

Assessing the Possibility of RUNX1 Related Predisposition in Myeloid Neoplasms in a Somatic Cancer Setting

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INTRODUCTION

- The World Health Organization recently released an update to the classification of hematopoietic neoplasms, which includes a new diagnostic category of myeloid neoplasms with germline predisposition (WHO, 2016). Although such germline predispositions are rare in the hematologic realm, it is important as a commercial lab that we are aware of the possibility of germline mutations that could be detected through somatic based genetic testing.
- Genes identified as having possible germline predisposition to myeloid neoplasms are: CEBPA, DDX41, RUNX1, ANKRD26, ETV6 and GATA2. For the purpose of our study we focused only on Runt-related transcription factor 1 (RUNX1) germline mutations, which predispose to familial platelet disorder with associated myeloid malignancy (WHO, 2016).
- RUNX1 is included on our somatic myeloid testing panel. Patients with germline RUNX1 mutations often have mild-to-moderate thrombocytopenia from birth, with or without bleeding tendency. In carriers of the mutation, the likelihood of developing MDS or AML is approximately 35% to 44% (Owen et al, 2008, Godley, 2014).
- The purpose of our study was to observe how often we are seeing RUNX1 alterations in younger patients and at allele frequencies suggestive of a germline mutation. This study also allowed us to analyze the content of our reports in regards to detecting RUNX1 alterations that appear to be possibly present in the germline.

METHODS

- For our study we isolated the number of RUNX1 alterations we have detected through our somatic next generation sequencing on myeloid neoplasms. We then isolated the RUNX1 alterations that were present at an allele frequency that could be suggestive of a germline mutation (40-60% allele frequency). We also gathered clinical information on the top ten youngest patients with RUNX1 alterations.

RESULTS

- A total of 274 RUNX1 alterations have been detected in our lab out of the total ~4,300 myeloid cases analyzed over the last 16 months. A single RUNX1 alteration was detected in 11% of patients and the remainder had other gene alterations detected (Table 1).
- Diagram 1 shows that the majority of RUNX1 alterations were disease associated. Table 2 shows that the majority of individuals with RUNX1 alterations were between 60-90 years of age. Table 2 also shows that the most common category of disease was a suspected myeloid neoplasm. Of the cases where a diagnosis was known, RUNX1 alterations were most commonly seen in myelodysplastic syndrome and acute myeloid leukemia (Table 2).
- The youngest patient we saw with a RUNX1 variant was 12 years old while the oldest patient was 94 years old, with a median age of 71.
- Of the 274 alterations reported, 94 were found to have an allele frequency suggestive of being a germline alteration (40-60%). Of the RUNX1 alterations seen at an allele frequency suggestive of a germline alteration the majority were seen in patients between the age of 65 to 80. However, there were also four outliers in Graph 1 that were less than age 30 with a RUNX1 alteration. Of the 10 youngest patients with suggestive germline RUNX1 alterations 8 alterations were unclear variants and 2 were disease associated (Table 3). In addition, in this group of young patients with possible germline RUNX1 alterations six cases were identified to have suspected myeloid neoplasms and two were reported to have acute myeloid leukemia (Table 3).

Table 1: Other gene alterations detected in patients that were found to have a RUNX1 alteration.

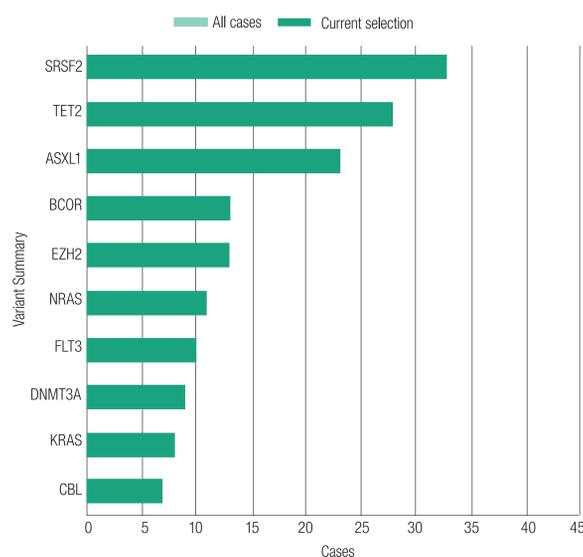


Diagram 1: Proportion of RUNX1 unclear variants versus disease associated alterations.

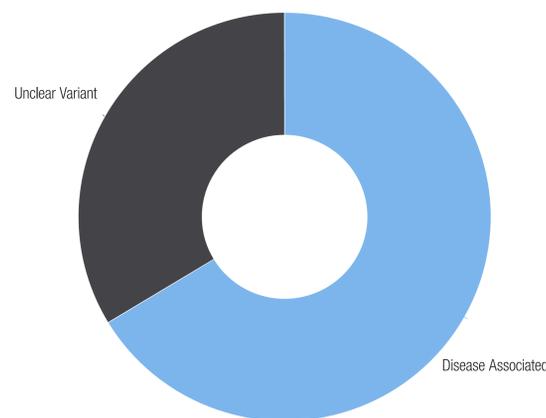
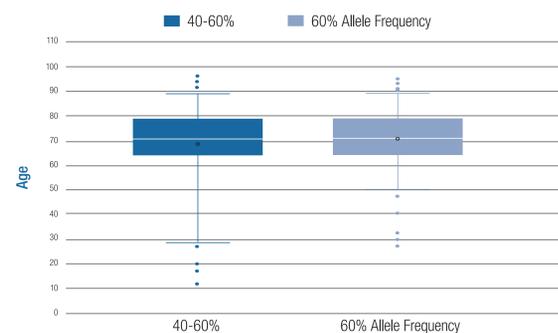
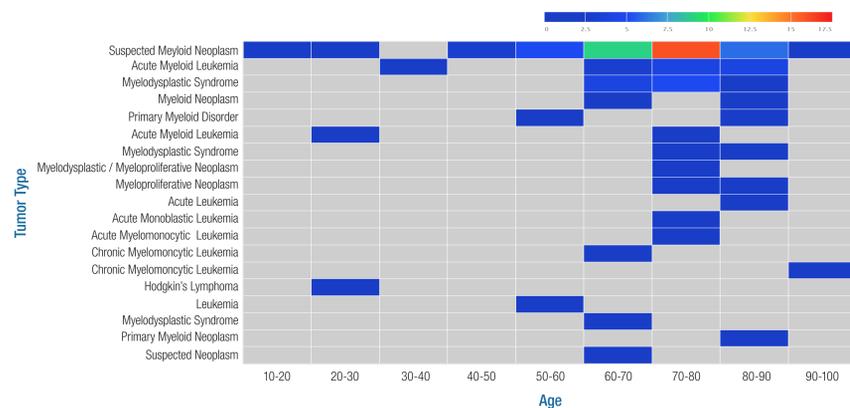


Table 2: Tumor types seen in cases with RUNX1 alterations, separated by age.



Graph 1: Average age of individuals with RUNX1 alterations seen at an allele frequency suggestive of a germline mutation.

Table 3: Clinical information of the top ten youngest patients identified to have a RUNX1 alteration between 40-60% allele frequency.

RUNX1 Alteration	Classification	Allele Frequency (%)	Age	Gender	Test Ordered	Other Gene Alterations Detected	Tumor Type	Other Clinical Information
p.L56S	Unclear variant	57.01	12	Female	Myeloid Disorder Sequencing Panel	ZRSR2	Suspected Myeloid Neoplasm	The patient has a history of severe congenital neutropenia with documented ELANE mutation
p.L56S	Unclear variant	53.3	17	Female	Myeloid Disorder Sequencing Panel	JAK2	Suspected Myeloid Neoplasm	Thrombocytosis
p.S424L	Unclear variant	54.65	20	Male	Myeloid Disorder Sequencing Panel	-	Hodgkin's Lymphoma	None
p.M133I	Unclear variant	44.12	27	Male	Myeloid Disorder Sequencing Panel	CSF3R, SRSF2	Acute Myeloid Leukemia	None
p.?	Disease Associated	41.78	28	Male	AML Sequencing Panel	-	Suspected Myeloid Neoplasm	None
p.G199E	Unclear Variant	41.97	30	Female	AML Sequencing Panel	-	Acute Myeloid Leukemia	Leukocytosis
p.V452G	Unclear variant	47.87	41	Female	Myeloid Disorder Sequencing Panel	-	Suspected Myeloid Neoplasm	Pancytopenia. BM morphology normocellular marrow (50%) with maturing trilineage hematopoiesis, with no evidence of overt or high grade myelodysplasia, acute leukemia, or lymphoma seen
p.S28del	Unclear variant	52.56	46	Female	Myeloid Disorder Sequencing Panel	FLT3	Suspected Myeloid Neoplasm	None
p.H242Afs*14	Disease Associated	42.3	46	Male	MDS Sequencing Panel	EZH2	Suspected Myeloid Neoplasm	Reported diagnosis acute myeloid leukemia
p.M52K	Unclear variant	52.89	50	Female	MDS Sequencing Panel	-	Leukemia	Reported diagnosis of new leukemia

DISCUSSION

- The major finding of our study was that suspected germline RUNX1 alterations were seen in both young patients and in patients with a myeloid neoplasm, usually either acute myeloid leukemia or myelodysplastic syndrome. However, it is important to note that older age and lack of family history of disease or clinical history of disease does not necessarily rule out a germline predisposition in these cases. It is also important to note that due to variations in the tumor burden as well as the presence of other genetic alterations and preferential detection of alleles, the allele frequency of ~50% is not definitive enough to confirm a germline mutation. Although germline mutations were not confirmed with skin biopsy cultured fibroblast genetic testing, these findings still identify a need to inform doctors and patient's about the possibility of a germline predisposition.
- One major difficulty comes with RUNX1 variants of unknown significance, however these alterations could also be considered related to a germline predisposition. Identifying RUNX1 variants of uncertain significance that appear to be present in the germline may help identify other relevant disease associated RUNX1 alterations.
- The WHO guidelines suggest genetic counseling for any patient with acute leukemia or MDS with a first-degree or second-degree relative with AML, acute lymphocytic leukemia, or MDS. They also suggest keeping in mind other non-hematological characteristics that may be suggestive of germline predispositions to myeloid malignancies (WHO, 2016).
- Following these guidelines, our group suggests that if a RUNX1 alteration is found at an allele frequency suggestive of a germline mutation (~50%) we should include in our report a statement regarding the possibility of a germline predisposition syndrome, regardless of age, family history and medical history. Currently, for RUNX1 suspected germline mutations this statement reads "The World Health Organization now recognizes that germline RUNX1 alterations can predispose to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), which may be another possibility for this particular patient given that the allele frequency is close to 50% (WHO, 2016). If clinically indicated, testing on a skin biopsy may be considered in order to determine whether this represents a germline abnormality."
- Acknowledging such predisposition related conditions allows for appropriate clinical management and surveillance. The awareness of these predispositions will also allow for earlier detection of disease and potential development of prevention options.

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