High-resolution single-nucleotide polymorphism array-profiling in myeloproliferative neoplasms identifies novel genomic aberrations

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ABSTRACT

Single-nucleotide polymorphism arrays allow for genome-wide profiling of copy-number alterations and copy-neutral runs of homozygosity at high resolution. To identify novel genetic lesions in myeloproliferative neoplasms, a large series of 151 clinically well characterized patients was analyzed in our study. Copy-number alterations were rare in essential thrombocythemia and polycythemia vera. In contrast, approximately one third of myelofibrosis patients exhibited small genomic losses (less than 5 Mb). In 2 secondary myelofibrosis cases the tumor suppressor gene *NF1* in 17q11.2 was affected. Sequencing analyses revealed a mutation in the remaining *NF1* allele of one patient. In terms of copy-neutral aberrations, no chromosomes other than 9p were recurrently affected. In conclusion, novel genomic aberrations were identified in our study, in particular in patients with myelofibrosis. Further analyses on single-gene level are necessary to uncover the mechanisms that are involved in the pathogenesis of myeloproliferative neoplasms.

Key words: single-nucleotide polymorphism, myeloproliferative neoplasms, polycythemia vera, *NF1* allele.

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Introduction

Recently, single-nucleotide polymorphism (SNP) arrays have been successfully applied to identify cryptic oncogenic lesions harboring genes and gene mutations in malignant hematologic diseases such as acute lymphoblastic or acute myeloid leukemia.[1,2] In myeloproliferative neoplasms (MPN), detection of acquired uniparental disomy (UPD) in chromosome 9p using microsatellite mapping led to the identification of the gain-of-function mutation V617F in *JAK2*.[3] Thus, we hypothesized that SNP-arrays that allow for the simultaneous detection of copy-number alterations (CNAs) and copy-number neutral aberrations (runs of homozygosity, ROH) at high resolution are appropriate to search for novel genomic aberrations in MPN patients. In our study, we used 250K SNP-arrays for genetic profiling of 151 clinically well characterized MPN patients: essential thrombocythemia (ET, n=45), polycythemia vera (PV, n=45), primary myelofibrosis (PMF, n=47), post-ET myelofibrosis (n=6), and post-PV MF (n=8). To our knowledge, this is the first report about a large MPN patient cohort investigated on the 250K SNP-array platform.

Design and Methods

All patients included in the study gave written informed consent for molecular analyses. The study was approved by the local ethical committee according to the Declaration of Helsinki. DNA was extracted from peripheral blood granulocytes and hybridized on 250K *Nsp* or *Sty* arrays according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). Hybridizations resulting in SNP call-rates lower than 90% were excluded from data analysis. SNP profiles were evaluated using a Hidden Markov Model and Copy Number Analyzer for Affymetrix GeneChip Mapping arrays (CNAG) version 2.0 software.[4] CNAs and ROH were identified by normalization to a set of 30 reference samples (unmatched pair analysis). Regions described as copy number variants (CNVs) in the database of the UCSC Human Genome Browser Gateway were excluded from the analysis. In selected cases, germline material was used to validate findings (matched pair analysis). Complete SNP-array data of our study are accessible at a gene expression omnibus (www.ncbi.nlm.nih.gov/projects/geo/, accession number GSE18197). Clinical characteristics and mutation status of the patients (*JAK2* V617F and *MPL* W515L) are given in the Online Supplementary Appendix.

Results and Discussion

Unpaired SNP-profiling revealed CNAs in 15% of PV, 31% of ET, 33% of post-ET MF, 53% of PMF, and 62% of post-PV MF patients, respectively. With regard to chromosomal abnormalities known from conventional cytogenetic analysis, the most frequent observed abnormalities were deletions in 20q11-q13 (n=10), gain of 9p / trisomy 9 (n=6), partial or total

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gain of 1q (n=4), and trisomy 8 (n=4), followed by loss of 5q11-q13 and 13q12-q21 in single cases (Table 1). While 20q losses occurred in all MPN subgroups, gain of 9p / trisomy 9 was restricted to PV and secondary MF patients, and trisomy 8 to PMF patients. Gain of 1q was observed in one PV, one ET, and 2 PMF cases. In addition, SNP-profiling revealed two recurrent small CNAs that were undetectable in cytogenetic studies: 11 ET and 2 PV patients showed small gains in 10q11.22 (1.5-2.7 Mb), whereas one post-ET and one post-PV MF case exhibited microdeletions in 17q11.2 (1.6 and 2.7 Mb, respectively) encompassing the tumor suppressor gene *Neurofibromatosis-1* (*NF1*).

To validate these findings, matched pair analysis using germline and tumor material was performed in a subset of these cases. Skin biopsies were taken from 2 patients with 10q11 gain and from one patient with 17q11 loss. SNP-profiles revealed that 10q11 gains represented CNVs in both cases, whereas the tumor-specific origin of cryptic deletion in 17q11 was confirmed (Figure 1). The disclosure of the 10q11 CNV reduced the frequency of detected CNAs in ET and PV to 11% each. Furthermore, fluorescence *in situ* hybridization performed in both cases with loss in 17q11 showed monoallelic *NF1* deletion in nearly 100% of the metaphase and interphase cells. Mutation analysis revealed a single base pair deletion in exon 5 (c.528delT) leading to a stop codon in the post-PV MF patient, whereas in the post-ET case no sequence variations were detected. With regard to the clinical phenotype in both patients MF was secondary to a prior ET or PV, respectively. Both cases were characterized by a progressive clinical course and both patients underwent allogeneic stem cell transplantation. Of note, the patient with monoallelic *NF1* deletion reached a sustained complete remission, whereas the patient with biallelic *NF1* alteration transformed into acute myeloid leukemia three months after transplantation and died.

NF1 is associated with the hereditary von Recklinghausen's neurofibromatosis. It has been shown that these patients have an increased risk of developing various tumors including myeloid leukemias.[5] *NF1* functions as a negative regulator of the RAS signal transduction pathway, and loss of *NF1* can lead to a progressive myeloproliferative disorder.[6] Cases with cryptic deletions in 17q11 encompassing *NF1* have been identified in several recent studies on adult and childhood acute myeloid and T-lymphoblastic leukemia patients as well as in PMF patients.[7-10] In addition, Balgobind *et al.* identified in 3 out of 4 childhood leukemia patients with monallelic *NF1* deletions truncating mutations in the remaining allele, that resulted in biallelic inactivation of the gene.[9] Taken together, these data strongly suggest a pathogenic role of *NF1* loss in a subset of leukemia patients. However, to date it is unclear whether loss of *NF1* triggers leukemic transformation in MPN patients.

In addition, in 14 of the 47 PMF cases and in one SMF patient, 18 microdeletions ranging from 0.1 to 2.2 Mb in size affected regions other than 17q11. However, all findings were restricted to single cases. For instance, in one PMF patient a biallelic loss affecting a 0.5 Mb segment in 12q24.11-12 harboring the gene *CUTL2* (*cut-like 2*) was observed; the remaining 17 microdeletions represented monoallelic aberrations. In ET and PV patients only one microdeletion was observed. These data might reflect the genomic instability and heterogeneity of MF.

ROH were detectable in 14% of ET, 17% of post-ET MF,

Table 1. Copy number alterations (CNAs) in 151 patients.

	Chromosome	Size (Mb)	CNAs (n)	UPN	*JAK2* V617F
CNAs in ET: 5/45 pts (11%)			**6**		
Gain	1q21-q32	61.7	1	192	-/-
Gain	3q27-q28	1.3	1	199	-/-
Gain	10q11	3.2	1	203	-/-
Gain	16p13	1.9	1	170	+/-
Loss	10p12	0.5	1	203	-/-
Loss	20q11-q13	14.7	1	193	-/-
CNAs in PV: 5/45 pts (11%)			**6**		
Gain	1q	N/A	1	60	+/-
Gain	7p21	0.3	1	69	+/-
Gain / Trisomy	9p/9	N/A	2	60, 62	+/- +/-
Loss	20q11-q13	13.1/13.8	2	37, 45	+/+ +/+
CNAs in PMF: 25/47 pts (53%)			**35**		
Gain	1q	N/A	2	129, 130	-/- +/-
Gain	3q26-qter	29.0	1	104	-/-
Gain	6p11-p12	0.5	1	117	+/-
Trisomy	8	N/A	4	21, 101, 105, 134	+/- -/- -/- +/-
Gain	18p11	0.4	1	127	-/-
Trisomy	21	N/A	1	108	+/-
Loss	2p12-p14	5.2	1	109	+/-
Loss	2q36	0.6	1	15	+/-
Loss	3p24	0.5	1	116	+/-
Loss	3p26-pter	7.3	1	104	-/-
Loss	3q26	0.3	1	134	+/-
Loss	7p21	0.4	1	105	-/-
Loss	7q	N/A	1	130	+/-
Loss	8p22	0.1	1	119	-/-
Loss	8p23	0.2	1	129	-/-
Loss	8q22	0.5	1	9	-/-
Loss	10q21	0.1	1	162	-/-
Loss	12q15	0.2	1	125	+/-
Loss	12q21	0.5	1	16	-/-
Loss*	12q24	2.2	1	162	-/-
Loss	12q24	1.6	1	103	-/-
Loss	13q12	1.6	1	131	+/-
Loss	13q12-q21	31.8	1	11	-/-
Loss	15q21	0.9	1	123	+/-
Loss	17q22	0.5	1	134	+/-
Loss	19p13	0.2	1	109	+/-
Loss	20q11-q13	12.2-25.8	5	14, 18, 119, 123, 133	+/- -/- -/- +/- -/-
CNAs in SMF: 7/14 pts (50%)			**12**		
Gain	3p24-p25	1.5	1	118	+/-
Trisomy	9	N/A	4	26, 28, 29, 118	+/- +/- +/- +/-
Loss	5q11-q13	18.0	1	28	+/-
Loss	9q33	1.0	1	30	+/+
Loss	17p13	0.7	1	30	+/+
Loss**	17q11	1.6/2.7	2	23, 30	-/- +/+
Loss	20q11-q13	22.0 / 18.6	2	102, 118	+/+ +/-

**encompassing a biallelic deleted segment in 12q24.11-12 (0.5 Mb); **identified as tumor-specific aberration using dermal cells as germline control; ET: essential thrombocythemia; PV: polycythemia vera; PMF: primary myelofibrosis; SMF: secondary myelofibrosis; pts: patients; UPN: unique patient number; -/-, JAK2 WT; +/- heterozygous, +/+ homozygous JAK2 V617F.*

27% of PMF, 61% of PV, and 62% of post-PV MF cases, respectively. However, these frequencies included interstitial as well as terminal ROH. Based on the findings of recent studies that suggest that acquired UPDs in myeloid malignancies comprise at least one terminal end of the chromosome, we excluded interstitial ROH from data analysis.[11] Consequently, ROH were restricted to the following chromosomal segments (Table 2): 9p24-pter (6.3-44.1 Mb; n=30) encompassing the *JAK2* locus, 4q13-qter (116.1 Mb, n=1), 7q22-qter (57.3 Mb, n=1), and 11q14-qter (53.3 Mb, n=1). Of note, mutations in the genes *TET2* (*tet oncogene family member 2*) or *CBL* (*Casitas B-lineage lymphoma*) were absent in cases affecting chromosomal bands 4q24 or 11q23, respectively, as investigated by DNA sequencing. ROH in 9p mainly occurred in PV (21/45) and post-PV MF (5/8) patients, followed by 3 PMF patients and a single post-ET MF case. Interestingly, all patients with 9p ROH harbored the JAK2 V617F mutation, and 93% (28/30) exhibited an allele burden greater than 50% indicating a homozygous mutational status. To summarize, unpaired SNP-array analysis did not allow the identification of recurrent ROH other than 9p in our study of 151 MPN patients. Chromosomes 4q, 7q, and 11q were affected sporadically, whereas ROH in 9p was present in 20% of MPN cases.

Taken together, high-resolution genetic profiling in a large cohort of BCR/ABL-negative MPN cases did not reveal novel recurrent CNAs or ROH. However, 30% of PMF patients and 14% of SMF exhibited small genomic losses (0.1-2.7 Mb) most of which had not been previously described. Two recent microarray studies impressively demonstrated that rare microdeletions may be of great importance and pinpoint genes being frequently

Table 2. Runs of homozygosity (ROH) in 151 patients.

	Chromosome	Size (Mb)	ROH (n)	UPN	*JAK2* V617F
ROH in ET: none					
ROH in PV: 22/45 pts (49%)			22		
ROH	4q13-qter	116.1	1	52	+/+
ROH	9p	6.3-44.1	21	34-43, 45, 46, 48-51, 55, 61, 64, 70, 71	+/+ [19] +/- [2]
ROH in PMF: 5/47 pts (11%)			5		
ROH	7q22-qter	57.3	1	105	-/-
ROH	9p	17.4-33.9	3	19, 108, 120	+/+ +/+ +/+
ROH	11q14-qter	53.3	1	104	-/-
ROH in secondary MF: 6/14 pts (43%)			6		
ROH	9p	17.7-38.7	6	22, 30, 31, 33, 102, 122	+/+ +/+ +/+ +/+ +/+ +/+

ET, essential thrombocythemia, PV, polycythemia vera; PMF, primary myelofibrosis; MF, myelofibrosis; pts, patients; UPN, unique patient number; -/-, JAK2 WT; +/- heterozygous, +/+ homozygous JAK2 V617F.

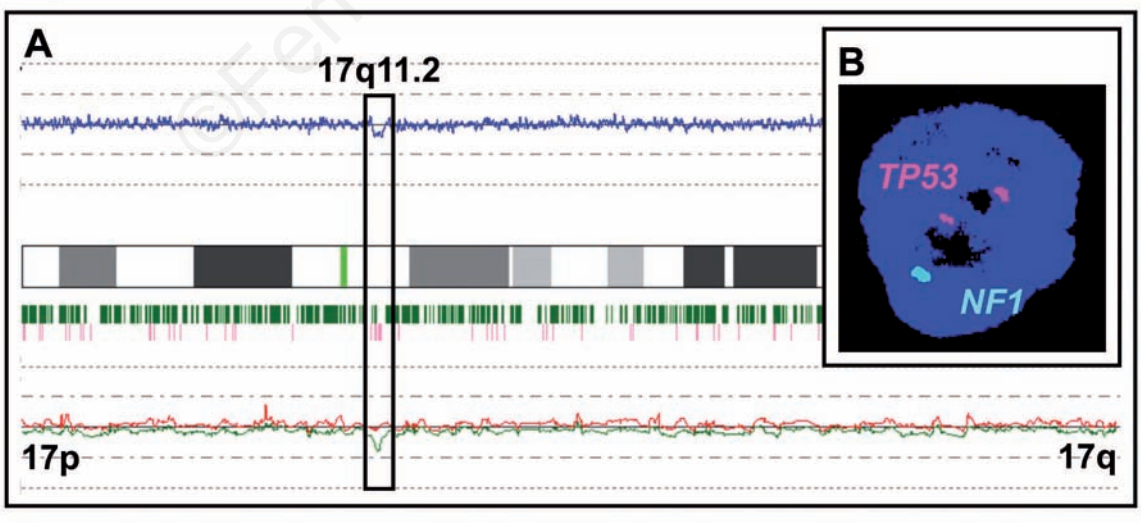

Figure 1. (A) Matched-pair SNP-array profile of one post-essential thrombocythemia myelofibrosis patient with microdeletion in 17q. Allele-based analysis at the bottom of the figure differentiates SNP calls derived from germline (red) and tumor (green) material. Overlay of both profiles (blue) reveals one stretch of tumor-specific loss of heterozygosity in 17q11.2 (1.6 Mb). (B) Validation by fluorescence *in situ* hybridization with a representative cell from the same patient. One green fluoresence signal using a *Neurofibromatosis-1*- (*NF1*-) specific DNA-probe indicates monoallelic loss of the gene, whereas two red signals obtained with a *TP53*-specific control probe demonstrate the existence of both alleles.

involved.[12,13] Investigating MPN patients with common progenitor cell characteristics, Delhommeau *et al.* observed two acquired UPDs in 4q22-qter and one 0.3 Mb loss in 4q24.[12] This microdeletion encompassed *TET2* as a single gene and led to the identification of somatic *TET2* mutations in 12% (24/198) of MPN cases. The 250K SNP-array study by Langemeijer *et al.* revealed a single 0.8 Mb deletion in 4q24 among 102 myelodysplastic syndrome (MDS) patients.[13] As investigated by genomic sequencing, 26% (27/102) of MDS cases exhibited TET2 mutations. Thus, both studies underline the potential relevance of sporadic non-recurrent findings.

Consequently, our data provide important information that allow the selection of appropriate candidate genes for further investigation at single-gene level. In addition, future studies need to validate the impact of *NF1* mutations or genetic alterations in other non-recurrently affected genes such as *CUTL2* in a large number of MPN patients. Within this context, the use of higher resolution platforms (e.g. 6.0 SNP-arrays) and application of high-throughput sequencing techniques, as well as studies on epigenetic mechanisms, might further contribute to unravel the genetic changes underlying the pathogenesis of MPN.

Authorship and Disclosures

FS performed research, analyzed data and wrote the paper. LB and MG analyzed data. MH, SK, CM and SS performed research. HD and KD designed research, analyzed data and wrote the paper.

The authors reported no potential conflicts of interest.

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