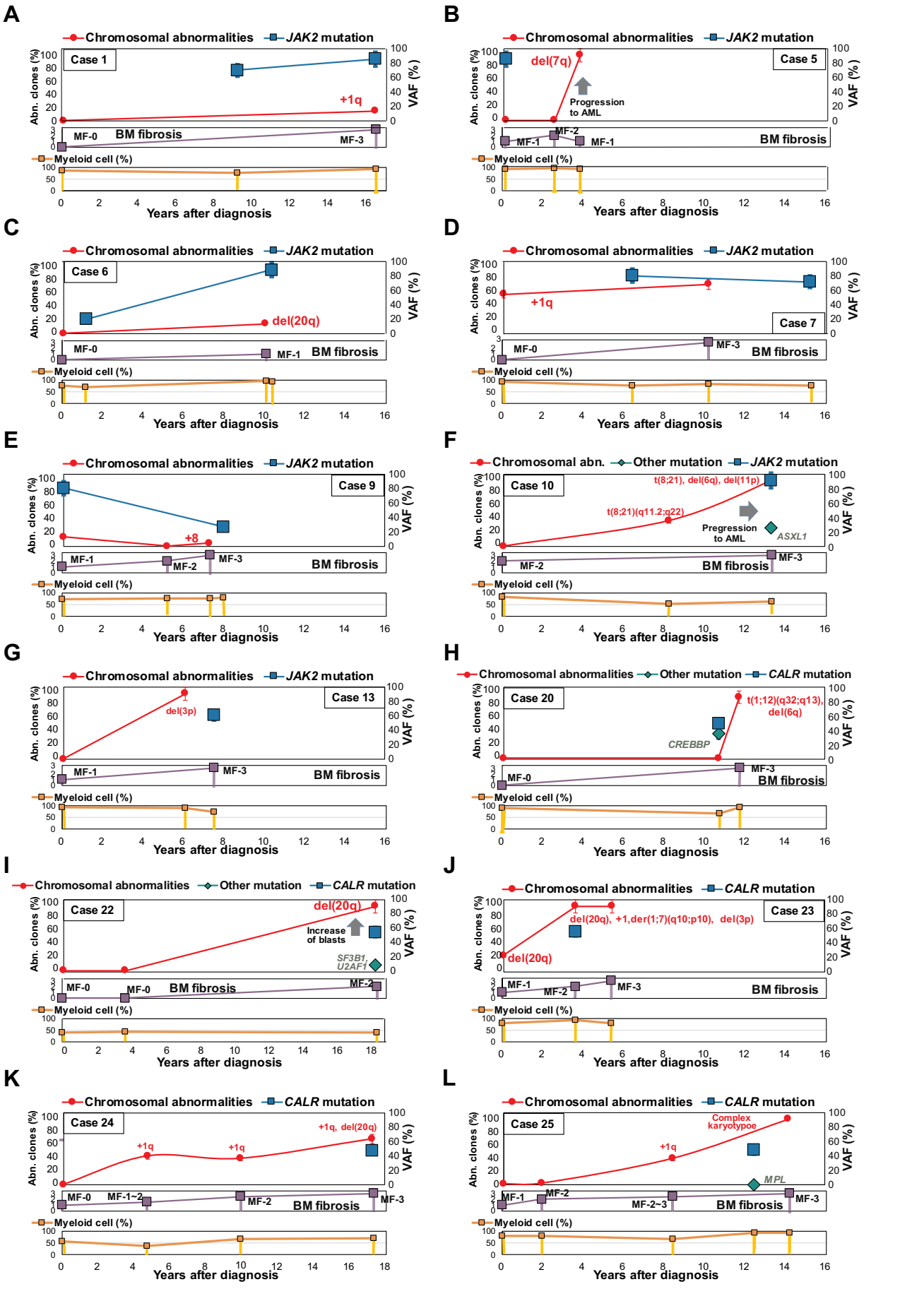

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Fig. 2. The percentage of metaphases with chromosomal abnormalities and the variant allele frequencies (VAFs) of *JAK2* or *CALR* mutations are plotted according to years after diagnosis for 12 patients (A–L) for whom mutation burden data were available and cytogenetic abnormalities were detected. The bone marrow fibrosis results were presented as MF-0 to MF3, and the percentages of myeloid cells in the peripheral blood when cytogenetic and molecular testing was performed are also presented.

(q10;p10),+8[14]/46,XY[6]). Ultimately, 21/28 patients (75.0%) presented cytogenetic abnormalities during the overall follow-up period. Twelve patients (57.1%) had single abnormalities, 3 patients (14.3%) had two abnormalities, 3 patients had three abnormalities, and 3 patients had ≥5 chromosomal abnormalities. Therefore, 6 patients (28.6%) had complex karyotypes. The +1q and del(20q) abnormalities were most frequently found (8/28 patients, 28.6%), followed by del(6p) (4/28, 14.3%) and trisomy 8 and del(7q) (each in 3/28 patients, 10.7%). Approximately 25% of del(20q) and +1q appeared as secondary abnormalities, and approximately 75% of del(6p) appeared as secondary abnormalities after the appearance of other chromosomal abnormalities (Fig. 1B). The frequency of +1q was not significantly different between *JAK2*- and *CALR*-mutated patients (4/19 vs 3/6; $P = 0.169$). Del(20q) was more frequent in *CALR*-mutated patients (4/6, 66.7%) than in *JAK2*-mutated patients (3/19, 15.8%, $P = 0.016$). Two patients with *CALR* mutations presented del(20q) at initial diagnosis, and 4 patients developed del(20q) during disease progression. The temporal changes in chromosomal abnormalities and percentages of abnormal metaphases and *JAK2* and *CALR*-mutant allelic burdens in each patient are presented in Fig. 2. The metaphases with cytogenetic abnormalities usually demonstrated an increasing trend over time, and the bone marrow fibrosis progressed accordingly. In addition, the targeted sequencing results of follow-up samples from 11 patients revealed additional molecular abnormalities in 6 patients in genes including *ASXL1*, *TP53*, and *SF3B1* (in ≥2 patients) and *CREBBP*, *U2AF1*, *MPL*, *NRAS*, *GNAS*, *RUNX1* (each in 1 patient) (Supplemental Table S1). These additional abnormalities had lower variant allele frequencies (VAFs) than the VAFs of *JAK2* or *CALR*. The copy number analysis of off-target reads in the targeted sequencing data had variable results according to each sample and each cytogenetic locus and were not exactly matched with the G-banding karyotype results. However, the initial cytogenetic abnormalities that were present in all the metaphases tended to be matched with the copy number analysis results by CopywriteR (Supplemental Table S1). In Case 10, G-banding analysis showed an abnormal karyotype of 46,XX,del(6)(q22),t(8;21)(q11.2;q22),del(11)(p13) [20] in a follow-up sample. NGS analysis of these samples revealed a *JAK2* V617F VAF of 90.9%, which suggested uniparental disomy. The two single nucleotide variants (SNVs) in exon 6 and exon 19 of the *JAK2* gene had VAFs of 84.5% and 85.7%, which suggested uniparental disomy in much of the *JAK2* gene. The additional *ASXL1* G646fs mutation had a VAF of 28.3%, which suggested that the *ASXL1* mutation was a secondary abnormality. The copy number analysis of NGS data demonstrated partial copy loss in 6q and 11p, which matches the karyotype results, and copy loss in 12q, which was not a detectable locus in the G-banding analysis (Fig. 3A). Although the total metaphase analysis revealed cytogenetic abnormalities, copy number abnormalities in 6q and 11p were partially observed (Fig. 3A). In Case 22, del(20q) emerged as a clonal change, which was clearly observed in the NGS data (Fig. 3B). In these samples, *U2AF1* Q157R and *SF3B1* K700E mutations were observed with VAFs of 10.4% and 5.1%, respectively.

3.3. Prognostic impact of cytogenetic abnormalities

The OS of patients was investigated according to the presence of chromosomal abnormalities at initial diagnosis and the presence of overall chromosomal abnormalities either at diagnosis or follow-up periods (Fig. 4). Both in the analysis of all patients and PMF patients, the chromosomal abnormalities at initial diagnosis were associated with poor OS (*P* values, 0.021 and 0.046, respectively; Fig. 4A and C). However, the OS of patients who presented with chromosomal abnormalities at any point in the follow-up period did not differ from that of patients who persistently had normal karyotypes (Fig. 4B and D). These results were the same as those for the progression of fibrosis; the presence of chromosomal abnormalities at initial diagnosis was associated with earlier bone marrow fibrosis (Fig. 4E); however, patients who presented with chromosomal abnormalities at any point during the follow-up period did not have differences in the rate of fibrosis progression compared with patients with persistently normal karyotypes (Fig. 4F). Patients with the chromosomal abnormalities of del(20q) and del(9q) at initial diagnosis had worse OS than patients with +1q, trisomy 8, and normal karyotypes at initial diagnosis ($P < 0.001$), although the number of samples was very small (Fig. 4G). The overall presence of specific chromosomal abnormalities during follow-up was not significantly associated with OS (Fig. 4H).

4. Discussion

The prognostic impact of karyotype on MPN, especially on PMF, has been investigated in many studies, and investigation of a large population of patients resulted in the classification of karyotypic abnormalities into unfavorable and favorable cytogenetic abnormalities [7,14,15]. In the DIPSS-plus system for PMF, the unfavorable abnormalities included +8, −7/7q−, i(17q), inv.(3), −5/5q−, 12p− or 11q23 rearrangement, and all other abnormalities were considered favorable [9]. In a recently revised cytogenetic risk group, favorable cytogenetic abnormalities were normal karyotypes, sole 20q−, sole 13q−, sole +9, sole −Y, sole sex chromosome abnormality, and sole chromosome 1 translocation/duplication. Cytogenetic abnormalities with very high risk included single/multiple monosomy 7, inv.(3)/3q21, i(17q), 12p−/12p11 abnormalities, 11q−/11q23 abnormalities, and autosomal trisomies other than +8 or +9 [11]. In these risk classifications, the chromosomal abnormalities at initial diagnosis were assessed for their prognostic impact. However, molecular and cytogenetic abnormalities at initial diagnosis change over time in parallel to the disease course of malignancies [18]. Because a patient can be diagnosed either at an early stage or at an advanced stage, the profiles of cytogenetic abnormalities might be different according to the timing of the disease course; consequently, the prognostic impact of cytogenetic abnormalities might be different. In this study, to observe the clonal evolution of cytogenetic abnormalities in each patient according to their clinical and molecular profiles and phase of disease progression, we investigated long-term changes in the cytogenetic profiles of several individual patients. At initial diagnosis, only approximately 30% of patients had cytogenetic abnormalities, and the frequency was not significantly different between PV and PMF patients. Most patients with chromosomal abnormalities had single abnormalities (87.5%). Cytogenetic abnormalities, including trisomy 8, del(20q), +1q, and del(9q), were found in these patients. These abnormalities, except del(9q), were well known and common cytogenetic abnormalities in MPN, and we suggest that these abnormalities might be considered initial clonal cytogenetic changes in the pathogenesis of MPN. Subsequent analysis of follow-up samples revealed that more patients with normal karyotypes gained cytogenetic abnormalities, and additional cytogenetic abnormalities appeared in patients who initially had single abnormalities. Approximately 50% of patients had new cytogenetic abnormalities or additional clonal changes. The +1q and del(20q) were present at high frequencies during follow-up and at the initial diagnosis. They presented both as primary abnormalities and secondary changes that appeared after the initial diagnosis. A recently published paper by Marcellino et al. analyzed the data from a large cohort of 1294 MPN

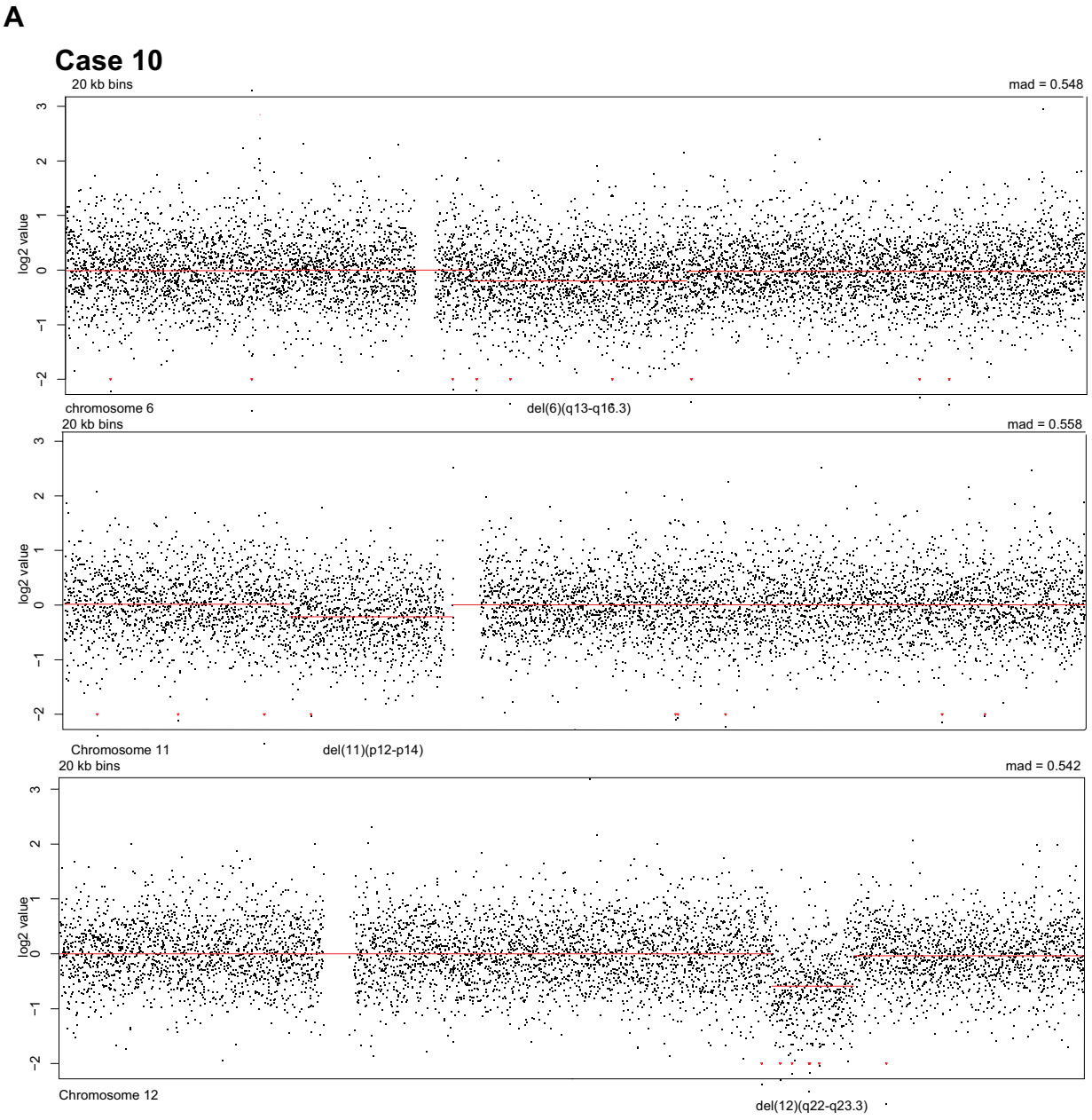

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Fig. 3. The off-target copy number analysis of next-generation sequencing (NGS) data in two patients presented (A) partial copy loss in 6q, 11p, and 12q, (B) and copy loss of 20q.

Table 2
Cytogenetic abnormalities in the 28 patients with myeloproliferative neoplasms (MPNs) according to molecular abnormalities.

Variable	Total MPN (n = 28)	JAK2-mutated MPN total (n = 19)	JAK2-mutated PMF (n = 13)	CALR-mutated PMF (n = 6)	*P*-value*
Chromosomal abnormalities at initial diagnosis, n (%)	8/26 (30.8)	6/17 (35.3)	5/12 (41.7)	1/6 (16.7)	0.394
trisomy 8	3/26 (11.5)	2/17 (11.8)	1/13 (7.7)	0 (0)	0.472
del(20q)	2/26 (7.7)	1/17 (5.9)	1/13 (7.7)	1/6 (16.7)	0.503
+1q	2/26 (7.7)	1/17 (5.9)	1/13 (7.7)	0 (0)	0.584
del(9q)	2/26 (7.7)	2/17 (11.8)	2/13 (15.4)	0 (0)	0.472
del(7q)	1/26 (3.8)	0 (0)	0 (0)	0 (0)	0.407
Chromosomal abnormalities either at initial diagnosis or during follow-up, n (%)	21 (75.0)	14 (73.7)	9 (69.2)	5 (83.3)	0.630
del(20q)	8 (28.6)	3 (15.8)	2 (15.4)	4 (66.7)	0.016
+1q	8 (28.6)	4 (21.1)	2 (15.4)	3 (50.0)	0.169
del(6p)	4 (14.3)	2 (10.5)	2 (15.4)	2 (33.3)	0.184
trisomy 8	3 (10.7)	2 (10.5)	1 (7.7)	0 (0)	0.407
del(7q)	3 (10.7)	1 (5.3)	0 (0)	1 (16.7)	0.369
del(6q)	2 (7.1)	1 (5.3)	1 (7.7)	1 (16.7)	0.369
del(9q)	2 (7.1)	2 (10.5)	2 (9.1)	0 (0)	0.407
del(3p)	2 (7.1)	1 (5.3)	1 (7.7)	1 (16.7)	0.369

Data are presented as the number of cases (percentage) unless otherwise indicated.
Abbreviations: MPN, myeloproliferative neoplasm; n, number; PMF, primary myelofibrosis; PV, polycythemia vera.
* *P* values were calculated using the χ^2 test between the *JAK2*-mutated and *CALR*-mutation PMF patients.

patients specifically focusing on +1q and abnormalities at chromosome 12 and 17, and showed that the frequency of del(20q) and +1q in MPN were 7% and 6%, respectively [27]. In the study, +1q was shown to be more prevalent in more advanced forms of MPNs than in early stage MPNs such as PV and ET. These results are consistent with our results.

The timing of the clonal evolution of cytogenetic abnormalities was variable in each patient. When the data from all patients was summarized, additional chromosomal abnormalities after initial diagnosis were detected after approximately 8 years in PMF patients and 10 to 13 years in PV patients. In addition to the increase in the number and types of cytogenetic abnormalities over time, the proportion of metaphases with abnormal clones also tended to increase over the course of the disease. Many patients had cytogenetic abnormalities in a portion of metaphases when initially diagnoses; however, several years later, when the disease progressed to overt bone marrow myelofibrosis or the blast phase, almost all metaphases had cytogenetic abnormalities. A previous study by Marcellino et al. presented a case with PV and longitudinal data over the course of 16 years. This patient presented with the additional gain of +1q 7 years after the initial diagnosis of iso (9p) clone, and this timing was similar to the clonal change in our summarized results.

Cytogenetic changes had a possible association with molecular abnormalities. There were no statistically significant differences in the distribution of cytogenetic abnormalities at initial diagnosis among *JAK2*- and *CALR*-mutated patients. However, when all cytogenetic abnormalities were considered in both the initial and follow-up samples, del(20q) was significantly more frequent in patients with *CALR* mutations than in patients with *JAK2* mutations. In half the *CALR*-mutated patients, del(20q) was gained as a secondary abnormality after the appearance of other abnormalities. In previous studies on other myeloid malignancies, del(20q) was reported to be associated with other abnormalities, such as in myelodysplastic syndrome (MDS) in which del (20q) was frequently observed in *U2AF1*-mutated patients [25,28]. Our results suggested a possible association between the *CALR* mutation and the gain of del(20q) during the clonal evolution of malignant cells; however, the number of patients was too small to obtain conclusive results. A previous study reported that the *JAK2* V617F mutation was associated with +1q, and *CALR* or *MPL* mutations are rarely found in MPN patients with +1q [27]. However, our results showed that there were no statistically significant differences in the frequency of which genes were mutated in conjunction with +1q, although the number of patients was small for conclusive results.

The follow-up samples of a subset of patients were analyzed with NGS, and molecular abnormalities including in *JAK2*, *CALR*, and *MPL* were found in most tumor cells, with VAFs > 50%. In some samples, uniparental disomy (copy neutral loss of heterozygosity) in the genetic loci of the *JAK2* gene was found, which is a well-known abnormality involving *JAK2* in MPNs [29]. In addition to these abnormalities, several other molecular abnormalities, including in genes such as *ASXL1*, *TP53*, and *SF3B1*, were also detected, and these molecular abnormalities had lower VAFs, which suggests that these abnormalities occurred after the initial molecular changes. However, the VAFs were estimated in the whole peripheral blood and bone marrow, in which clonal cells and non-clonal cells are variably mixed in each sample; therefore, the estimation of VAFs may not accurately represent the true mutant allele burden. When the NGS data were used for copy number analysis using off-target reads analysis, these data were not perfectly matched with the karyotyping results. In some patients with multiple cytogenetic abnormalities, the depth of copy number changes was different according to the cytogenetic abnormalities, and an additional cytogenetic change could be found.

When the prognostic impacts of cytogenetic abnormalities were investigated, only the presence of cytogenetic abnormalities at initial diagnosis was associated with shorter OS, and the appearance of cytogenetic abnormalities at any point in the follow-up period was not associated with OS. In addition, patients with cytogenetic abnormalities at initial diagnosis presented earlier progression to fibrosis. Although the number of patients was very small, the initial presence of del(20q) or del(9q) was associated with worse OS than the initial presence of partial trisomy 1q or trisomy 8. Because del(20q) is a generally favorable prognostic factor, our results were different than those in previous studies. Because the number of patients was very small, the statistical power might be limited. Among the initial cytogenetic abnormalities, there were no abnormalities that belonged to the very high risk group, and among the initial cytogenetic abnormalities with deletions, there were more adverse prognostic effects than among the cytogenetic abnormalities with chromosomal gains. Among the total cytogenetic abnormalities that were found throughout the follow-up period, del(20q) and partial trisomy 1q did not present significant prognostic differences.

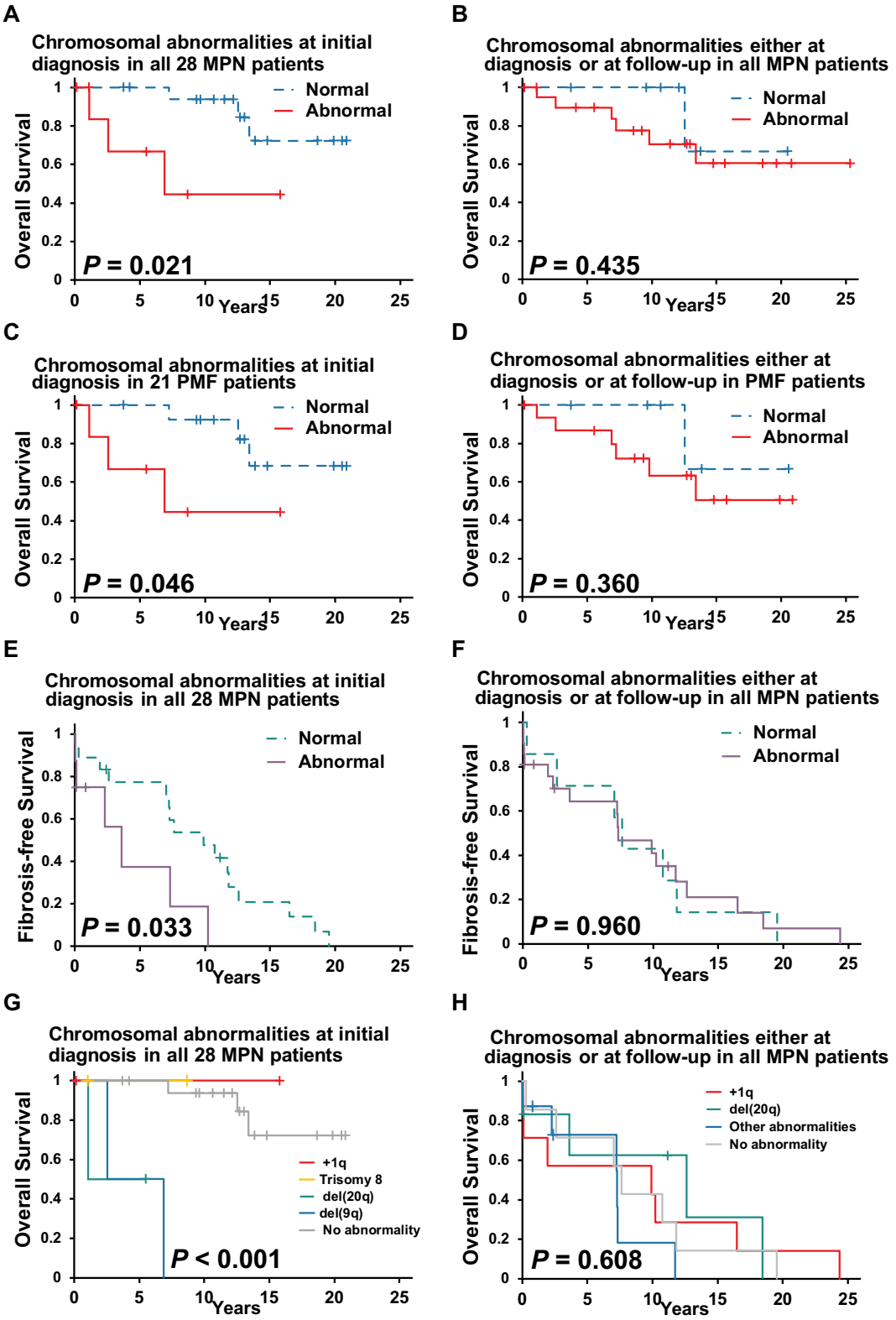

Fig. 4. Overall survival of 28 myeloproliferative neoplasm (MPN) patients (A) according to the presence of cytogenetic abnormalities at initial diagnosis and (B) according to the presence of chromosomal abnormalities either at diagnosis or at follow-up. Overall survival of the subgroup of 21 primary myelofibrosis (PMF) patients (C) according to the presence of cytogenetic abnormalities at initial diagnosis and (D) according to the presence of chromosomal abnormalities either at diagnosis or at follow-up. Fibrosis-free survival of 28 patients (E) according to the presence of cytogenetic abnormalities at initial diagnosis and (F) according to the presence of chromosomal abnormalities either at diagnosis or at follow-up. Overall survival of all patients according to the presence of specific cytogenetic abnormalities either (G) at diagnosis or (H) at diagnosis or during the follow-up period.

This study has limitations; it included a small number of samples and was conducted in a single center. In addition, the follow-up diagnostic tests were not performed as prospective schedules, but they were conducted when patients' laboratory data or clinical conditions were changed and disease progression was suspected. Therefore, the timing of follow-up tests was variable among patients, and the exact timing of the gain of novel cytogenetic and molecular abnormalities could not be investigated.

In conclusion, for many MPN patients, cytogenetic abnormalities clonally evolved during disease progression and were associated with the molecular characteristics. The association of cytogenetic abnormalities with MPN pathogenesis and cytogenetic evolution may provide interesting insights into the disease.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Authors' contributions

Seon Young Kim shared responsibility for the study design, data management and statistical analyses and helped to write the manuscript. Mosae Koo, Yumi Park, Jinsook Lim, Jimyung Kim, Ik-Chan Song, Deog-Yeon Jo, Gye Cheol Kwon, and Sun Hoe shared responsibility for the study design, data interpretation and manuscript revision, providing important intellectual content. All authors read and approved the final manuscript.

References

[1] S.H. Swerdlow, E. Campo, N.L. Harris, S.A. Pileri, E.S. Jaffe, H. Stein, J. Thiele, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, International Agency for Research on Cancer, 2017.

[2] J. Nangalia, A.R. Green, Myeloproliferative neoplasms: from origins to outcomes, Blood 130 (2017) 2475–2483.

[3] P. Lundberg, A. Karow, R. Nienhold, R. Looser, H. Hao-Shen, I. Nissen, S. Girsberger, T. Lehmann, J. Passweg, M. Stern, C. Beisel, R. Kralovics, R.C. Skoda, Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms, Blood 123 (2014) 2220–2228.

[4] N. Gangat, J. Strand, T.L. Lasho, C.M. Finke, R.A. Knudson, A. Pardanani, C.Y. Li, R.P. Ketterling, A. Tefferi, Cytogenetic studies at diagnosis in polycythemia vera: clinical and JAK2V617F allele burden correlates, Eur. J. Haematol. 80 (2008) 197–200.

[5] M. Sever, A. Quintas-Cardama, S. Pierce, L. Zhou, H. Kantarjian, S. Verstovsek, Significance of cytogenetic abnormalities in patients with polycythemia vera, Leuk. Lymphoma 54 (2013) 2667–2670.

[6] G. Tang, J.E. Hidalgo Lopez, S.A. Wang, S. Hu, J. Ma, S. Pierce, W. Zuo, A.A. Carballo-Zarate, C.C. Yin, Z. Tang, S. Li, L.J. Medeiros, S. Verstovsek, C.E. Bueso-Ramos, Characteristics and clinical significance of cytogenetic abnormalities in polycythemia vera, Haematologica 102 (2017) 1511–1518.

[7] F. Cervantes, B. Dupriez, A. Pereira, F. Passamonti, J.T. Reilly, E. Morra, A.M. Vannucchi, R.A. Mesa, J.L. Demory, G. Barosi, E. Rumi, A. Tefferi, New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment, Blood 113 (2009) 2895–2901.

[8] C.S. Tam, L.V. Abruzzo, K.I. Lin, J. Cortes, A. Lynn, M.J. Keating, D.A. Thomas, S. Pierce, H. Kantarjian, S. Verstovsek, The role of cytogenetic abnormalities as a prognostic marker in primary myelofibrosis: applicability at the time of diagnosis and later during disease course, Blood 113 (2009) 4171–4178.

[9] K. Hussein, A.D. Pardanani, D.L. Van Dyke, C.A. Hanson, A. Tefferi, International prognostic scoring system-independent cytogenetic risk categorization in primary myelofibrosis, Blood 115 (2010) 496–499.

[10] E. Wassie, C. Finke, N. Gangat, T.L. Lasho, A. Pardanani, C.A. Hanson, R.P. Ketterling, A. Tefferi, A compendium of cytogenetic abnormalities in myelofibrosis: molecular and phenotypic correlates in 826 patients, Br. J. Haematol. 169 (2015) 71–76.

[11] A. Tefferi, M. Nicolosi, M. Mudireddy, T.L. Lasho, N. Gangat, K.H. Begna, C.A. Hanson, R.P. Ketterling, A. Pardanani, Revised cytogenetic risk stratification in primary myelofibrosis: analysis based on 1002 informative patients, Leukemia 32 (5) (2018) 1189–1199.

[12] N. Gangat, A. Tefferi, G. Thanarajasingam, M. Patnaik, S. Schwager, R. Ketterling, A.P. Wolanskyj, Cytogenetic abnormalities in essential thrombocythemia: prevalence and prognostic significance, Eur. J. Haematol. 83 (2009) 17–21.

[13] M. Sever, H. Kantarjian, S. Pierce, N. Jain, Z. Estrov, J. Cortes, S. Verstovsek, Cytogenetic abnormalities in essential thrombocythemia at presentation and transformation, Int. J. Hematol. 90 (2009) 522–525.

[14] F. Passamonti, F. Cervantes, A.M. Vannucchi, E. Morra, E. Rumi, A. Pereira, P. Guglielmelli, E. Pungolino, M. Caramella, M. Maffioli, C. Pascutto, M. Lazzarino, M. Cazzola, A. Tefferi, A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment), Blood 115 (2010) 1703–1708.

[15] N. Gangat, D. Caramazza, R. Vaidya, G. George, K. Begna, S. Schwager, D. Van Dyke, C. Hanson, W. Wu, A. Pardanani, F. Cervantes, F. Passamonti, A. Tefferi, DIPSS plus: a refined dynamic international prognostic scoring system for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 29 (2011) 392–397.

[16] T. Klampfl, A. Harutyunyan, T. Berg, B. Gisslinger, M. Schalling, K. Bagienski, D. Olcaydu, F. Passamonti, E. Rumi, D. Pietra, R. Jager, L. Pieri, P. Guglielmelli, I. Iacobucci, G. Martinelli, M. Cazzola, A.M. Vannucchi, H. Gisslinger, R. Kralovics, Genome integrity of myeloproliferative neoplasms in chronic phase and during disease progression, Blood 118 (2011) 167–176.

[17] J. Nangalia, T.R. Green, The evolving genomic landscape of myeloproliferative neoplasms, Hematology Am. Soc. Hematol. Educ. Program 2014 (2014) 287–296.

[18] C. Hahm, H.J. Huh, Y.C. Mun, C.M. Seong, W.S. Chung, J. Huh, Genomic aberrations of myeloproliferative and myelodysplastic/myeloproliferative neoplasms in chronic phase and during disease progression, Int. J. Lab. Hematol. 37 (2015) 181–189.

[19] J. Thiele, H.M. Kvasnicka, F. Facchetti, V. Franco, J. van der Walt, A. Orazi, European consensus on grading bone marrow fibrosis and assessment of cellularity, Haematologica 90 (2005) 1128–1132.

[20] Y. Oh, I.C. Song, J. Kim, G.C. Kwon, S.H. Koo, S.Y. Kim, Pyrosequencing-based quantitative measurement of CALR mutation allele burdens and their clinical implications in patients with myeloproliferative neoplasms, Clin. Chim. Acta 483 (2018) 183–191.

[21] J. McGowan-Jordan, M. Schmid, A. Simons, S.K. GmbH, Iscn 2016: An International System for Human Cytogenomic Nomenclature (2016). Reprint of: Cytogenetic and Genome Research, vol. 148, S. Karger AG2016, 2016.

[22] A.V. Jones, S. Kreil, K. Zoi, K. Waghorn, C. Curtis, L. Zhang, J. Score, R. Seear, A.J. Chase, F.H. Grand, H. White, C. Zoi, D. Loukopoulos, E. Terpos, E.C. Vervessou, B. Schultheis, M. Emig, T. Ernst, E. Lengfelder, R. Hehlmann, A. Hochhaus, D. Oscier, R.T. Silver, A. Reiter, N.C. Cross, Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders, Blood 106 (2005) 2162–2168.

[23] J.S. Ha, Y.K. Kim, S.I. Jung, H.R. Jung, I.S. Chung, Correlations between Janus kinase 2 V617F allele burdens and clinicohematologic parameters in myeloproliferative neoplasms, Ann. Lab. Med. 32 (2012) 385–391.

[24] S.Y. Kim, K. Im, S.N. Park, J. Kwon, J.A. Kim, D.S. Lee, CALR, JAK2, and MPL mutation profiles in patients with four different subtypes of myeloproliferative neoplasms: primary myelofibrosis, essential thrombocythemia, polycythemia vera, and myeloproliferative neoplasm, unclassifiable, Am. J. Clin. Pathol. 143 (2015) 635–644.

[25] S.Y. Kim, K. Kim, B. Hwang, K. Im, S.N. Park, J.A. Kim, S.M. Hwang, D. Bang, D.S. Lee, The high frequency of the U2AF1 S34Y mutation and its association with isolated trisomy 8 in myelodysplastic syndrome in Asians, but not in Caucasians, Leuk. Res. 61 (2017) 96–103.

[26] T. Kuilman, A. Velds, K. Kemper, M. Ranzani, L. Bombardelli, M. Hoogstraat, E. Nevedomskaya, G. Xu, J. de Ruiter, M.P. Lolkema, B. Ylstra, J. Jonkers, S. Rottenberg, L.F. Wessels, D.J. Adams, D.S. Peeper, O. Krijgsman, CopywriteR: DNA copy number detection from off-target sequence data, Genome Biol. 16 (2015) 49.

[27] B.K. Marcellino, R. Hoffman, J. Tripodi, M. Lu, H. Kosiorek, J. Mascarenhas, R.K. Rampal, A. Dueck, V. Najfeld, Advanced forms of MPNs are accompanied by chromosomal abnormalities that lead to dysregulation of TP53, Blood Adv. 2 (2018) 3581–3589.

[28] T.A. Graubert, D. Shen, L. Ding, T. Okeyo-Owuor, C.L. Lunn, J. Shao, K. Krysiak, C.C. Harris, D.C. Koboldt, D.E. Larson, M.D. McLellan, D.J. Dooling, R.M. Abbott, R.S. Fulton, H. Schmidt, J. Kalicki-Veizer, M. O'Laughlin, M. Grillot, J. Baty, S. Heath, J.L. Frater, T. Nasim, D.C. Link, M.H. Tomasson, P. Westervelt, J.F. DiPersio, E.R. Mardis, T.J. Ley, R.K. Wilson, M.J. Walter, Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes, Nat. Genet. 44 (2011) 53–57.

[29] C. O'Keefe, M.A. McDevitt, J.P. Maciejewski, Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies, Blood 115 (2010) 2731–2739.