

ASXL1 and STAG2 are common mutations in GATA2 deficiency patients with bone marrow disease and myelodysplastic syndrome

Robert R. West,¹ Katherine R. Calvo,² Lisa J. Embree,¹ Weixin Wang,² Laura M. Tuschong,¹ Thomas R. Bauer Jr,¹ Desiree Tillo,³ Justin Lack,⁴ Stephenie Droll,² Amy P. Hsu,⁵ Steven M. Holland,⁵ and Dennis D. Hickstein¹

¹Immune Deficiency-Cellular Therapy Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health (NIH), Bethesda MD; ²Department of Laboratory Medicine, NIH Clinical Center, Bethesda, MD; ³Genomics Core, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD; ⁴Advanced Biomedical Computational Science, Frederick National Laboratory for Cancer Research, Frederick, MD; and ⁵Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

Key Points

- Somatic mutations in chromatin- and cohesin-related genes, particularly *STAG2*, *ASXL1*, and *DNMT3A*, are common in *GATA2* deficiency.
- Mutations in other common myeloid malignancy-related genes, including splicing factors, are conspicuously rare in *GATA2* deficiency.

Patients with *GATA2* deficiency harbor de novo or inherited germline mutations in the *GATA2* transcription factor gene, predisposing them to myeloid malignancies. There is considerable variation in disease progression, even among family members with the same mutation in *GATA2*. We investigated somatic mutations in 106 patients with *GATA2* deficiency to identify acquired mutations that are associated with myeloid malignancies. Myelodysplastic syndrome (MDS) was the most common diagnosis (~44%), followed by *GATA2* bone marrow immunodeficiency disorder (G2BMID; ~37%). Thirteen percent of the cohort had *GATA2* mutations but displayed no disease manifestations. There were no correlations between age or sex with disease progression or survival. Cytogenetic analyses showed a high incidence of abnormalities (~43%), notably trisomy 8 (~23%) and monosomy 7 (~12%), but the changes did not correlate with lower survival. Somatic mutations in *ASXL1* and *STAG2* were detected in ~25% of patients, although the mutations were rarely concomitant. Mutations in *DNMT3A* were found in ~10% of patients. These somatic mutations were found similarly in G2BMID and MDS, suggesting clonal hematopoiesis in early stages of disease, before the onset of MDS. *ASXL1* mutations conferred a lower survival probability and were more prevalent in female patients. *STAG2* mutations also conferred a lower survival probability, but did not show a statistically significant sex bias. There was a conspicuous absence of many commonly mutated genes associated with myeloid malignancies, including *TET2*, *IDH1/2*, and the splicing factor genes. Notably, somatic mutations in chromatin-related genes and cohesin genes characterized disease progression in *GATA2* deficiency.

Introduction

GATA2 deficiency is a bone marrow failure syndrome resulting from germline mutations in the transcription factor gene *GATA2*. It was first described in 2010 as MonoMAC syndrome, to reflect 2 common disease manifestations: monocytopenia and *Mycobacterium avium* complex.^{1,2} It was also described as dendritic cell, monocyte, and B- and NK-cell lymphoid deficiency³; Emberger syndrome with lymphedema and monosomy 7⁴; and familial myelodysplastic syndrome (MDS)/acute myelogenous

Submitted 23 April 2021; accepted 29 June 2021; prepublished online on *Blood Advances* First Edition 16 September 2021; final version published online 31 January 2022. DOI 10.1182/bloodadvances.2021005065.

Original data are available in response to an e-mail request to the corresponding author. All genomic data from this study have been deposited in the dbGaP database (<https://www.ncbi.nlm.nih.gov/gap>; accession number phs002311.v1.p1).

The full-text version of this article contains a data supplement.

© 2022 by The American Society of Hematology. Licensed under Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0), permitting only noncommercial, nonderivative use with attribution. All other rights reserved.

leukemia (AML).⁵ Patients with *GATA2* deficiency frequently present with a hypocellular bone marrow, monocytopenia and B- and NK-cell lymphopenia, and opportunistic infections, particularly human papilloma virus and mycobacterial infections.^{6,7} However, there is significant variability in other features of the disease, including peripheral lymphedema (Emberger syndrome), deafness, lupus-like autoimmunity, and pulmonary alveolar proteinosis, among others. The age at disease onset ranges considerably from pediatric to midlife, even within the same family, whereas other individuals with *GATA2* mutations can remain asymptomatic throughout life. *GATA2* deficiency penetrance is incomplete, with estimates ranging from ~50% to 80%.⁸⁻¹⁰ Like penetrance, the rate of disease progression varies significantly, even among siblings in the same family, from a few years to decades.

Progression from immunodeficiency to hypocellular MDS is common in *GATA2* deficiency, together with the appearance of cytogenetic abnormalities such as trisomy 8 and monosomy 7. Although a minority of patients progress to AML or proliferative chronic myelomonocytic leukemia (CMML), myeloid progression is an ominous development in *GATA2* deficiency. In cases of non-*GATA2* MDS and AML, disease progression is thought to be driven by the accumulation of somatic mutations. There is a consensus that a few dozen common mutations constitute a “hit list” for leukemia progression.¹¹ These mutations tend to sort into 4 specific categories: transcription factors, chromatin modifiers, splicing factors, and signal transduction proteins. Somatic mutations in *ASXL1*, a chromatin modifier, are relatively common in non-*GATA2* MDS/AML.¹¹ Two reports on *GATA2* deficiency noted a high frequency of *ASXL1* mutations in patients with clonal progression.^{12,13} Recently, applications of genomic techniques in *RUNX1*, *GATA2* deficiency, and *CEBPA* (CCAAT/enhancer-binding protein- α) mutations have suggested a high frequency of somatic *ASXL1* mutations and have identified *STAG2* as another commonly mutated gene, in contrast with a low frequency of other MDS/AML-associated genes.¹⁰

We applied next-generation sequencing techniques to blood and bone marrow samples of 106 patients with *GATA2* deficiency, to identify acquired mutations that may drive myeloid progression of the disease. Genetic findings were compared with cytogenetics, biometric parameters, and *GATA2* mutations. *ASXL1*, *DNMT3A*, and *STAG2* mutations were the most common somatic ones; mutated *BCOR* and *SETBP1* mutations were also recurrent, but less frequent. Mutations in the other most common genes associated with MDS/AML (*TET2*, *IDH1/2* and the splicing factor genes) were notably rare or absent in *GATA2* deficiency.

Methods

Patient samples

The clinical protocols were reviewed and approved by the Institutional Review Board of the National Institutes of Health, Clinical Research Center. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Declaration of Helsinki of 1975, as revised in 2008. All participants gave written informed consent. Patients in this study were enrolled in National Institute of Allergy and Infectious Diseases clinical trial 13-I-1057 (registered on clinicaltrials.gov as NCT01905826) and National Cancer Institute clinical trial 13C-0132 (NCT01861106). Peripheral blood and bone marrow samples were collected by using standard techniques. Clinical Laboratory Improvement Amendments–certified sequencing was performed on peripheral blood to identify *GATA2* mutations. The *GATA2* variant allele

frequency (VAF) in coding mutations was >40% in DNA sequencing (described in the next section), consistent with germline mutations, either de novo or inherited (the exception was patient 100, for whom VAF data were not available). The patients were also evaluated for signs typical of MonoMAC syndrome to presume germline mutations. Genomic DNA (gDNA) for somatic mutation sequencing was isolated from peripheral blood leukocytes or bone marrow mononuclear cells by using the Puregene Blood Core Kit or DNeasy Blood and Tissue Kits (Qiagen Sciences, Germantown, MD). Standard metaphase cytogenetic analysis was performed on bone marrow aspirates. Survival probabilities were determined from Kaplan-Meier curves with significance determined with the Mantel-Cox log-rank test (Prism, version 9.0.0; GraphPad).

DNA sequencing

Sequencing was performed on gDNA isolated from peripheral blood leukocytes and/or bone marrow aspirates. Whole exome sequencing (WES) is described in the supplemental Methods. Targeted gene panel sequencing was performed with the Illumina TruSight Myeloid Panel, Integrated DNA Technologies xGen Lockdown Probes, or Qiagen Myeloid Neoplasms Panel (supplemental Table 1), and variants were called using the CCBP Pipeliner (Center for Cancer Research Collaborative Bioinformatics Resource; National Cancer Institute), omitting the deduplication filter. Details for sequencing, alignment, and quality control metrics are provided in the supplemental Methods. *ASXL1* exons 12 and 13 (National Center of Biotechnology Information: NM_015338) were initially genotyped by direct DNA sequencing, as described previously.¹³ DNA sequences were analyzed with (MacVector, version 12.0; MacVector, Inc, Cary, NC). Mutations in *ASXL1* were confirmed with independent polymerase chain reaction and primer sets. Statistical analyses and graphing were performed when indicated, using Prism, version 9.0.0. Statistical analyses were not performed when “n” was deemed too low.

Variant analysis

Somatic variants were analyzed by using MuTect2 with additional sorting parameters to exclude common population variants and variants of unknown significance. Variants were removed from further analysis based on a population VAF >0.1% in the ExAC/gnomAD or dbSNP (build 152) databases, sequencing read coverage of <20 reads by WES or <100 reads for targeted arrays, alternative read coverage of <5 reads by WES or <10 reads for targeted arrays, and/or <5% total reads. Mutations were considered to be pathogenic if damaging or deleterious scores by PolyPhen II or SIFT were consistent, or the Combined Annotation Dependent Depletion (CADD) score was >25, according to the CADD tool (<https://cadd.gs.washington.edu>).¹⁴ Variants were considered likely to be somatic if the VAF was <35% or was 70% on the X chromosome of the male participants. Other variants were considered probable germline and were not included in subsequent analyses. Variant bam files were also reviewed with the Integrative Genomics Viewer (IGV; version 2.6.2, <http://software.broadinstitute.org>), and additional sequence analysis was performed with MacVector, version 17.0.

Results

Patient population characteristics

We examined the biometrics and other parameters (age, sex, *GATA2* mutation, diagnosis, cytogenetics, and survival) in 106 individuals

bearing germline mutations in the *GATA2* gene ($n = 98$) or with symptoms consistent with MonoMAC syndrome ($n = 8$; Table 1). These symptoms include hypocellular bone marrow with monocytopenia, B- and NK-cell lymphopenia and recurrent opportunistic infections. The total cohort consisted of 40 males (38%) and 66 females (62%) of similar age (32.7 ± 15.4 years) from 77 different families (Table 1; supplemental Figure 1). There were 42 different heterozygous *GATA2* mutations from 72 different families and unidentified *GATA2* mutations but with the MonoMAC syndrome from 5 families (Table 1). There were no patients with homozygous or compound heterozygous *GATA2* mutations in this cohort.

The patients were divided into 5 separate categories based on their bone marrow and clinical status: asymptomatic (Asym), *GATA2* bone marrow immunodeficiency disorder (G2BMID),⁶ MDS, AML, and CMML (Figure 1A). Asymptomatic individuals had normal complete blood counts (CBCs), normal bone marrow pathology, and no history of recurrent or life-threatening infections. Patients with G2BMID had a hypocellular marrow with cytopenia, but no definitive evidence of dysplasia. MDS was characterized by dysplasia, ineffective hematopoiesis, and other associated factors, including abnormal cytogenetics. Patients with AML or CMML were frequently grouped together and are designated AML/CMML for analysis because of low sample numbers.

MDS was the most common bone marrow diagnosis in this cohort (44.3%; $n = 47$), followed by G2BMID (36.8%; $n = 39$), normal (13.2%; $n = 14$), and AML/CMML (5.7%; $n = 6$; Figure 1A). Age ranged from 9.4 to 80.9 years, and clinical status ranged from asymptomatic to advanced proliferative leukemia (Figure 1B). Patients with MDS were significantly older (32.0 ± 11.4 years) than those with G2BMID (25.3 ± 10.2 years; Figure 1B). This study also included 6 patients with overt leukemia: 3 with AML and 3 with proliferative CMML. Together, the patients with AML and CMML were younger than those with MDS, with the exception of an 81-year-old man with CMML. The diagnoses were not significantly biased by sex (supplemental Figure 1).

Fourteen patients had pathogenic *GATA2* mutations or were carriers of MonoMAC syndrome based on an extended family pedigree (supplemental Figure 2), but these patients remained asymptomatic throughout the course of the study. The asymptomatic group was significantly older than the group with clinical symptoms, with an average age of 55.9 ± 13.2 years vs 29.2 ± 12.3 years ($n = 92$; Figure 1B).

Bone marrow transplant

Hematopoietic stem cell transplant (HSCT) represents the definitive treatment of *GATA2* deficiency. Seventy-six percent of the patients (70 of 92) underwent HSCT (Figure 1C), and 84% (59 of 70) were alive and disease-free after HSCT. Of the 24% (22 of 92) of affected patients who did not receive transplants, 77% (17 of 22) were alive at the end of the study (Table 1). Detailed descriptions of HSCT in patients with *GATA2* deficiency are presented elsewhere (Parta et al¹⁵ and "HSCT with *GATA2* deficiency: influence of donor stem cell source and posttransplantation cyclophosphamide"; Nichols-Vinueza et al.¹⁶).

Inheritance

GATA2 deficiency may be inherited through an existing germline mutation in a parent or as a de novo germline mutation first

expressed in the patient (see "Methods"). The *GATA2* mutation inheritance pattern was investigated as a factor for disease development and progression. The patients in this cohort were classified as familial if they had a first-degree relative with the same *GATA2* mutation or de novo if neither biological parent tested positive for a patient's *GATA2* mutation. Cases that did not have the data for either of these scenarios were classified as "unknown." An inheritance pattern was established for 89 patients in 60 families. Familial inheritance was observed in 62% (37 of 60) and de novo inheritance in 38% (23 of 60; Figure 1D) of the cases. The inheritance mode could not be confirmed in 17 families. The inheritance mechanism did not affect the bone marrow diagnosis (Table 1; Figures 1D and 4A).

Cytogenetics

Cytogenetic analysis remains a key diagnostic component in myeloid malignancies. Cytogenetic analysis was performed on samples from 92 patients with disease manifestations, and at least 1 abnormality was found in 43% of the cases (Table 1). The most common abnormalities were trisomy 8 (23%; 21 of 92) and monosomy 7 (12%; 11 of 92; Figure 2A). Other cytogenetic abnormalities were found in 18% (16 of 92) of patients, including 5 with der(1;7)(q10;p10). The other cytogenetic abnormalities were concurrent with trisomy 8 in 6 cases and monosomy 7 in 1 case. The presence of more than 1 type of cytogenetic abnormality was found in 11% of patients (10 of 92).

Cytogenetic abnormalities were rare in G2BMID (5%; 2 of 39) and common in MDS (68%; 32 of 47). However, because certain cytogenetic abnormalities define MDS in the setting of persistent cytopenia (eg, monosomy 7),¹⁷ the percentage is inherently higher in the MDS group than in G2BMID. Cytogenetic abnormalities were the most common in patients with AML or CMML (5 of 6). The presence of cytogenetic abnormalities was not biased by patient age (normal, 27.6 ± 12.8 years; abnormal, 31.4 ± 11.6 years; Student *t* test; $P = .1586$; Figure 2B). Monosomy 7 was more common in the male patients (25%; 8 of 33) vs the female patients (5%; 3/59; supplemental Figure 2A). All other cytogenetic abnormalities did not vary significantly between the sexes. Cytogenetic abnormalities did not significantly affect survival ($P = .3637$; Figure 2C).

Clinical diagnosis and survival

The survival rate for all patients with G2BMID, MDS, and AML/CMML was 82% (72 of 92), and the survival was 100% for asymptomatic patients ($n = 14$; Figure 2D). The asymptomatic group, consisting of family members of affected individuals, also lacked cytogenetic abnormalities (13 of 14 tested). Therefore, this group was considered separately in subsequent analyses of cytogenetic and mutational backgrounds. The survival probability for patients with G2BMID was not significantly higher than for those with MDS ($P = .2119$), but the AML/CMML group survival was significantly lower than all other groups (Figure 2D). Overall survival was not affected by sex or age (supplemental Figure 3B-C).

Somatic mutations

The acquisition of somatic mutations in oncogenic and tumor-suppressor genes is thought to drive leukemic transformation.¹¹ Therefore, we used whole-exome and myeloid leukemia-based targeted gene array sequencing on peripheral blood and bone marrow samples to identify acquired somatic mutations. An average of

Table 1. Diagnoses, cytogenetics, and mutations in patients with GATA2 deficiency

Pt	Family pedigree	GATA2 mutation	Disease	Cytogenetics	Sex	Inheritance	BMT	Survival	Common mutations	Total filtered variants
1	35.III.3	c.1061C>T; p.T354M	MDS	Trisomy 8	M	Familial	Yes	Deceased	0	1
2	25.II.1	c.1017 + 572C>T	G2BMID	Normal	M	Familial	No	Deceased	ASXL1, PTPN11, SMC1A, STAG2	5
3	8.I.1	c.243_244delAinsGC; p.G82fs	G2BMID	Normal	M	De novo	Yes	Alive	DNMT3A, STAG2	4
4	12.I.1	c.1084_1095del12; p.R362del4	MDS	Monosomy 7, trisomy 21	M	Unknown	Yes	Deceased	0	10
5	17.I.1	c.1061C>T; p.T354M	MDS	Trisomy 8	M	Familial	Yes	Alive	0	3
6	17.II.1	c.1061C>T; p.T354M	G2BMID	Normal	M	Familial	Yes	Alive	STAG2	5
7	26.I.1	c.302delG; p.G101fs	CMML	del(11)(q13q23)	F	Unknown	Yes	Deceased	ASXL1, CBL, DNMT3A	13
8	34.II.2	c.1116_1130del15; p.C373del5	MDS	Monosomy 7	M	Familial	Yes	Deceased	STAG2	5
9	34.I.1	c.1116_1130del15; p.C373del5	G2BMID	Normal	F	Familial	No	Alive	0	5
10	31.II.2	c.1187G>A; p.R396Q	G2BMID	Normal	M	Familial	Yes	Alive	STAG2	4
11	27.I.1	c.586_593dup; p.G199fs	MDS	del(9)(q13q22)	F	Familial	Yes	Alive	ASXL1	6
12	39.I.2	c.988C>T; p.R330X	MDS	Trisomy 8	F	Familial	Yes	Alive	0	4
13	39.I.1	c.988C>T; p.R330X	MDS	Trisomy 8	F	Familial	Yes	Alive	STAG2	2
14	15.I.1	c.1186C>T; p.R396W	G2BMID	Normal	F	De novo	Yes	Alive	STAG2	4
15	38.I.1	c.417dupT; p.V140Cfs	G2BMID	Normal	F	De novo	Yes	Alive	ASXL1, JAK2	12
16	9.II.1	c.1192C>T; p.R398W	MDS	Normal	M	Familial	No	Deceased	BCOR, SRSF2, STAG*	12
17	9.III.2	c.1192C>T; p.R398W	Asymptomatic	Normal	M	Familial	No	Alive	0	4
18	41.I.1	c.1009C>T; p.R337X	G2BMID	Normal	F	De novo	Yes	Alive	ASXL1, DNMT3A, SMC1A	14
19	42.I.1	c.1186C>T; p.R996W	G2BMID	Normal	M	Familial	Yes	Alive	KDM2A	2
20	14.I.1	c.1187G>A; p.R396Q	G2BMID	Normal	F	De novo	No	Deceased	ASXL1, STAG2	9
21	146.I.1	c.1082G>A; p.R361H	MDS	Normal	M	Familial	No	Deceased	DNMT3A, STAG2	7
22	159.I.1	Unknown	AML	Trisomy 8, Monosomy 13	F	Unknown	Yes	Deceased	KMT2A, MECOM	14
23	48.II.1	c.1017 + 572C>T	Asymptomatic	Normal	F	Familial	No	Alive	CUX1, DNMT3A, KMT2A,* U2AF1	7
24	126.III.4†	Unknown	MDS	Trisomy 8	F	Familial	Yes	Alive	0	1
25	48.III.14	c.1017 + 572C>T	G2BMID	der(1;14)(q10;p10), trisomy 21	F	Familial	Yes	Alive	STAG2	1
26	126.III.23†	Unknown	MDS	Trisomy 8	M	Familial	Yes	Alive	STAG2*	3
27	126.II.8†	Unknown	Asymptomatic	Normal	F	Familial	No	Alive	0	1
28	126.II.4†	Unknown	Asymptomatic	Normal	M	Familial	No	Alive	0	0
29	256.I.1	c.1123C>T; p.L375S	AML	Trisomy 8, trisomy 20	F	De novo	Yes	Deceased	NRAS	5
30	218.I.1	c.1114G>A; p.A372T	MDS	Trisomy 8	F	Fe novo	No	Deceased	DNMT3A	10
31	129.I.1	c.802G>T; p.G268X	MDS	del(13)(q12q14)	F	Unknown	No	Alive	0	2
32	233.I.1	c.803delG; p.G268fs	G2BMID	Normal	F	De novo	Yes	Alive	0	11

Patient (Pt) number and Family pedigree designation are listed chronologically as the samples were collected. The survival time is from first sample collection until the end of the study. Common mutations are those found in ≥3 patients in this study or >5% in other studies of myeloid malignancies. Mutations in patient 78 have also been described in Molina et al.⁶⁷ NA indicates that WES was not performed (supplemental Table 1).

*Two different variants were found in the gene for the indicated patient.

†The pedigree for family 126 is given in supplemental Figure 3.

#GATA2 mutations unique to our study.

Table 1. (continued)

Pt	Family pedigree	GATA2 mutation	Disease	Cytogenetics	Sex	Inheritance	BMT	Survival	Common mutations	Total filtered variants
33	203.I.1	c.1021delG; p.A341Pfs	G2BMID	Normal	M	Familial	Yes	Alive	DNMT3A	6
34	47.I.1	c.1084C>T; p.R362X	Asymptomatic	Normal	F	Familial	No	Alive	0	1
35	270.I.1	c.1082G>A; p.R361H	G2BMID	Normal	M	Familial	Yes	Alive	BCOR	7
36	49.III.2	c.1084C>T; p.R362X	G2BMID	Normal	F	Familial	Yes	Alive	0	3
37	281.I.1	c.1186C>T; p.R396W	MDS	inv(9)(p12q13)	F	Unknown	Yes	Deceased	ASXL1	5
38	50.II.2	c.1128C>A; p.Y376X	MDS	Normal	F	Familial	Yes	Alive	STAG2	3
39	50.II.1	c.1128C>A; p.Y376X	MDS	der(17)(q10;p10), trisomy 8	M	Familial	Yes	Alive	STAG2	14
40	293.I.1	Unknown	AML	der(5)t(5;13)(q13q13)	M	Unknown	Yes	Alive	0	5
41	291.I.1	c.1084C>T; p.R362X	MDS	Monosomy 7	M	De novo	Yes	Alive	STAG2	2
42	216.I.1	c.1192C>T; p.R398W	MDS	der(17)(q10;p10), 1+der(17)(q10;q10), trisomy 8, monosomy X	F	Unknown	Yes	Alive	ASXL1	7
43	51.III.1	c.1192C>T; p.R398W	G2BMID	Normal	F	Familial	Yes	Deceased	ASXL1	6
44	51.II.1	c.1192C>T; p.R398W	Asymptomatic	Normal	M	Familial	No	Alive	0	2
45	52.II.4	c.1082G>C; p.R361P	G2BMID	Normal	F	Familial	No	Alive	BCL9	2
46	52.II.5	c.1082G>C; p.R361P	G2BMID	Normal	F	Familial	No	Alive	0	4
47	52.I.4	c.1082G>C; p.R361P	MDS	Normal	M	Familial	Yes	Deceased	STAG2	4
48	330.I.1	c.898dupG; p.A300Gfs	MDS	Normal	F	Familial	Yes	Deceased	ASXL1, BCOR	5
49	335.III.1	c.1017 + 572C>T	MDS	Normal	F	Familial	Yes	Alive	0	1
50	340.II.1	c.1159_1160dupAC; p.M388fs	G2BMID	Monosomy 7, der(17)(q10;p10), del(13)(q12q22)	M	Familial	Yes	Alive	STAG2#	3
51	324.I.1	c.1018delT; p.S340fs	MDS	Trisomy 8	F	De novo	Yes	Alive	STAG2	6
52	342.I.1	c.1017 + 572C>T	MDS	Monosomy 7	M	Unknown	Yes	Alive	SAMD9, SETBP1, U2AF1	7
53	53.V.4	c.1017 + 572C>T	G2BMID	Normal	F	Familial	No	Alive	0	2
54	53.V.3	c.1017 + 572C>T	MDS	Normal	F	Familial	Yes	Alive	ETV6	3
55	350.I.1	c.248delA; p.O89Rfs#	MDS	Normal	F	De novo	Yes	Alive	0	4
56	349.II.4	c.1192C>T; p.R398W	MDS	trp(1)(q21q32)	F	Familial	No	Alive	DNMT3A, *U2AF1	11
57	347.I.1	c.1017 + 2T>A	MDS	Normal	F	De novo	Yes	Alive	STAG2	5
58	351.I.1	c.1192C>T; p.R398W	MDS	Normal	F	De novo	Yes	Alive	0	2
59	333.II.3	c.1018-50_1143 + 247del; p.S340-N381del	MDS	Trisomy 8	M	Familial	Yes	Alive	DNMT3A, STAG2*	6
60	337.I.1	c.921dupG; p.R308Afs#	G2BMID	Normal	F	Unknown	Yes	Alive	STAG2	12
61	360.I.1	c.1024_1025insGCCG; p.A342Gfs	G2BMID	Normal	F	Unknown	Yes	Alive	STAG2	2
62	357.II.1	c.1017 + 1G>T#	MDS	Monosomy 7	F	Familial	Yes	Alive	RUNX1	4

Patient (Pt) number and Family pedigree designation are listed chronologically as the samples were collected. The survival time is from first sample collection until the end of the study. Common mutations are those found in ≥3 patients in this study or >5% in other studies of myeloid malignancies. Mutations in patient 78 have also been described in Molina et al.⁶⁷ NA indicates that WES was not performed (supplemental Table 1).
 BMT, bone marrow transplant.
 #Two different variants were found in the gene for the indicated patient.
 †The pedigree for family 126 is given in supplemental Figure 3.
 #GATA2 mutations unique to our study.

Table 1. (continued)

Pt	Family pedigree	GATA2 mutation	Disease	Cytogenetics	Sex	Inheritance	BMT	Survival	Common mutations	Total filtered variants
63	357.I.1	c.1017 + 1G>T#	MDS	Trisomy 8	F	Familial	No	Alive	ASXL1	6
64	362.I.1	c.247C>T; p.Q83X#	MDS	Monosomy 7	F	De novo	Yes	Alive	STAG2*	5
65	n/a	c.1084C>T; p.R362X	G2BMID	Normal	F	De novo	No	Deceased	NOTCH2	6
66	n/a	c.680_683del; p.S227fs#	G2BMID	Normal	F	De novo	Yes	Alive	STAG2	4
67	365.I.1	c.1081C>T; p.R361C	MDS	der(1;7)(q10;p10), trisomy 8	M	Familial	Yes	Alive	STAG2*	7
68	368.I.1	c.1061C>T; p.T354M	Asymptomatic	Unknown	F	Familial	No	Alive	DNMT3A	7
69	370.I.1	unknown	MDS	Normal	F	Unknown	Yes	Deceased	ASXL1, RUNX1	10
70	375.I.2	c.1192C>T; p.R398W	MDS	(1)(1;15), Trisomy 8	F	Familial	Yes	Alive	ASXL1, CUX1, RAD21	10
71	n/a	c.1150delA; p.R384fs#	G2BMID	Normal	M	De novo	Yes	Alive	0	2
72	378.I.1	c.1021 delG; p.A341Pfs	MDS	Normal	M	unknown	Yes	Alive	BCOR	5
73	379.I.1	c.1277C>G; p.S426C#	G2BMID	Normal	F	Unknown	No	Alive	0	3
74	367.I.1	unknown	MDS	Trisomy 8	F	Unknown	Yes	Alive	0	2
75	382.I.2	c.1061C>A; p.T354K	MDS	Normal	F	Familial	Yes	Alive	0	7
76	283.II.1	c.58C>T; p.Q20X#	MDS	der(1;7)(q10;p10)	F	Familial	Yes	Alive	SETBP1, STAG2	5
77	n/a	c.1061C>T; p.T354M	G2BMID	Normal	F	De novo	Yes	Alive	0	3
78	4.III.5	c.1017 + 572C>T	CMML	Monosomy 7	M	familial	Yes	Alive	ASXL1, SETBP1, U2AF1	26
79	384.I.1	c.1192C>T; p.R398W	MDS	Monosomy 7	F	Familial	Yes	Alive	ASXL1	4
80	n/a	c.1192C>T; p.R398W	G2BMID	Normal	F	Familial	No	Alive	0	2
81	393.II.1	c.1061C>T; p.T354M	G2BMID	Normal	F	Familial	No	Alive	0	9
82	n/a	c.840delT; p.K281Sfs#	G2BMID	Normal	F	De novo	Yes	Alive	0	3
83	390.I.1	c.1009C>T; p.R337X	G2BMID	Normal	F	De novo	Yes	Alive	ASXL1, STAG2	4
84	394.I.1	c.1114G>A; p.A372T	G2BMID	Normal	M	Unknown	Yes	Alive	0	6
85	349.IV.5	c.1192C>T; p.R398W	G2BMID	Normal	M	Familial	Yes	Alive	0	1
86	349.III.16	c.1192C>T; p.R398W	MDS	Trisomy 8	F	Familial	Yes	Alive	0	4
87	349.IV.7	c.1192C>T; p.R398W	G2BMID	Normal	F	Familial	No	Alive	0	4
88	1.IV.1	c.1192C>T; p.R398W	G2BMID	Normal	F	Familial	Yes	Alive	0	3
89	1.III.2	c.1192C>T; p.R398W	Asymptomatic	Normal	M	Familial	No	Alive	DNMT3A	5
90	389.I.1	c.1024_1025insG; p.A342Gfs	G2BMID	Normal	F	Unknown	Yes	Alive	0	8
91	4.III.2	c.1017 + 572C>T	G2BMID	Normal	F	Familial	No	Alive	DNMT3A, FLT3	NA
92	37.I.1	c.1081C>T; p.R361C	MDS	Normal	F	De novo	Yes	Alive	0	NA
93	48.II.6	c.1017 + 572C>T	Asymptomatic	Normal	F	Familial	No	Alive	0	NA
94	48.II.2	c.1017 + 572C>T	Asymptomatic	Normal	F	Familial	No	Alive	0	NA
95	n/a	c.1187G>A; p.R396Q	MDS	Normal	F	Familial	Yes	Alive	ASXL1	NA
96	4.III.1	c.1017 + 572C>T	Asymptomatic	Normal	M	Familial	No	Alive	0	NA

Patient (Pt) number and Family pedigree designation are listed chronologically as the samples were collected. The survival time is from first sample collection until the end of the study. Common mutations are those found in ≥3 patients in this study or >5% in other studies of myeloid malignancies. Mutations in patient 78 have also been described in Molina et al.⁶⁷ NA indicates that WES was not performed (supplemental Table 1).
 BMT, bone marrow transplant.
 #Two different variants were found in the gene for the indicated patient.
 *The pedigree for family 126 is given in supplemental Figure 3.
 #GATA2 mutations unique to our study.

Table 1. (continued)

Pt	Family pedigree	GATA2 mutation	Disease	Cytogenetics	Sex	Inheritance	BMT	Survival	Common mutations	Total filtered variants
97	4.I.1	c.1017+572C>T	CMML	Normal	M	Familial	No	Alive	0	NA
98	17.II.2	c.1061C>T; p.T354M	G2BMID	Normal	M	Familial	No	Alive	0	NA
99	48.II.8	c.1017+572C>T	Asymptomatic	Normal	M	Familial	No	Alive	0	NA
100	4.III.4	c.1017+572C>T	MDS	Monosomy 7, Trisomy 8	M	Familial	Yes	Alive	0	NA
101	n/a	c.839delC; p.P280Lfs	MDS	Trisomy 8	M	Unknown	No	Alive	0	NA
102	6.I.1	c.1017+512del28	Asymptomatic	Normal	M	Familial	No	Alive	0	NA
103	335.II.1	c.1017+572C>T	Asymptomatic	Normal	F	Familial	No	Alive	0	NA
104	n/a	c.1084C>T; p.R362X	G2BMID	Normal	M	De novo	No	Alive	STAG2*	5
105	277.I.1	c.1187G>A; p.R396Q	MDS	Trisomy 8	F	De novo	Yes	Alive	0	4
106	368.II.1	c.1061C>T; p.T354M	MDS	Monosomy 7	M	Familial	Yes	Alive	0	NA

Patient (Pt) number and Family pedigree designation are listed chronologically as the samples were collected. The survival time is from first sample collection until the end of the study. Common mutations are those found in ≥ 3 patients in this study or $>5\%$ in other studies of myeloid malignancies. Mutations in patient 78 have also been described in Molina et al.⁶⁷ NA indicates that WES was not performed (supplemental Table 1).
 BMT, bone marrow transplant.

*Two different variants were found in the gene for the indicated patient.

†The pedigree for family 126 is given in supplemental Figure 3.

#GATA2 mutations unique to our study.

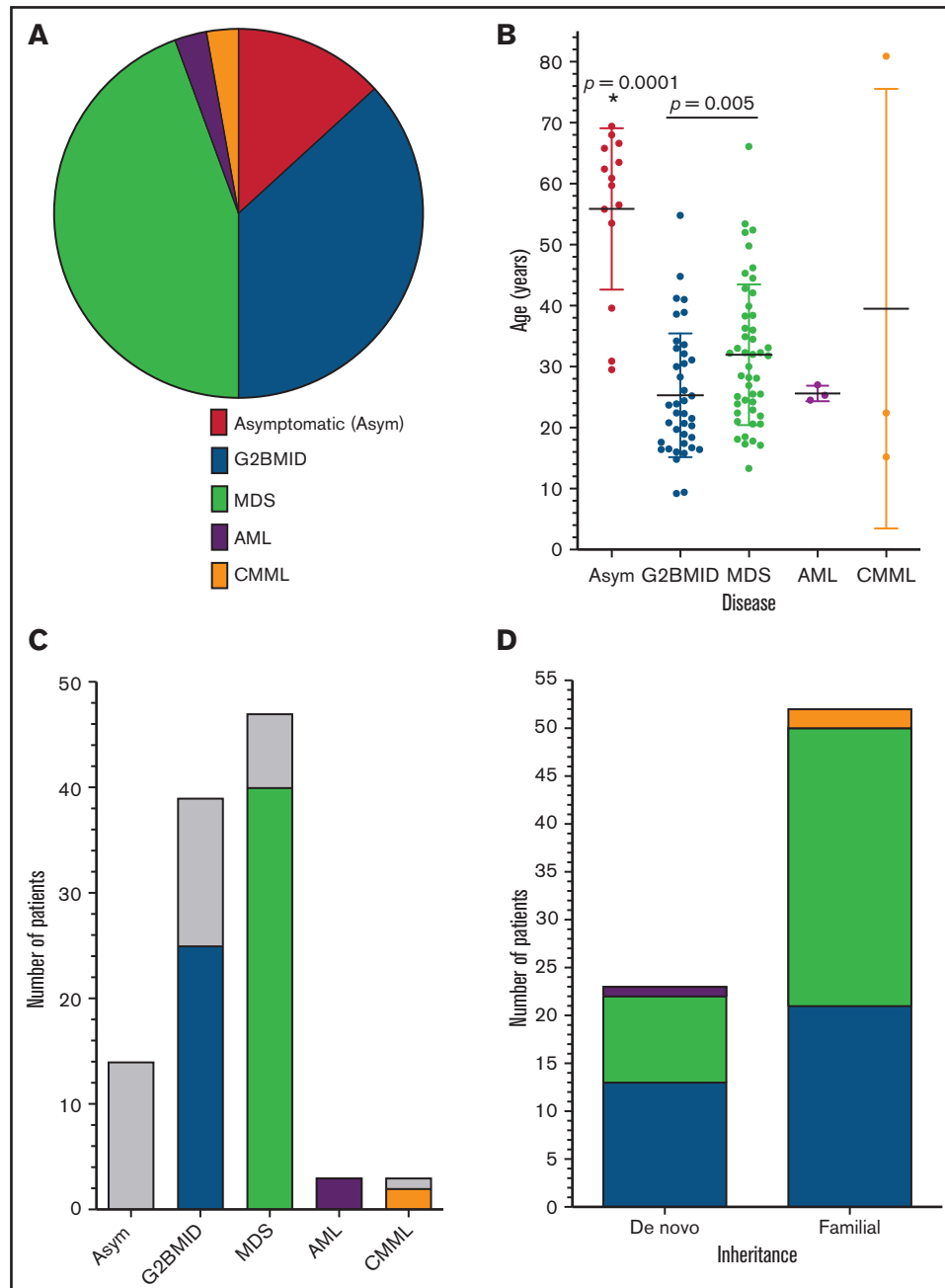


Figure 1. Patients with GATA2 deficiency by diagnosis, age and inheritance. (A) Bone marrow diagnosis. The relative frequency of bone marrow diagnoses among patients with GATA2 deficiency/MonoMAC ($n = 106$). (B) Diagnosis vs age. Plot of patient age at sample collection for each diagnosis category. The means and standard deviations are shown: Asym $n = 14$, G2BMID $n = 39$, MDS $n = 47$, AML $n = 3$, CMML $n = 3$. *Significant differences by ANOVA; P -values, by Student t test. (C) Patients with HSCT for each diagnosis category. Patients with HSCT are indicated according to diagnosis and color in the legend to panel A and those without are in gray. (D) Diagnosis vs mode of inheritance. The number of patients with de novo or familial inheritance with each bone marrow diagnosis as indicated by the color scheme.

5.4 ± 4.0 filtered variants per sample were found among all samples by WES, with a range of 0 to 26 mutations per sample (Figure 3A; Table 1; supplemental Table 2). The patient in the AML/CMML group averaged a higher number of mutations (12.6 ± 8.6) than the other diagnoses (5.2 ± 3.2), but there was no significant difference among the other categories (analysis of variance [ANOVA], $P = .2990$). Gene mutations common in non-GATA2 MDS/AML, including *TET2*, *EZH2*, *IDH1/2*, *RUNX1*, *TP53*, and splicing factors (*SF3B1*, *SRSF2*,

U2AF1, *ZRSR2*), were rare or absent in this cohort with GATA2 deficiency (Table 1).

ASXL1 and *STAG2* were highly recurrent mutations (Figure 3B). *ASXL1* mutations were found in 16% ($n = 17$ of 106) of all patients, with the most common mutation being c.1934_1935insG, p.646Wfs*12 (supplemental Table 2). *STAG2* mutations were observed in 26% ($n = 28$) of the 106 patients; the location of these

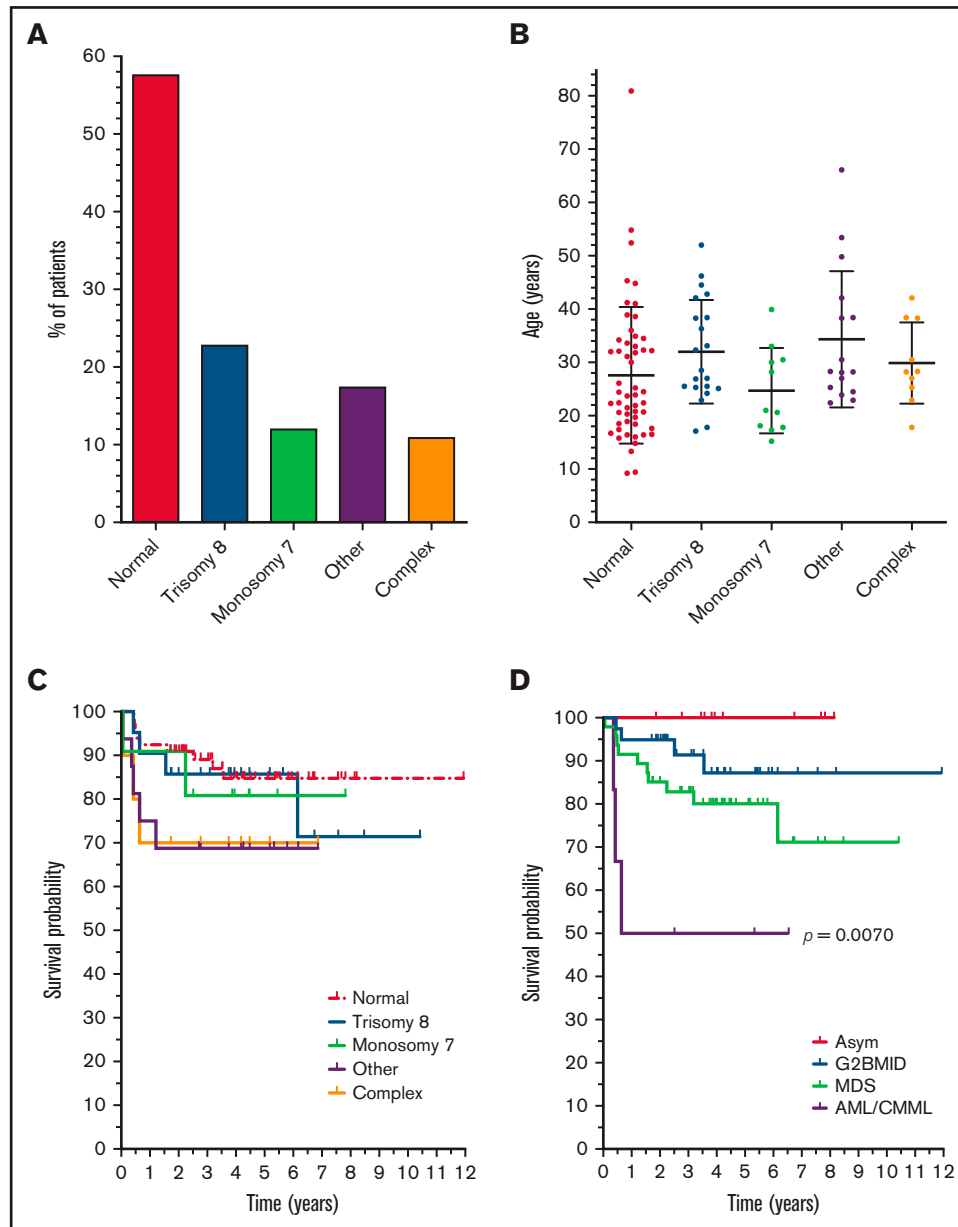


Figure 2. Cytogenetic abnormalities in GATA2 deficiency. (A) Cytogenetic findings. Percentages of patients with the indicated cytogenetic abnormalities. The number of patients with trisomy 8 or monosomy 7 includes all patients with this abnormality, independent of other abnormalities. “Other,” abnormalities other than trisomy 8 or monosomy 7; “complex,” more than 1 abnormality in the same patient, including trisomy 8 and/or monosomy 7. (B) Cytogenetics vs age. The age distribution of cytogenetic findings, with the mean ages and standard deviations indicated (ANOVA, $P = .1069$). (C) Cytogenetics vs survival. Kaplan-Meier survival probability as a function of cytogenetics. Classifications are as in panel A. Survival is from the initial sample collection to the end of the study. (D) Diagnosis vs survival. Kaplan-Meier survival probability as a function of bone marrow diagnosis. Patients with AML or CMML were combined because of the low number of cases. Each graph shows the survival time from sample collection until the end point. Mantel-Cox test significance is shown for survival probability among the samples.

mutations was highly variable, but nearly all were truncation or splicing mutations. Mutations in *DNMT3A* ($n = 12$), *BCOR* ($n = 4$), and *SETBP1* ($n = 3$) were also found. When pooled, mutations in the splicing factors *U2AF1* and *SRSF2* were found in 5 patients, but no mutations were found in *SF3B1* or *ZRSR2*. Mutations in *CUX1*, *KMT2A*, *RUNX1* and *SMC1A* were each found in two patients (Table 1). At least 1 cohesin- or chromatin-related gene mutation was found in ~50% (54) of the 106 patients.

Age was not a significant factor in the occurrence of *STAG2* or *ASXL1* mutations (ANOVA; $P = .0896$; Figure 3C), although *DNMT3A* mutations occurred in older individuals than did mutations in *ASXL1* or *STAG2*. However, *ASXL1* mutations were highly enriched in female patients (23% of female vs 5% of male patients; Fisher’s exact test, $P = .016$), whereas *STAG2* mutations were found more often in male patients (38% of male vs 20% female patients; Figure 3D). Abnormal cytogenetics were as

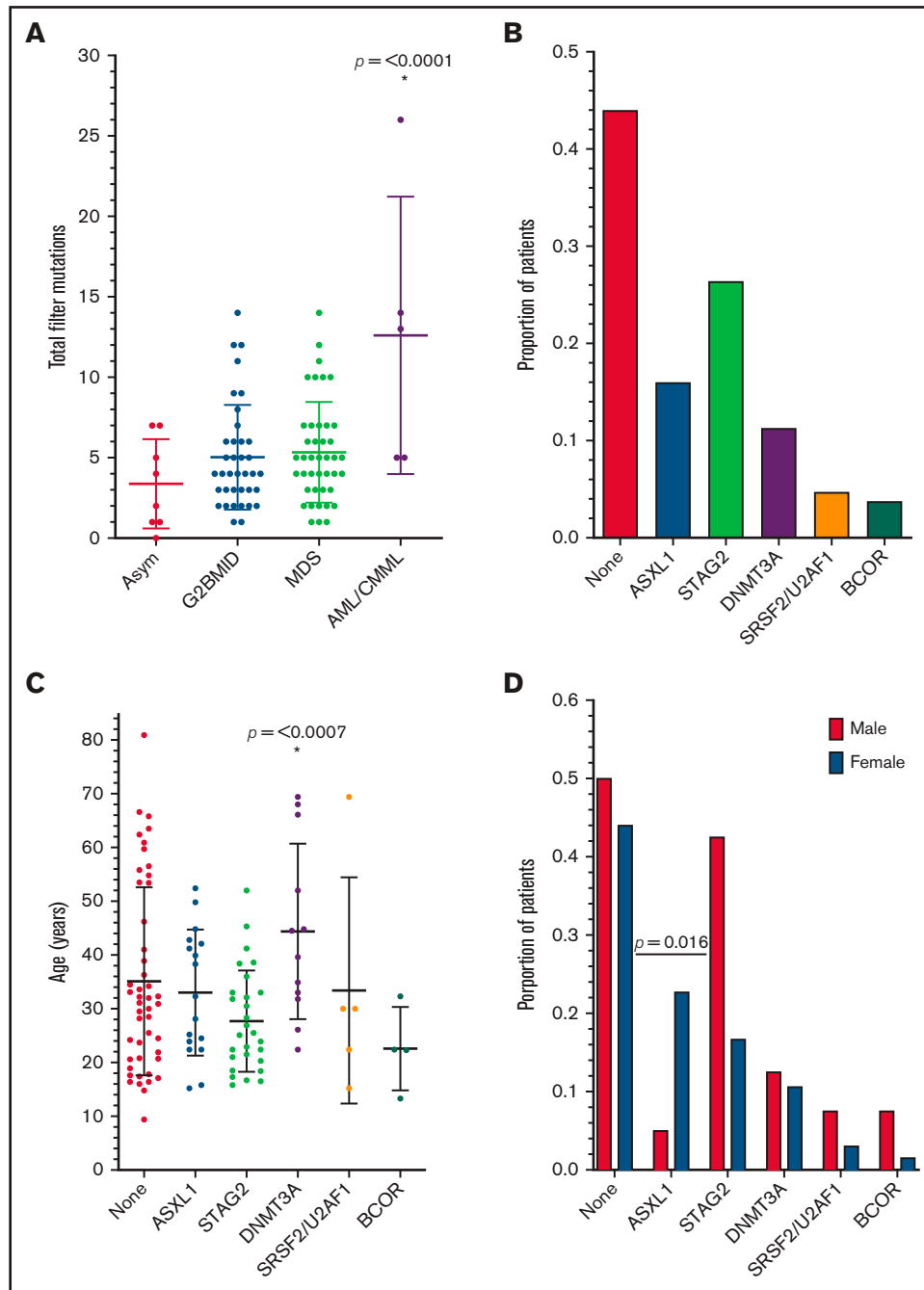


Figure 3. Somatic mutations in patients with GATA2 deficiency. (A) Somatic mutations vs diagnosis. The total number of WES somatic variants per patient for each diagnosis category. The mean and standard deviation are shown. (B) Frequency of recurrent somatic mutations. The proportion of patients with the most frequently recurrent somatic mutations. (C) Recurrent mutations vs patient age. The age distribution of patients with each somatic mutation with the mean age and standard deviation indicated. (D) Recurrent mutations vs sex. The percent frequency of recurrent somatic mutations by patient sex. "None" indicates the lack of any recurrent somatic mutations. *Significant difference by ANOVA.

frequent among the patients with either *ASXL1* (47%; n = 8 of 17) or *STAG2* (44%; 12 of 27) mutations, as they were in patients who lacked either of the mutated genes (Fisher's exact test: *ASXL1*, $P = .4216$; *STAG2*, $P = .4902$). Neither mutated *ASXL1* or *STAG2* was present in asymptomatic patients but, critically, the mutations occurred similarly in patients with G2BMID or MDS (χ^2 test,

$P = .5534$; Table 1; Figure 4A). These data suggest that aberrant clones with malignancy-associated mutations are common in the premalignant G2BMID state. Patients with mutations in *ASXL1*, *DNMT3A*, or *STAG2* all had lower probabilities of survival (Figure 4B). The total number of variants did not correlate with patients' age, but 6 to 10 variants had a lower survival probability (Figure 4C).

leads to disruption of normal hematopoiesis and the loss of hematopoietic stem cells in the bone marrow, which may create a Darwinian selection gate for “breakaway” stem cells, in which acquired somatic mutations provide a proliferation and/or survival advantage. Consistent with this effect, patients with *GATA2* deficiency can progress from hypocellular marrow, presumably putting stem cells under replicative stress, to hypercellular marrow, suggesting clonal hematopoiesis. Clonal hematopoiesis precedes myeloid malignancy, and mutations in *ASXL1* and *DNMT3A* are common in patients with *GATA2* deficiency and also in clonal hematopoiesis.¹⁹

This study was conducted to identify somatic mutations that lead to disease progression in patients with *GATA2* deficiency/MonoMAC syndrome and to correlate these findings with biometric and other parameters. We recorded the patients’ age, sex, clinical features, cytogenetics and survival and compared the results with genetic profiles based on germline *GATA2* mutations and acquired somatic mutations. Survival probabilities across the variables did not differ when patients who had undergone HCST were considered separately from patients who had not.

Several factors limit the ability of next-generation DNA sequencing to identify clinically important somatic mutations. Therefore, we used a conservative strategy to predict somatic mutations. The thresholds used in the analysis process were informed approximations, and the inclusion/exclusion boundaries were therefore inherently imprecise. The conservative estimations we used were meant to produce a plausible mutation list while minimizing the false positives that, once reported, tend to linger in databases. Our results are undoubtedly also affected by unknown patient enrollment biases, particularly patients evaluated for HSCT.

Somatic mutations in *GATA2* were not identified in this study and are generally rare (1% to 5%) in myeloid malignancies, with the exception of biallelic mutations in *CEBPA* (*CEBPA*^{bi}).²⁰⁻²⁶ In these cases, 18% to 40% of *CEBPA*^{bi}-bearing patients have a somatic mutation in *GATA2*.²⁷⁻³¹ However, *CEBPA* mutations were not detected in this cohort, indicating that *CEBPA*^{bi}/*GATA2*-bearing patients are not among the patients with *GATA2* deficiency in this study.

Patients with *GATA2* deficiency typically present with symptoms of immunodeficiency with hypocellular marrows in adolescence and show signs of myeloid malignancy in early adulthood.^{8,10,32-35} However, we identified a distinct population of patients who do not develop symptoms of the disease at the typical age or progress into a diseased state as they age. These asymptomatic patients were identified by family screening after the proband tested positive for *GATA2* mutation, constituted ~13% of the cohort, and have been reported elsewhere at ~10% to 30% of the studied cohort.^{6,8,33,36-38} In this and other studies, the asymptomatic group was the oldest, suggesting that a mutation in *GATA2* alone is necessary but not sufficient for the development of disease. Indeed, the most critically ill patients are the youngest group. Asymptomatic individuals in this and other studies generally do not have the common somatic mutations associated with patients who have *GATA2* deficiency with active disease or with non-*GATA2* MDS/AML,^{6,37} although there was 1 striking exception. Three asymptomatic individuals with *DNMT3A* mutations were also exceptions. The mechanism underlying the incomplete penetrance of *GATA2* mutations remains unknown. Among symptomatic patients, there is a trend for older patients to have MDS rather than G2BMID, as previously reported.^{6,37} In this study, the presence of somatic mutations was similar in both the G2BMID and MDS groups, as noted previously,⁶ indicating that clonal

hematopoiesis is present at an early stage of immunodeficiency and cytopenia with hypoplastic marrow, even before the manifestation of MDS or myeloid malignancy. Moreover, the mutant allele frequencies (MAFs) for the common somatic mutations did not segregate by bone marrow diagnosis.

We determined the cytogenetics in patients with *GATA2* deficiency, to identify relative risk factors for HSCT. We found that 44% of affected patients had abnormal cytogenetics, with trisomy 8 dominating (24%), followed by monosomy 7 (12%). The frequency of these anomalies has been reported, although in most other studies monosomy 7 was more common than trisomy 8.^{6,9,32,33,39-43} McReynolds and colleagues also reported trisomy 8 as the most common cytogenetic finding in a small cohort of patients with *GATA2*.⁶ A small subset of the patients in our study has been previously reported; however, our study is considerably larger and includes a broader range of disease. The overall frequency of abnormal cytogenetics is similar to non-*GATA2* MDS/AML,⁴⁴ but the frequency of trisomy 8 is notably higher in *GATA2* deficiency. The der(1;7)(q10;p10) chromosomal abnormality was present in 5 patients in our study and in those with *GATA2* deficiency in other studies.^{9,45} It is a rare myeloid abnormality usually associated with Shwachman-Diamond syndrome.⁴⁶ The der(1;7)(q10;p10) frequently occurs with trisomy 8 in non-*GATA2* MDS,⁴⁷ and it was present in 3 of 5 cases in our study and in a patient with *GATA2* deficiency in another study.⁴⁵ It results in a 7q- genotype, but its inclusion with monosomy 7 did not change survival probability. In our studies, monosomy 7/7q- did not correlate with significantly lower survival probability, in contrast to non-*GATA2* MDS. Complex cytogenetic findings were also more frequent in our study (~11%) than in other studies.^{6,9,32,33,39-43}

We investigated the acquisition of somatic mutations in patients with *GATA2* deficiency to better understand and predict disease progression. Patients with *GATA2* deficiency have a low number of unique somatic mutations compared with those with other myeloid malignancies. In particular, there is a conspicuous absence of the “usual suspects” of somatic mutations found in non-*GATA2* MDS/AML, including the splicing factor *SF3B1*, and *TET2*, and *IDH1/2*. A recent study of 1809 patients with MDS showed *TET2* to be the most common mutation (27%) followed by mutations in the splicing factor *SF3B1* (23%).⁴⁸ Other studies have found a very high frequency of splicing factor mutations (~65%) and DNA methylation mutations (~47%) in MDS.^{49,50} The lack of *SF3B1* mutations is consistent with the absence of ringed sideroblasts among the patients with *GATA2*-deficient MDS. The lack of other common MDS/AML mutations in *GATA2* deficiency, particularly *TET2* and splicing factor mutations, has been found in other studies.^{6,9,37,38,43}

The 2 significant exceptions to this trend are *ASXL1* and *STAG2*. Mutations in *ASXL1* are very common in all myeloid malignancies and are present in ~20% of patients with *GATA2* deficiency.^{21,22,24,26,44,50-52} A mutation in *STAG2* is less common in hematological malignancies (2% to 8%),^{21,22,24,26,44,50,51,53,54} but it was the most common somatic mutation in patients with *GATA2* deficiency (~25%).

The *ASXL1* mutations in non-*GATA2* MDS/AML are found in older patients, with a significant bias toward males.⁵⁵ However, the *ASXL1* mutations in *GATA2* deficiency did not show an age bias and were found far more frequently in female patients. We reported this trend in a previous study of a smaller patient population.¹³ *ASXL1* mutations associate with *TET2*, *U2AF1*, *RUNX1*, *SETBP1*, *STAG2*, and *EZH2* in non-*GATA2* MDS/AML.^{22,48,49,55,56} However, we did not observe

this association in patients with GATA2 deficiency. Some studies report that cohesin mutations are mutually exclusive with adverse cytogenetics,⁵⁷ but we did not observe any significant correlation with mutations in either *ASXL1* or *STAG2* and abnormal cytogenetics. Pastor and colleagues found a high incidence of mutated *ASXL1* concomitant with monosomy 7,⁴³ but that was also not observed in our cohort. In our study, *ASXL1* mutations had a negative effect on survival probability, as we reported previously.¹³ Mutated *ASXL1* and cohesin mutations are also negative prognosticators across all myeloid malignancies.^{22,55,57}

A high frequency of mutations in *STAG2* is a particular feature of GATA2 deficiency compared with other hematological malignancies. *STAG2* mutations have been reported in a small number of patients with GATA2 deficiency^{6,58,59}; however, they now emerge as the leading somatic mutation in this disease. As seen in non-GATA2 MDS/AML and solid tumors,⁵⁷ the *STAG2* mutations in GATA2 deficiency were almost exclusively truncating mutations. Seven patients had 2 variant alleles of *STAG2*, with 6 of 7 of the patients being male. Given that *STAG2* is on the X chromosome, 13 of 14 mutations are splice site or truncations, and the MAFs are very distinct, suggests 2 independent clonal populations in these patients. Curiously, the MAFs of the 2 *STAG2* alleles were approximately the same in the female patient and in the male patient with a missense mutation, leaving the possibility of either 2 separate clones or compound heterozygous clones in these individuals. In non-GATA2-associated patients with MDS, *STAG2* mutations have shown concurrence with *ASXL1*, *RUNX1*, and *BCOR*^{49,50,60} mutations, but this concurrence was not observed in our study. McReynolds and colleagues found *STAG2* solely in MDS patients⁶; however, we found both *ASXL1* and *STAG2* mutations in similar frequencies in patients with G2BMID and those with MDS.

STAG2 mutations are also very common in myeloid leukemia–Down syndrome.^{61,62} *STAG2* was the most common cohesin mutation in our cohort, but there were also 1 *RAD21* and 2 *SMC1A* mutations. These other cohesin gene mutations were more frequent in patients with myeloid leukemia–Down syndrome and included *RAD21*, *SMC1A*, and also *SMC3* and *CTCF*. These 2 groups of patients also share a conspicuous lack of the most common MDS/AML mutations, including those in *TET2*, *IDH1/2*, and *SF3B1*.

The other less frequently recurring mutations include *DNMT3A*, *BCOR*, and *SETBP1*; the splicing genes *SRSF2* and *U2AF1*; and the histone modifiers *KMT2A* and *KDM2A*. *DNMT3A*, *BCOR*, and *SETBP1* have been reported in patients with GATA2 deficiency, along with *NRAS* and *RUNX1*.^{6,9,38,40,43,63-66}

In summary, GATA2 deficiency is characterized by a novel pattern of somatic mutations with a strong bias toward chromatin- and cohesin-related genes and a notable absence of nearly all other common MDS/AML-associated somatic mutations. The 3 most common mutations are *STAG2*, *ASXL1*, and *DNMT3A*. The significance of mutations in *ASXL1* and *STAG2* on the GATA2 mutation background and their relevance to myeloid transformation remain a topic for future investigation.

Acknowledgments

The authors thank the National Cancer Institute (NCI) Center for Cancer Research (CCR) Genomics Core for their assistance in direct and array DNA sequencing.

This work was supported, in whole or (in part), by federal funds from NCI, National Institutes of Health (NIH; HHSN261200800001E; ZIABC010870). The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. This research was supported in part by the Intramural Research Program of the NIH, NCI, CCR, and the National Institute of Allergy and Infectious Diseases and the NIH Clinical Center Research Award for Staff Clinicians (RASCL) program.

Authorship

Contribution: R.R.W. designed the research, performed the experiments and data analysis, and drafted the manuscript; K.R.C. performed the experiments, provided critical revision of the manuscript for important intellectual content, and reviewed the histopathology; L.J.E. and W.W. performed the experiments and data analysis; L.M.T. performed the experiments; T.R.B. and D.T. performed the data analysis and provided critical revision of the manuscript for important intellectual content; J.L. performed data analysis; S.D. performed the experiments; A.P.H. and S.M.H. provided critical revision of the manuscript for important intellectual content; and D.D.H. supervised the study and drafted the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: R.R.W., 0000-0002-2079-0922; T.R.B., 0000-0001-5614-7333; D.T., 0000-0003-3568-6148; S.D., 0000-0002-0655-3576; A.P.H., 0000-0001-6841-2122.

Correspondence: Dennis D. Hickstein, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Bldg 10/CRC, Room 3-3142, Bethesda, MD 20892; e-mail: hicksted@mail.nih.gov.

References

1. Hsu AP, Sampaio EP, Khan J, et al. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood*. 2011;118(10):2653-2655.
2. Vinh DC, Patel SY, Uzel G, et al. Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. *Blood*. 2010;115(8):1519-1529.
3. Bigley V, Haniffa M, Doulatov S, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. *J Exp Med*. 2011; 208(2):227-234.
4. Ostergaard P, Simpson MA, Connell FC, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). *Nat Genet*. 2011;43(10):929-931.

5. Hahn CN, Chong CE, Carmichael CL, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet.* 2011;43(10):1012-1017.
6. McReynolds LJ, Yang Y, Yuen Wong H, et al. MDS-associated mutations in germline GATA2 mutated patients with hematologic manifestations. *Leuk Res.* 2019;76:70-75.
7. Kallen ME, Dulau-Florea A, Wang W, Calvo KR. Acquired and germline predisposition to bone marrow failure: Diagnostic features and clinical implications. *Semin Hematol.* 2019;56(1):69-82.
8. Spinner MA, Sanchez LA, Hsu AP, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood.* 2014;123(6):809-821.
9. Wlodarski MW, Collin M, Horwitz MS. GATA2 deficiency and related myeloid neoplasms. *Semin Hematol.* 2017;54(2):81-86.
10. Brown AL, Hahn CN, Scott HS. Secondary leukemia in patients with germline transcription factor mutations (RUNX1, GATA2, CEBPA). *Blood.* 2020;136(1):24-35.
11. Gilliland DG. Molecular genetics of human leukemias: new insights into therapy. *Semin Hematol.* 2002;39(4 suppl 3):6-11.
12. Bödör C, Renneville A, Smith M, et al. Germ-line GATA2 p.THR354MET mutation in familial myelodysplastic syndrome with acquired monosomy 7 and ASXL1 mutation demonstrating rapid onset and poor survival. *Haematologica.* 2012;97(6):890-894.
13. West RR, Hsu AP, Holland SM, Cuellar-Rodriguez J, Hickstein DD. Acquired ASXL1 mutations are common in patients with inherited GATA2 mutations and correlate with myeloid transformation. *Haematologica.* 2014;99(2):276-281.
14. Rentsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res.* 2019;47(D1):D886-D894.
15. Parta M, Shah NN, Baird K, et al. Allogeneic hematopoietic stem cell transplantation for GATA2 deficiency using a busulfan-based regimen. *Biol Blood Marrow Transplant.* 2018;24(6):1250-1259.
16. Nichols-Vinueza DX, Parta M, Shah NN, et al. Donor source and post-transplantation cyclophosphamide influence outcome in allogeneic stem cell transplantation for GATA2 deficiency. *Br J Haematol.* 2021 Sep 27. doi: 10.1111/bjh.17840. Epub ahead of print. PMID: 34580862.
17. Swerdlow S, Campo E, Harris NL, et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Vol. 2. 4th ed. Lyon, France: IARC Publications; 2017;97-106.
18. Bresnick EH, Jung MM, Katsumura KR. Human GATA2 mutations and hematologic disease: how many paths to pathogenesis? *Blood Adv.* 2020;4(18):4584-4592.
19. Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. *Nature.* 2018;559(7714):400-404.
20. Fasan A, Eder C, Haferlach C, et al. GATA2 mutations are frequent in intermediate-risk karyotype AML with biallelic CEBPA mutations and are associated with favorable prognosis. *Leukemia.* 2013;27(2):482-485.
21. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia.* 2014;28(2):241-247.
22. Kishtagari A, Levine RL, Viny AD. Driver mutations in acute myeloid leukemia. *Curr Opin Hematol.* 2020;27(2):49-57.
23. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med.* 2016;374(23):2209-2221.
24. Tate JG, Bamford S, Jubb HC, et al. COSMIC: the catalogue of somatic mutations in cancer. *Nucleic Acids Res.* 2019;47(D1):D941-D947.
25. Vedula RS, Cheng MP, Ronayne CE, et al. Somatic GATA2 mutations define a subgroup of myeloid malignancy patients at high risk for invasive fungal disease. *Blood Adv.* 2021;5(1):54-60.
26. Wan Z, Han B. Comparison and implications of mutational profiles of myelodysplastic syndromes, myeloproliferative neoplasms, and myelodysplastic/myeloproliferative neoplasms: a meta-analysis. *Front Oncol.* 2020;10(2077):579221.
27. Konstandin NP, Pastore F, Herold T, et al. Genetic heterogeneity of cytogenetically normal AML with mutations of CEBPA. *Blood Adv.* 2018;2(20):2724-2731.
28. Lavallée VP, Kros J, Lemieux S, et al. Chemo-genomic interrogation of CEBPA mutated AML reveals recurrent CSF3R mutations and subgroup sensitivity to JAK inhibitors. *Blood.* 2016;127(24):3054-3061.
29. Ping N, Sun A, Song Y, et al. Exome sequencing identifies highly recurrent somatic GATA2 and CEBPA mutations in acute erythroid leukemia. *Leukemia.* 2017;31(1):195-202.
30. Theis F, Corbacioglu A, Gaidzik VI, et al. Clinical impact of GATA2 mutations in acute myeloid leukemia patients harboring CEBPA mutations: a study of the AML study group. *Leukemia.* 2016;30(11):2248-2250.
31. Tien FM, Hou HA, Tsai CH, et al. GATA2 zinc finger 1 mutations are associated with distinct clinico-biological features and outcomes different from GATA2 zinc finger 2 mutations in adult acute myeloid leukemia. *Blood Cancer J.* 2018;8(9):87.
32. Wlodarski MW, Hirabayashi S, Pastor V, et al; EWOG-MDS. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. *Blood.* 2016;127(11):1387-1397, quiz 1518.
33. Donadieu J, Lamant M, Fieschi C, et al; French GATA2 study group. Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients. *Haematologica.* 2018;103(8):1278-1287.
34. Calvo KR, Vinh DC, Maric I, et al. Myelodysplasia in autosomal dominant and sporadic monocytopenia immunodeficiency syndrome: diagnostic features and clinical implications. *Haematologica.* 2011;96(8):1221-1225.
35. Ganapathi KA, Townsley DM, Hsu AP, et al. GATA2 deficiency-associated bone marrow disorder differs from idiopathic aplastic anemia. *Blood.* 2015;125(1):56-70.

36. Brambila-Tapia AJL, García-Ortiz JE, Brouillard P, et al. GATA2 null mutation associated with incomplete penetrance in a family with Emberger syndrome. *Hematology*. 2017;22(8):467-471.
37. Al Seraihi AF, Rio-Machin A, Tawana K, et al. GATA2 monoallelic expression underlies reduced penetrance in inherited GATA2-mutated MDS/AML. *Leukemia*. 2018;32(11):2502-2507.
38. Haddox CL, Carr RM, Abraham RS, et al. Phenotypic heterogeneity associated with germline GATA2 haploinsufficiency: a comprehensive kindred study. *Leuk Lymphoma*. 2019;60(13):3282-3286.
39. Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. *Br J Haematol*. 2015;169(2):173-187.
40. Fisher KE, Hsu AP, Williams CL, et al. Somatic mutations in children with GATA2-associated myelodysplastic syndrome who lack other features of GATA2 deficiency. *Blood Adv*. 2017;1(7):443-448.
41. Hirabayashi S, Wlodarski MW, Kozyra E, Niemeyer CM. Heterogeneity of GATA2-related myeloid neoplasms. *Int J Hematol*. 2017;106(2):175-182.
42. Nováková M, Žaliová M, Suková M, et al. Loss of B cells and their precursors is the most constant feature of GATA-2 deficiency in childhood myelodysplastic syndrome. *Haematologica*. 2016;101(6):707-716.
43. Pastor V, Hirabayashi S, Karow A, et al. Mutational landscape in children with myelodysplastic syndromes is distinct from adults: specific somatic drivers and novel germline variants. *Leukemia*. 2017;31(3):759-762.
44. Awada H, Thapa B, Visconte V. The genomics of myelodysplastic syndromes: origins of disease evolution, biological pathways, and prognostic implications. *Cells*. 2020;9(11):2512.
45. Kurata T, Shigemura T, Muramatsu H, Okuno Y, Nakazawa Y. A case of GATA2-related myelodysplastic syndrome with unbalanced translocation der(1;7)(q10;p10). *Pediatr Blood Cancer*. 2017;64(8):e26419.
46. Dror Y. Shwachman-Diamond syndrome. *Pediatr Blood Cancer*. 2005;45(7):892-901.
47. Bruyre H. der(1;7)(q10;p10). *Atlas Genet Cytogenet Oncol Haematol*. 2016;20(9):496-498.
48. Nagata Y, Makishima H, Kerr CM, et al. Invariant patterns of clonal succession determine specific clinical features of myelodysplastic syndromes. *Nat Commun*. 2019;10(1):5386.
49. Nagata Y, Maciejewski JP. The functional mechanisms of mutations in myelodysplastic syndrome. *Leukemia*. 2019;33(12):2779-2794.
50. Ogawa S. Genetics of MDS. *Blood*. 2019;133(10):1049-1059.
51. Patnaik MM, Lasho TL. Genomics of myelodysplastic syndrome/myeloproliferative neoplasm overlap syndromes. *Hematology (Am Soc Hematol Educ Program)*. 2020;2020(1):450-459.
52. Panuzzo C, Signorino E, Calabrese C, et al. Landscape of Tumor suppressor mutations in acute myeloid leukemia. *J Clin Med*. 2020;9(3):802.
53. Kon A, Shih LY, Minamino M, et al. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. *Nat Genet*. 2013;45(10):1232-1237.
54. Thota S, Viny AD, Makishima H, et al. Genetic alterations of the cohesin complex genes in myeloid malignancies. *Blood*. 2014;124(11):1790-1798.
55. Asada S, Fujino T, Goyama S, Kitamura T. The role of ASXL1 in hematopoiesis and myeloid malignancies. *Cell Mol Life Sci*. 2019;76(13):2511-2523.
56. Kakosaiou K, Panitsas F, Daraki A, et al. ASXL1 mutations in AML are associated with specific clinical and cytogenetic characteristics. *Leuk Lymphoma*. 2018;59(10):2439-2446.
57. Heimbruch KE, Meyer AE, Agrawal P, Viny AD, Rao S. A cohesive look at leukemogenesis: The cohesin complex and other driving mutations in AML. *Neoplasia*. 2021;23(3):337-347.
58. Ding LW, Ikezoe T, Tan KT, et al. Mutational profiling of a MonoMAC syndrome family with GATA2 deficiency. *Leukemia*. 2017;31(1):244-245.
59. Loyola VBP, Hirabayashi S, Pohl S, et al. Somatic genetic and epigenetic architecture of myelodysplastic syndromes arising from GATA2 deficiency [abstract]. *Blood*. 2015;126(13). Abstract 299.
60. Makishima H, Yoshizato T, Yoshida K, et al. Dynamics of clonal evolution in myelodysplastic syndromes. *Nat Genet*. 2017;49(2):204-212.
61. Labuhn M, Perkins K, Matz S, et al. Mechanisms of progression of myeloid preleukemia to transformed myeloid leukemia in children with Down syndrome [published correction appears in *Cancer Cell*. 2019;36(3):340]. *Cancer Cell*. 2019;36(2):123-138.e10.
62. Yoshida K, Toki T, Okuno Y, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders [published correction appears in *Nat Genet*. 2013;45(12):1516]. *Nat Genet*. 2013;45(11):1293-1299.
63. Alfayez M, Wang SA, Bannon SA, et al. Myeloid malignancies with somatic GATA2 mutations can be associated with an immunodeficiency phenotype. *Leuk Lymphoma*. 2019;60(8):2025-2033.
64. Kim N, Choi S, Kim SM, et al. Monozygotic twins with shared de novo GATA2 mutation but dissimilar phenotypes due to differential promoter methylation. *Leuk Lymphoma*. 2019;60(4):1053-1061.
65. McReynolds LJ, Zhang Y, Yang Y, et al. Rapid progression to AML in a patient with germline GATA2 mutation and acquired NRAS Q61K mutation. *Leuk Res Rep*. 2019;12:100176.
66. Wang X, Muramatsu H, Okuno Y, et al. GATA2 and secondary mutations in familial myelodysplastic syndromes and pediatric myeloid malignancies. *Haematologica*. 2015;100(10):e398-e401.
67. Molina JC, Asare JM, Tuschong L, et al. Venetoclax/decitabine for a pediatric patient with chronic myelomonocytic leukemia. *Pediatr Blood Cancer*. 2021;68(3):e28865.