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Germline duplication of *ATG2B* and *GSKIP* genes is not required for the familial myeloid malignancy syndrome associated with the duplication of chromosome 14q32

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To the Editor,

We read with interest the recent Perspective by Tawana and colleagues¹ discussing the advantages and disadvantages of upfront genetic testing for inherited susceptibility to myeloid neoplasms for all patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). At least 10% of patients with myeloid neoplasms are believed to have a genetic predisposition to MDS/AML¹. One additional consideration for implementing universal genetic testing for myeloid malignancies is the still incomplete understanding of genotype-phenotype correlations for the more recently described and emerging syndromes. Recently, Saliba et al.² identified a novel type of myeloid neoplasm

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Author contributions

D.B. and E.H. designed the study and performed clinical record review, D.B. performed genomic analyses using WES, SNP-A, and WGS analyses, N.S. performed Sanger sequencing, J.M. and D.L. performed targeted somatic mutation analysis and provided invaluable banked patient samples, S.C. and T.O. contributed hematopoiesis expertise, D.B., N.S., J.M., D.L., T.O., S.C. and E.H. analyzed and interpreted the data, D.B. wrote the manuscript. All authors revised and approved the manuscript.

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predisposition in four large West Indian families, where a germline tandem duplication of a 700 kilobase (kb) region on chromosome 14q32 conferred a highly penetrant form of a myeloid neoplasm inherited in an autosomal dominant manner. The duplicated region encompassed five protein-coding genes, *TCL1A*, *GSKIP*, *ATG2B*, *BDKRB1*, and *BDKRB2*. Extensive correlative studies concluded that malignant predisposition in these families was due to a germline duplication of the *GSKIP* and *ATG2B* genes and not the other genes in the duplicated region. Based on these findings, the recommended testing for familial myeloid neoplasms has been expanded to include duplications of the *GSKIP* and *ATG2B* genes¹. Here we report a North American family with an autosomal dominant predisposition to myeloid neoplasms and a duplication of chromosome 14q32 that does not involve the *GSKIP* and *ATG2B* genes, suggesting that duplication of *GSKIP* and *ATG2B* may not be required for this autosomal dominant myeloid neoplasm predisposition syndrome.

We identified a North American family of Caucasian ancestry with three affected generations who presented with myeloid neoplasms similar to the four families reported by Saliba et al. (Figure 1A, Table 1). The proband (IV-3) developed a *JAK2* V617F mutation-positive primary myelofibrosis at the age of 22, and eight years later developed AML. The proband's father (III-2) was diagnosed with myelodysplastic syndrome with ringed sideroblasts and multilineage dysplasia at the age of 64 years and rapidly progressed to AML within months of presentation. The proband's paternal grandmother (II-2) died of acute leukemia at the age of 37 years. Whole exome sequencing (WES) of skin fibroblast DNA from the proband's father (III-2) detected no genetic lesions associated with known familial myeloid neoplasms. However, single nucleotide polymorphism array (SNP-A) analysis identified a germline duplication on chromosome 14q32. The same chromosome 14q32 duplication was also present in the proband (IV-3) and was absent in the unaffected relatives. The duplicated region partially overlapped the duplication reported by Saliba et al.; however, among the five genes duplicated in the West Indian families, only *TCL1A* was duplicated in our family.

To characterize the duplicated region more precisely, we used whole genome sequencing (WGS) in combination with polymerase chain reaction (PCR) amplification and Sanger sequencing to map the breakpoints of the rearrangement (Figure 1B). The 1.8 Mb tandem duplication included 29 protein-coding genes and had a 56 kb region in common with the duplication reported by Saliba et al. The shared region included *TCL1A*, a portion of a noncoding transcript *BX247990*, and three regions enriched for the H3K27 histone acetylation marks (Figure 1C). *GSKIP*, *ATG2B*, *BDKRB1* and *BDKRB2* genes were not duplicated (Figure 1C). Another family in Australia with similar autosomal dominant myeloid malignancy predisposition was also found to have a duplication of chromosome 14q32 that did not include *GSKIP* and *ATG2B*³ (Figure 1C).

Because *GSKIP* and *ATG2B* are not included in the common duplicated region shared by these families, our data suggest that duplication of *GSKIP* and *ATG2B* is not required for the familial myeloid neoplasm syndrome associated with duplication of 14q32. There could be several explanations for our findings in light of experimental observations reported by Saliba and colleagues. It is possible that the duplication in our family could affect the expression of *ATG2B* and *GSKIP* genes through long-range regulatory interactions.

Alternatively, the primary evidence for the involvement of *ATG2B* and *GSKIP* is the reduction of the number and size of colony forming unit-megakaryocyte progenitor colonies (CFU-MK) when both *ATG2B* and *GSKIP* were knocked down in normal and patient-derived CD34+ cells and induced pluripotent stem cells (iPSc)-derived megakaryocyte progenitors². Although a hematopoietic phenotype caused by a lower expression of *ATG2B* and *GSKIP* suggests that these genes play an important role in megakaryopoiesis, this observation does not fully establish the etiologic role of these genes in causing malignant predisposition in the families. Conversely, Saliba et al. evaluated expression of *TCL1A* in patient-derived Epstein Barr Virus (EBV)-transformed B lymphocyte cell lines and normal CD34+ cells, but not in primary hematopoietic cells from the affected individuals². However, *TCL1A* is expressed in several early myeloid progenitors^{4, 5}, and studies in B cells or CD34+ cells may not fully recapitulate the role of *TCL1A* in myelopoiesis. Based on the synergistic role of *ATG2B* and *GSKIP* genes in megakaryopoiesis, it is likely that duplication of *ATG2B* and *GSKIP* in the West Indies families affects their hematopoietic phenotype. Such differences in additional duplicated genes likely shape the spectrum of myeloid neoplasms in families with various duplications of 14q32, possibly explaining the higher prevalence of the classical myeloproliferative phenotype in West Indian families, whereas the North American and Australian families have more myelodysplasia and acute leukemia.

In conclusion, germline duplication of chromosome 14q32 is a recently described genetic determinant of a highly penetrant autosomal-dominant predisposition to myeloid neoplasms². Our findings, combined with others, show that duplication of *GSKIP* and *ATG2B* is not required for this malignancy syndrome, and point to the duplication of *TCL1A*, the only protein-coding gene duplicated in all six families, as a potential alternative driver of this predisposition syndrome. Studies are needed to determine the mechanism of myeloid neoplasm predisposition caused by the duplications of chromosome 14q32 and to assess whether a similar mechanism may contribute to the pathogenesis of sporadic myeloid neoplasms. Notably, our results suggest that until the mechanism of malignant predisposition associated with duplication of chromosome 14q32 is further clarified, diagnostic testing for this syndrome should include the larger region of chromosome 14q32 and should not be limited to the *GSKIP* and *ATG2B* genes.

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Figure 1.

A) A pedigree of a North American family with an autosomal dominant inheritance of predisposition to myeloid neoplasms. The black symbol represents cases with myeloid neoplasms, detailed in Table 1; the gray symbol indicates other types of hematologic malignancy, in this case, lymphoma not otherwise specified. B) The genetic characterization of the 1.8Mb tandem duplication on chromosome 14q32 in the proband's father (III-2). The breakpoints were characterized by whole genome sequencing (WGS) as shown within the Integrative Genomics Viewer in the bottom panel. The breakpoints of the duplication are indicated by the red color of the aberrant reads and an increased read depth in the region of the duplication. All breakpoints were additionally validated by Sanger sequencing, with the corresponding chromatographs shown in the middle panel. Sequence data have been deposited at the European Genome-phenome Archive (EGA), which is hosted by the EBI and the CRG, under accession number EGAS00001003111. All coordinates are based on hg19. C) Schematic alignment of the 14q32 duplications in the West Indian, North American and Australian families. Vertical lines indicate that the 56Kb shared region encompasses the *TCL1A* but not the *ATG2B* and *GSKIP* genes. The ~700 kb duplication in the Australian family is depicted based on the report by Hahn et al.³ An expanded view of the shared region is illustrated in the bottom panel based on the annotation from the UCSC Genome Browser and includes *TCL1A* and a part of the noncoding transcript *BX247990*. The regions of enrichment of histone H3K27 acetylation marks are shown as pink peaks in the bottom panel.

Table 1.

Clinical and pathologic characteristics of the affected individuals

Patient	Gender	Diagnosis	Age at Diagnosis (Yrs)	Leukemic Transformation	Time to leukemic transformation (Yrs)	Molecular Profile Somatic Mutation (% allele frequency)	Cytogenetics	Current Status
II-2	F	AML	37	N/A	0	N/A	N/A	died
III-2	M	MDS-RS-MLD	64	AML	0.25	<i>SF3B1</i> p.K700E (43%), <i>FLT3</i> p.G583_L601dup (8%), <i>SRSF2</i> p.P95H (49%), <i>RUNX1</i> p.T178Sfs*34 (36%), <i>RUNX1</i> p.S226* (5%)	46,XY, +Y,t(1;3)(p36.3;q21) [20]	died
IV-3	M	PMF	22	AML	8	<i>JAK2</i> p.V617F (91%), <i>EZH2</i> splice p.? (21%), <i>ASXL1</i> p.E728Kfs*16 (10%), <i>IDH2</i> p.R140Q (30%), <i>ETV6</i> p.R399C (9%)	45,XY,t(3;21)(q26.2;q22), -7[20]	died

Yrs, years; AML, acute myeloid leukemia; MDS-RS-MLD, myelodysplastic syndrome with ringed sideroblasts with multilineage dysplasia; PMF, primary myelofibrosis.