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CBL, *CBLB*, *TET2*, *ASXL1*, and *IDH1/2* mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia

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Progression of chronic myelogenous leukemia (CML) to accelerated (AP) and blast phase (BP) is because of secondary molecular events, as well as additional cytogenetic abnormalities. On the basis of the detection of *JAK2*, *CBL*, *CBLB*, *TET2*, *ASXL1*, and *IDH1/2* mutations in myelodysplastic/myeloproliferative neoplasms, we hypothesized that they may also contribute to progression in CML. We screened these genes for mutations in 54 cases with CML (14 with chronic phase, 14 with AP, 20 with myeloid, and 6 with nonmyeloid BP). We identified 1 *CBLB* and 2 *TET2* mutations in AP, and 1 *CBL*, 1 *CBLB*, 4 *TET2*, 2 *ASXL1*, and 2 *IDH* family mutations in myeloid BP. However, none of these mutations were found in chronic phase. No cases with *JAK2*V617F mutations were found. In 2 cases, *TET2* mutations were found concomitant with *CBLB* mutations. By single nucleotide polymorphism arrays, uniparental disomy on chromosome 5q, 8q, 11p, and 17p was found in AP and BP but not involving 4q24 (*TET2*) or 11q23 (*CBL*). Microdeletions on chromosomes 17q11.2 and 21q22.12 involved tumor associated genes *NF1* and *RUNX1*, respectively. Our results indicate that *CBL* family, *TET2*, *ASXL1*, and *IDH* family mutations and additional cryptic karyotypic abnormalities can occur in advanced phase CML. (*Blood*. 2011;117(21):e198-e206)

Introduction

Loss of heterozygosity (LOH) because of acquired uniparental disomy (UPD) is a commonly observed chromosomal lesion in myelodysplastic/myeloproliferative neoplasms (MDS/MPNs).[1,2] Mapping recurrent areas of LOH may aid in the identification of genes harboring mutations, as shown for UPD9p and *JAK2*V617F mutations.[3,4] *CBL* mutations, often found in a homozygous constellation associated with UPD11q23.3, were most commonly detected in MDS/MPN, including chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia.[2,5-7] Ring finger domain mutations of *CBL* family members abrogate their ability to ubiquitinate and inactivate phosphorylated receptor tyrosine kinases.[8-11]

TET2 mutations are ubiquitous in myeloid malignancies, including MPN, MDS/MPN, MDS, and secondary acute myelogenous leukemia (sAML) and can occur in heterozygous or hemizygous forms, as well as in homozygous forms specifically in the context of UPD4q24.[12-16] The TET family of proteins may be involved in the conversion of methylcytosine to hydroxymethylcytosine.[17] It is thereby possible that TET proteins regulate the maintenance of methylation-based silencing or prevent aberrant hypermethylation.

Mutations of the polycomb-associated gene *ASXL1* were observed in myeloid malignancies, including CMML[18,19] and chronic myelogenous leukemia (CML)[20]; unlike *TET2* and *CBL* mutations, mutations in *ASXL1* are mostly heterozygous. However, similar to *TET2* mutations, *ASXL1* mutations may be lead to epigenetic dysregulation. ASXL1 is associated with LSD1, which is involved in histone H3K4 demethylation and thereby chromatin remodeling.[21]

Somatic mutations of isocitrate dehydrogenases (*IDH1* and *IDH2*), initially described in CNS tumors,[22,23] were also found in primary AML[24,25] and in sAML evolved from MPN, but not in chronic phase (CP) MPN.[26] This distribution pattern suggests a role of *IDH1/2* mutations in disease progression.

Although translocations resulting in a *BCR/ABL1* fusion gene invariably characterize CML, we stipulated that in analogy to other MDS/MPN entities, *JAK2*V617F, *TET2*, *ASXL1*, *CBL*, and *IDH* family mutations may also occur in CML, either contributing to phenotypic heterogeneity within *BCR/ABL1*-associated chronic myeloid disorders or as secondary events leading to their malignant progression to accelerated phase (AP) or blast phase (BP). Similarly, a higher level of resolution of cytogenetic testing as achieved by single nucleotide polymorphism array (SNP-A)–based karyotyping may show additional chromosomal abnormalities associated with stepwise progression.[27,28] Consequently, this study focuses on the combined analysis of additional chromosomal lesions and mutations identified in patients with AP and BP and the association of these defects with clinical features.

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Table 1. Patients' characteristics and *CBL* family, *TET2*, *ASXL1*, and *IDH* family mutations (N = 54)

Phase	WHO diagnosis	Cases with variants			
		CBL family	*TET2*	*ASXL1*	*IDH* family
Aggressive	AP (N = 14)	1	2	0	0
	BP (myeloid) (N = 20)	2	4	2	2
	BP (nonmyeloid) (N = 6)	0	0	0	0
Chronic	CP (N = 14)	0	0	0	0

WHO indicates World Health Organization; AP, accelerated phase; BP, blast phase; and CP, chronic phase.

Methods

Patients

Informed consent for sample collection was obtained according to protocols approved by institutional review boards of the Cleveland Clinic, Johns Hopkins University, and University of California Los Angeles Medical Center. BM aspirates were collected from 54 patients with 14 CP, 14 AP, and 26 BP (20 myeloid and 6 nonmyeloid; Table 1). Diagnosis was assigned according to classification criteria by the World Health Organization. Because serial samples were not available, only cross-sectional results were reported.

Metaphase cytogenetics

Cytogenetic analysis was performed on marrow aspirates or peripheral blood or both according to standard methods; 20 metaphase spreads were examined per patient. Chromosome preparations were G-banded with the use of trypsin and Giemsa, and karyotypes were described according to the International System for Cytogenetic Nomenclature.[29]

SNP-A analysis

Affymetrix Genome-Wide Human SNP Array 6.0 and Illumina HumanCytoSNP-12 were used for SNP-A analysis of BM DNA as previously described.[30] Sufficient DNA was available from 26 of 40 patients with AP (N = 12) and BP (N = 14). Briefly, signal intensity was analyzed, and SNP calls were determined with GeneChip Genotyping Analysis Software Version 4.0 (GTYPE). Genotyping console v3.0 (Affymetrix) and KaryoStudio (Illumina) were used for analysis of 6.0 arrays and HumanCytoSNP-12, respectively. Germline-encoded copy number variations and nonclonal areas of UPD were excluded from further analysis with the use of a bioanalytic algorithm, based on lesions identified by SNP-A[13,31] in an internal control series (N = 1003) and reported in the Database of Genomic Variants (http://projects.tcag.ca/variation/). Size and location criteria (telomeric > 8.7 Mb and interstitial ≥ 25 Mb in size) were used for identification of somatic UPD as previously described.[7] The reference genome used for annotation was NCBI36/hg18 (March 2006).[32]

JAK2, *CBL* family, *TET2*, *ASXL1*, and *IDH* family mutational screening

We checked mutational status of *JAK2*, *CBL* family, *TET2*, *ASXL1*, and *IDH* family genes in all 54 enrolled patients (Table 1). Screening for the *JAK2*V617F mutation was performed with a DNA tetraprimer amplification refractory mutation system assay as previously described.[2,33] For *CBL* (exons 8-9), *CBLB* (exons 9-10), *TET2* (all coding exons), *ASXL1* (exon 12), *IDH2* (exon 2), and *IDH2* (exon 4), direct genomic sequencing was performed as previously described.[7,13,26,34] Bidirectional sequencing was performed by standard techniques with the use of an ABI 3730xl DNA analyzer (Applied Biosystems). All mutations were scored as pathogenic on the basis of the observation that they were not detected in normal samples and were not found in a publically available SNP database,[35] or they were not reported as SNPs in previous publications.

Statistical analysis

For comparison of the clinical features between disease groups, categorical and continuous variables were analyzed with the Fisher exact test and Mann-Whitney *U* test, respectively. Overall survival was analyzed with Kaplan-Meier statistics and compared by log-rank test and generalized Wilcoxon test.

Results

CBL family, *ASXL1*, *TET2*, and *IDH* family mutations in CML

When we performed mutational screening for *JAK2*, *CBL* family, *TET2*, *ASXL1*, and *IDH* family genes in CML, we identified mutations in a number of AP CML. One *CBLB* and 2 *TET2* mutations in AP (N = 14), and 1 *CBL*, 1 *CBLB*, 4 *TET2*, 2 *ASXL1*, and 2 *IDH* family mutations in myeloid BP (N = 20). In contrast, when patients with CP (N = 14) were screened, no mutations were found (Tables 1-2). *JAK2*V617F mutation was not present in any case. Interestingly, *TET2* mutations were concomitantly present in 2 cases with *CBLB* mutations. Similarly, 1 myeloid BP case was characterized by an *ASXL1* nonsense mutation as well as an *IDH1* mutation (Table 2). An overview of the mutations found in the affected genes is shown in Figure 1. Mutant cases and corresponding cytogenetic features are presented in Table 2.

SNP-A–based detection of accessory karyotypic abnormalities in AP and BP

In addition to mutations, progression of CML may also be associated with the acquisition of additional, previously cryptic, chromosomal abnormalities. SNP-A allows for the identification of not only submicroscopic copy number changes but also UPD, not amenable to detection with the use of routine metaphase cytogenetics. FISH or reverse transcription PCR confirmed the presence of a *BCR/ABL1* fusion gene in all of these patients. We focused on additional karyotypic abnormalities other than t(9;22) in AP and BP. With the use of metaphase cytogenetics, additional chromosomal aberrations were found in 21 of 40 patients (53%). Additional unbalanced (deletion and addition) defects, balanced (translocation and inversion) defects, and both were found in 28%, 15%, and 10% of cases, respectively (Figure 2). The most common recurrent defects included abnormalities on chromosomes 3 (15%) and 22 (15%), as well as common lesions on chromosomes 7 (10%), 8 (8%), 9 (8%), 11 (8%), and 18 (8%).

SNP-A–based karyotyping was available on patients with AP (N = 12) and BP (N = 14). For the purpose of this study, we only included lesions that did not overlap with either copy number variations or germline-encoded regions of homozygosity detected in an internal control cohort or external databases (see "SNP-A analysis"); 21 gains, 18 losses, and 4 regions of UPD were identified in the patients (Tables 3-5). In general, SNP-A confirmed the results of metaphase cytogenetics with regard to known unbalanced defects. Additional copy number abnormalities, including microdeletions, were also found in 58% of all examined cases (67% and 50% in AP and BP, respectively) by SNP-A.

Recurrent lesions were detected on chromosomes 1, 8, 9, 17, and 22 by SNP-A. However, these lesions were overlapping those detected by metaphase cytogenetics. Microdeletions on chromosomes 17q11.2 (3.84 Mb) and 21q22.12 (1.21 Mb), which were undetectable by metaphase cytogenetics, involved tumor-associated genes *NF1* and *RUNX1*, respectively (Figure 3; Table 4). In 1 patient with AP, a microdeletion of 11q23.3 was present, but no

Table 2. Characteristic of *CBL* family, *TET2*, *ASXL1*, and *IDH* family mutation positive patients

Patient no.; diagnosis	*CBL*	*CBLB*	*TET2*	*ASXL1*	*IDH1*	*IDH2*	Treatment	Metaphase cytogenetics	SNP-A			*BCR-ABL1* mutation
									Gain	Loss	UPD	
16; AP	WT	c., 1072−103 del (intron8-exon9) (hetero)*	T1114Nfs (hetero)*	WT	WT	WT	Allo BMT, imatinib mesylate	45,XY,inv(3)(q21q26.2), t(5;6)(q11.2;p23), t(9;22)(q34;q11.2), inv(11)(p15q21), −18[20]	N	whole 18	N	T315I
20; AP	WT	WT	G1719R (hetero)*	WT	WT	WT	Imatinib mesylate	46,XX,t(9;22)(q34;q11.2)[10]/ 47,XX,+8[3]/46,XX[2]	NE	NE	NE	NE
30; BP (myeloid)	WT	R463W (hetero)*	C1298W (hetero)*	WT	WT	WT	Imatinib mesylate, dasatinib	45,X,−Y,del(5)(q22q23), der(7)(7qter- > 7p12::22q13- > 22qter), der(9)(9pter- > 9q34::22q11.2−.22q13::7p22 > 7p13), der(22)t(9;22)(q34;q11.2)[19]	18p11.21	7q11.21, whole Y	N	V299L
34; BP (myeloid)	WT	WT	WT	E1102D (hetero)*	WT	WT	Hydroxyurea, IFN, allo BMT	47,XX,+8,t(9;22)(q34;q11.2), i(17)(q10)[20]	Whole 8, 17p11.2qter	17p13.3p11.2	N	NE
37; BP (myeloid)	P395A (hetero)*	WT	WT	WT	WT	WT	Imatinib mesylate, cytarabine, idarubicin	46,XY,t(9;11)(9qter- > 9q24:: 11q25- > 11q14;11pter- > 11q14::9p24- > 9pter), t(9;22)(q34;q11.2)[20]	N	N	N	WT
39; BP (myeloid)	WT	WT	E350K (hetero)*	WT	WT	WT	NE	46,XY,t(9;22)(q34;q11.2)[20]	NE	NE	NE	NE
40; BP (myeloid)	WT	WT	S1556Y (hetero)*	WT	WT	WT	Hydroxyurea, IFN, cytarabine, imatinib mesylate, dasatinib	46,XY,t(1;12)(p22;p13), t(9;22)(q34;q11.2)[2]/48, sl,+8,+der(22)t(9;22)[18]	1p22.2p13.3, 1q25.3q32.1, 1q32.3q41, whole 8, 9q34.12q34.3, 22q11.1q11.23	9q34.11q34.12, 22q11.23q12.3	N	M244V
42; BP (myeloid)	WT	WT	R1465X (hetero)*	WT	WT	WT	Imatinib mesylate	46,XY,t(9;22)(q34;q11.2)[1]/45, idem,−7[19]	N	Whole 7	N	NE
44; BP (myeloid)	WT	WT	WT	W796X (hetero)*	R132C (hetero)*	WT	Hydroxyurea, IFN, mithramycin	46,XY,t(9;22)(q34;q11.2)[3]/47, idem,+8,der(22)t(1;22)(q21;p11.2)[16]	1q21.2qter, whole 8	N	11p15.5p12, 17q11.2qter	NE
46; BP (myeloid)	WT	WT	WT	WT	WT	R140Q (hetero)*	Hydroxyurea	46,XY,t(9;22)(q34;q11.2)[20]	NE	NE	NE	NE

SNP-A indicates single nucleotide polymorphism arrays; UPD, uniparental disomy; AP, accelerated phase; WT, wild type; BMT, bone marrow transplantation; NE, not evaluated; N, negative; and BP, blast phase.
*Heterozygous mutation.

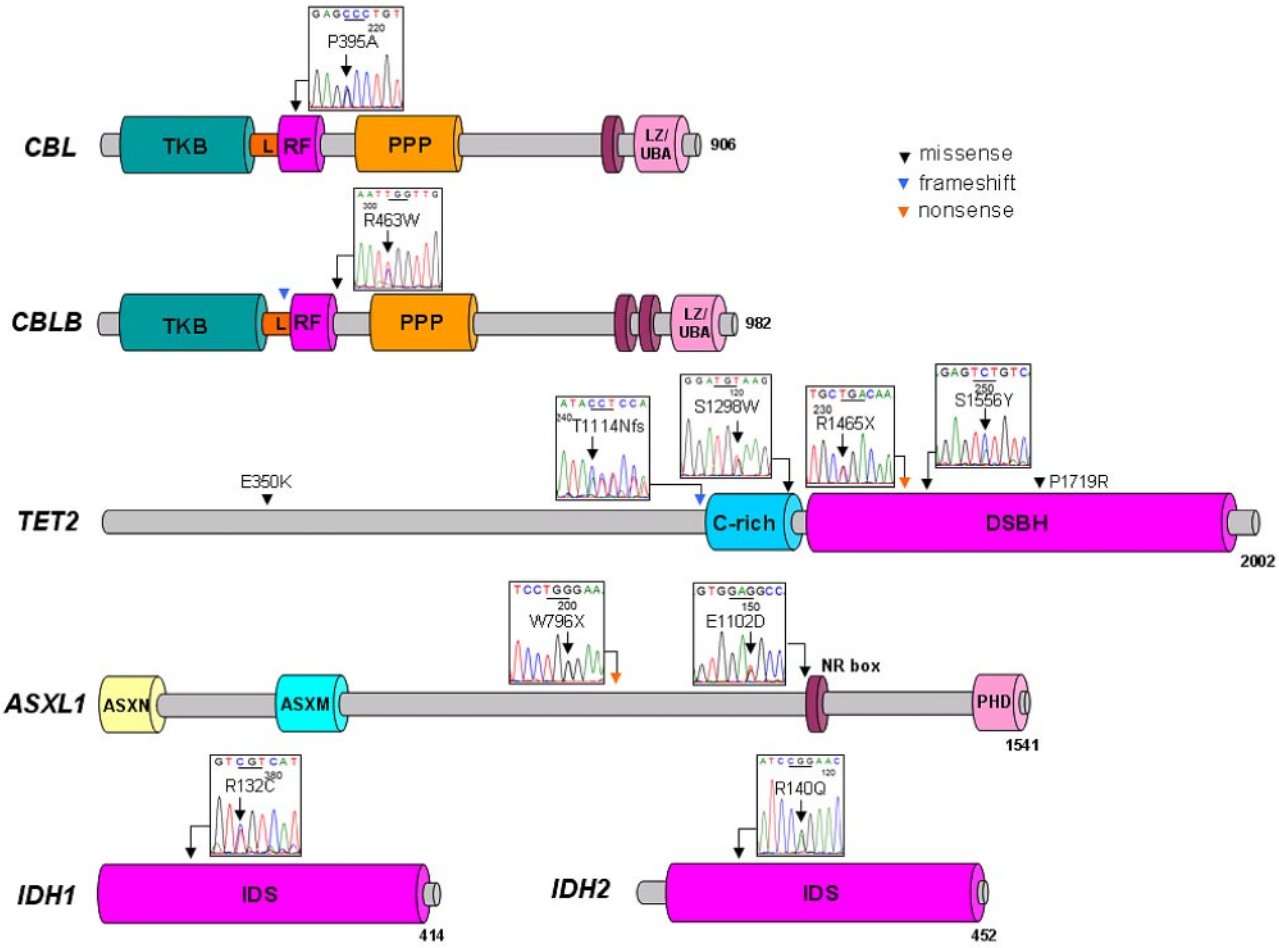

Figure 1. Distribution of mutations in tested genes in 10 patients with CML. Schematic representation shows the main domains, primarily the tyrosine kinase binding (TKB) domain, linker sequence (L), RING finger domain (RF), proline-rich region (PPP), and leucine zipper (LZ)/ubiquitin-associated domain (UBA) of the CBL family, cysteine (C)–rich domain, and double-stranded β helix (DSBH) domain of TET2, additional sex comb (ASX) N-terminal (ASXN) domain, ASX-middle (ASXM) domain, nuclear receptor coregulator binding (NR box) motif, plant homeo domain (PHD) of ASXL1, and isocitrate dehydrogenase superfamily (IDS) region of IDH1/2. Genomic sequencing of protein-coding regions and splice sites showed missense (black), nonsense (orange), and frameshift mutations (blue) in *CBL*, *CBLB*, *TET2*, *ASXL1*, *IDH1*, and *IDH2* genes. All base pair changes identified occurred in a heterozygous constellation.

mutation in *CBL*, mapping to this interval, was detected. Deletions flanking the *ABL1* and *BCR* genes (chromosome 9 and 22) were observed in 2 cases with der(22)t(9;22) or der(9)t(9;22) by metaphase cytogenetics and were previously described by others.[36,37] Gains, including whole chromosome 8 and 17q24.3 (0.76 Mb), were found in 3 cases (Table 3). Regions of UPD included UPD5q, 8q, 11p, and 17q, but no UPD involving 11q (*CBL*) and 4q (*TET2*) regions were found confirming the heterozygous nature of the corresponding mutations (Figure 3; Table 5). Of note is that none of the cases showed the presence of somatic UPD9p (associated with homozygous *JAK2*V617F mutation).

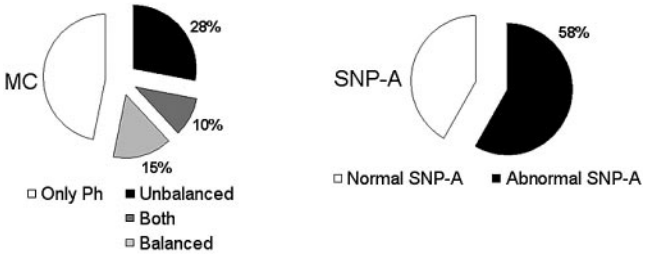

Figure 2. Chromosomal regions affected in patients with AP and BP. The left pie diagram shows proportion of patients with chromosome aberrations detected by standard metaphase cytogenetics (MC) [only Philadelphia chromosome (Ph; white), unbalanced (black), balanced (light gray), and both classes of abnormalities (dark gray)] in persons with AP and BP (N = 40), whereas the right pie chart represents the abnormalities by SNP-A karyotyping [normal (white) or abnormal (black); N = 26].

Clinical characteristics in patients with *CBL* family, *TET2*, *ASXL1*, and *IDH* family mutations

Newly detected molecular lesions associated with AP and BP may change the biology and thereby clinical features of affected cases. Mutant and wild-type (WT) cases did not differ by age or duration of the disease. Although karyotypic abnormalities were detected by SNP-A in all cases with *TET2* mutations, there was no significant difference in the frequencies between mutant and WT groups (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Clinically, there was no significant difference in the treatment regimen administered to patients with and without specific mutations. All patients with *CBL* family and 4 of 5 patients with *TET2* mutations were resistant to imatinib mesylate. However, dasatinib was effective in a patient with a mutation of *TET2*. Overall survival of patients with mutations did not differ from patients without mutations (median survival of progressed CML: 49, 36, 46, 67, and 41 months in patients with *CBL* family, *TET2*, *ASXL1*, *IDH* family,

Table 3. Copy number gain lesions detected by SNP-A and affected candidate genes (N = 26)

Patient no.	Disease	Chromosome	Start	Stop	Length, Mb	Affected candidate genes
40	Myeloid BP	1p22.2p13.3	90311409	107802971	17.49	*HFM1, CDC7, TGFBR3, BRDT, GLMN, RPAP2, GFI1, EVI5, PTBP2, DPYD, SNX7, AGL, VCAM1, EXTL2, DPH5*
44	Myeloid BP	1q21.2q44	148187512	247169378	98.98	*VPS45, APH1A, ECM1, MCL1, ENSA, ARNT, SETDB1, GABPB2, PIP5K1A, PI4KB, TCHH, PGLYRP4, ILF2, INTS3, IL6R, MUC1, ARHGEF2, CD1, SLAMF6, ATF6, NOS1AP, DDR2, NUF2, PBX1, MPZL1, NME7, PRRX1, RABGAP1L, FmABL2, MR1, DHX9, EDEM3, HMCN1, UCHL5, TROVE2, KCNT2, ASPM, CRB1, PTPRC, NR5A2, PKP1, PTPN7, PTPRV, LGR6, PPP1R12B, JARID1B, BTG2, SOX13, MDM4, PCTK3, ELK4, SRGAP2, IKBKE, RASSF5, MAPKAPK2, IRF6, TRAF5, RD3, PTPN14, USH2A, RRP15, TGFB2, DUSP10, TLR5, TP53BP2, FBXO28, WDR26, WNT9A, NUP133, ARID4B, CEP170, AKT3, SMYD3, AHCTF1, NLRP3*
40	Myeloid BP	1q25.3q41	183864117	218274672	34.41	*HMCN1, UCHL5, TROVE2, KCNT2, ASPM, CRB1, PTPRC, NR5A2, PKP1, PTPN7, PTPRV, LGR6, PPP1R12B, JARID1B, BTG2, SOX13, MDM4, PCTK3, ELK4, SRGAP2, IKBKE, RASSF5, MAPKAPK2, IRF6, TRAF5, RD3, PTPN14, USH2A, RRP15, TGFB2*
23	AP	2q33.1q37.3	202918925	242678246	39.76	*NRP2, FASTKD2, IDH1, IKZF2, IGFBP2, USP37, WNT6, PTPRN, EPHA4, PAX3, CUL3, PID1, FBXO36, PSMD1, ECEL1, GIGYF2, INPP5D, USP40, SH3BP4, MLPH, HES6, HDAC4*
27	AP	6q11.1q12	61949077	63513069	1.56	*KHDRBS2*
34	Myeloid BP	8	166818	146263890	146.1	Trisomy 8
44	Myeloid BP	8	166818	146263890	146.1	Trisomy 8
40	Myeloid BP	8	166818	146263890	146.1	Trisomy 8
40	Myeloid BP	9q34.12q34.3	132658747	140164310	7.51	*ABL1, MED27, SETX, TTF1, DDX31, VAV2, NOTCH1*
19	AP	9q34.12q34.3	132672428	140164310	7.49	*ABL1, MED27, SETX, TTF1, DDX31, VAV2, NOTCH1*
25	AP	10q11.21	43543186	44823480	1.28	*CXCL12, RASSF4*
36	Myeloid BP	11p15.2p12	13226368	43097900	29.87	*SOX6, PTPN5, BBOX1, LGR4, METT5D1, ELP4, PAX6, WT1, HIPK3, COMMD9, TRAF6*
25	AP	15q21.1	43920344	46958155	3.04	*SEMD6D, DUT, SEP152*
34	Myeloid BP	17p11.2q25.3	22095317	78640854	56.55	Whole 17q duplication
23	AP	17q21.31q25.3	41700840	78640854	36.94	*WNT3, WNT9B, CDC27, SP2, CDK5RAP3, CBX1, HOXB family, ABI3, SPOP, SAMD14, ABCC3, CROP, SPAG9, HLF, AKAP1, SEPT4, RPS6KB1, BRIP1, MAP3K3, SMURF2, AXIN2, PRKCA, MAP2K6, SOX9, SDK2, NUP85, GRB2, LLGL2, CDK3, JMJD6, BIRC5, SOCS3, CARD14, RAPTOR, AATK*
47	Myeloid BP	17q24.3	66201540	66965287	0.76	*BC039327*
16	AP	18	79 140	76117153	76.04	Trisomy 18
30	Myeloid BP	18p11.21	12321501	13522208	1.2	*SLMO1, SPIRE1, CEP76, PTPN2, SEH1L, SEP192*
27	AP	18q12.2q12.3	33414141	41421419	8.01	*KC6, PIK3C3, RIT2, SETBP1*
40	Myeloid BP	22q11.1q11.23	14449498	21959552	7.51	*GAB4, BID, HIRA, GNB1L, MED15, PI4KA, CRKL, BCR, MAPK1*
19	AP	22q11.1q11.23	14919628	21880556	6.96	*GAB4, BID, HIRA, GNB1L, MED15, PI4KA, CRKL, BCR, MAPK1*

BP indicates blast phase; and AP, accelerated phase.

or no mutations, respectively; supplemental Table 1). Of note is that *BCR-ABL1* kinase domain mutations were detected in 9 of 10 patients with imatinib mesylate resistance. In these 9 cases, 3 *TET2* and 2 *CBLB* mutations were detected. In 1 patient resistant to imatinib mesylate without *BCR-ABL1* kinase domain mutation, *CBL* mutation was present (Table 2).

Discussion

Mutations in *JAK2*, *CBL*, *CBLB*, *TET2*, *ASXL1*, *IDH1*, and *IDH2* have been found in various myeloid malignancies, including MDS/MPN, MPN, and sAML. This is the comprehensive mutational analysis of these genes in CML, based on the theory that these mutations may contribute to the progression of CML to AP and BP. Among the genes studied, *TET2* was the most frequently mutated, and the mutations were distributed in AP and BP.

CBL family mutations found in AP and BP were located in or next to the ring finger domain or linker sequence, similar to those observed in MDS/MPN. These regions are completely conserved in *CBL* orthologs as well as in 2 other human *CBL* family members. Of 3 *CBL* family mutations, 2 were missense substitutions and 1 was a frameshift variation with deletion of 144 bp in intron 8 and exon 9. Previously, in CMML and related disorders we identified UPD11q and associated homozygous *CBL* mutations.[2,6,38] However, we also noted that heterozygous mutations or alterations of

Table 4. Copy number loss lesions detected by SNP-A and affected candidate genes (N = 26)

Patient no.	Disease	Chromosome	Start	Stop	Length, Mb	Affected candidate genes
25	AP	1p34.3	35028712	36757321	1.73	*PSMB2, CLSPN, STK40, CSF3R*
26	AP	6q14.2q21	83951101	110175307	26.22	*PRSS35, SNAP91, NT5E, SNHG5, RARS2, CNR1, MAP3K7, EPHA7, TSG1, FHL5, FBXL4, USP45, SIM1, GRIK2, HACE1, BVES, PRDM1, ATG5, PDSS2, SCML4, SNX3, FOXO3, SESN1, FIG4, WASF1*
42	Myeloid BP	7	153672	158821424	158.67	Monosomy 7
25	AP	8q11.1q11.21	47062007	49246034	2.18	*CEBPD, PRKDC, MCM4*
34	Myeloid BP	9p21.3p13.2	20934558	37208231	16.27	*PTPLAD2, IFNA5, KLHL9, MTAP, CDKN2A, ELAVL2, TUSC1, PLAA, IFT74, TEK, IFNK, ACO1, APTX, SMU1, NFX1, NOL6, PRSS3, CNTFR, GALT, VCP, FANCG, TESK1, RECK, CLTA, GNE, MELK, PAX5*
40	Myeloid BP	9q34.11q34.12	130686250	132652879	1.97	*PHYHD1, SH3GLB2, DOLPP1, PPP2R4, ASB6, PIG12, FNBP1, ASS1, FUBP3, ABL1*
28	AP	9q34.11q34.12	130900242	132604796	1.7	*ASB6, PIG12, FNBP1, ASS1, FUBP3, ABL1*
27	AP	10q22.1q22.2	73539669	76313833	2.77	*DNAJB12, ANXA7, SEC24C, CAMK2G, VCL, AP3M1, ADK*
26	AP	11p15.5p15.2	1613884	14397978	12.78	*H19, IGF2, ASCL2, TSSC4, CDKN1C, NAP1L4, NUP98, RRM1, ILK, TPP1, NLRP10, STK33, ST5, WEE1, SBF2, AMPD3, MRVI1, EIF4G2, DKK3, TEAD1, ARNTL, RRAS2*
27	AP	11q23.3	115868656	119126150	3.26	*BACE1, FXYD2, FXYD6, IL10RA, MLL, DDX6, BCL9L, CBL*
26	AP	15q21.2q22.2	49041794	58605542	9.56	*DMXL2, SCG3, TMOD2, LEO1, MAPK6, BCL2L10, GNB5, RAB27A, CCPG1, DYX1C1, NEDD4, TCF12, GCOM1, ADAM10, SLTM, BNIP2, ANXA2*
34	Myeloid BP	16p11.2	32045466	33703188	1.66	*TP53TG3*
34	Myeloid BP	17p13.3p11.2	18901	18868271	18.85	*GLOD4, ABR, YWHAE, SMYD4, METT10D, GARNL4, ITGAE, MINK1, CAMTA2, DHX33, NLRP1, XAF1, BCL6B, CLEC10A, TP53, JMJD3, HES7, AURKB, PIK3R5, NTN1, GAS7, MAP2K4, ELAC2, NCOR1, TNFRSF13B, NT5M, PEMT, DRG2, ALKBH5, LLGL1, SHMT1, FBXW10, PRPSAP2*
27	AP	17q11.2	24504173	28343137	3.84	*GOSR1, NF1, SUZ12, CDK5R1*
23	AP	21q22.12	34958486	36169173	1.21	*RUNX1*
28	AP	22q11.23	21963842	22551295	0.59	*BCR, IGLL1, Ini1*
40	Myeloid BP	22q11.23q12.3	21963842	35542482	13.58	*BCR, IGLL1, Ini1, Chk2, XBP1, KREMEN1, EWSR1, NF2, LIF, DUSP18, LIMK2, PATZ1, EIF4ENIF1, YWHAH, TOM1, MCM5, RASD2, RBM9,*
30	Myeloid BP	Y	3259468	23370508	20.11	Whole Y chromosome deletion

AP indicates phase; and BP, blast phase.

other closely related E3 ubiquitin ligases, such as *CBLB* and *CBLC*, may be found in some patients with otherwise indistinguishable morphologic features.[7] Moreover, heterozygous *CBL* mutations are also described in juvenile myelomonocytic leukemia.[5,39,40] In this report, *CBL* family mutations were present only in aggressive forms of CML. In contrast, they were found in both CMML and CMML-derived sAML,[7] suggesting that heterozygous *CBL* family mutations may play an initiating role in MDS/MPN but are accessory in the pathogenesis of evolution into aggressive phase of CML.

In all patients with *TET2* mutations, additional chromosomal lesions were found by SNP-A. Of the 6 *TET2* mutations identified, 4 (67%) were missense substitutions, 1 (17%) was frameshift, and 1 (17%) produced a stop codon and were located within the N-terminus as well as in a conserved DSBH 2OG-Fe(II)–dependent dioxygenase domain. The presence of nonsense and frameshift mutations suggests that these changes result in inactivation, consistent with putative tumor suppressor functions, whereas heterozygous mutations indicate that the WT allele is not completely protective. In other myeloid malignancies, the distribution pattern and types of mutations are similar,[13,15,16] but they occur in both homozygous and heterozygous configurations. Because no *TET2* mutations were identified in CP, these mutations might represent an additional pathogenic event. Recently, *TET2* mutations were shown to be a late event in MDS, MDS/MPN, and MPN, because they are rarely present in low-grade MDS and frequent in secondary AML[13,41,42] in contrast to the initial findings that mutations in *TET2* may precede acquisition of a *JAK2* mutation.[12] Consequently, the role of *TET2* mutations as initial or cooperating events is not settled and may differ in various myeloid malignancies.

ASXL1 mutations are reported in 11% of MDS, 43% of CMML, and 47% of secondary AML.[18,43] Recently, *ASXL1* mutations were seen in both BP as well as CP,[20] whereas in our series these mutations were detected in only AP and BP. Combining our findings with the previously reported results, *ASXL1* mutations were seen in 4 of 48 CP samples and 5 of 47 BP cases. Consequently, it is still uncertain whether *ASXL1* mutations constitute an early or a secondary event. Because knockout mice did not develop myeloid malignancies,[44] *ASXL1* mutations might cooperate with other defects such as the *BCR-ABL1* fusion gene or other mutations.

IDH family mutations confer an enzymatic gain of function that increases 2-hydroxyglutarate; consequently, heterozygous acquisition of these mutations may be sufficient to facilitate malignant progression.[45,46] We have detected canonical *IDH* mutations [R132 (*IDH1*) and R140 (*IDH2*)] in aggressive stages of CML analogous to gliomas and AML.[23,26] This suggests their secondary role in acquisition of a more malignant phenotype.

In isolated case reports, *JAK2*V617F mutations were found in patients with CML.[47] In some instances, the *JAK2*V617F-positive

Table 5. UPD lesions detected by SNP-A and affected candidate genes (N = 26)

Patient no.	Disease	Chromosome	Start	Stop	Length, Mb	Affected candidate genes
22	AP	5q22.1q33.3	110815528	157807809	46.99	*APC, IRF1, TCF7, SKP1, CDKL3, PHF15, TIFAB, GFRA3, JMJD1B, ETF1, NRG2, HBEGF, ANKHD1, SRA1, HDAC3, NDFIP1, SPRY4, FGF1, ARHGAP26, NR3C1, TCERG1, PPP2R2B, PPARGC1B, CSF1R, PDGFRB, CDX1, RPS14, IRGM, ANXA6, FAT2, SPARC, ATOX1, G3BP1, HAND1, MRPL22, MED7, SOX30, CLINT1*
22	AP	8q21.2q24.3	87129788	146263890	59.13	*WWP1, RIPK2, MTG8, GEM, RAD54B, GDF6, RPL30, STK3, YWHAZ, GRHL2, RRM2B, UBR5, BAALC, FZD6, ZFPM2, EIF3E, EBAG9, CSMD3, TRPS1, EIF3H, MED30, SAMD12, TNFRSF11B, NOV, ENPP2, TAF2, DSCC1, ZHX1/2, ANXA13, MYC, WISP1, Cap43, NDRG1, ST3GAL1, CHRAC1, PTK2, PTP4A3, BAI1, JRK, MAFA, MAPK15, PUF60, FBXL6, NFKBIL2, RECQL4*
44	Myeloid BP	11p15.5p12	194228	41346378	41.15	*DUSP8, RASSF7, H19, IGF2, ASCL2, TSSC4, CDKN1C, NAP1L4, NUP98, RRM1, ILK, TPP1, NLRP10, STK33, ST5, WEE1, SBF2, AMPD3, MRVI1, EIF4G2, DKK3, TEAD1, ARNTL, RRAS2, SOX6, PTPN5, BBOX1, LGR4, METT5D1, ELP4, PAX6, WT1, HIPK3, COMMD9, TRAF6*
44	Myeloid BP	17q11.2q25.3	27492835	78640854	51.15	Whole 17q

AP indicates accelerated phase; and BP, blast phase.

clone evolved when *BCR-ABL1* allelic burden decreased after treatment for CML.[48,49] However, larger studies suggest that *JAK2*V617F mutations do not occur in CML.[50] In agreement with this study, neither this mutation nor UPD9p was found in our cohort of patients; thus, it may be safe to conclude that *JAK2*V617F mutations are less common in CML than other genes examined in this study.

In 3 cases we observed a combination of mutations in 2 genes. The coexistence of *CBLB* and *TET2* mutations in 2 cases suggests that these might be mutually supportive of each other in the AP phase of CML or that sequential clonal events lead to stepwise progression and more aggressive disease. We also found a combination of *IDH1* and *ASXL1* mutations in 1 of our patients with BP, suggesting that both mutations contribute to the clonal advantage.

Most of the *CBL*, *TET2*, and *ASXL1* mutations were unique. Similarly, frameshift and nonsense mutations are unlikely to be germline variants. Theoretically, it is possible that some of the missense alterations found here represent novel germline polymorphisms.

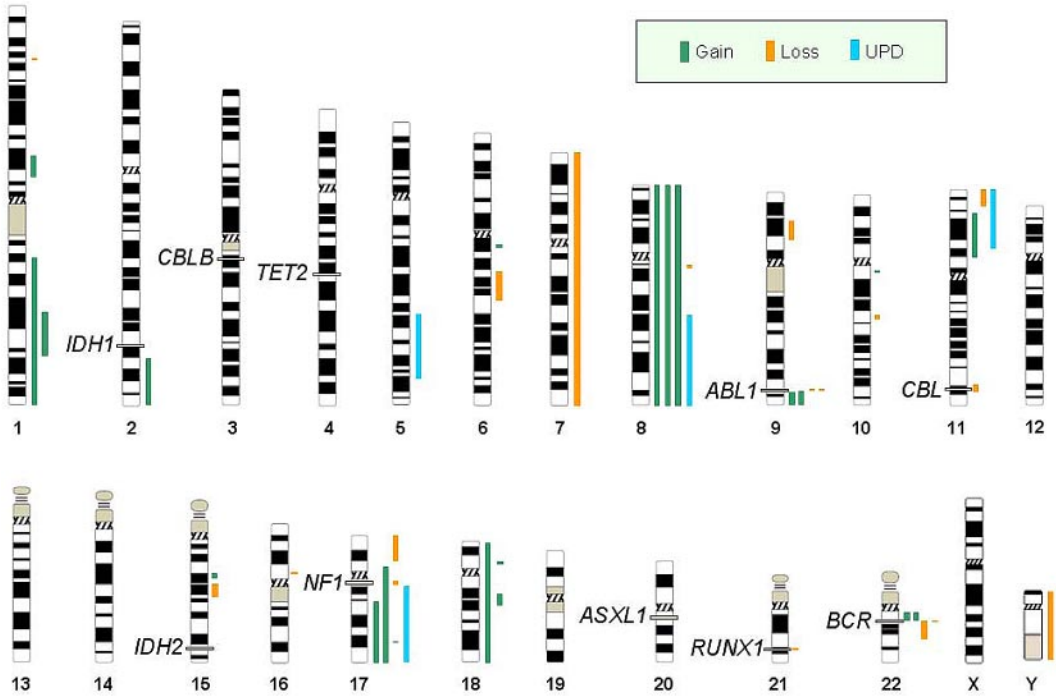

Figure 3. Overview of all genetic aberrations found by SNP-A karyotyping in 26 patients with AP and BP. Each line represents chromosomal aberration: green indicates copy number gains; orange, losses; and blue, regions of UPD. Exact location of *IDH1*, *CBLB*, *TET2*, *ABL1*, *CBL*, *IDH2*, *NF1*, *ASXL1*, *RUNX1*, and *BCR* are noted on chromosomes 2q, 3q, 4q, 9q, 11q, 15q, 17q, 20q, 21q, and 22q, respectively.

However, they were not found in any SNP databases and also not encountered in any of our previous sequencing studies (> 400 patients sequenced to date) or described by others, suggesting that their frequency would be exceedingly low.

Additional nonrandom chromosomal abnormalities (eg, affecting chromosome 8, 17, 19, and 22) are often observed during progression of CML to aggressive phase.[51] To date, 3 different groups reported SNP-A–based surveys of chromosomal lesions in CML. One analyzed 45 tyrosine kinase inhibitor-resistant CML cases and showed that several acquired regions of UPD and recurrent deletions, as well as duplication of the *BCR-ABL1* gene and trisomy 8, previously found by metaphase cytogenetics.[52] Although in our study SNP-A results were available in a representative subset of patients, the findings are in agreement with previous studies.[20,52] We demonstrate that some defects are recurrent, for example, microdeletions in 9q and 22q and a recurrent copy number gain on 17q. Most importantly, we showed regions of LOH associated with oncogenes and proto-oncogenes, such as deletion of 17p13.3p11.2, containing the *TP53* locus, as previously reported in myeloid BP.[53] In 2 cases with lymphoid phenotype studied by SNP-A, we were unable to find previously described homozygous deletion of *IKZF1*,[54] but a heterozygous deletion 9p21.3p13.2 in *CDKN2A* region was present in a patient with myeloid BP. However, no cryptic recurrent lesions were identified by SNP-A, and in general shared defects were detectable by both metaphase cytogenetics and SNP-A karyotyping

Although there was a general concordance between SNP-A and metaphase cytogenetics in most examined cases, some differences were found. Discordance is probably related to the differences in the sensitivity of these methods as previously described.[27,30] For example, in patient with AP (no. 30), del(5)(q22q23) in 19 metaphases was not detected by SNP-A. We conceived this study fully aware that the SNP-A technology is not designed to replace metaphase cytogenetics.

When clinical features were studied, there was no significant difference in overall survival between patients with mutations and WT. This may be because of the generally poor prognosis of patients with CML with advanced malignancy refractory to imatinib mesylate. A screen for *BCR-ABL1* tyrosine kinase mutations helped to identify a patient resistant to imatinib mesylate with a mutation of *CBL* who had a WT *BCR-ABL1* kinase domain, suggesting that mutations in other genes might contribute to refractoriness to tyrosine kinase inhibitors. However, it is probable that imatinib mesylate resistance and progression to AP or BP may be controlled by separate molecular events.

It is possible that, because of limited sensitivity of Sanger sequencing, mutations in these genes are present in a much higher proportion of patients. For example, although we detected *CBL* mutations in 13%-15% of CMML cases with the use of routine sequencing, application of next-generation sequencing resulted in a higher detection rate of 25%.[55] Clearly, our report does not settle this issue, and the mutation status of genes investigated in our study should be prospectively and serially evaluated in a larger cohort of patients with the use of deep sequencing approaches.

In conclusion, *CBL* family, *TET2*, *ASXL1*, and *IDH* family mutations as well as additional unbalanced chromosomal abnormalities not seen by metaphase cytogenetics can occur in advanced phase CML with myeloid phenotype. These mutations probably represent secondary lesions that contribute to aggressive features in AP and BP.

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Authorship

Contribution: H.M., A.M.J., and J.P.M. designed and performed research, analyzed data, and wrote the paper; M.A.M., A.A., and R.P. designed research and wrote the paper; C.O., S.D., H.C., C.P., J.N., H. Szpurka, and E.H. performed research; and B.P., H. Siddaiah, and M.S. analyzed data.

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