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RESEARCH ARTICLE

Characteristics of myeloproliferative neoplasms in patients exposed to ionizing radiation following the Chernobyl nuclear accident

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Abstract

Myeloproliferative neoplasms (MPNs) driver mutations are usually found in *JAK2*, *MPL*, and *CALR* genes; however, 10%-15% of cases are triple negative (TN). A previous study showed lower rate of *JAK2* V617F in primary myelofibrosis patients exposed to low doses of ionizing radiation (IR) from Chernobyl accident. To examine distinct driver mutations, we enrolled 281 Ukrainian IR-exposed and unexposed MPN patients. Genomic DNA was obtained from peripheral blood leukocytes. *JAK2* V617F, *MPL* W515, types 1- and 2-like *CALR* mutations were identified by Sanger Sequencing and real time polymerase chain reaction. Chromosomal alterations were assessed by oligo-SNP microarray platform. Additional genetic variants were identified by whole exome and targeted sequencing. Statistical significance was evaluated by Fisher's exact test and Wilcoxon's rank sum test (R, version 3.4.2). IR-exposed MPN patients exhibited a different genetic profile vs unexposed: lower rate of *JAK2* V617F (58.4% vs 75.4%, $P = .0077$), higher rate of type 1-like *CALR* mutation (12.2% vs 3.1%, $P = .0056$), higher rate of TN cases (27.8% vs 16.2%, $P = .0366$), higher rate of potentially pathogenic sequence variants (mean numbers: 4.8 vs 3.1, $P = .0242$). Furthermore, we identified several potential drivers specific to IR-exposed TN MPN patients: *ATM* p.S1691R with copy-neutral loss of heterozygosity at 11q; *EZH2* p.D659G at 7q and *SUZ12* p.V71 M at 17q with copy number loss. Thus, IR-exposed MPN patients represent a group with distinct genomic characteristics worthy of further study.

1 | INTRODUCTION

Ionizing radiation (IR) is a well-established human carcinogen that can cause genetic mutations or copy number alterations. Exposure to IR is associated with the induction of solid cancers and hematological malignancies including myeloproliferative neoplasms (MPNs).[1,2] On April 25-26, 1986, the Chernobyl nuclear power plant accident at reactor 4 occurred in Ukraine, classified as a level 7 event by the International Nuclear Event Scale. Over 500 000 people were involved in clean-up work following the accident during 1986-1991. Nearly 400 million people resided in territories that were contaminated with dangerous levels of radioactivity from April to July 1986. About 5 million people still live with dangerous levels of radioactive contamination in Ukraine, Belarus, and European Russia, thus the hematologic effects of IR are of ongoing concern.

Philadelphia-chromosome negative chronic MPNs are clonal diseases of hematopoietic stem cells that encompass polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Today the cause of MPNs can be identified on the molecular level in the majority of cases.[3,4] Acquired somatic mutations that drive the myeloproliferative phenotype usually arise in a mutually exclusive manner in 3 known genes: *Janus Kinase 2 (JAK2)*, *Thrombopoietin Receptor (MPL)*, and *Calreticulin (CALR)*, all of which activate JAK/STAT signaling.[4–6] The most frequent mutation of the *JAK2* gene, *JAK2* V617F, is associated with >95% of PV cases and with 50%-60% of ET and PMF cases.[5–8] The frequencies of the *CALR* gene mutations in patients with ET and PMF vary from 14% to 31% and from 12% to 35% of cases, respectively, as shown in meta-analysis of 19 studies.[9] A small proportion, 5%-10%, of MPN patients has activating mutations in the *MPL* gene.[5] However, somatic mutations of *JAK2*, *MPL*, or *CALR* gene are absent in 10%-15% of MPN patients, and this group is in a molecular diagnostic gap.[4,5,10,11] Thus, other molecular mechanisms might be involved in disease development in this group of patients. Numerous additional mutations have been described in MPNs, which can be mutually exclusive or co-exist with 1 of the 3 usual driving mutations. These include mutations affecting genes involved in cell signaling (*c-CBL*, *SOCS*, *LNK*, *PTPN11*, *FLT3*, *KIT*, *KRAS*, and *NF1*), tumor suppression (*CEBPA*, *NPM1*, and *RUNX1*), RNA maturation and slicing (*SF3B1*, *SRSF2*, and *U2AF1*), epigenetic regulation (*ATRX*, *DAXX*, *TET2*, *DNMT3A*, *EZH2*, and *ASXL1*), or leukemic transformation (*IDH1/2*, *NRAS/KRAS*, *TP53*, and *RUNX1*).[12–16]

Rapidly proliferating hematopoietic tissue is the most radiosensitive in a human body. When exposed to IR at doses in excess of 1-2 Gy, the sequence of predictable events appears within hours or days and is referred to as acute radiation syndrome which includes hematopoietic, gastrointestinal and neurovascular subsyndromes. The severity and duration of bone marrow depression is dose dependent at doses >1 Gy (deterministic or nonstochastic effects). Late IR effects caused by lower doses can manifest months or years after irradiation and do not exhibit a dose threshold (nondeterministic or stochastic effects). This study is focused on these late stochastic IR effects. The fundamental alterations (DNA base damages and changes, DNA–DNA and DNA–protein cross linking, single-, and double-strand breaks) that lead to late effects occur immediately after exposure. Radiogenic acute leukemia is one of the best studied neoplasms with the shortest minimal latent period and peak-incidence after IR exposure (2 and 5-7 years, respectively). In contrast, the minimal latent period for IR-induced multiple myeloma is approximately 20 years with unknown peak-incidence time after exposure. For IR-induced MPNs the latent period and peak-incidence after exposure are unknown.[17,18]

In this study, we provide a molecular and cytogenetic characterization of patients who developed MPN's following exposure to IR during the Chernobyl nuclear accident, in comparison with other Ukrainian MPN patients who did not report significant exposure to IR. A preliminary study of the same cohort of patients showed that the *JAK2* V617F mutation was less common in IR-exposed PMF patients than in unexposed PMF patients.[1,19] Thus, we hypothesized that IR-exposed MPN patients would exhibit a lower rate of the usual driver mutations, but would exhibit novel mutations and genomic alterations which could contribute to MPN development and evolution. In this study, we confirmed this hypothesis and identified combinations of sequence variants and copy number variants that may serve as drivers of MPN in IR-exposed patients.

2 | METHODS

2.1 | Patients and controls

The study included 281 MPN patients diagnosed in different clinics of Ukraine between 2009 and 2016 and referred to the National Research Center for Radiation Medicine of Ukraine (Kyiv, Ukraine). The group of patients included 90 MPN patients exposed to IR during the Chernobyl nuclear accident in Ukraine and 191 IR-unexposed MPN patients. In the majority of cases, the cleanup workers received 20-500 mSv, while permanent residents in the radionuclide contaminated territories received average total dose 5.9-31 mSv (≤0.5-5 mSv/year).[20] The controls included 96 female patients with average age 64 years (range: 49-79) and 25 years after IR exposure (dose range: 8-650 mSv), who were not diagnosed with any oncological condition at the moment of DNA sampling; and 89 IR-unexposed Ukrainians: 34 males and 55 females with average age 45 years (range: 19-77) who were not diagnosed with oncological conditions or other severe diseases and considered as healthy at the moment of DNA sampling. The patients and controls provided written informed consent in accordance with the Declaration of Helsinki. The study was approved by the local National Research Center for Radiation Medicine (Kyiv, Ukraine) and Dana-Farber/Harvard Cancer Center (Boston, USA) ethics committees. World Health Organization (WHO) 2016 MPN diagnostic criteria were used to classify the types of MPN. Clinical data (complete blood count, spleen size, history of thrombosis, transfusion dependence, transformation to acute leukemia) were also analyzed.

2.2 | Samples

Blood samples were obtained from MPN patients and controls and processed by density gradient centrifugation to obtain the peripheral blood mononuclear cells (PBMCs). PBMCs were used immediately for genomic DNA extraction with Quiamp DNA extraction kit (Qiagen,

Hilgen, Germany) or innuPREP Mini DNA extraction kit (Analytik Jena, Jena, Germany). Remaining PBMCs were stored at −20°C. DNA samples for 26 MPN patients were extracted from frozen PBMCs.

2.3 | Detection of usual driver mutations

Real time polymerase chain reaction (RT-PCR) TaqMan Assay was used for *JAK2* V617F mutation detection according to the method described[21] (Supporting Information Table S1, Methods). Patients negative for *JAK2* V617F mutation were tested for *MPL* and *CALR* gene mutations. Sanger Sequencing was used to detect *MPL* gene mutations. A 212-bp fragment containing *MPL* exon 10 sequence was amplified. Purified products were bidirectionally sequenced using forward and reverse nested primers as described[20] at the Boston Children's Hospital IDDRC Molecular Genetics Core Facility (Supporting Information Table S1, Methods). The chromatograms of MPL exon 10 were analyzed using SeqMan Pro 14 software (DNASTAR, Madison, Wisconsin, USA). Sequence NM_005373.2 was used as reference. Type 1-like *CALR* gene mutation was detected by RT-PCR and Melting Point Analysis. A 134-bp fragment containing *CALR* exon 9 was amplified and the melting point analysis was performed using Applied Biosystems 7500 Fast Real-Time PCR System. The result was analyzed using 7500 Software version 2.0.4. RT-PCR TaqMan Assay was used to detect type 2-like *CALR* gene mutation. Specific oligonucleotide probes were designed using NM_004343.3 sequence in Primer3Plus software (Supporting Information Table S1). PCR was performed as described in Supporting Information Methods.

2.4 | High-density array assay

Copy-number alterations and copy-neutral loss of heterozygosity (cnLOH) were assessed on the PBMC DNA from 30 PMF patients using the high-density CytoScan HD microarray platform (Affymetrix, Santa Clara, California, USA), which includes 2.67 million probes. DNA digestion, labeling, and hybridization were performed following the manufacturer's recommendations. The Chromosome Analysis Suite software version 3.1 (Affymetrix) based on the genome assembly version GRCh37/hg19 was used to analyze the data considering at least 50 markers over 200 kb (50 markers over 100 kb for oncology regions) for gains, 30 markers over 50 kb (15 markers over 20 kb for oncology regions) for losses, and cnLOH with a minimum length of 5 Mb (3 Mb for oncology regions). All genomic alterations were visually inspected and confirmed, and regions with poor quality were excluded.

2.5 | Whole-exome sequencing

Whole-exome sequencing (WES) was performed at the Broad Institute on the PBMC DNA from the same 30 PMF patients. Somatic variants from 30 samples were called using MuTect 2 (M2) over the ICE intervals. The germline DNA for these tumor samples were not available, thus germline events and sequencing noise were filtered using a panel of normals gathered from 406 samples and the HapMap 20-plex as an unmatched normal. Called variants that existed in 5% or more of ~60 000 samples in ExAC were filtered out (Supporting Information Methods). As a reference, GRCh37/hg19 human genome assembly was used. The sequence variants within coding regions were analyzed. To avoid sequence variants that were detected as a result of unfaithful PCR amplification or sequencing, a sequence variant was only further analyzed when the following conditions were met: (1) the variant was detected in at least 5 (absolute) reads; and (2) the variant was detected in >5% of all reads at the variant site.

2.6 | Targeted DNA sequencing

Targeted sequencing of suspected driver genes was performed on PBMC DNA from 28 MPN patients negative for usual driver mutations in 3 genes *(JAK2*, *MPL*, and *CALR)*, 96 IR-exposed Ukrainian subjects without oncologic diseases, and 89 unexposed healthy Ukrainian controls. The group of unexposed healthy individuals was analyzed to identify germline single nucleotide polymorphisms (SNPs) or sequence variants that may be unique to the Ukrainian population. 309 cancer-related genes were chosen for targeted sequencing, including genes with sequence variants detected by WES and genes located in the regions of genomic alterations identified by high-density CytoScan HD microarray assay and classified as cancer-related according to Catalog of Somatic Mutations in Cancer (COSMIC) database. Genomic DNA libraries were generated from PBMC DNA using SureSelectXT2 Target Enrichment System for Illumina Paired-End Multiplexed Sequencing (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions, using the SureSelect design wizard. DNA from the 89 healthy IR-unexposed controls were pooled and indexed as a single sample, while the other samples were processed individually. The libraries were sequenced with the Illumina HiSeq platform, 150-bp paired-end configuration using Illumina HiSeq 4000 System. The raw sequencing data was processed to remove any adaptor, PCR primers and low-quality reads using trimmomatic program.[22] The quality of the reads was checked using FastQC program.[23] High-quality reads were aligned against human genome using Burrows-Wheeler Alignment algorithm in paired manner.[24] As a reference, GRCh38 human genome assembly was used. Variant calling was performed using Haplotype caller function in GATK workflow.[25] Further annotation of variants was performed using the ANNOVAR tool.[26] Nonsynonymous nucleotide variants with adequate read depth (at least 5 variant reads and variant read count >5% of reads) were compared between MPN patients and controls (healthy IR-exposed and unexposed groups).

2.7 | Statistical analysis

Statistical significance for categorical variables and continuous variables were evaluated by Fisher's exact test and Wilcoxon's rank sum test using R, version 3.4.2.

3 | RESULTS

3.1 | Demographic and clinical features of MPN patients

We studied 281 patients (106 PV patients, 58 ET patients, 108 PMF patients and 9 MPN, unclassifiable). Supporting Information Tables S2

and S3 report the demographic, clinical, and hematologic features of IR-exposed and unexposed MPN patients at the time of diagnosis, classified by disease subtype according to the World Health Organization (WHO) classification of 2016.[27] Overall, 151 males and 128 females were enrolled in the study. There were more males than females among IR-exposed MPN patients ($P < .0001$), the same pattern of sex distribution was observed in disease subtypes of IR-exposed MPN patients including PV, ET, and PMF patients. This is consistent with more males being involved in the cleanup work of the Chernobyl nuclear accident consequences. IR-exposed MPN patients were older ($P < .0001$) at the time of diagnosis with median age 60 years (range 11-79), than IR-unexposed MPN patients with median age 53 years (range 19-87). The median age at the time of exposure to low doses of IR was 35 years (range 0-57). After IR-exposure, the median time to MPN diagnosis was 25 years (range 2-30 years).

The clinical features of both groups, IR-exposed and unexposed MPN patients (cases of thrombosis, splenomegaly, transfusion dependence, transformation to acute leukemia), were similar and comparable to the reported data in retrospective studies.[10,28–31] However, more PMF IR-exposed patients were transfusion dependent (32.4%), than PMF unexposed patients (14.1%) ($P = .042$), indicating enhanced severity of radiation-associated PMF. No significant difference in the rate of transformation to acute leukemia was observed (Supporting Information Table S2). However, for most MPN patients the follow up period was <10 years from diagnosis, which may not be sufficient time to observe leukemia transformation.

3.2 | Frequencies of the usual driver mutations in *JAK2*, *MPL*, and *CALR* genes in IR-exposed and IR-unexposed MPN patients

We next carried out a series of studies to identify the spectrum of genomic alterations in IR-exposed vs unexposed MPN patients (Figure 1A). We first tested for the presence of the *JAK2* V617F mutation. There were significantly more *JAK2* V617F-positive cases (75.4%) among IR-unexposed MPN patients, compared with IR-exposed patients (58.4%) ($P = .0077$) (Figure 1B; Supporting Information Table S4). This did not appear to reflect a higher prevalence of PV among IR-unexposed MPN patients as there was also a trend toward higher *JAK2* mutation frequency in IR-unexposed vs exposed patients with PV (92.9% vs 65%, $P = .1014$). MPN patients who were *JAK2* V617F-negative were tested for *MPL* and *CALR* gene mutations (Figure 1B; Supporting Information Table S4). We did not observe a significant difference in the rate of MPL or *CALR* type 2-like mutations in IR-exposed vs unexposed MPN patients, but the rate of *CALR* type 1-like mutation was significantly increased in IR-exposed MPN patients compared with unexposed MPN patients (12.2% vs 3.1%, $P = .0056$). This increase in *CALR* type 1-like mutation rate held true for PMF and ET subtypes: (18.9% vs 4.2% and 33.3% vs 6.5%, $P = .0279$ and .0298, respectively). Consistent with our prior hypothesis, we observed a higher rate of MPNs that are triple negative (TN) for *JAK2*, *MPL*, and *CALR* mutations (TN MPN) in the IR-exposed vs unexposed patients (27.8% vs 16.2%, $P = .0366$). The *JAK2* V617F and *CALR* mutation frequencies in unexposed to IR MPN patients in this study were similar to the reported data.[9,32–37] The frequency of *MPL* W515 gene mutation was lower in MPN patients in our study, in comparison to published data.[5,38–40]

3.3 | Genetic variants identified in IR-exposed and IR-unexposed PMF patients

WES was performed on the PBMC DNA from 30 PMF patients (13 IR-exposed and 17 unexposed PMF patients). WES identified previously reported and unreported nonsynonymous nucleotide variants considered as pathogenic or potentially pathogenic based on COSMIC, dbSNP, 1000Genoms, ClinVar, Varsome, and UniProt databases. Overall, excluding usual mutations in *JAK2*, *MPL*, and *CALR* genes, there were more pathogenic or potentially pathogenic sequence variants identified in IR-exposed than unexposed PMF patients. The mean number of pathogenic or potentially pathogenic nonsynonymous variants detected in IR-exposed PMF patients was 4.8 (range 1-9), while the mean number of nonsynonymous nucleotide variants detected in unexposed PMF patients was 3.1 (1-8), ($P = .0242$). Among recurrently affected genes in IR-exposed PMF patients were *ASXL1*, *U2AF1*, *PEG3*, *LAMB4*, *NF1*, *JARID2*, *EZH2*, *DNMT3A*, *TET2*, and *ATM*, in addition to *JAK2* and *CALR* (Figure 2; Supporting Information Tables S5 and S6). In IR-unexposed patients recurrently affected were genes *ASXL1*, *EZH2*, *PEG3*, *RTEL1*, *SUZ12*, *RBBP8*, *BRCA2*, *FLT3LG*, and *ATM*, in addition to *JAK2*, *MPL*, and *CALR*. Interestingly, *ASXL1* gene was affected more frequently in IR-exposed (5 cases, 38.5%) vs unexposed (3 cases, 17.6%) PMF patients. However, this difference does not reach statistical significance ($P = .242$) (Figure 2A,B; Supporting Information Tables S5 and S6).

Among the subset of IR-exposed patients negative for mutations in *JAK2*, *MPL*, and *CALR* (TN), recurrently affected genes were *EZH2*, *DNMT3A*, *TET2*, and *ATM*. Genes affected in single cases in this IR-exposed TN subset were *KIT*, *SUZ12*, *CUX1*, *KRAS*, *UMODL1*, *CBL*, and *SF3B1* (Figure 2A). Overall, the gene functions most frequently affected in these 30 PMF patients are involved in signal transduction, DNA damage, splicing, and epigenetic regulation (Figure 2C).

3.4 | Copy number alterations and cnLOH in IR-exposed and IR-unexposed PMF patients

Analysis of copy-number alterations and cnLOH for 30 PMF patients was assessed by High-Density Array Assay in addition to WES. This revealed frequent alterations, but no significant difference in the rates of copy-number loss, copy-number gain, cnLOH, or multiple chromosomal alterations, respectively in the IR-exposed vs unexposed groups (30.8% vs 47.1%, $P = .4651$; 15.4% vs 17.6%, $P = 1$; 38.5% vs 70.6%, $P = .1376$; and 30.8% vs 47.1%, $P = .4651$) (Figure 2D and Table 1). The most common chromosomal abnormalities were cnLOH at 1p, 9p, 11q, and copy-number loss at 7q, 13q. These findings are consistent with published data indicating that the most frequent chromosomal abnormalities in MPNs are LOH at 1p, 9p, 14q, gain of chromosome 8, 9, 14, gain at 1q, and loss at 11q, 13q, 18p, 20q, while loss at 5q, 7p, 7q, and LOH at 17q have been observed in single cases.[41,42]

The cnLOH we found at 1p in IR-unexposed PMF patients duplicated the pathogenic *MPL* W515R mutation in 2 out of 4 cases and

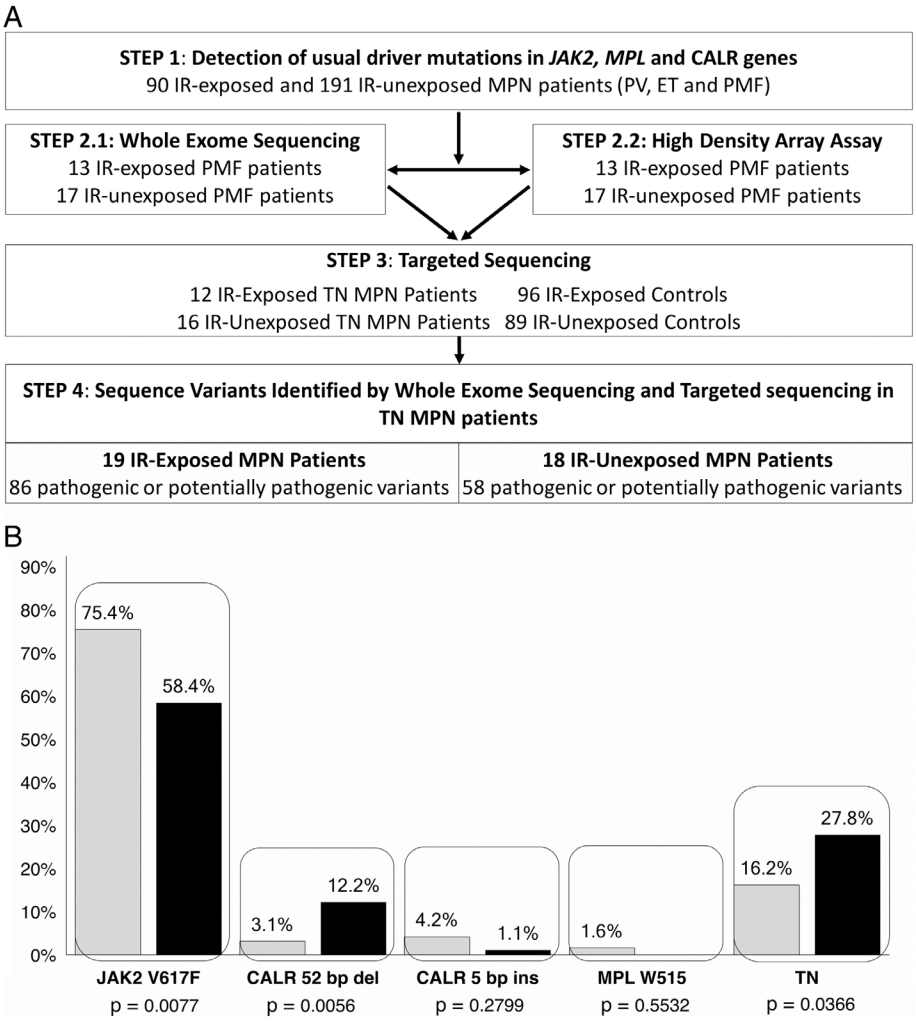

FIGURE 1 A, Study flow chart. B, Frequencies of *JAK2* V617F, *CALR* type 1- (52 bp del) and 2-like (5 bp ins), *MPL* W515 and negative cases for these mutations (TN) in IR-exposed (black) and IR-unexposed (white) MPN patients. IR, Ionizing radiation; MPN, Myeloproliferative neoplasms

also a novel *MPL* P222S variant in 1 case. In contrast, the 1p alterations did not involve the *MPL* gene in IR-exposed cases. Copy-number LOH at 19p duplicated the pathogenic *CALR* K385 f. mutation in 1 out of 2 cases in IR-exposed PMF patients and did not affect chromosome 19 in IR-unexposed PMF patients. This supports our results suggesting that *CALR* gene alterations contribute more and *MPL* gene alterations less to the disease development in IR-exposed MPN patients.

Copy-number losses of *EZH2* at 7q (ID 846) and *SUZ12* at 17q (ID 818), both components of polycomb repressive complex 2 (PRC2), were identified in conjunction with nonsynonymous missense variants in IR-exposed PMF patients who were negative for usual driver mutations in the *JAK2*, *MPL*, and *CALR* genes. This may suggest the contribution of impaired PRC2 (by loss-of-function alterations) to MPN development. Copy-neutral LOH involving *ATM* gene at 11q with identified nonsynonymous missense variant in another TN IR-exposed patient (ID 740) suggests homozygous loss of *ATM* function and subsequent DNA damage repair defects. In the same patient (ID 740) cnLOH at 11q duplicated an additional missense mutation *CBL* R420G (89% allele frequency) with enhancing cell survival capacity, but without confirmed influence on cell proliferation.[43] Finally, copy-number loss of *TP53* at 17p was identified in 1 *JAK2*-positive (24% allele frequency) unexposed PMF patient in conjunction with a likely pathogenic missense variant p.C176Y (c.527G > A) (23% allele frequency), which suggests its contribution to the disease evolution due to loss of DNA repair function.

3.5 | Targeted sequencing of candidate driver genes in TN MPN patients

Based on the WES and high-density array assay data we identified 309 genes that were altered in at least 1 case and may be pathogenic. To identify novel sequence variants that may drive MPN development in TN patients, we performed targeted sequencing of the 309 genes on an additional cohort of IR-exposed (12) and unexposed (16) TN MPN patients' DNA. In parallel we similarly examined 89 IR-unexposed healthy controls to identify germline SNPs or sequence variants that may be unique to the Ukrainian population. We also examined 96 IR-exposed healthy controls to further identify novel SNPs or sequence variants and potentially find evidence of subclinical MPN (Figure 1A).

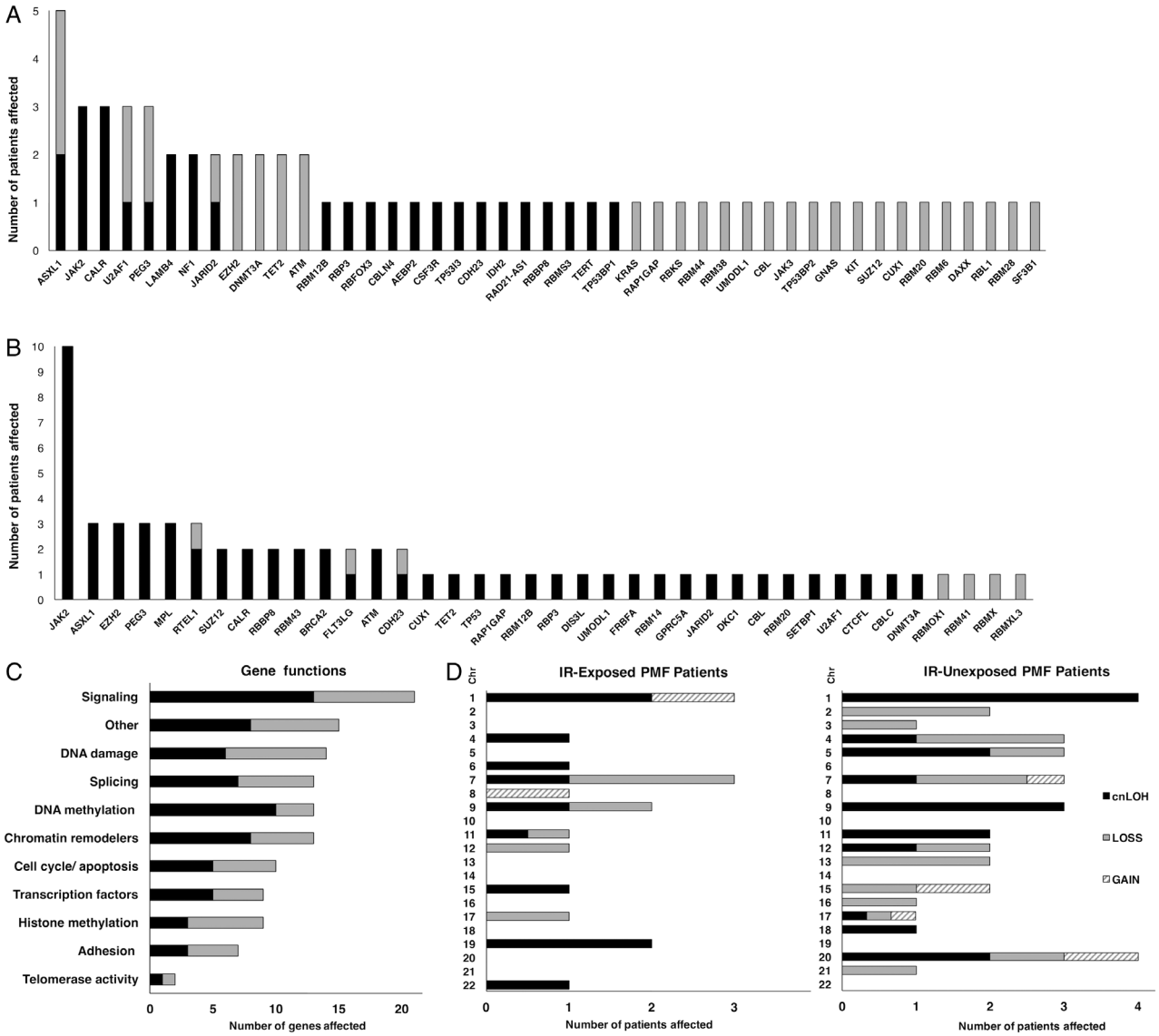

FIGURE 2 Frequencies of genes with variants considered as pathogenic or potentially pathogenic in (A) IR-exposed and (B) IR-unexposed PMF patients with known driver mutation (black) and in TN patients (white) as identified by WES of PBMC. C, Pathways potentially affected in IR-exposed (black) and unexposed (white) PMF patients. D, Copy-number alterations and cnLOHin 13 PMF patients previously exposed to IR and in 17 unexposed PMF patients. IR, Ionizing radiation; PMF, primary myelofibrosis; TN, patients negative for usual driver mutations in *JAK2*, *MPL*, and *CALR* genes; Chr, chromosome; cnLOH, copy-nutral loss of heterozygosity; LOSS, copy-number loss; GAIN: copy-number gain

Overall, there were 133 nonsynonymous sequence variants (Supporting Information Tables S7-S9) in the 28 TN MPN patients with a range of 1-11 sequence variants per sample. It should be noted that despite filtering sequence variants, many variants had allele frequencies around 50% that could be clonal mutations, but we are not able to exclude that these are rare germline sequence variants.

IR-exposed and unexposed TN MPN patients did not differ regarding mean number of sequence variants per patient, 4.5 (range 1-9) and 4.9 (1-11), respectively (P = .3208). In contrast, TN IR-unexposed patients displayed a higher mean number of sequence variants per patient (4.9, range 1-11) vs nonTN IR-exposed patients (3, range 1-8) (P = .0112), suggesting a greater role for DNA damage in the group of IR-unexposed TN MPN patients. Overall, combining the targeted sequencing data from the IR-exposed and unexposed patients, genes affected in TN MPN patients were involved in signal transduction, transcriptional regulation, DNA damage, and splicing (Figure 3B).

3.6 | Recurrently affected genes in TN IR-exposed MPN patients

We next combined WES and Targeted Sequencing data to focus on TN cases (negative for usual mutations in *JAK2*, *MPL*, and *CALR* genes) (Figure 3A). First, we asked whether there are recurrently affected genes that are specific to TN IR-exposed MPN patients. Several potentially pathogenic sequence variants were detected exclusively in TN IR-exposed MPN patients in the following genes: *KIT*, *JAK3*, *USP6*, *RBPJL*, and *RBM44* (Figure 3A). One PMF patient (ID 547) harbored *KIT* D816V missense mutation with 16% allele frequency reported in patients with systemic mastocytosis.[44]

TABLE 1 Copy-number alterations and cnLOH in PMF patients

Chr	Patients's ID	Exposure to IR	Region altered	Type of alteration	Size, Mb	Genes within the regions	Pathogenic or potentially pathogenic variants[a]
1	740	Exp	1p36.32p36.22, 1q32.3q41	cnLOH	12.8	*RBP7*	
	818	Exp	1p36.33p34.3	cnLOH	34.8	*RAP1GAP*, *RBP7*	
	615	Exp	1q21.1q44	GAIN	103.8	*RBBP5*, *RBM34*, *TP53BP2*	
	842	Un	1p34	cnLOH	1	*MPL*	*MPL* c.1543 T > A p.W515R
	848	Un	1p36.33p33	cnLOH	48.2	*CSF3R*, *MPL*, *RAP1GAP*, *RBP7*	*MPL* c.1543 T > A p.W515R
	983	Un	1p33p32.3	cnLOH	3		
	702	Un	1p36.36p22.1	cnLOH	93.6	*CSF3R*, *JAK1*, *MPL*, *RAP1GAP*, *RBMXL1*, *RBP7*, *RPL5*	*MPL* c.664C > T p.P222S
2	638	Un	2p22.2, 2p23.3, 2p25.3, 2q35	LOSS	3.3	*DNMT3A*, *TP53I3*	
	702	Un	2q22.3q23.3	LOSS	3.9		
3	852	Un	3p14.2	LOSS	0.3		
4	740	Exp	4q31.23q31.3	cnLOH	4.9	*FBXW7*	
	1131	Un	4p16.3	LOSS	1.8		
	926	Un	4q31.3	LOSS	0.364		
	724	Un	4q22.3q23	cnLOH	3.5	*RAP1GDS1*	
5	724	Un	5p13.2q11.2	cnLOH	9.7		
	638	Un	5p, 5q multiple alterations[b]	LOSS	134.4	*IRF1*, *RBM22*, *NPM1*, *DDX41*	
	852	Un	5p15.2p14.3	cnLOH	6.1		
6	740	Exp	6q21q22.31	cnLOH	15.2		
7	740	Exp	7q21.3	cnLOH	4.3		
	818	Exp	7q22.3q36.2	LOSS	47.9	*BRAF*, *EZH2*, *LAMB4*, *POT1*	
	846	Exp	7q35q36.2	LOSS	6.8	*EZH2*	*EZH2* c.1976A > G p.D659G
	638	Un	7q11.23, 7q11.23q21.11, 7q21.2q21.3	GAIN	12.9	*RBM48*	
	638	Un	7q21.11q21.2, 7q21.3q36.3	LOSS	71.7	*BRAF*, *CUX1*, *EZH2*, *LAMB4*, *POT1*, *RBM33*	
	702	Un	7q21.3q31.31	LOSS	25.1	*CUX1*, *EZH2*, *LAMB4*	
	842	Un	7q36.1q36.2	cnLOH	3.3		
8	846	Exp	8p23.3q24.3	GAIN	146.4	*CSMD1*, *RAD21-AS1*, *RBM12B*, *RUNX1T1*, *TP53INP1*	
9	615	Exp	9p24.3p13.3	cnLOH	35.9	*FANCG*, *JAK2*	*JAK2* c.1849G > T p.V617F
	818	Exp	9q32q33.1	LOSS	5.5		
	724	Un	9p24.3p13.1, 9q34.2q34.3	cnLOH	42.4	*FANCG*, *JAK2*	*JAK2* c.1849G > T p.V617F
	539	Un	9p24.3p23	cnLOH	13.7	*JAK2*	*JAK2* c.1849G > T p.V617F
	702	Un	9q21.11q21.13	cnLOH	6.5		
11	740	Exp	11p15.5p15.4	LOSS	1.7		
	740	Exp	11q13.2q25	cnLOH	67.7	*ATM*, *CBL*,	*ATM* c.5071A > C p.S1691R, *CBL* c.1258C > G p.R420G
	1131	Un	11q12.3q13.2, 11q13.3q25	cnLOH	72.1	*ATM*, *CBL*, *RBM7*, *TP53AIP1*, *RBM14*, *RBM4B*	*CBL* c.1139 T > C p.L380P
	1008	Un	11q23.3q24.1	cnLOH	6.4	*CBL*	
12	615	Exp	12p13.33p11.1	LOSS	34.7	*AEBP2*, *GPRC5A*, *KRAS*	*AEBP2* c.198_199insG p.G66 fs
	638	Un	12q multiple alterations[c]	LOSS	12.5	*SH2B3*, *NCOR2*	
	926	Un	12q21.2q21.31	cnLOH	4.1		
13	702	Un	13q12.3q14.3	LOSS	19.8	*BRCA2*, *RB1*	
	638	Un	13q14.13q14.3	LOSS	4.8	*RB1*	

(*Continues*)

TABLE 1 (Continued)

Chr	Patients's ID	Exposure to IR	Region altered	Type of alteration	Size, Mb	Genes within the regions	Pathogenic or potentially pathogenic variants[a]
15	740	Exp	15q23q24.2	cnLOH	7.7		
	743	Un	15q13.3	GAIN	0.433		
	1014	Un	15q24.1q24.2	LOSS	1.4		
16	743	Un	16q23.1	LOSS	0.174		
17	818	Exp	17p13.3q21.2	LOSS	39.6	*PRPF8*, *RAP1GAP2*, *SUZ12*, *TP53*	*SUZ12* c.211G > A
	638	Un	17p13.1p11.2	cnLOH	9.1		
	638	Un	17p13.3p13.1, 17q21.31q21.32, 17q21.33	LOSS	13.3	*PRPF8*, *RAP1GAP2*, *TP53*	*TP53* c.527G > A p.C176Y
	638	Un	17q23.2q25.3	GAIN	22.5	*RBFOX3*, *SRSF2*	
18	702	Un	18q12.2q21.1	cnLOH	12.4	*SETBP1*	
19	538	Exp	19p13.3p12	cnLOH	22.9	*CALR*, *CALR3*, *ELANE*, *JAK3*, *ZSWIM4*	*CALR* c.1154_1155insTTGTC p.K385 fs
	740	Exp	19q12q13.12	cnLOH	3.5	*CEBPA*	
20	904	Un	20p13	GAIN	0.226		
	842	Un	20p13p12.3	cnLOH	7	*RAD21L1*, *RBCK1*	
	702	Un	20q11.21q13.13	LOSS	18.8	*ASXL1*, *RBL1*, *RBM12*, *RBM39*, *RBPJL*, *TP53INP2*, *TP53RK*	
	743	Un	20q13.13q13.33	cnLOH	15.7	*CBLN4*, *CTCFL*, *DIDO1*, *GNAS*, *RTEL1*, *TP53RK*	
21	724	Un	21q11.2	LOSS	0.335		
22	703	Exp	22q12.1q12.3	cnLOH	4.4		

Abbreviations: PMF, primary myelofibrosis; Chr, Chromosome; ID, identification; IR, ionizing radiation; Exp, exposed to IR PMF patients; Un, unexposed to IR PMF patients; cnLOH, copy-neutral loss of heterozygosity; GAIN, copy-number gain; LOSS, copy-number loss; Mb, megabase.

[a] Pathogenic or potentially pathogenic variants identified by WES.

[b] 5p15.32p15.31, 5q11.1q11.2, 5q11.2, 5q11.2q12.1, 5q12.1q12.3, 5q12.3, 5q12.3q21.3, 5q21.3q22.1, 5q22.2q23.1, 5q23.1q31.3, 5q31.3, 5q31.3q33.1, 5q33.2q33.3, 5q33.3q34, 5q34, 5q34q35.1, 5q35.1q35.3, 5q35.3, 5p14.2p14.1, 5p14.3.

[c] 12q12q13.11, 12q13.11q13.12, 12q13.3q14.1, 12q21.2, 12q22, 12q24.11q24.12, 12q24.23q24.31, 12q24.33.

3.7 | Recurrently affected genes in TN IR-exposed and IR-unexposed MPN patients

We next identified recurrently affected genes that were exclusive to TN cases (IR-exposed or unexposed), which included *SF3B1*, *ATMIN*, *BCORL1*, *CSMD1*, *SEPT9*, *RBM6*, and *MEGF6*. One of the identified *SF3B1* variants was a missense mutation K700E with 32% allele frequency in an unexposed TN PMF patient (ID 1007). K700E, the most frequently reported mutation in the *SF3B1* gene, has been identified in chronic lymphocytic leukemia, myelodysplastic syndrome, breast, and pancreatic cancers.[45–48]

3.8 | Recurrently affected genes in TN IR-exposed and IR-unexposed MPN patients also identified in nonTN cases

Several recurrently affected genes in TN MPN patients (IR-exposed or unexposed) were also involved in MPN patients with usual driver mutations in *JAK2*, *MPL* or *CALR* genes. Among them were *ASXL1*, *ATM*, *DNMT3A*, *EZH2*, *SUZ12*, *TET2*, *RTEL1*, *SETBP1*, *U2AF1*, *CSF3R*, and *UMOD1* (Figure 3A). Although mutations of *ASXL1* and *DNMT3A* were found in cases with usual driver mutations, they were more frequent in TN MPN patients, and only in the IR-exposed TN MPN patients. *ASXL1* variants were found in 5 (29.4%) IR-exposed TN cases with allele frequencies varying from 27% to 56%, and DNMT3A variants were found in 3 (17.6%) IR-exposed TN cases with allele frequencies varying from 26% to 48%.

Two sequence variants of the recurrently affected *CSF3R* gene were identified in IR-exposed (ID 1360) and unexposed (ID 879) TN ET patients. *CSF3R* T618I (observed in ID 1360 with 37% allele frequency) is a highly prevalent and well-studied specific mutation in chronic neutrophilic leukemia.[49,50] The other patient (ID 879) had a previously undescribed missense variant *CSF3R* G415R (46% allele frequency) and in addition *TP53* V31I variant (45% allele frequency) which has been previously identified in patients with acute myeloid leukemia.[51]

Finally, noncanonical mutations in *JAK2* gene were identified in 2 TN cases. *JAK2* R938Q missense variant with 45% allele frequency was detected in a IR-exposed TN PMF patient (ID 1283) The *JAK2* R938Q somatic mutation was reported previously in a hereditary thrombocythemia case and B-cell acute lymphoblastic leukemia case.[52,53] An IR-exposed PV patient (ID 1887) exhibited a frameshift variant *JAK2* K539 f. of uncertain biological significance with 23% allele frequency.

We also identified potentially pathogenic variants in a healthy 68-years old female who was exposed to IR (documented dose 50 mSv). These were missense *U2AF1* p.Q157P (c.470A > C) with

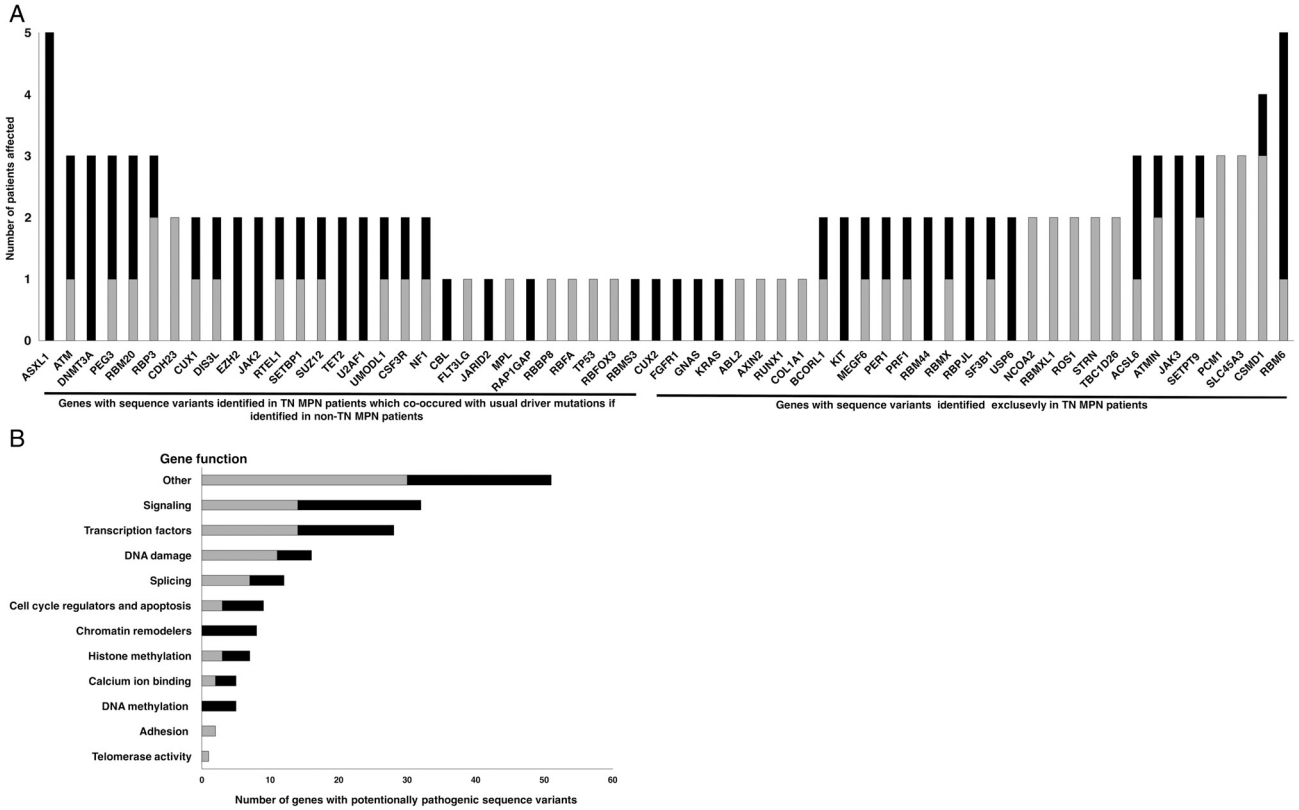

FIGURE 3 A, Frequencies of genes with sequence variants identified by WES and targeted sequencing in IR-exposed (black) and unexposed (white) MPN patients negative for usual driver mutations in *JAK2*, *MPL*, and *CALR* genes. B, Pathways potentially affected in 12 IR-exposed (black) and 16-unexposed (white) MPN patients negative for usual driver mutations in *JAK2*, *MPL*, and *CALR* genes. IR, ionizing radiation; MPN, myeloproliferative neoplasms

23% allele frequency and nonsense *DNMT3A* p.C368X (c.C1104A) mutations with 21% allele frequency. *U2AF1* p.Q157P (c.470A > C) was previously reported in patients with MDS[54,55] and PMF.[56] Interestingly, a daughter of this woman was diagnosed with acute leukemia. We did not identify any potential pathogenic variants in healthy IR-unexposed controls.

4 | DISCUSSION

In this study, we evaluated the effect of prior exposure to IR during the Chernobyl nuclear accident on the clinical characteristics and genomic profiles of MPN patients in Ukraine. IR-exposed MPN patients exhibited a higher mean age and were predominantly men, which likely reflect the time elapsed from the Chernobyl disaster and the male sex-bias of cleanup workers. The clinical profiles of the IR-exposed and unexposed patients were similar, although the rate of transfusion-dependence was slightly higher among IR-exposed MPN patients. We found that IR-exposed MPN patients exhibited a different genetic profile from that of unexposed MPN patients: a lower rate of *JAK2* V617F mutation, a higher rate of type 1-like *CALR* mutation, a higher rate of TN cases, and a higher rate of potentially pathogenic sequence variants. No difference in the rates of chromosomal alterations in the IR-exposed and unexposed groups may be related to the fact that some of the patients who developed chromosomal alterations immediately following IR exposure >30 years ago developed leukemia and did not survive to be included in this cohort.

Using Next Generation Sequencing, we identified a spectrum of affected genes with sequence variants in both IR-exposed and -unexposed MPN patients. TN IR-exposed and unexposed MPN patients did not differ regarding median numbers of sequence variants per patient and exhibited mutations in several of the same genes. This may suggest a common molecular basis of TN MPNs in Ukrainian IR-exposed patients with a documented history of irradiation and unexposed MPN patients without such history, but for whom we could not exclude the possibility of exposure to IR due to external (visiting contaminated with radionuclides territories, traveling by flight or undergoing specific medical examinations) or internal irradiation (contaminated food, using radioactive substances for medical purposes).

Nevertheless, in IR-exposed TN MPN patients, in comparison to unexposed TN MPN cases and healthy controls, we identified several nonsynonymous sequence variants in conjunction with copy number alteration of the corresponding chromosomal regions that we propose as potential drivers of MPN development. Among these genetic variants, *ATM* p.S1691R (c.5071 A > C) variant (91% allele frequency) with cnLOH at 11q22.3 was identified in 1 TN IR-exposed PMF patient (ID 740) diagnosed 13 years after IR-exposure at the age of 47. Patient's DNA was collected at the age of 58 when he presented with anemia (HGB 88 g/L), thrombocytopenia (80×10^9/L), leukocytosis (18.7×10^9/L), increased level of LDH (589 U/L), palpable

splenomegaly (218 mm by ultrasound), and hepatomegaly (191 mm by ultrasound) and was erythrocyte-transfusion dependent. Previously the same *ATM* S1691R variant was reported in an Ataxia-Telangiectasia family[57] and in patients with chronic lymphocytic leukemia, including 1 case with LOH at *D11S2179*,[58,59] breast cancer,[60–62] and melanoma,[63] but not in MPN patients.

Missense mutations in *EZH2* at 7q36.1 and *SUZ12* at 17q11.2 with copy number loss were also identified separately in TN IR-exposed PMF patients (ID 846 and 818, respectively). Loss-of-function mutations and cytogenetic alterations of *EZH2* and *SUZ12* impair function of PRC2 and are frequently reported in MPNs/MDS.[64–66] Missense mutation *EZH2* p.D659G (c.1976 A > G) at 7q36.1 with copy number loss (66% allele frequency) was identified in a 69-years old IR-exposed TN PMF (ID 846) patient. The patient was diagnosed 26 years after IR-exposure and presented with mild anemia (HGB 125 g/L), leukocytosis (14.8 $\times$ 10^9/L), increased level of LDH (907 U/L), palpable splenomegaly (171 mm by ultrasound) and later became RBC-transfusion dependent. Frequently described in MPNs, loss-of-function mutations of *EZH2* gene (~10%) are mostly early events in leukemogenesis and associated with a poor prognosis and MPN phenotype modifications.[65,67]

Missense mutation *SUZ12* p.V71 M (c.211G > A) at 17q11.2 with copy number loss and 50% allele frequency was identified in a 58 years old TN PMF patient 25 years after IR-exposure (ID 818). The patient presented with mild anemia (HGB 125 g/L), leukopenia (1.9 $\times$ 10^9/L), palpable splenomegaly (249 mm by ultrasound) and hepatomegaly (187 mm by ultrasound). *SUZ12* gene mutations in MPNs are relatively rare (1.4%[66]; 1.6%[68]), although in the range of *MPL* gene mutation frequencies.

This study provides the first extensive chromosomal and genomic characterization of patients who developed MPN's following exposure to IR and the Chernobyl nuclear disaster in particular. The limitations of this study include the retrospective nature and the relatively small number of samples evaluated by WES and HD Array Assay, which limited the statistical power to discern the effect of IR on the incidence of specific subgroups of MPN, specific nonsynonymous nucleotide variants, or specific copy number alterations. Another limitation was that the only DNA obtained was from PBMC's, which likely included both somatic and germline contributions, as a germline source of DNA was not available. To overcome this limitation, we performed targeted sequencing of DNA from healthy Ukrainian controls, to filter out germline nonsynonymous variants that occur frequently in the general Ukrainian population. Supporting the argument that the nonsynonymous sequence variants in potential MPN driver genes that we identified in MPN patients were somatic, rather than germline, none of these sequence variants were identified in unexposed healthy controls. Despite these limitations, we have identified a wide spectrum of nonsynonymous sequence variants among IR-exposed and unexposed MPN patients. We found that while TN IR-exposed and unexposed MPN patients often exhibited similar DNA alterations suggesting a common molecular basis for MPN development, there were unusual genetic variants specific to some of the IR-exposed TN MPN patients and that TN MPN is more common among IR-exposed MPN patients. These findings indicate that IR exposure contributes particularly to the development of TN MPN, which in turn is driven by a diversity of oncogenic genomic alterations.

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SUPPORTING INFORMATION

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