Clonal Hematopoiesis in Normal Individuals is Not Random and Likely Reflects Early MDS

#4269

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Introduction

Clonal hematopoiesis, as determined by the presence of *TET2, ASXL1,* and *DNMT3* mutation, is believed to be increasingly common in normal individuals as they age. This phenomenon is currently referred to as clonal hematopoiesis of indeterminate potential (CHIP). It has been reported that a few of these patients will develop myeloid neoplasms that begin as myelodysplastic syndrome (MDS) and may evolve into acute myeloid leukemia (AML). However, the clinical relevance of this particular subset of gene mutations (*TET2, ASXL1,* and *DNMT3A*) in healthy individuals is unclear as there are more than 50 genes that have been reported to be involved in myeloid neoplasms. *FLT3* and *NPM1* mutations have been reported in de *novo* AML patients and may occur as a secondary mutations on an MDS background. In order to clarify the significance of *TET2, ASXL1,* and *DNMT3* in CHIP and their relationship to MDS and AML, we assessed the prevalence of *TET2, ASXL1,* and *DNMT3* genes in patients with *NPM1* and *FLT3* mutations. In addition, since *IDH1/2* gene are rarely mutated with *TET2*, we also studied the frequency of mutations of various myeloid genes in this group of patients.

Methods

A total of 6390 consecutive bone marrow aspirate samples or peripheral blood samples submitted with clinical impression of AML, MDS, or MPN between January 2014 and mid 2017 were tested for mutations in myeloid genes using next generation sequencing (NGS). We used the TruSight Myeloid Panel (Illumina, San Diego, CA) for detecting missense mutations and fragment length analysis (FLA) for detecting ITD in *FLT3* and large indels in *CALR*. DNA was extracted from samples using the QIAamp DNA Mini Kit. This NGS testing covers mutations in 54 myeloid-related genes. The average depth of sequencing was 10,000x.

Results

In our database of consecutively tested patients, there were 311 patients with *FLT3* mutations, 318 patients with NPM1 mutation, and 467 patients with IDH1/2 mutation. In addition, there were 308 patients diagnosed as MDS. All these patients tested for mutations in all 54 myeloid-related genes. The median age of patients in *FLT3*, MDS, *IDH1/2*, and *NPM1* groups was 65, 75, 69, and 66, respectively. The top 4 most frequently mutated in genes in the FLT3-positive patients, which present AML, were *NPM1, DNMT3, WT1, and RUNX1*. In patients with NPM1 mutations, also representing AML, the top 4 most mutated genes were *FLT3, IDH2, NRAS, and PTPN11*. In the *IDH1/2* mutated patients, the top 4 mutated genes were *DNMT3A, SRSF2, ASXL1, and NPM1*. As expected in the MDS group, the top 4 mutated genes were *TET2, DNMT3A, ASXL1, and SRSF2*.

Frequency of Co-mutated Genes in Sub-Cohorts: NPM1+, FLT3+, IDH1/2+, and MDS

Mutated Gene	CHIP: VAF<20% VAF	CHIP: VAF<20% VAF
DNMT3A	***32.88%***	***51.87%***
TP53	***22.71%***	***35.83%***
SF3B1	***18.31%***	***28.88%***
TET2	***16.27%***	***25.67%***
ASXL1	***14.92%***	***23.53%***
JAK2	5.93%	9.36%
U2AF1	4.92%	7.75%
MYD88	4.24%	6.68%
CEBPA	2.71%	4.28%
KRAS	2.71%	4.28%
RUNX1	2.54%	4.01%
BRAF	2.20%	3.48%
IDH2	2.20%	3.48%
NRAS	2.20%	3.48%
FLT-3	2.03%	3.21%
BCOR	1.53%	2.41%
SRSF2	1.53%	3.21%
ZRSR2	1.36%	2.14%
IDH1	1.19%	1.87%

Top table shows the frequency of mutations in various genes detected in patients with CHIP. The bottom tables show the frequency of co-mutated genes in various groups. The *NPM1+* (318) and *FLT3+* (311) represent AML. The *IDH1/2+* (467) representing confirmed myeloid neoplasms. The MDS (308) group who were confirmed by VAF>20%. As shown, the most commonly mutated genes in CHIP as the same genes mutated in MDS. In contrast, patients with *FLT-3* and *NPM1*, which are more likely to represent AML as well as patients with *IDH2/1* are more likely to have mutation pattern different from that of CHIP and MDS.

NPM1-Positive		*FLT-3-Positive*		*IDH1/2-Positive*		*MDS*	
N=318		*N=311*		*N=467*		*N=308*	
Gene	**%**	**Gene**	**%**	**Gene**	**%**	**Gene**	**%**
DNMT3A	**47.81%**	NPM1	44.09%	**DNMT3A**	**34.26%**	**TET2**	**31.29%**
FLT3-ITD	30.31%	**DNMT3A**	**40.26%**	**SRSF2**	**27.62%**	**DNMT3A**	**28.39%**
FLT3-KD	20.31%	**TET2**	**17.25%**	**ASXL1**	**22.48%**	**ASXL1**	**25.16%**
IDH2	19.06%	WT1	14.70%	NPM1	22.06%	**SRSF2**	**17.10%**
NRAS	17.81%	RUNX1	14.38%	RUNX1	19.70%	**SF3B1**	**14.52%**
PTPN11	16.56%	**ASXL1**	**12.46%**	NRAS	9.64%	RUNX1	10.97%
IDH1	15.00%	IDH2	11.18%	**TET2**	**7.71%**	TP53	10.32%
TET2	**14.38%**	NRAS	11.18%	FLT3-ITD	7.28%	NRAS	7.74%
WT1	6.56%	IDH1	6.71%	BCOR	6.64%	U2AF1	5.81%
KRAS	5.94%	PTPN11	6.39%	JAK2	6.64%	IDH2	5.48%
SRSF2	**5.31%**	BCOR	5.75%	TP53	6.64%	JAK2	5.48%
ASXL1	**5.00%**	CEBPA	4.47%	STAG2	6.21%	SETBP1	4.84%
CEBPA	4.69%	STAG2	3.83%	PTPN11	5.35%	ZRSR2	4.84%
SF3B1	**2.50%**	**SF3B1**	**3.51%**	FLT3 KD	4.93%	CBL	4.19%

Variation in Age Between Various Groups

Groups	Valid N	Mean	Median
FLT3	311	62	65
MDS	308	72	75
Normal	332	63	65
IDH	467	68	69
NPM1	318	64	66
CHIP	590	67	70

Key Points

- A few of patients with CHIP will develop myeloid or lymphoid neoplasms.
- We considered a patient having CHIP when a mutation in one gene is detected and the VAF is <20%.
- Patients samples were referred for molecular testing on patients with clinical suspicion of hematologic neoplasm due to anemia, thrombocytopenia, or neutropenia.
- A total of 7497 samples were tested between 2014 and mid 2017. Of these 4075 had one mutation in one or more genes.
- Only 590 of 7497 (8%) total tested and 14% of cases with mutations showed a mutation in one gene with VAF<20%. Of these 374 (63%) had VAF <10%.
- Mutation pattern in cases with CHIP in this population of patients, who have one or more cytopenia, is more suggestive of early MDS.
- Unusually high rate of TP53 mutation is detected in this group of patients.
- A high percentage of patients have MYD88 mutations, suggesting lymphoid rather than myeloid neoplasm in this sub-cohort.

Conclusion

The demonstration that mutations in *TET2, DNMT3A,* and *ASXL1* are most common in patients presenting with MDS, as well as in CHIP, but not in patients presenting with acute myeloid neoplasms, suggests that CHIP likely represents early MDS. It is possible that mutations in these genes in few hematopoietic cells may not be adequate for manifestation of clinical MDS, but the presence of these mutations in large number of cells (high VAF) or the accumulation of mutations in additional genes is necessary for clinical disease. Therefore the clinical relevance of CHIP should always be considered in conjunction with other mutations and with VAF.