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High Frequency of Copy-Neutral Loss of Heterozygosity in Patients with Myelofibrosis

Milton Rego de Paula Junior[a] Alexandre Nonino[d–f]
Juliana Minuncio Nascimento[a, e] Raphael S. Bonadio[b] Aline Pic-Taylor[b]
Silviene F. de Oliveira[b] Rinaldo Wellerson Pereira[d] Cintia do Couto Mascarenhas[d]
Juliana Forte Mazzeu[a, c]

[a]Programa de Pós-graduação em Ciências Médicas, [b]Departamento de Genética e Morfologia, Instituto de Ciências Biológicas, and [c]Faculdade de Medicina, Universidade de Brasília, [d]Programa de Pós-Graduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília, [e]Hospital de Base de Brasília, Secretaria de Estado de Saúde, and [f]CETTRO – Centro de Câncer de Brasília, Brasília, Brazil

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Keywords
Chromosome microarray · Chromosome rearrangements · Copy-neutral loss of heterozygosity · *JAK2* V617F mutation · Myelofibrosis

Abstract
Myelofibrosis is the rarest and most severe type of Philadelphia-negative classical myeloproliferative neoplasms. Although mutually exclusive driver mutations in *JAK2*, *MPL*, or *CALR* that activate JAK-STAT pathway have been related to the pathogenesis of the disease, chromosome abnormalities have also been associated with the phenotype and prognosis of the disease. Here, we report the use of a chromosomal microarray platform consisting of both oligo and SNP probes to improve the detection of chromosome abnormalities in patients with myelofibrosis. Sixteen patients with myelofibrosis were tested, and the results were compared to karyotype analysis. Driver mutations in *JAK2*, *MPL*, or *CALR* were investigated by PCR and MLPA. Conventional cytogenetics revealed chromosome abnormalities in 3 out of 16 cases (18.7%), while chromosomal microarray analysis detected copy-number variations (CNV) or copy-neutral loss of heterozygosity (CN-LOH) alterations in 11 out of 16 (68.7%) patients. These included 43 CN-LOH, 14 deletions, 1 trisomy, and 1 duplication. Ten patients showed multiple chromosomal abnormalities, varying from 2 to 13 CNVs or CN-LOHs. Mutational status for *JAK2*, *CALR*, and *MPL* by MLPA revealed a total of 3/16 (18.7%) patients positive for the *JAK2* V617F mutation, 9 with *CALR* deletion or insertion and 1 positive for *MPL* mutation. Considering that most of the CNVs identified were smaller than the karyotype resolution and the high frequency of CN-LOHs in our study, we propose that chromosomal microarray platforms that combine oligos and SNP should be used as a first-tier genetic test in patients with myelofibrosis.

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Juliana Forte Mazzeu
Faculdade de Medicina
Universidade de Brasília
Brasília, DF 70910-900 (Brazil)
E-Mail julianamazzeu@unb.br

Pathogenesis of myelofibrosis (MF), the rarest and most severe type of Philadelphia-negative classical myeloproliferative neoplasms, is not thoroughly understood. MF is characterized by bone marrow fibrosis, extramedullary hematopoiesis, and abnormal cytokine expression, with consequent hepatosplenomegaly and cytopenia. The disease can occur de novo as primary (PMF) or result from late progression of polycythemia vera (PV), or essential thrombocythemia (ET), in these cases termed as post-PV MF or post-ET MF, respectively. Both conditions are associated with thrombohemorrhagic events, constitutional symptoms, and leukemic transformation [Tam et al., 2009; Visani et al., 2011; Brecqueville et al., 2014; Vainchenker and Kralovics, 2017].

A clonal expansion of a single hematopoietic stem cell, driven by a somatic mutation, culminates in chronic myeloproliferation and atypical megakaryocytic hyperplasia. The clonally expanded megakaryocytes shed abnormal growth factors that induce non-clonal fibroblastic proliferation and hyperactivity, ultimately leading to bone marrow fibrosis. A defective hematopoietic microenvironment has been postulated as necessary for the development of the clonal hematopoietic stem cell and extramedullary hematopoiesis [Lataillade et al., 2008; Hussein et al., 2009; Boiocchi et al., 2013; Mughal et al., 2014; Vainchenker and Kralovics, 2017].

Cytogenetic abnormalities detected by karyotyping can be found in 32–48% of the patients, the most prevalent being 20q–, 13q–, and abnormalities of chromosome 1, although several other numerical and structural rearrangements have also been described [Djordjevic et al., 2007; Tefferi et al., 2008; Hussein et al., 2009; Visani et al., 2011; Boiocchi et al., 2013]. Poor prognostic is attributed to +8, –7/7q, i(17q), inv(3), –5/5q–, 12p–, 11q23 rearrangements, complex karyotypes, or monosomy. On the other hand, 20q–, 13q–, or +9 do not appear to impact survival in MF [Tefferi et al., 2014].

More than 85% of PMF patients have a mutually exclusive mutation in one of the following 3 genes: *JAK2* (60–65%), *MPL* (5%), or *CALR* (20–25%). All of these, referred to as "driver" mutations, activate JAK-STAT pathway [Cazzola and Kralovics, 2014]. The type of driver mutation, however, may have prognostic impact: e.g., the *CALR* mutations appear to have a favorable impact on survival, while triple negative patients have worse outcomes [Cazzola and Kralovics, 2014; Tefferi et al., 2014].

Several other mutations have been implicated in the pathogenesis and progression of PMF, including *TET2*, *DNMT3A*, and *IDH1/2* (involved in DNA methylation); polycomb repressor complex 1 and 2 members and *IDH1/2* (histone modifiers); *TP53, CUX1*, *IKZF1*, *FOXP1*, *ETV6*, and *RUNX1* (transcription factors); *NF1*, *NRAS*, *KRAS*, *LNK*, *CBL*, and *FLT3* (signaling proteins) as well as *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2* (splicing factors). Notably, *TET2* and *DNMT3A* may play an important role in disease initiation and progression; *EZH2*, *ASXL1*, and *SRSF2* are associated with poor prognosis, and *TP53* is associated with progression to leukemia in MF patients [Carbuccia et al., 2009; Brecqueville et al., 2014; Vainchenker and Kralovics, 2017].

Detection of chromosome imbalances using conventional karyotyping (or G-banding) is limited due to the method's low resolution of only 5–10 Mb. On the other hand, DNA array-based methods, such as SNP arrays, increased the resolution up to the base-pair level, enabling studies of copy number variants (CNVs) and acquired UPD. CNVs are amplified or deleted regions ranging in size from intermediate (1–50 kb) to large (50 kb–3 Mb) and are recognized as a major source of human genome variability [Sebat et al., 2004; da Silva et al., 2016]. Importantly, CNVs are involved in cancer development and can increase during tumor progression, influencing phenotype and prognosis.

Acquired UPDs are loss-of-heterozygosity (LOH) regions occurring without concurrent changes in the gene copy number; a phenomenon also reported as copy-neutral LOH (CN-LOH). These defects are attributed to mitotic recombination in somatic cells and have been recognized in a variety of neoplasms [O'Keefe et al., 2010; Visani et al., 2011; Hahm et al., 2015].

Implementation of chromosomal microarrays in MF have been reported in few studies with a detection rate of imbalances of 44% [Tefferi et al., 2008], 69% [Rumi et al., 2011], 54% [Brecqueville et al., 2014], and 56% [Hahm et al., 2015]. However, most of the abovementioned used array-CGH platforms without SNPs and therefore were unable to detect CN-LOHs.

Here, we report the importance of using a chromosomal microarray platform consisting of both oligo and SNP probes to improve the detection of chromosome abnormalities in patients with MF evidencing the high frequency of CN-LOH.

Material and Methods

Sample

The study included 16 Brazilian patients, 13 with PMF and 3 with post-ET MF, consisting of 57% females with ages varying from 46 to 84 (median age of 57) and 43% males with ages from 35

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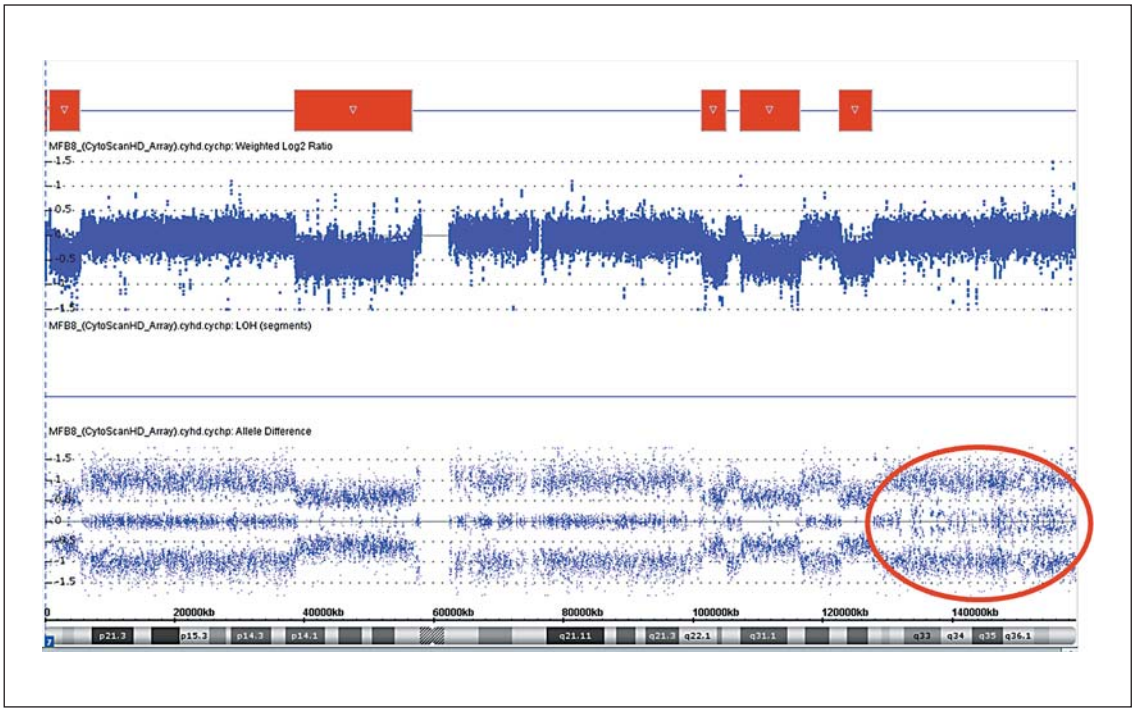


Fig. 1. Graphical representation of a complex rearrangement of chromosome 7 consisting of multiple deletions (red boxes) and a mosaic CN-LOH (circle) in patient 4.

to 81 (median age of 59). All patients were diagnosed according to WHO 2008 criteria. DNA was obtained from peripheral blood leucocytes and extraction was performed using Puregene® (Qiagen, Germany) according to the manufacturer's instructions.

Conventional Cytogenetics

Routine cytogenetic analysis with conventional G-banding techniques was performed from bone marrow aspirate at nonaffiliated diagnostic laboratories.

Array Comparative Genomic Hybridization

The presence of submicroscopic chromosomal alterations was investigated by microarray analysis using the Affymetrix CytoScan™ HD (14 samples) or Affymetrix 750K (2 samples). These platforms consist of microarrays containing non-polymorphic probes for CNVs from coding and noncoding regions of the human genome as well as polymorphic SNP probes. All procedures were carried out as instructed by the manufacturer. The hybridized slides bearing DNA, and marked with biotin, were analyzed by a GeneChip® Scanner 3000 7G (Affymetrix) and the Chromosome Analysis Suite (ChAS) Software (Affymetrix). Calling of the abnormalities considered a minimum of (a) 50 consecutively duplicated probes, (b) 25 consecutively deleted probes, and (c) segments of LOH larger than 5 Mb. Smaller alterations involving cancer-associated genes were also investigated. Common CNVs were considered constitutional/likely benign. Candidate genes mapped to large altered segments were defined based on disease-associated OMIM genes, previously related to myeloid neoplasms and/or other types of cancers.

Detection of JAK2, CALR *and* MPL *Driver Mutations*

Allele specific PCR for detection of the *JAK2* V617F mutation was performed using standard PCR protocols, as described by Campbell et al. [2006]. Screening for *CALR, MPL* as well as *JAK2* mutations was performed by MLPA using kit P420 according to manufacturer's protocol. This kit is designed to detect mutations in *JAK2* (V617F, N542_E543del and E543_D544del), *CALR* (L367fs*46, type 1 deletion and K385fs*47, type 2 insertion), and *MPL* (W515K and W515L) with an allele burden >10%.

Results

Cytogenetic Studies

G-banding analysis detected in 3/16 (18.7%) patients with MF (Table 1). One patient has trisomy 8, one has a partial deletion of chromosome 13, and the third patient has a complex karyotype involving chromosomes 7, 12, and 20.

Chromosomal microarray analysis (CMA) detected copy-number or LOH alterations in 11 out of 16 (68.7%) patients (Table 1); these included 43 CN-LOH, 14 deletions, 1 trisomy, and 1 duplication. One patient presented a single abnormality, a chromosome 8 trisomy. The other 10 patients showed multiple chromosomal abnormali-

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Table 1. Genomic alterations identified by CMA in patients with myelofibrosis

Patient	Age, years	Sex	Type	CMA results	Karyotype	Mapped cancer genes	Size, kb	Driver mutations
1	84	F	primary	1q24.2q25.2(170,365,327–176,346,512) hmz	46,XX	*FASLG*	5,981	*JAK2*
				1q41q42.13(222,161,988–229,516,511) hmz			7,355	
				4q28.3q34.3(133,645,289–179,928,406) hmz		*TLR2*, *PALLD*	46,283	
				12q13.2q24.33(56,002,232–133,778,166) hmz		*CDK4*, *SRGAPi*, *SH2B3*, *ALDH2*, *PTPN11*, *POLE1*	77,776	
				17q21.2q24.3(39,624,325–69,311,217) hmz		*STAT5B*, *BRCA1*, *PHB*, *NME1*, *MPO*, *RAD51C*, *PPM1D*, *BRIPI*, *PRKCA*, *PRKAR1A*	29,687	
				17p12p11.1(13,675,000–22,217,883) hmz			8,543	
				17q11.1q12(25,309,336–36,049,552) hmz		*NF1*, *RAD51D*, *HNF1B*	10,74	
				Xq21.33q23(97,629,749–111,888,527) hmz			14,259	
2	62	F	primary	1p22.3p13.1(86,010,458–116,805,160) hmz	46,XX,del(13)	*RBM15*, *NRAS*	30,795	*MPL*
				11p15.5p12(372,355–41,907,116) mos hmz			331	
				12q24.31(122,161,101–122,266,479)×1		*SETD1B*	109	
				13q13.1q21.33(33,571,752–69,638,023)×1		*KL*, *FOXO1A*, *RB1*	36,066	
				14q12(27,970,825–29,004,858)×3		*LINC00645 (MIR3171)*	1,034	
				19q13.33(49,274,808–49,513,502)×1			239	
3	61	F	primary	Trisomy 8	46,XY+8		146,138	*CALR*.del
4	48	F	primary	3p26.2p24.3(3,807,650–22,204,563) hmz	46,XX,del(7)(q22)add(20)(q13)	*OGG1*, *VHL*	18,397	*CALR*.del
				7p22.3p22.1(861,359–5,499,980)×1	46,XX,del(12)(q21)add(20)(q10)	*MAD1L1*	4,639	
				7p14.1p11.2(38,652,686–56,755,583)×1	46,XX,add(20)(q13)	*EGFR*	18,103	
				7q22.3q31.2(107,395,740–116,562,288)×1	46,XX,del(7)(q22)del(12)(q21)add(20)(q2)		9,167	
				7q22.1q22.3(101,428,262–105,113,953)×1			3,686	
				7q31.32q32.1(122,748,812–127,779,114)×1		*POT1*	5,030	
				12p13.2p12.3(12,109,324–18,729,898)×1		*CDKN1B*	6,620	
				12q21.2(76,448,583–80,275,907)×1			3,827	
				12q21.31q22(81,467,393–95,908,234)×1			14	
				18q11.2q12.1(24,767,485–32,456,354) hmz			8	
				20q13.13q13.2(47,848,257–53,158,589)×1			5	
5	68	M	primary	11q24.1q25(121,768,985–132,498,284) hmz	46,XY	*OPCML*	10,729	*CALR*.del
6	59	M	primary	1p36.33p21.1(849,466–26,290,550) mos hmz	46,XY		823	negative
7	47	F	primary	1p12p11.2(120,553,551–120,624,056)×1	46,XX	*NOTCH2*	71	*CALR*.inst
				Xq13.1q21.1(70,848,162–77,290,593) hmz			6	
9	46	F	primary	1p35.2p34.2(30,478,995–43,548,398) hmz	46,XX	*RSPO1*	13,069	negative
				9q33.1q33.2(117,808,079–124,657,456) hmz			6,849	
				11q23.3q24.3(120,972,517–128,650,029) hmz			7,648	
				13q14.13q14.2(46,722,034–49,139,504)×1		*RB1*	2,417	
				Xq22.1q22.3(99,700,785–106,311,924) hmz			6,611	

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Table 1 (continued)

Patient	Age, years	Sex	Type	CMA results	Karyotype	Mapped cancer genes	Size, kb	Driver mutations
12	66	M	Pos-ET	4p15.32p15.1(17,707,300–30,328,979) hmz	46,XY		12,622	*JAK2*
				5q35.1q35.3(171,766,824–180,692,321) hmz			8,925	
				9p24.3p13.3(216,123–34,431,079) hmz		*JAK2*, *MTAP*, *CDKN2A*	34,215	
				12q22q23.2(95,963,706–102,978,696) hmz			7,015	
				19p13.3p13.12(260,911–14,742,924) hmz		*STK11*, *SMARCA4*, *CALR*, *LYL1*	14,482	
				22q11.1q11.22(16,888,899–23,275,341) hmz			6,386	
14	69	M	primary	2p13.1p11.2(74,139,628–89,129,064) hmz			14,989	*JAK2*
				6q22.31q23.2(122,165,276–134,129,708) hmz			11,964	
				7q31.32q36.3(122,153,555–155,964,676) hmz		*POT1*, *SMOH*, *BRAF*	33,811	
				11p14.3p13(24,549,526–35,437,736) hmz		*LMO2*	10,888	
15	48	F	primary	1p33p31.1(47,858,053–69,907,944) hmz	46,XX		22	*CALR*.del
				2p13.2q13(71,835,787–113,889,134) hmz		*TMEM127*, *BUB1*, *IL1B*, *ILRN*	42,053	
				2q11.1q13(95,341,387–113,889,134) hmz			18,548	
				3q11.1q22.1(93,536,053–131,849,410) hmz			38,313	
				3p13q22.1(72,568,037–131,849,410) hmz		*GATA2*	59,281	
				6p22.3p12.3(15,735,087–49,880,005) hmz		*DEK*, *GGB*	34,145	
				7q21.11q22.1(83,470,313–103,219,027) hmz			19,749	
				9q33.1q33.2(120,027,912–125,306,402) hmz			5,278	
				10q25.1q26.13(107,670,340–124,523,169) hmz		*MX11*	16,853	
				11q12.1q13.5(56,930,833–76,123,829) hmz		*CCNDI*, *NUMA1*	19,193	
				11p11.2p11.12(46,304,337–51,563,636) hmz			5,259	
				15q14q21.3(39,625,545–57,874,494) hmz		*BUB1B*	18,249	
				16p13.2p13.11(9,182,576–16,159,628) hmz			6,977	
				Xp11.3p11.1(43,815,818–58,337,890) hmz			14,522	
				Xp21.1p11.4(34,218,865–40,790,309) hmz			6,571	
				Xq11.1q13.1(61,932,503–68,422,477) hmz			6,490	
8	57	M	primary	normal	46,XY			*CALR*.del
10	81	M	primary	normal	46,XY			negative
11	67	F	primary	normal	46,XX			*CALR*.inst
13	38	M	primary	normal	46,XY			*CALR*.inst
16	35	M	primary	normal	46,XY			*CALR*.del

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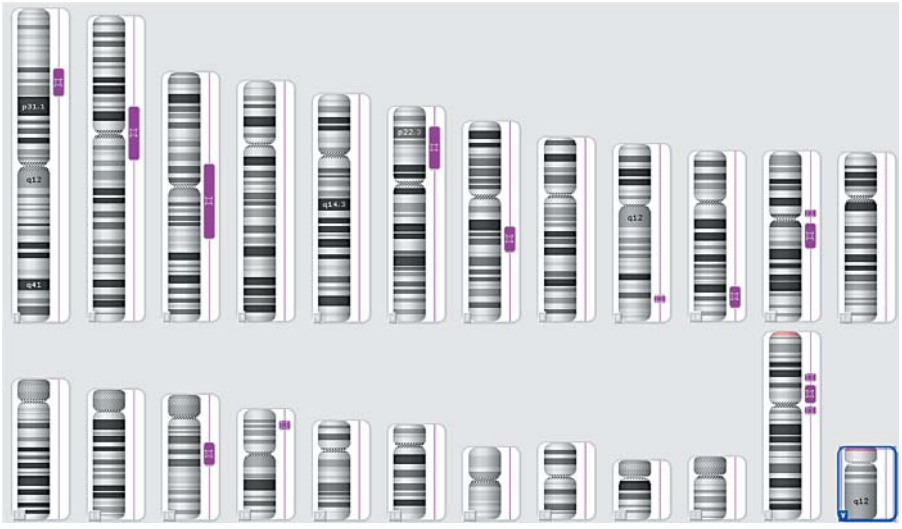


Fig. 2. Graphical representation of several CN-LOH of different chromosomes (indicated by purple blocks) found by chromosomal microarray analysis in patient 15.

ties, varying from 2 to 13 CNVs or CN-LOHs. CMA did not disclose any alterations in 5 patients 8, 10, 11, 13, and 16.

CMA Detection Rate of Numerical and Structural Chromosome Imbalances

Presumed pathogenic CNVs were identified in 6/16 patients (37.5%). One patient had a single copy number imbalance without CN-LOH. Regarding the 14 deletions detected, 7 were identified in a single patient who had 2 complex chromosome rearrangements involving chromosomes 7 and 12 (Fig. 1). All patients with deletions also had at least 1 CN-LOH. Only 1 microduplication was identified in a patient with multiple imbalances and CN-LOHs.

Detection Rate of Copy-Neutral LOH

We identified 43 CN-LOH in our sample, distributed among 10 patients, ranging from 1 to 13 segments of CN-LOH (Fig. 2). Six patients had CN-LOH without copy number imbalances. One patient presented a single CN-LOH of chromosome 11 without any other CN-LOH or imbalance. CN-LOH size varied from 5.2 to 823 Mb, with a median size of 14.25 Mb. Three CN-LOH were in mosaic state, as inferred by the SNP distribution (Fig. 1).

Driver Mutation Status

Mutational status for *JAK2*, *CALR*, and *MPL* by MLPA revealed a total of 3/16 (18.7%) patients positive for the *JAK2* V617F mutation, 9 with *CALR* deletion or insertion, and 1 positive for *MPL* mutation (Table 1).

Discussion

Cytogenetic abnormalities play an important role in the pathogenesis of MF. In previous studies, one-third of the patients with MF showed karyotype abnormalities at diagnosis. In these patients, the number of alterations increased as the disease progressed [Hussein et al., 2009]. The most frequent abnormalities in MF are del(20q), del(13q), +8, +9, and abnormalities of chromosomes 1 and 7 [Tefferi et al., 2001; Visani et al., 2011]. However, conventional karyotyping has a low resolution of up to 5–10 Mb. Thus, for the detection of minor chromosomal alterations, techniques with higher resolution are required. One of the standard molecular cytogenetic techniques for screening submicroscopic chromosomal alterations is CMA [Gondek et al., 2008].

In our sample, conventional cytogenetics disclosed chromosome abnormalities in 3 patients, while chromosomal microarray detected imbalances or LOH in a total of 11 patients.

CMA was concordant with karyotype results in 5 normal cases and in the patient with trisomy 8. In the patient with a 13q deletion, CMA revealed other submicroscopic imbalances in chromosomes 12, 14, and 19 as well as 2 segments of CN-LOH. The third patient with karyotype alterations had a complex chromosome rearrangement involving chromosomes 7, 12, and 20 that could be clarified by microarray analysis. Two patients had a normal karyotype, however CMA disclosed a submicroscopic CNV and LOH. The remaining 6 cases presented only LOH without chromosomal imbalances.

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Previous studies showed the ability of such techniques in detecting novel genetic lesions in both myeloid and lymphoid disorders [Walker et al., 2006; Mohamedali et al., 2007; Visani et al., 2011; Sato-Otsubo et al., 2012]. Myeloid neoplasms are among the best targets for CMA as the results can be directly incorporated into their prognostic scores [Sato-Otsubo et al., 2012].

Our overall detection rate of abnormalities was 68%, close to previous reports. However, we had a low detection rate of CNVs (37.5%) compared to other studies (44–69%) [Tefferi et al., 2008; Rumi et al., 2011; Brecqueville et al., 2014; Hahm et al., 2015]. We could not identify any clinical bias between our study and those described in the literature that could reasonably explain this discrepancy. In addition, this difference should not be attributed to the limitation of CMA not to recognize chromosomal translocations [Tefferi et al., 2009] since those represent only 3–9% of the alterations in patients with MF [Tefferi et al., 2001; Hussein et al., 2009].

Driver mutations in *JAK2*, *CALR*, and *MPL* were identified in 13 patients. The frequency of *JAK2* V617F mutations in our sample is 18.7%, lower than previously described [Liang et al., 2017], with an increased frequency in *CALR* mutations (56.2%) and a single case with *MPL* mutation.

In recent years, a renewed interest in the molecular pathogenesis of myeloproliferative neoplasms was observed, notably after the discovery of *JAK2* V617F mutation. *JAK2* plays an important role in cell signaling and proliferation [Campbell et al., 2006; Tefferi et al., 2009; Vainchenker et al., 2017]. Mutations in this gene confer constitutive activation of the JAK-STAT and related pathways, promoting differentiation and proliferation of different cell lineages [Hahm et al., 2015]. It is still not understood whether the *JAK2* mutation is the first event in myeloproliferative neoplasms or secondary to other genetic and epigenetic events [Campbell et al., 2006; Tefferi et al., 2008, 2009].

Candidate genes potentially involved in disease initiation or progress are listed in Table 1. Since most patients present with several large alterations, we proposed the genes previously associated to hematological malignancies and other cancers as candidates. Sixty genes associated with cancer were identified, 11 of them previously described in hematological malignancies.

Patient 1 presents 8 CN-LOH segments of chromosomes 1q, 4q, 12q, 17q and Xq, in which several oncogenes have been mapped. For instance, the *PTPN11* (12q) and *STAT5B* (17q) genes have been associated with leukemia and non-Hodgkin lymphomas [Chen et al., 2013; Liang et al., 2017]. Although the test for *JAK2* V617F mutation was positive, we did not identify CN-LOH of chromosome 9p.

Patient 2 has a complex set of rearrangements: 3 deletions (13q, 14q, and 19q), 1 duplication (14q), 1 CN-LOH (1p), and 1 mosaic CN-LOH (11p). The presence of a mosaic CN-LOH and non-mosaic rearrangement points to multiple cell lineages in the sample. The deletion in chromosome 13 includes *RB1*, a gene previously associated with multiple cancers including MF [Tam et al., 2009; Brecqueville et al., 2014].

The single alteration present in patient 3 is a trisomy of chromosome 8, a common chromosome abnormality associated with an unfavorable prognosis in MF [Hussein et al., 2009; Brecqueville et al., 2014; Mehrotra et al., 2015].

CMA analysis for patient 4 identified 2 complex rearrangements with multiple deletions in chromosomes 7 and 12. The *MAD1L1* gene, mapped to a deleted segment in chromosome 7p, is a checkpoint gene, whose deregulation is associated with chromosomal instability. Rare mutations in this gene have been reported in lymphoma, colon, and lung cancers [Tsukasaki et al., 2001]. This patient also presents with a mosaic CN-LOH of chromosome 12 and two others in chromosomes 3 and 18 (Fig. 1).

Patient 7 shows a deletion in the region encompassing the *NOTCH2* gene. The Notch signaling cascade is essential for cell proliferation and differentiation [Ranganathan et al., 2011]. A deregulated Notch pathway is found in various types of cancer such as T-cell leukemia, breast, and prostate cancers [Yuan et al., 2014].

CN-LOH of chromosome X including the *GATA* gene was identified in patient 15. The *GATA* family is essential to the development of many cell lineages, including hematopoietic, cardiac, and endodermal. *GATA1*, *GATA2*, and *GATA3* are needed for normal hematopoiesis, and their mutations are implicated on a variety of blood disorders, including MF [Dos Santos et al., 2011; Hahm et al., 2015; Mehrotra et al., 2015].

In addition, we identified 1 patient with LOH involving the *NUMA* gene. This leads to the disruption of the retinoic acid signaling pathway, and myeloid differentiation is arrested at the promyelocytic stage [Zhang et al., 2000].

CN-LOH as well as CNVs constitute markers of clonality. Previously undetected areas of clonal CN-LOH are frequently identified in various cancers, including hematologic malignancies [Maciejewski and Mufti, 2008]. However, their prognostic significance is still poorly understood, and the implementation of CMA as a routine

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test will give further evidence to establish its role on the pathogenesis of the disease.

In our cohort, 1 patient has a *JAK2* mutation and CN-LOH of chromosome 9p, possibly leading to homozygosity of the *JAK2* mutation. Interestingly, this patient also has a CN-LOH that includes the *CALR* gene despite not having the *CALR* driver mutation, as they have been shown to be mutually exclusive. No CN-LOH of the overlapping segments of *JAK2*, *CALR*, and *MPL* were identified in the remaining patients.

However, our results show that LOH recurrently harbors genes involved in cell differentiation and proliferation.

In addition, our study highlights the importance of a thorough analysis of chromosomal microarrays in patients with clonal neoplasms for visual detection of mosaic CN-LOHs, as it may not be indicated by the analysis software.

Chromosomal microarrays are already used as a first-tier test for patients with intellectual disability and/or congenital malformations due to the higher resolution. Considering that most of the CNVs identified were smaller than the karyotype resolution and the high frequency of CN-LOHs in our study, we recommend that chromosomal microarray platforms that combine oligos and SNP should be a first-tier genetic test in patients with MF.

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Statement of Ethics

The study was performed in accordance with the Helsinki declaration and was approved by local ethics committee.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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