

COPY NUMBER ALTERATIONS AND COPY-NEUTRAL LOSS OF HETEROZYGOSITY IN UKRAINIAN PATIENTS WITH PRIMARY MYELOFIBROSIS

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Aim: To examine frequencies and spectrum of genomic alterations in Ukrainian patients diagnosed with primary myelofibrosis (PMF). *Materials and Methods*: We enrolled 30 Ukrainian patients diagnosed with PMF who were previously tested for usual mutations in myeloproliferative neoplasms driver genes (*JAK2, MPL* and *CALR*). Genomic DNA samples were obtained from peripheral blood leukocytes of these patients. Copy number alterations and copy-neutral loss of heterozygosity (cnLOH) were assessed using a high-density Cyto-Scan HD microarray platform. Statistical significance was evaluated by the Fisher exact test. *Results*: We identified frequent genomic alterations, but no significant difference in the rates of copy-number loss, copy-number gain, cnLOH, or multiple genomic alterations were found in the groups of PMF patients that were positive for one of the usual mutations in driver genes or negative for such mutations (33.3% and 55.6%, p = 0.4181, 19.0% and 11.1%, p = 1.0000, 61.9% and 44.4%, p = 0.4434, 33.3% and 55.6%, p = 0.4181, respectively). The most frequent alterations were cnLOH at 1p36-1p22, 9p24.3-9p13.3 and 11q12.3-11q25; copy number loss at 7q21-7q36.3 and 13q12.3-13q14.3. Copy number alterations and cnLOH commonly affected the *EZH2*, *LAMB4*, *CBL*, *CUX1*, *ATM*, *RB1* and *TP53* genes, in addition to *JAK2*, *MPL* and *CALR*. *Conclusion*: We demonstrated the spectrum of genomic alterations in the groups of the Ukrainian PMF patients with or without the usual mutations in the specific driver genes. We identified several potential genes, which may be involved in the myeloproliferative neoplasms development and their phenotype modification (*EZH2*, *LAMB4*, *CBL*, *CUX1*, *ATM*, *RB1* and *TP53*). *Key Words*: primary myelofibrosis, copy number alterations, copy-neutral loss of heterozygosity, driver mutation.

Primary myelofibrosis (PMF) is a clonal disorder of early hematopoietic stem cells manifesting as bone marrowfibrosis and pancytopenia and classified as BCR-ABL-negative myeloproliferative neoplasm (MPN) [1]. PMF patients are characterized by a severe disease course and worse prognosis, compared with patients of other MPN subtypes: polycythemia vera (PV) and essential thrombocythemia (ET). MPNs are driven by acquired somatic mutations of *JAK2*, *CALR* and *MPL* genes in more than 85% of cases [2].

Studies of chromosomal alterations in MPN patients revealed that they occurred more frequently in PMF patients (50%) compared with PV (15%) and ET (5%) patients. Some alterations were detected recurrently: deletions of 20q, 18q, 13q and 12p; trisomy of chromosomes 8 and 9; copy number gain at 1p, 9p, 17q; and various translocations [3, 4]. Chromosomal alterations in PMF patients, such as trisomy of chromosome 8, deletions of 7, 7q, 5, 5q, 12p, inversion of chromosome 3 are considered as an unfavorable karyotype and used for the risk stratification for these patients [5].

Improved methods for genomic alterations studies, from standard karyotyping with limited sensitivity to the high-resolution single-nucleotide polymorphism (SNP) arrays, allowed detection of short regions of copy number alterations and copy-neutral loss

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*Correspondence: E-mail: larysa.poluben@gmail.com *Abbreviations used*: cnLOH – copy-neutral loss of heterozygosity; ET – essential thrombocythemia; MPN – myeloproliferative neoplasm; PMF – primary myelofibrosis; PV – polycythemia vera; SNP – single-nucleotide polymorphism. of heterozygosity (cnLOH). Thus, oncogenic microalterations can be studied, in particular, in MPN patients. This increases the search power for identification of potentially involved genes in MPN development (as 10–15% of MPN patients are negative for known mutations of the specific driver genes) [2, 5–7].

The aim of the study was to examine frequencies and spectrum of genomic alterations (copy number alterations and cnLOH) in Ukrainian PMF patients.

MATERIALS AND METHODS

30 Ukrainian PMF patients, previously tested for mutations in MPN driver genes (JAK2, MPL and CALR), were enrolled in the study. There were 13 JAK2 V617Fpositive, three MPL W515-positive and five positive for CALR gene mutations PMF patients. Nine PMF patients were negative for these mutations. Each patient signed an informed consent in accordance with the Declaration of Helsinki. The study was approved by the local Ethical Committee at the National Research Center for Radiation Medicine (Kyiv, Ukraine). DNA samples were obtained from peripheral blood leukocytes of PMF patients, using a Quiamp DNA extraction kit (Qiagen, Hilgen, Germany). Copy number alterations and cnLOH were assessed, using a High-density Affymetrix CytoScan HD oligo-SNP microarray platform (Affymetrix, Santa Clara, CA, US). Digested and labeled genomic DNA was hybridized to 2.67 million probes according to manufacturer's recommendations. The Chromosome Analysis Suite (ChAS) software version 3.1 (Affymetrix) was used to analyze the data. The genome assembly version GRCh37/hg19 was used as a reference. All genomic alterations were visually inspected and confirmed, and regions with poor quality were excluded. The regions with at least 50 markers (over 200 kb (50 markers over 100 kb for leukemia regions) were considered for gains, 30 markers over 50 kb (15 markers over 20 kb for leukemia regions) — for losses, and a minimum length of 5 Mb (3 Mb for leukemia regions) — for cnLOH. An online catalog of human genes and genetic disorders Online Mendelian Inheritance in Man (OMIM) was used to identify leukemia-related genes within the altered regions. Statistical significance was evaluated by the Fishers exact test.

RESULTS AND DISCUSSION

Copy-number alterations and cnLOH were frequently found in Ukrainian PMF patients (Table, Fig. 1). There were 71.4% (15/21) of cases with genomic alterations among PMF patients positive for one of known driver gene (*JAK2, MPL* or *CALR*) mutations and 55.6% (5/9) of cases — among PMF patients negative for these mutations (p = 0.4311). However, here was no significant difference in the rates of copy-number loss, copy-number gain, cnLOH, or multiple genomic alterations, respectively, in these groups of PMF patients (33.3% and 55.6%, p = 0.4181; 19.0% and 11.1%, p = 1.0000; 61.9% and 44.4%, p = 0.4434; 33.3% and 55.6%, p = 0.4181). The most frequently altered regions were cnLOH at 1p36-1p22 (N = 6), 9p24.3-9p13.3 (N = 3) and 11q12.3-11q25 (N = 3); copy number loss at 7q21-7q36.3 (N = 5) and 13q12.3-13q14.3 (N = 2). Our findings are consistent with published data [3, 4, 7]. CnLOH at 9p, 1p and 19p duplicated the usual driver gene (*JAK2, MPL* or *CALR,* respectively) mutations in 33.3% (7/21) of cases among the subset of PMF patients that were positive for one of these mutations. The length of these altered regions varied from 13.7 to 93.6 Mb (Table, Fig. 2). A shorter genomic fragment (1 Mb) of cnLOH with duplicated *MPL* gene mutation was visually detected in a PMF patient (ID 842) and considered as leukemogenic.

In PMF patients, the most frequently affected genes due to copy number alterations and cnLOH in addition to *JAK2*, *MPL* and *CALR* were *EZH2*, *LAMB4*, *CBL*, *CUX1*, *ATM*, *RB1* and *TP53* genes (Fig. 3). Copy number losses of *EZH2* at 7q36.1 were detected in three PMF patients negative for usual driver gene mutations and in one *JAK2* V617F-positive PMF patient. Epigenetic regulator *EZH2* is a member of Polycomb Repressive Complex 2 which is involved in H3K27 trimethylation. Loss of function mutations and cytogenetic alterations of *EZH2* are frequently observed in MPN patients. Recent studies showed that *EZH2* alterations may be early events in leukemogenesis [8, 9].

Other studies demonstrated that *EZH2* loss can dramatically modify the myeloproliferative phenotype reducing survival in the presence of *JAK2* V617F muta-



Fig. 1. Genomic alterations in Ukrainian PMF patients positive (*a*) and negative (*b*) for usual mutations in MPN driver genes (*JAK2, MPL* and *CALR*). LOSS — copy number loss; GAIN — copy number gain



Fig. 2. The length of altered regions: a - cnLOH at chromosomes carrying usual mutations of known MPN driver genes; b - the region with cnLOH at 9p24.3p13.1 (35.9 Mb) in *JAK2* V617F-positive PMF patient (patient's ID 615)

Induited Display 22368.22 (ad2.3q41 Coll allefault Mo DBP7 Dipole 1 1 146 1166.32104.3 cntol N 103.8 RBP56, RBM34, PF33BP2 55 412 MPL 110.33104.3 cntol N 103.8 RBP56, RBM34, PF33BP2 55 412 MPL 110.3333.3 cntol N 103.8 RBBP56, RBM34, PF33BP2 55 412 MPL 110.336, 3622.1 cntol N 48.2 CSF3R, MPL, RAP16AP, RBM31, RBP7, RPL5 100 983 JAK2 1222, 223.23, 23.2 LOSS 3.3 55 57 702 TN 1263.36/22.1 cntol N 4.3 DMM134, TB97, RPL5 100 183 JAK2 2122.2, 202.3 cntol N 4.9 FBXW7 100 193 GA JAK2 LOSS 0.34 100 101 194 MA YA 401.3 LOSS 0.344 RF1, RBM22, NPM1, DDX41 24-89 20 JAK2 59, 59, 59, 59, 59, 59, 59, 59, 50, 50, 50, 50,	Chr	Patients's ID	Driver gene	Region altered	Type	Size,	Genes within the regions	Altered DNA
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652 JAK2 5p15.2p14.3 cnL0H 6.1 100 7 740 TN 6q212.31 cnL0H 4.3 100 818 TN 7q23.3 cnL0H 4.3 100 818 TN 7q25.q36.2 LOSS 47.9 BRAF, EZH2, LAMB4, POT1 21 846 TN 7q12.3q1.31 CALN 12.9 RBM48 41-65 702 TN 7q12.3q31.31 LOSS 71.7 BRAF, CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 842 MPL 7q36.1q36.2 cnL0H 3.3 100 58 844 TN 8q32.qg3.1 LOSS 5.5 20 20 724 JAK2 9p24.3p13.3 cnL0H 32.9 FANCG, JAK2 30, 100 733 JAK2 9p24.3p13.3 cnL0H 42.4 FANCG, JAK2 30, 100 724 JAK2 9p24.3p2.qg2.3 cnL0H 6.7 74 74 74 740 TN <td< td=""><td></td><td>638</td><td>JAK2</td><td>5p, 5q multiple alterations</td><td>LOSS</td><td>134.4</td><td>IRF1, RBM22, NPM1, DDX41</td><td>24-88</td></td<>		638	JAK2	5p, 5q multiple alterations	LOSS	134.4	IRF1, RBM22, NPM1, DDX41	24-88
6 740 TN 6q21q22.31 cnLOH 15.2 100 7 740 TN 7q21.3 cnLOH 4.3 00 818 TN 7q22.3g86.2 LOSS 47.9 BRAF, EZH2, LAMB4, POT1 21 846 TN 7q21.3g21.11, GAN 12.9 RBM48 41–65 702 TN 7q21.3g36.3 LOSS 25.1 CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 702 TN 7q21.3g1.31 LOSS 25.5 FANCG, JAK2 100 8 846 TN 8p23.3g24.3 GANN 146.4 CSMD1, RAD21-AS1, RBM12B, RUNX1T1, TPS3INP1 76 9 615 JAK2 9p24.3p13.3 cnLOH 35.9 FANCG, JAK2 100 702 TN 9q24.3p13.1 9034.2q34.3 cnLOH 13.7 JAK2 30,100 711 740 TN 11913.2q32.5 cnLOH 6.7 71 71 740 TN 11913.3q25 cnLOH <td></td> <td>852</td> <td>JAK2</td> <td>5p15.2p14.3</td> <td>cnLOH</td> <td>6.1</td> <td></td> <td>100</td>		852	JAK2	5p15.2p14.3	cnLOH	6.1		100
7 740 TN 7q21.3 cnL0H 4.3 100 818 TN 7q25036.2 LOSS 6.8 EZH2 92 638 JAK2 7q11.23, 7q11.23q21.11, GAN 12.9 RBM48 41-65 702 TN 7q21.3q36.3 LOSS 6.8 EZH2 92 638 JAK2 7q11.23, 7q13.3q3.31 LOSS 71.7 BRAF, CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 702 TN 8p21.3q31.31 LOSS 25.5 CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 842 MPL Tq36.1q36.2 cnL0H 3.3 100 100 818 TN 9q32.3q24.3 cnL0H 3.5 FANCG, JAK2 100 724 JAK2 9p24.3p23 cnL0H 13.7 JAK2 30, 100 733 JAK2 9p24.3p24 cnL0H 13.7 JAK2 30, 100 71 TM 9q21.11q21.13 cnL0H 13.7 JAK2 30, 100 <tr< td=""><td>6</td><td>740</td><td>TN</td><td>6q21q22.31</td><td>cnLOH</td><td>15.2</td><td></td><td>100</td></tr<>	6	740	TN	6q21q22.31	cnLOH	15.2		100
818 TN Tq22.3q36.2 LOSS 47.9 BRAF, E2H2, LAMB4, POT1 21 846 TN Tq35q36.2 LOSS 6.8 E2H2 92 638 JAK2 Tq11.23, Tq11.23q21.11, Cq11.23q21.3 GAIN 12.9 RBM48 41-65 638 JAK2 Tq21.123, Tq21.3q61.3 LOSS 25.1 CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 702 TN Tq21.3q61.3 LOSS 25.1 CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 842 MPL Tq36.1q36.2 cnL0H 3.3 PARA CXMD1, RAD21-AS1, RBM12B, RUNXITI, TP53INP1 76 9 615 JAK2 924.3p13.3 cnL0H 35.5 FANCG, JAK2 30, 100 702 TN 9q21.11q21.13 cnL0H 13.7 JAK2 41 702 TN 9q21.11q21.32 cnL0H 13.7 ATM, CBL, 100 113 JAK2 9p24.3p23 cnL0H 13.7 ATMCG, JAK2 30, 100 1700 TN	7	740	TN	7q21.3	cnLOH	4.3		100
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		818	TN	7q22.3q36.2	LOSS	47.9	BRAF, EZH2, LAMB4, POT1	21
		846	IN	/q35q36.2	LOSS	6.8	EZH2	92
638 JAK2 7q21.11q21.2, 7q21.3q36.3 LOSS 71.7 BRAF, CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 702 TN 7q21.3q36.3 LOSS 25.1 CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 842 MPL 7q36.1q36.2 CILOH 3.3 CUX1, EZH2, LAMB4, POT1, RBM33 56, 57 9 615 JAK2 9p24.3p13.3 CuLOH 3.5 FANCG, JAK2 30, 100 818 TN 9q23q33.1 LOSS 5.5 20 724 JAK2 9p24.3p12 cuLOH 42.4 FANCG, JAK2 30, 100 101 702 TN 9q21.11q21.13 cuLOH 6.5 100 11 740 TN 11q12.3q13.2 cuLOH 7.7 ATM, CBL, RBM7, TP53AIP1, RBM48, RBM4B 26, 100 100 JAK2 11q12.3q32.1 cuLOH 7.7 ATM, CBL, RBM7, RBM48, RBM4B 26, 100 113 JAK2 12q multiple alterations LOSS 3.4.7 AEBP2, GPRC5A, KRAS 93 638 JAK2		638	JAK2	/q11.23, /q11.23q21.11, 7a21 2a21 3	GAIN	12.9	KBM48	41-65
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		638	JAK2	7a21.11a21.2. 7a21.3a36.3	LOSS	71.7	BRAF. CUX1. EZH2. LAMB4. POT1. RBM33	56.57
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		702	TN	7g21.3g31.31	LOSS	25.1	CUX1, EZH2, LAMB4	55
8 846 TN 8 p23.3q24.3 pp24.3p13.3 cnLOH GAIN 146.4 pp24.3p13.3 cnLOH CSMD1, RAD21-AS1, RBM12B, RIJNX1T1, TP53INP1 76 pANCG, JAK2 90 p04.3p13.3 pp14.3p13.3 pp24.3p13.3 pp24.3p13.3 pp24.3p23.2 cnLOH CSMD1, RAD21-AS1, RBM12B, RIJNX1T1, TP53INP1 76 pANCG, JAK2 100 724 JAK2 9p24.3p13.3 pp24.3p13.2 pp24.3p23 cnLOH 42.4 FANCG, JAK2 30, 100 723 JAK2 9p24.3p13.3 pp24.3p23 cnLOH 42.4 FANCG, JAK2 30, 100 702 TN 9q21.11q21.13 pp24.3p23 cnLOH 6.5 100 11 740 TN 11q13.3q25 cnLOH 6.7 ATM, CBL, RBM7, TP53AIP1, RBM14, RBM4B 26, 100 100 1131 JAK2 11q12.3q13.2, 11q13.3q25 cnLOH 72.1 ATM, CBL, RBM7, TP53AIP1, RBM14, RBM4B 26, 100 100 133 TO2 TN 12q21.3q14.3 LOSS 12.5 SH2B3, NCOR2 47-61 926 TN 12q21.2q21.31 cnLOH 4.1 100 13 702 TN 13q12.3q14.3 LOSS <		842	MPL	7q36.1q36.2	cnLOH	3.3		100
9 615 JAK2 9p24.3p13.3 cnLOH 35.9 FANCG, JAK2 100 818 TN 9q24.3p13.1, 9q34.2q34.3 LOSS 5.5 20 724 JAK2 9p24.3p13.1, 9q34.2q34.3 cnLOH 42.4 FANCG, JAK2 30, 100 539 JAK2 9p24.3p13.1, 9q34.2q34.3 cnLOH 13.7 JAK2 41 702 TN 9q21.11q21.13 cnLOH 6.5 71 70 111 740 TN 11q15.5p15.4 LOSS 1.7 ATM, CBL, RBM7, TP53AiP1, RBM14, RBM4B 26, 100 1008 JAK2 11q23.3q24.1 cnLOH 6.4 CBL, BBM7, TP53AiP1, RBM14, RBM4B 26, 100 12 615 JAK2 12q multiple alterations LOSS 34.7 AEBP2, GPRC5A, KRAS 93 926 TN 12q21.2q1.31 cnLOH 4.1 100 103 13 702 TN 13q24.12q4.1 LOSS 1.4 80 638 JAK2 13q41.13q14.3	8	846	TN	8p23.3q24.3	GAIN	146.4	CSMD1, RAD21-AS1, RBM12B, RUNX1T1, TP53INP1	76
818 TN 9q32q33.1 LOSS 5.5 20 724 JAK2 9p24.3p13.1, 9q34.2q34.3 cnLOH 42.4 FANCG, JAK2 30, 100 539 JAK2 9p24.3p23 cnLOH 42.4 FANCG, JAK2 41 702 TN 9q21.11q21.13 cnLOH 6.5 100 11 740 TN 11q15.2q25 cnLOH 6.7 ATM, CBL, RBM7, TP53AIP1, RBM14, RBM4B 26, 100 1018 JAK2 11q12.3q13.2, 11q13.3q25 cnLOH 6.4 CBL 100 1131 JAK2 11q12.3q13.2, 11q13.3q25 cnLOH 6.7 ATM, CBL, RBM7, TP53AIP1, RBM14, RBM4B 26, 100 1008 JAK2 12q13.3g11.1 LOSS 3.4.7 AEBP2, GPRC5A, KRAS 93 638 JAK2 12q13.3g14.3 LOSS 12.5 SH2B3, NCOR2 47-61 926 TN 12q2t.2q21.31 cnLOH 4.1 100 13 55 740 TN 15q22g24.2 cnLOH 7.7 14<	9	615	JAK2	9p24.3p13.3	cnLOH	35.9	FANCG, JAK2	100
724 JAK2 9p24.3p13.1, 9q34.2q34.3 cnLOH 42.4 FANCG, JAK2 30, 100 539 JAK2 9p24.3p23 cnLOH 13.7 JAK2 41 702 TN 9q21.1tq21.13 cnLOH 6.5 100 11 740 TN 11p15.5p15.4 LOSS 1.7 71 740 TN 11q12.3q13.2, 11q13.3q25 cnLOH 67.7 ATM, CBL, RBM7, TP33AIP1, RBM14, RBM4B 26, 100 1018 JAK2 11q23.3q24.1 cnLOH 6.4 CBL 100 12 615 JAK2 12q13.3g11.1 LOSS 34.7 AEBP2, GPRC5A, KRAS 93 638 JAK2 12q multiple alterations LOSS 12.5 SH2B3, NCOR2 47-61 926 TN 12q21.2q21.31 cnLOH 4.1 100 13 55 638 JAK2 13q14.13q14.3 LOSS 9.8 BRCA2, RB1 80 638 JAK2 15q13.3 GAIN 0.433 9		818	TN	9q32q33.1	LOSS	5.5		20
539 JAK2 9p24.3p23 cnLOH 13.7 JAK2 41 702 TN 9q21.11q21.13 cnLOH 6.5 100 11 740 TN 11p15.5p15.4 LOSS 1.7 ATM, CBL, 100 111 740 TN 11q13.2q25 cnLOH 67.7 ATM, CBL, RBM7, TP53AIP1, RBM14, RBM4B 26, 100 1008 JAK2 11q23.3q24.1 cnLOH 6.4 CBL 100 12 615 JAK2 12q13.3g11.1 LOSS 34.7 AEBP2, GPRC5A, KRAS 93 926 TN 12q21.31 cnLOH 4.1 100 13 702 TN 13q12.3q14.3 LOSS 19.8 BRCA2, RB1 80 638 JAK2 13q14.13q14.3 LOSS 1.4 100 35 15 740 TN 15q23.4 ColLH 7.7 100 743 CALR 15q23.3 GAIN 0.433 91 101 104		724	JAK2	9p24.3p13.1, 9q34.2q34.3	cnLOH	42.4	FANCG, JAK2	30, 100
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		539	JAK2	9p24.3p23	cnLOH	13.7	JAK2	41
11 740 IN 11p15.5p15.4 LOSS 1.7 ATM, CBL, 110 740 TN 11q13.2q25 cnLOH 67.7 ATM, CBL, RBM7, TP53AIP1, RBM14, RBM4B 26, 100 1008 JAK2 11q12.3q13.2, 11q13.3q25 cnLOH 67.7 ATM, CBL, RBM7, TP53AIP1, RBM14, RBM4B 26, 100 1008 JAK2 12p13.33p11.1 LOSS 34.7 AEBP2, GPRC5A, KRAS 93 638 JAK2 12q multiple alterations LOSS 12.5 SH2B3, NCOR2 47-61 926 TN 12q21.2q1.31 cnLOH 4.1 100 100 638 JAK2 13q14.3q14.3 LOSS 4.8 RB1 35 15 740 TN 15q23q24.2 cnLOH 7.7 100 743 CALR 15q13.3 GAIN 0.433 91 1014 JAK2 15q24.1q24.2 LOSS 1.4 46 16 743 CALR 15q23.1 LOSS 3.0 PRPF8, RAP1GAP2, SUZ12, TP53 19 638 JAK2 17p13.1p11.2 cnLOH 9.1		702	IN	9q21.11q21.13	CNLOH	6.5		100
140 IN I1q13.2q25 ChLOH 07.7 AIM, CBL, 100 1131 JAK2 11q12.3q13.2, 11q13.3q25 ChLOH 72.1 ATM, CBL, RBM7, TP53AIP1, RBM14, RBM4B 26, 100 100 JAK2 11q12.3q13.2, 11q13.3q25 ChLOH 6.4 CBL 100 12 615 JAK2 12q13.33p11.1 LOSS 34.7 AEBP2, GPRC5A, KRAS 93 638 JAK2 12q multiple alterations LOSS 12.5 SH2B3, NCOR2 47–61 926 TN 12q2.3q14.3 LOSS 19.8 BRCA2, RB1 80 638 JAK2 13q14.13q14.3 LOSS 19.8 BRCA2, RB1 80 638 JAK2 13q14.13q14.3 LOSS 1.4 91 100 13 702 TN 15q23q24.2 cnLOH 7.7 100 91 1014 JAK2 15q24.1q24.2 LOSS 1.4 46 100 1014 JAK2 15q24.1q24.2 LOSS 0.174 100 100 638 JAK2 17p13.3p13.1, 17q21.3lq2	11	740		11p15.5p15.4	LUSS	1.1		/1
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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		1008	JAKZ	11023 3024 1	CILOH cnl OH	6.4	AIM, CBL, RBM7, IP33AIP1, RBM14, RBM4B CRI	20, 100
12 613 JAK2 12 protoprint 100 100 100 13 702 TN 13 q12.3q14.3 LOSS 19.8 BRCA2, RB1 80 638 JAK2 13 q14.13 q14.3 LOSS 4.8 RB1 35 15 740 TN 15 q23 q24.2 cnLOH 7.7 100 743 CALR 15 q24.1q24.2 LOSS 1.4 46 16 743 CALR 16 q23.1 LOSS 0.174 81 17 818 TN 17 p13.3p13.1, 17q21.31q21.32, LOSS 13.3 PRPF8, RAP1GAP2, SUZ12, TP53 50-55 16 38 JAK2 17 q23.2q25.3 GAIN 22.5 RBF0X3, SRSF2 <	12	615	JAK2	11423.3424.1 12n13 33n11 1		347	AFRP2 GPRC54 KRAS	03
936 TN 12q21.2q21.31 cnLOH 4.1 100 13 702 TN 13q12.3q14.3 LOSS 19.8 BRCA2, RB1 80 638 JAK2 13q14.13q14.3 LOSS 4.8 RB1 35 15 740 TN 15q23q24.2 cnLOH 7.7 100 743 CALR 15q13.3 GAIN 0.433 91 1014 JAK2 15q24.1q24.2 LOSS 1.4 46 16 743 CALR 16q23.1 LOSS 0.174 81 17 818 TN 17p13.3q21.2 LOSS 39.6 PRPF8, RAP1GAP2, SUZ12, TP53 19 638 JAK2 17p13.1p11.2 cnLOH 9.1 100 100 638 JAK2 17q21.3q25.3 GAIN 22.5 RBF0X3, SRSF2 54 18 702 TN 18q12.2q21.1 cnLOH 12.4 SETBP1 100 19 538 CALR	12	638	.14K2	12g multiple alterations	1055	12 5	SH2R3 NCOR2	47_61
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638 JAK2 13q14.13q14.3 LOSS 4.8 RB1 35 15 740 TN 15q23q24.2 cnLOH 7.7 100 743 CALR 15q13.3 GAIN 0.433 91 1014 JAK2 15q24.1q24.2 LOSS 1.4 46 16 743 CALR 16q23.1 LOSS 0.174 81 17 818 TN 17p13.3q21.2 LOSS 39.6 PRPF8, RAP1GAP2, SUZ12, TP53 19 638 JAK2 17p13.3p13.1, 17q21.31q21.32, LOSS 13.3 PRPF8, RAP1GAP2, TP53 50-55 17q21.33 - - 100 100 100 638 JAK2 17q23.2q25.3 GAIN 22.5 RBFOX3, SRSF2 54 18 702 TN 18q12.2q21.1 cnLOH 12.4 SETBP1 100 19 538 CALR 19p13.3p12 cnLOH 22.9 CALR, CALR3, ELANE, JAK3, ZSWIM4 100 <td< td=""><td>13</td><td>702</td><td>TN</td><td>13a12.3a14.3</td><td>LOSS</td><td>19.8</td><td>BRCA2. RB1</td><td>80</td></td<>	13	702	TN	13a12.3a14.3	LOSS	19.8	BRCA2. RB1	80
15 740 TN 15q23q24.2 cnLOH 7.7 100 743 CALR 15q13.3 GAIN 0.433 91 1014 JAK2 15q24.1q24.2 LOSS 1.4 46 16 743 CALR 16q23.1 LOSS 0.174 81 16 743 CALR 16q23.1 LOSS 0.174 81 17 818 TN 17p13.3q21.2 LOSS 39.6 PRPF8, RAP1GAP2, SUZ12, TP53 19 638 JAK2 17p13.3p13.1, 17q21.31q21.32, LOSS 13.3 PRPF8, RAP1GAP2, TP53 50-55 17q21.33 17q21.33 100 12.4 SETBP1 100 19 538 CALR 19p13.3p12 cnLOH 12.4 SETBP1 100 19 538 CALR 19p13.3p12 cnLOH 22.9 CALR, CALR3, ELANE, JAK3, ZSWIM4 100 19 538 CALR 10p12q13.12 cnLOH 3.5 CEBPA 100	-	638	JAK2	13g14.13g14.3	LOSS	4.8	RB1	35
743 CALR 15q13.3 GAIN 0.433 91 1014 JAK2 15q24.1q24.2 LOSS 1.4 46 16 743 CALR 16q23.1 LOSS 0.174 81 17 818 TN 17p13.3q21.2 LOSS 39.6 PRPF8, RAP1GAP2, SUZ12, TP53 19 638 JAK2 17p13.1p11.2 cnLOH 9.1 100 638 JAK2 17q21.31q21.32, LOSS 13.3 PRPF8, RAP1GAP2, SUZ12, TP53 50-55 17q21.33 17q21.31q21.32, LOSS 13.3 PRPF8, RAP1GAP2, TP53 50-55 17q21.33 17q21.31q21.32, LOSS 13.3 PRPF8, RAP1GAP2, TP53 50-55 17q21.33 17q21.31q21.32, LOSS 13.3 PRPF8, RAP1GAP2, TP53 50-55 18 702 TN 18q12.2q21.1 cnLOH 12.4 SETBP1 100 19 538 CALR 19p13.3p12 cnLOH 22.9 CALR, CALR3, ELANE, JAK3, ZSWIM4 100 740 TN 19q12q1	15	740	TN	15q23q24.2	cnLOH	7.7		100
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17 818 TN 17p13.3q21.2 LOSS 39.6 PRPF8, RAP1GAP2, SUZ12, TP53 19 638 JAK2 17p13.1p11.2 cnLOH 9.1 100 638 JAK2 17p13.3p13.1, 17q21.31q21.32, LOSS 13.3 PRPF8, RAP1GAP2, SUZ12, TP53 50-55 638 JAK2 17q23.2q25.3 GAIN 22.5 RBFOX3, SRSF2 54 18 702 TN 18q12.2q21.1 cnLOH 12.4 SETBP1 100 19 538 CALR 19p13.3p12 cnLOH 22.9 CALR, CALR3, ELANE, JAK3, ZSWIM4 100 740 TN 19q12q13.12 cnLOH 3.5 CEBPA 100 20 904 CALR 20p13 GAIN 0.226 81 100 702 TN 20q11.21q13.13 LOSS 18.8 ASXL1, RBL1, RBM39, RBPJL, TP53INP2, TP53RK 80 704 TN 20q13.13q13.33 cnLOH 7 RAD21L1, RBCK1 100 702 TN <t< td=""><td>16</td><td>743</td><td>CALR</td><td>16q23.1</td><td>LOSS</td><td>0.174</td><td></td><td>81</td></t<>	16	743	CALR	16q23.1	LOSS	0.174		81
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		743	CAIR	20013.13013.33	cnl OH	15.7	CBLN4, CTCFL, DIDO1, GNAS, RTFI 1, TP53RK	100
21 724 JAK2 21011.2 LUSS 0.335 53	21	724	JAK2	21g11.2	LOSS	0.335		53
22 703 JAK2 22q12.1q12.3 cnLOH 4.4 100	22	703	JAK2	22q12.1q12.3	cnLOH	4.4		100

Table. Copy-number alterations and cnLOH in PMF patients

Note: Chr - chromosome; GAIN - copy-number gain; LOSS - copy-number loss; Mb - megabase.

*Percentage of DNA with identified cnLOH, GAIN or LOSS among studied DNA sample.

tion. During disease initiation stage, the cooperation between *EZH2* alterations and *JAK2* V617F mutations increases the ability of *JAK2* V617F-positive stem cells to self-renewal [10]. Interestingly, the mentioned *JAK2* V617-positive PMF patient (ID 638) with coexisting *EZH2* loss at 7q36.1 had multiple chromosome alterations indicating genomic instability potentially caused by this harmful initial combination (Fig. 4). This patient had additional copy number losses at 2p (involving epigenetic regulator *DNMT3A* and *TP53I3* gene which cooperates with p53 in cell death control); 5p and 5q; 12q (involving *SH2B3* gene which assists in JAK2-signaling regulation); 13q and 17q (involving well-studied across different malignancies *RB1* and *TP53* genes, respectively). Altered DNA burden for these regions with copy number losses ranged from 24 to 88%, but most of them were closed to 40–50%, suggesting their relation to the same leukemogenic cell clone. Proto-oncogene *CBL* encodes E3 ubiquitin ligase which negatively regulated JAK2-signaling due to JAK2 molecules ubiquitination and degradation. Even though, there is no evidence confirming ability of *CBL* gene mutations to drive disease, it was shown that they increase cell proliferation due to hypersensitivity to cytokines [11]. In the study we observed two *JAK2* V617-positive PMF cases with *CBL* homozygous loss, indicating that impaired ubiquitination of signaling molecules might



PMF patients positive for one of usual driver mutations in JAK2, MPL or CALR genes

PMF patients negative for usual driver mutations in JAK2, MPL or CALR genes

Fig. 3. Frequencies of affected genes with cnLOH and LOSS in Ukrainian PMF patients positive and negative for usual mutations in MPN driver genes (*JAK2, MPL* and *CALR*). LOSS — copy number loss; GAIN — copy number gain



Fig. 4. Multiple genomic alterations (patient's ID 638)

give advantages to myeloproliferation. The LAMB4 gene variants were reported in studies on myeloid neoplasms previously, but their biological function remains unknown in MPN [12]. cnLOH of DNA-damage response ATM gene and copy number loss of TP53 suggest their contribution to the disease evolution due to loss of DNA repair function.

Another interesting gene was *POT1* which was deleted in two cases of PMF patients who harbored multiple genomic alterations (Table 1). This gene encodes a nuclear protein involved in the telomere maintenance, regulating its lengths, protecting chromosome ends from illegitimate recombination and abnormal chromosome segregation. Significantly shortened telomeres, activation of telomerase, and altered expression of telomere-associated proteins are common features of various hematologic malignancies [13].

Recent study reported the use of an oligonucleotide that targets the RNA template of a human telomerase reverse transcriptase and inhibits its activity in some PMF patients [14]. Thus, the role of impaired POT1 protein is most likely implemented in cooperation with other damaging genomic alterations.

CONCLUSIONS

The study demonstrates the spectrum of genomic alterations in Ukrainian PMF patients that are positive and negative for usual mutations in MPN driver genes (*JAK2, MPL* or *CALR*). We have identified several genes potentially involved in the disease development and phenotype modification (*EZH2, LAMB4, CBL, CUX1, ATM, RB1* and *TP53*).

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