

MYELOID NEOPLASIA

CME Article

Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents

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Key Points

- Germline *GATA2* mutations account for 15% of advanced and 7% of all primary pediatric MDS and do not influence overall survival.
- The majority (72%) of adolescents with MDS and monosomy 7 carry an underlying *GATA2* deficiency.

Germline *GATA2* mutations cause cellular deficiencies with high propensity for myeloid disease. We investigated 426 children and adolescents with primary myelodysplastic syndrome (MDS) and 82 cases with secondary MDS enrolled in 2 consecutive prospective studies of the European Working Group of MDS in Childhood (EWOG-MDS) conducted in Germany over a period of 15 years. Germline *GATA2* mutations accounted for 15% of advanced and 7% of all primary MDS cases, but were absent in children with MDS secondary to therapy or acquired aplastic anemia. Mutation carriers were older at diagnosis and more likely to present with monosomy 7 and advanced disease compared with wild-type cases. For stratified analysis according to karyotype, 108 additional primary MDS patients registered with EWOG-MDS were studied. Overall, we identified 57 MDS patients with germline *GATA2* mutations. *GATA2* mutations were highly prevalent among patients with monosomy 7 (37%, all ages) reaching its peak in adolescence (72% of adolescents with monosomy 7). Unexpectedly,

monocytosis was more frequent in *GATA2*-mutated patients. However, when adjusted for the selection bias from monosomy 7, mutational status had no effect on the hematologic phenotype. Finally, overall survival and outcome of hematopoietic stem cell transplantation (HSCT) were not influenced by mutational status. This study identifies *GATA2* mutations as the most common germline defect predisposing to pediatric MDS with a very high prevalence in adolescents with monosomy 7. *GATA2* mutations do not confer poor prognosis in childhood MDS. However, the high risk for progression to advanced disease must guide decision-making toward timely HSCT. (*Blood*. 2016;127(11):1387-1397)

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Disclosures

The authors, Associate Editor Mary C. Dinauer, and CME questions author Laurie Barclay, freelance writer and reviewer, Medscape, LLC, declare no competing financial interests.

Learning objectives

1. Describe the prevalence of germline *GATA2* mutations among children and adolescents with myelodysplastic syndrome (MDS), based on 2 consecutive prospective studies.

2. Compare other genetic features and clinical characteristics of germline *GATA2* mutation carriers with wild-type cases of MDS, based on 2 consecutive prospective studies and stratified analysis according to karyotype.

3. Determine the clinical implications of genetic findings on pediatric MDS, based on 2 consecutive prospective studies and stratified analysis according to karyotype.

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Introduction

The study of cancer genomes has identified a countless number of somatic gene mutations associated with malignancy.¹ Inherited genetic drivers predisposing to clonal evolution, are, however, less well understood. Myelodysplastic syndromes (MDSs) represent a heterogeneous group of clonal hematopoietic disorders occurring with various frequencies in all age groups. Significant differences between primary MDSs in children and adults are evident for morphology, cytogenetics, and therapeutic approaches. In children and adolescents, refractory cytopenia of childhood (RCC) represents the most common subtype usually associated with pancytopenia and hypocellular marrow^{2,3}; monosomy 7 is the most frequent aberration in children, whereas aberrations typically observed in adult MDS such as complex karyotypes or isolated del(5q) are rare.⁴ The somatic mutation landscape also differs between children and adults with myeloid diseases.⁵⁻⁷ Inherited genetic predisposition was thus far not considered a likely cause of de novo primary MDS, in contrast to MDS evolving from known inherited bone marrow failure (IBMF) syndromes such as Fanconi anemia or dyskeratosis congenita⁸ and the familial MDS/acute myeloid leukemia (AML) syndromes.^{9,10} The latter result from deficiencies of the hematopoietic transcription factors CEBPA, RUNX1,^{11,12} and *GATA2*.¹³ The spectrum of *GATA2*-related disease additionally involves immunodeficiencies¹⁴⁻¹⁶ and syndromic features such as congenital deafness and lymphedema originally defining Emberger syndrome.¹⁷ Patients present early in life with symptoms affecting hematopoietic, immune, and lymphatic systems, complicated by systemic infections and a high risk of developing MDS/AML^{18,19} However, no estimates exist on the prevalence and clinical significance of germline *GATA2* mutations within consecutive MDS cohorts. This makes it difficult giving advice on the expected outcomes and recommendations on evidence-based clinical care.

The *GATA2* gene encodes a chief hematopoietic transcription factor that through its 2 zinc fingers (ZFs) can occupy GATA DNA motifs in several thousand genes.^{20,21} Its major role in early hematopoiesis²²⁻²⁴ is attained by cooperating in a complex network of transcription factors in a dose-dependent manner.²³⁻²⁷ The *GATA2* germline mutational

landscape involves truncating mutations presumably resulting in loss of the second ZF (ZF2).¹⁵ In addition, missense mutations within ZF2 and noncoding variants in the +9.5-kb regulatory region of *GATA2* are thought to result in haploinsufficiency.^{13,17} Hence, the term *GATA2* deficiency or haploinsufficiency has been widely accepted to describe *GATA2*-spectrum disorders; in the following, both *GATA2*-mutated and *GATA2*-deficient are used interchangeably. It is noteworthy that somatic *GATA2* mutations in adult myeloid neoplasia can occur in both ZF regions^{28,29} and can have loss- or gain-of-function effects.^{28,30,31} In this study we aimed to assess the frequency of germline *GATA2* mutations that underlie pediatric MDS and to define clinical characteristics, therapeutic response, and prognostic consequences of this predisposition syndrome.

Methods

Study population

MDS was classified according to the World Health Organization criteria for pediatric MDS and included RCC and the advanced MDS subtypes, namely refractory anemia with excess blasts (RAEB) and RAEB in transformation (RAEB-t).³² We studied a consecutive cohort of 508 children and adolescents with material available for genetic testing (out of 544 enrolled) diagnosed with MDS in Germany between July 1, 1998 and June 30, 2013 and enrolled in the prospective studies 98 or 2006 (www.clinicaltrials.gov; #NCT00047268, #NCT00662090) of the European Working Group of MDS in Childhood (EWOG-MDS). Of the 508 patients, 426 had been diagnosed with primary MDS (including familial MDS defined as ≥ 1 first-degree relative suffering from hematological neoplasia) and 82 with MDS secondary to chemo- or radiation therapy or to acquired severe aplastic anemia. Patients with known IBMF disorders had been excluded from all analyses. For stratified analysis according to karyotype, we next extended the cohort of primary MDS by including a group of 108 additional patients registered in EWOG-MDS trials (Figure 1). The additional group was purposely biased

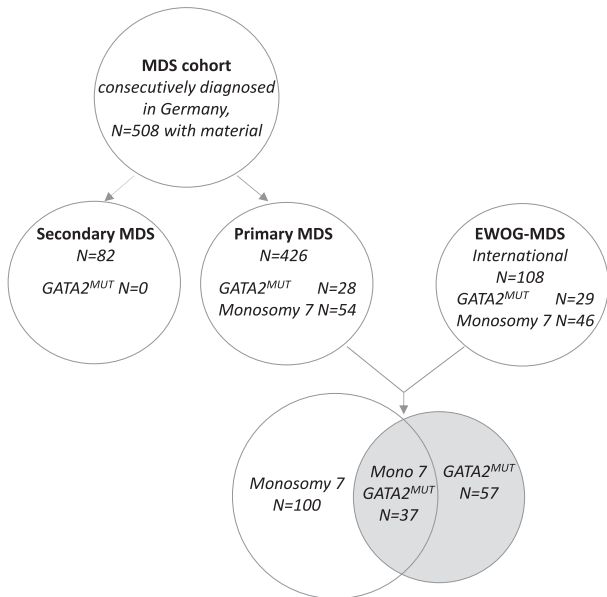


Figure 1. Composition of cohorts.

toward the major cytogenetic aberrations (monosomy 7 and trisomy 8) encountered in patients with GATA2 deficiency.

For metaphase karyotyping, chromosome banding analyses of diagnostic bone marrow (BM) specimens had been performed according to standard procedures.⁴ Hematopoietic stem cell transplantation (HSCT) was performed in accordance with the EWOG-MDS recommendations (www.ewog-mds.org). All studies were approved by the institutional ethics committees of the respective institutions. Written informed consent was obtained from patients or patients' guardians.

Genetic studies

Sanger sequencing of exons 2 to 6 and intron 4 in the GATA2 gene (NM_032638.4) was applied to DNA from BM or peripheral blood (PB) granulocytes. The presence of heterozygous polymorphisms ruled out a whole gene deletion and simplified selection of patients for copy number analysis (single nucleotide polymorphism array-based deletion analysis; Affymetrix 6.0, Santa Clara, CA). For germline investigations, nonmyeloid specimens were used according to the following priority: (1) cultured primary skin fibroblasts, (2) hair follicles, and (3) purified T cells. The feasibility of T-cell DNA to discriminate between germline and somatic events in patients prior to HSCT had previously been established for patients with somatic mutations such as ASXL1, detected only in the myeloid lineage but not in T cells (V.P., S.H., M.W.W., unpublished results, July 1, 2015). Novel GATA2 variants reported here were scored as likely pathogenic and disease associated according to the following criteria: (1) presence of identical mutation in other family member with GATA2-related MDS, (2) presence of known syndromic GATA2-related phenotype (eg, lymphedema in the affected individuals), (3) mutation previously reported in GATA2 deficiency, (4) novel mutation not present in controls in the Exome Aggregation Consortium browser (exac.broadinstitute.org) nor in the dbSNP database (ncbi.nlm.nih.gov/SNP) and exome variant server (evs.gs.washington.edu), and (5) high conservation across other species and high pathogenicity score (scale-invariant feature transform, mutation taster).

Statistical analysis

Overall survival (OS) was defined as the time from diagnosis to death or last follow-up, whereas event-free survival (EFS) was defined as the time from HSCT to death, disease recurrence, or last follow-up. The Kaplan-Meier method was used to estimate survival rates, and the 2-sided log-rank test was used to evaluate the equality of the survivorship functions in different subgroups. Time-to-event outcomes for relapse and transplant-related mortality (TRM) were estimated using cumulative incidence curves, using relapse and TRM as the respective

competing risks. The χ^2 test was used to examine the statistical significance of the association between GATA2 status and categorized factors. Fisher's exact test was calculated for 2 × 2 contingency analyses. Nonparametric statistics were used to test for differences in continuous variables in terms of mutational status (Mann-Whitney U test). All P values were 2 sided, and values <.05 were considered to be statistically significant. Software packages SPSS for Windows 20.0.0 (IBM Corp, New York, NY) and NCSS 2004 (NCSS, Kaysville, UT) were used.

Results

Prevalence and phenotype of GATA2-related MDS

BM or PB samples of 508 children and adolescents with primary or secondary MDS enrolled in 2 consecutive prospective studies were analyzed (Figure 1). In 28 of the 426 (7%) patients with primary MDS, 24 distinct GATA2 mutations were identified (Table 1). The constitutional nature of mutations was established in all cases tested. In contrast, no mutations were found among the 82 patients with secondary MDS. Accounting for the MDS subtype, GATA2 mutations were more prevalent in advanced MDS (15%, 13 of 85) compared with RCC (4%, 15 of 341, P < .01). GATA2 mutated (GATA2^{mut}) patients were older at diagnosis (median age, 12.3 vs 10.3 years; P < .01) and presented more often with advanced MDS (46% vs 18%, P < .01) and with monosomy 7 (70% vs 11%, P < .01; Table 1) compared with the GATA2 wild-type (GATA2^{WT}) group. Surprisingly, in the majority (71%) of the GATA2^{mut} group, MDS occurred sporadically without family history of hematologic malignancy.

Hematologic phenotype associated with GATA2 deficiency is determined by monosomy 7

Investigating PB findings of the consecutive cohort at diagnosis, we observed significantly higher hemoglobin concentrations and platelet counts, as well as higher numbers of myeloid precursors among patients with GATA2 deficiency compared with GATA2^{WT} (supplemental Table 1, available on the Blood Web site). Unexpectedly, there were more GATA2^{mut} cases with monocytosis (>1.0 × 10⁹/L) compared with GATA2^{WT} (P < .01).

Because the majority of GATA2^{mut} patients presented with MDS and monosomy 7, we reassessed the above findings in an extended

Table 1. Characteristics of primary MDS with GATA2 deficiency in a consecutive population-based cohort

Parameter		GATA2 ^{mut} (N = 28)	GATA2 ^{WT} (N = 398)	P value
Mutations	Total/distinct	28/24	0	
Age	Median (range), years	12.3 (5.2-17.4)	10.3 (0.2-18.1)	<.05
Sex	Males, N = 248	15 (54%)	233 (58%)	n.s.
	Females, N = 178	13 (46%)	165 (42%)	
Subtype	RCC, N = 341	15 (54%)	326 (82%)	<.01
	RAEB/RAEB-t, N = 85	13 (46%)	72 (18%)	
Karyotype*	Monosomy 7, † N = 54	19 (70%)	35 (11%)	<.01
	Structural complex, N = 9	0 (0%)	9 (3%)	
	Other, N = 30	1 (4%)‡	29 (9%)	
	Normal, N = 253	7 (26%)	246 (77%)	
Familial disease	Index patients (%)	8 (29%)	8 (2%)	<.01

n.s., not significant.

*Karyotypes at diagnosis were informative in 346 of 426 patients (GATA2 mutated: n = 27; wild type: n = 319).

†Includes monosomy 7 with 1 or 2 additional aberrations.

‡Includes 1 patient with der(1;7)(q10;p10) and trisomy 8.

Table 2. Cytogenetic abnormalities in GATA2-related childhood MDS

Karyotype	Patients, N (%) N = 56	MDS subtype	
		RCC	RAEB/RAEB-t
Monosomy 7*	38 (68%)	17	16/5
Der(1;7)(q10;p10)†	4 (7%)	1	3/0
Trisomy 8‡	5 (9%)	3	1/1
Normal	9 (16%)	9	0/0

Karyotypes were available for 56 of 57 GATA2-deficient patients.

*Includes 5 patients with an additional aberration [trisomy 8, trisomy 21, trisomy 22, del(12)(p11p13), del(18q)].

†Includes 2 patients with additional trisomy 8 and 1 patient each with trisomy 11 or a marker chromosome.

‡Includes 1 patient with a marker chromosome.

cohort purposely augmented with this cytogenetic category. Within the subgroup of patients with monosomy 7, hematologic parameters did not differ according to mutational status with the exception of a lower ratio of myeloid to erythroid cells in the BM of *GATA2*^{mut} patients (supplemental Figure 1; supplemental Table 2). Thus, the above differences in hematologic phenotype between *GATA2*^{mut} and *GATA2*^{WT} groups appears to have been determined by monosomy 7 rather than by *GATA2* mutations.

GATA2-related MDS is associated with der(1;7) and trisomy 8 in addition to monosomy 7

A total of 57 pediatric MDS patients with *GATA2* deficiency could be identified in the extended cohort (Figure 1). The abnormal karyotypes of these cases fall in only 3 categories: monosomy 7 in 68% of patients, unbalanced aberration der(1;7) (resulting in loss of the q-arm of chromosome 7) in 7%, and trisomy 8 in 9% of patients (Table 2). Neither del(5q) nor structurally complex aberrations were identified.

We next calculated the prevalence of *GATA2* deficiency within the specific cytogenetic categories. Of the 449 patients analyzed, 100 had monosomy 7 and 32 had trisomy 8. Within the monosomy 7 subgroup, one third (37%) carried *GATA2* mutations (Figure 2A), and the median age at diagnosis was significantly higher for *GATA2*^{mut} patients compared with the wild-type group (12.5 vs 4.2 years, $P < .01$; Figure 2B). Of note, the youngest child with *GATA2*-related MDS with monosomy 7 was 4.4 years old at time of diagnosis. The prevalence of *GATA2* mutations increased dramatically with age, reaching 72% in adolescents aged 12 to 19 years at diagnosis of MDS associated with monosomy 7 (Figure 2C). Among MDS patients with trisomy 8, 16% (5/32) had an underlying *GATA2* deficiency (Figure 2A). Their median age was 15.3 (11.4-17.1) years. The 4 patients with MDS and

der(1;7) translocation were also carriers of *GATA2* mutations (data not shown). Thus, 7% of the *GATA2*^{mut} patients carried a der(1;7) translocation (Table 2).

Landscape of germline GATA2 mutations in childhood MDS

In total, we identified 57 *GATA2*^{mut} patients with 44 distinct (31 novel) germline mutations (Figure 3A-B; Table 3) with the following predicted effects: 18 (16 novel) truncating frameshift/stop-gain in 22 patients; 5 (all novel) splice site in 6 patients; 13 (5 novel) missense in 17 patients; 4 (3 novel) noncoding intron 4 variants in 6 cases, and in 1 patient each a novel 30-bp in-frame deletion and a novel heterozygous germline deletion involving the entire *GATA2* locus: del(3)(q21.2q21.3), chr3:126 637 078-130 282 197(NCBI36/hg18). All missense mutations were confined to exons 5 and 6 (Figure 3A,C), corresponding to ZF2 (amino acids 347-398) with the exception of mutations C-terminal to ZF2: Glu415Lys (novel) and a previously reported mutation Ser447Arg³³ (Figure 3A). Both mutations affect highly conserved amino acids and were scored as deleterious by prediction algorithms. Noncoding mutations in intron 4 clustered between nucleotides +526 and +699 adjacent to the donor splice site c.1017 (Figure 3A, bottom). All the other mutations with predicted structural effect (nonsense, splice site, deletions) occurred randomly prior to the C-terminal end of ZF2. Two different truncating mutations, counted as novel in our cohort, had been previously reported in solid tumors in the COSMIC somatic mutation database; however, information on material source and germline status was inconclusive.³⁴ Finally, no correlation between the type of *GATA2* mutation and the hematologic phenotype was identified (data not shown).

Inheritance and penetrance

Detailed family history was available for 53 *GATA2*^{mut} patients. Familial MDS/AML had been identified in 12 study patients of 9 pedigrees (supplemental Figure 2A). All affected relatives were diagnosed with MDS/AML as children or young adults. The remaining 41 study patients with sporadic MDS showed unremarkable family history. For 11 of those, the unaffected family members (parents ± siblings) were investigated for the presence of *GATA2* mutations (supplemental Figure 2B). Although no silent carriers for coding mutations were identified, in 3 families with noncoding mutations found in the index case, the same variant was also present in the respective fathers who were clinically healthy. In an additional 7 patients with sporadic MDS and coding *GATA2* mutations who received HSCT from a matched sibling, the respective mutation was not identified in donor blood.

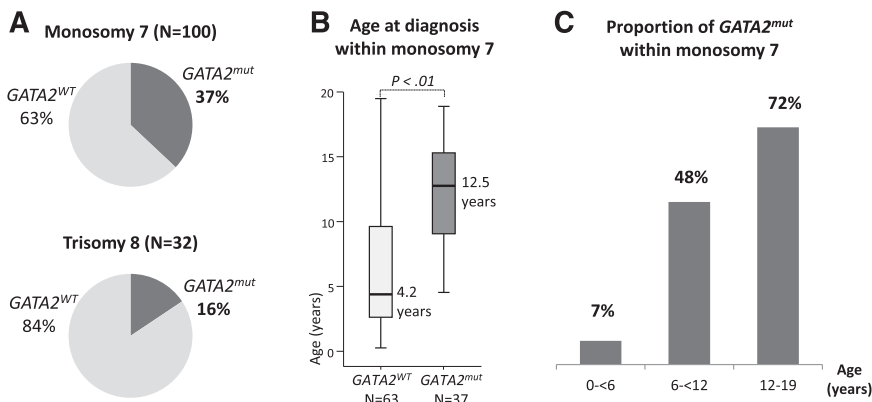


Figure 2. Prevalence of *GATA2* deficiency in MDS patients with monosomy 7 and trisomy 8. (A) Proportion of *GATA2* mutated and wild-type patients within subgroups of (top) monosomy 7 and (bottom) trisomy 8. (B) Boxplots with age distribution of patients with monosomy 7 according to *GATA2* mutational status. (C) Prevalence of *GATA2* deficiency in patients with monosomy 7 subgroup stratified by age groups.

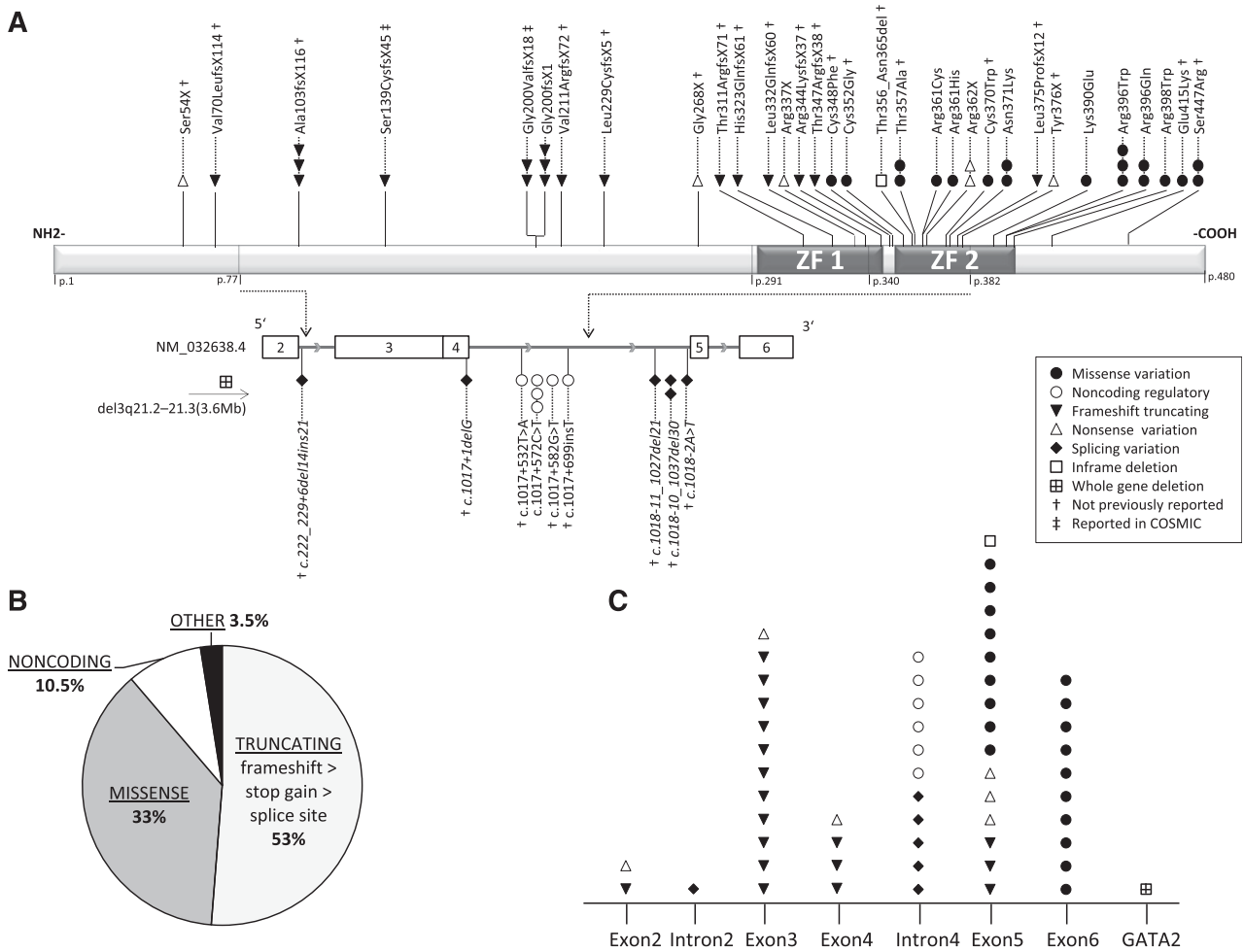


Figure 3. Landscape of GATA2 mutations in childhood MDS. (A) Distinct mutations identified in 57 patients are shown. The presumed functional effect of mutations is depicted by symbols as shown in the legend box. Top panel depicts the GATA2 protein structure with the known DNA-binding zinc fingers 1 (ZF1) and 2 (ZF2). GATA2 gene structure (NM_032638.4) is shown below and begins with exon 2 (first coding exon). Relevant introns represented by gray line are stretched out (ie, intron 4 that contains the regulatory sites E-box, GATA, and ETS). Italic font depicts splice site variants. Mutations previously reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) database were Ser139CysfsX45 in pancreas carcinoma (ID COSS2068125), and Gly200ValfsX18 in a patient with adenocarcinoma of the colon (ID COSS1650947); both mutations were confirmed as germline in our patients. (B) Pie chart depicting the proportion of patients with different mutation types; 53% of patients carry truncating mutations, among them, frameshift being the most common followed by stop gain and splice site mutations. (C) Distribution of distinct mutations identified in 57 patients in relation to their localization within the gene.

Nonhematologic phenotype in pediatric MDS cohort with GATA2 deficiency

Previously reported nonhematologic phenotypes of GATA2 deficiency (syndromic by means of Emberger-like features or signs of immunodeficiency associated with MonoMAC [monocytopenia, B and natural killer cell lymphopenia and mycobacterial, fungal, and viral infections]/DCML [dendritic cell, monocyte, and lymphocyte deficiency]) were identified in a total of 51% (29 of 57) of studied patients. In detail, 9% (5 of 57) of patients presented with deafness, 23% (13 of 57) with lymphedema/hydrocele, and 39% (22 of 57) with immunodeficiency; some patients presented with multiple symptoms. Additional novel clinical features recurrently present in the investigated cohort included anomalies of the urogenital tract in 12% (7 of 57) and relevant behavioral problems (eg, autism and aggressive behavior) in 19% (11 of 57) of GATA2^{mut} patients.

GATA2 deficiency is not a poor prognostic factor for OS

Among the 426 consecutively diagnosed children with primary MDS (Figure 1), 5-year OS was, as expected, significantly superior in the

RCC subgroup compared with the advanced MDS subgroup (91% vs 57%, *P* < .01; supplemental Figure 3A). When evaluating OS according to mutational status alone, the GATA2^{mut} group had an inferior OS compared with GATA2^{WT} (73% vs 84%, *P* < .05; supplemental Figure 3B). However, when accounting for MDS subtype and presence of monosomy 7, there was no difference in OS in relation to GATA2 mutational status (Figure 4A-B).

Stable disease is rare in GATA2-related MDS

Of the 57 children with MDS and GATA2 deficiency, 7 were not transplanted. Four of those children died of disease progression or infection after AML-like chemotherapy or supportive care. The remaining 3 patients with stable non-transfusion-dependent RCC are still alive without severe infectious complications 5 to 16 years from diagnosis (Table 3). Of note, 2 of these 3 patients had novel noncoding mutations in GATA2 intron 4 (D506 and D609).

Outcome of HSCT is not influenced by GATA2 mutational status

Among the cohort of 100 patients with monosomy 7 (Figure 1), 91 including 34 GATA2 mutation carriers had been transplanted. The

Table 3. Detailed mutational and clinical outcome data of pediatric patients with MDS and GATA2 deficiency

Patient no.	Sex	GATA2 mutation	Genotype	Age at dx (years)	Initial (highest) MDS subtype	Karyotype at dx	Therapy	Preparative regimen	Donor	SC source	Outcome
A044	F	FsX*	c.303delC	12.4	RCC	Der(1;7) add	HsCT	Bu-based	MSD	BM	CR (>9.5 y)
A056	M	Splice site*	c.1018-11_1027del21	16.1	RAEB	p.?	HsCT	Bu-based	MSD	BM	CR (>4.5 y)
B002	M	FsX*	c.968dupA	14.5	RCC(RAEB)	p.His323GlnfsX61	HsCT	Bu-based	MSD	PB	CR (>6.6 y)
B032	F	Missense*	c.1046G>T	12.7	RAEB-t	p.Cys348Phe	HsCT	Bu-based	MSD	BM	CR (>4.0 y)
C2041	M	Inframe deletion*	c.1066_1095del30	15.7	RCC(RAEB)	p.Thi356_Asn365del	HsCT	Bu-based	UD	BM	CR (>9.0 y)
C2053	M	FsX*	c.1035_1038dupCGGC	4.4	RAEB	p.Thr347ArgfsX38	HsCT	Bu-based	MSD	BM	CR (>7.2 y)
C2054	M	Intron 4	c.1017+572C>T	17	RCC	p.=	HsCT	Bu-based	MSD	BM	CR (>7.0 y)
C2061	M	Intron 4	c.1017+572C>T	17.5	RCC	p.=	HsCT	Bu-based	MSD	BM	CR (>3.9 y)
C2087	M	Splice site*	c.222_229+6del14ins21	11.4	RCC	p.?	HsCT	Treo-based	UD	PB	CR (>1.0 y)
D076	M	FsX*	c.627_630dupCGGC	12.5	RCC(RAEB)	p.Val211ArgfsX72	HsCT	Bu-based	UD	PB	CR (>13.4 y)
D1010	M	Stop gain*	c.1128C>G	12.7	RCC	p.Tyr376X	HsCT	Treo-based	UD	BM	CR (>0.4 y)
D314	F	FsX	c.599dupG	10	RAEB	p.Gly200fsX11	HsCT	Bu-based	UD	PB	Died, TRM (infection 3.5 mo after HSCT)
D342	M	FsX†	c.416_417delICT	16.6	RCC(RAEB)	p.Ser139CysfsX45	HsCT	Bu-based	UD	BM	Died, relapse 4 y after HSCT
D350	M	FsX*	c.207_208delICG	11	RAEB	p.Val70LeufsX114	HsCT	Bu-based	MSD	PB	CR (>5.3 y)
D415	F	Missense	c.1341C>A	13.6	RAEB	p.Ser447A>G	HsCT	Bu-based	UD	PB	CR (>7.0 y)
D427	F	Missense	c.1187G>A	12.9	RAEB	p.Arg396Gln	HsCT	Bu-based	MSD	PB	CR (>10.1 y)
D429	F	FsX†	c.599delG	16.3	RCC	p.Gly200ValfsX18	HsCT	RIC	UD	PB	CR (>9.6 y)
D479	F	Missense	c.1186C>T	16.8	RCC	p.Arg396Trp	HsCT	Other	UD	PB	CR (>4.9 y)
D569	F	FsX*	c.1031_1049del19	10.3	RCC	p.Arg344LysfsX37	HsCT	RIC	MSD	BM	CR (>5.0 y)
D612	M	FsX*	c.685delIC	7.5	RCC	p.Leu229CysfsX5	HsCT	Bu-based	MSD	BM	CR (>6.3 y)
D680	M	Splice site*	c.1018-10_1037del30	12.1	RCC	p.?	HsCT	Bu-based	UD	BM	CR (>6.5 y)
D726	F	FsX*	c.303delC	9.6	RAEB	p.Ala103fsX116	HsCT	Bu-based	UD	BM	CR (>6.3 y)
D731	F	FsX*	c.306delA	5.2	RAEB	p.Ala103fsX116	HsCT	Bu-based	UD	PB	CR (>4.3 y)
D794	F	Stop gain	c.1084C>T	6.1	RCC(RAEB)	p.Arg362X	HsCT	Bu-based	UD	BM	CR (>3.8 y)
D801	M	Missense	c.1187G>A	15.6	RCC	p.Arg396Gln	HsCT	Treo-based	UD	PB	CR (>1.0 y)
D807	M	Splice site*	c.1018-10_1037del30	13.8	RCC	p.?	HsCT	Treo-based	UD	BM	CR (>1.4 y)
D983	M	Intron 4*	c.1017+582G>T	13.4	RCC	p.=	HsCT	RIC	MSD	BM	CR (>1.9 y)
I112	F	Stop gain*	c.802G>A	17.1	RAEB-t	p.Gly268X	HsCT	Bu-based	UD	BM	Died, 1.4 y after HSCT
I198	M	FsX*	c.970_994dup25	18.6	RCC	p.Leu332GlnfsX60	HsCT	Bu-based	MSD	BM	Died, TRM (infection 6.5 mo after HSCT)
I301	M	Missense	c.1081C>T	10.7	RAEB	p.Arg361Cys	HsCT	n.a.	n.a.	n.a.	Died, TRM (GVHD 1.5 mo after HSCT)
I305	M	Stop gain*	c.161C>A	18	RAEB-t(MDR-AML)	p.Ser54X	HsCT	Treo-based	UD	BM	CR (>3.6 y)
I306	M	Missense	c.1186C>T	14.3	RCC	p.Arg396Trp	HsCT	Treo-based	UD	PB	CR (>2.8 y)
NL097	M	Missense	c.1341C>A	12.9	RCC(RAEB-t)	p.Ser447A>G	HsCT	Bu-based	MSD	BM	Died, TRM (infection 2 mo after HSCT)
NL113	F	Missense	c.1168A>G	12.8	RAEB	p.Lys390Glu	HsCT	Bu-based	UD	PB	CR (>6.2 y)
PL027	M	Stop gain	c.1009C>T	14.9	RCC	p.Arg337X	HsCT	Bu-based	UD	PB	CR (>5.9 y)
SC152	F	FsX	c.599dupG	12.2	RAEB	p.Gly200fsX11	HsCT	Bu-based	UD	BM	CR (>2.0 y)
D271	M	Missense	c.1186C>T	12.5	RAEB	p.Arg396Trp	HsCT	Bu-based	UD	PB	Relapse 1.8 y after 1.HSCT; CR after 2.HSCT (>4.2 y)

add, one additional cytogenetic aberration (excluding translocations); AML-t, AML-like therapy; Bu-based, busulfan-based conditioning; CR, complete remission (time from last HSCT); dx, diagnosis; fsX, frameshift truncating mutation; FUP, follow-up; Gene Δ, whole gene deletion; GF, graft failure; intron 4, noncoding mutation in intron 4; MDR-AML, MDS-related acute myeloid leukemia; MMFD, mismatched family donor; MSD, matched sibling donor; n.a., data not available; RIC, reduced intensity conditioning; SC source, stem cell source; TBI-based, total body irradiation-based conditioning; trunc, truncating; w&w, watch and wait; -7, monosomy 7; +8, trisomy 8; treo-based, treosulfan-based conditioning; UD, unrelated donor.

*Novel mutation, previously not reported.

†Mutation previously reported only in somatic setting (COSMIC database).

#Haploidentical transplantation.

Table 3. (continued)

Patient no.	Sex	GATA2 mutation	Genotype	Age at dx (years)	Initial (highest) MDS subtype	Karyotype at dx	Therapy	Preparative regimen	Donor source	SC source	Outcome
D492	M	Splice site*	c.1017+1delG	17.4	RCC(RAEB)	-7	HSCT	Bu-based	MMFD	BM	Relapse 2 y after 1.HSCT; Died, relapse 6 mo after 2.HSCT
D762	M	Missense	c.1113C>G	9.7	RAEB(RAEB-t)	-7	HSCT	Treo-based	UD	PB	Relapse 1.7 y after 1.HSCT; CR after 2.HSCT
D420	F	Gene Δ*	del3q21.2-21.3	15	RCC(RAEB-t)	-7	HSCT	TBI-based	MMFD\$	PB	CR (>7.3 y)
I126	F	Splice site*	c.1018-2A>T	5.1	RCC(RAEB)	-7	HSCT	TBI-based	MMFD\$	PB	CR (>10.2 y)
I199	M	FsX*	c.1124delT	7.8	RAEB(RAEB-t)	-7	HSCT	TBI-based	MMFD\$	PB	CR (>4.7 y)
D621	M	Missense	c.1192C>T	15.9	RCC	-7	HSCT	Other	MMFD\$	PB	GF after 1.HSCT; Died, TRM (GF/microangiopathy after 2.HSCT)
D147	M	Stop gain	c.1084C>T	14.2	RCC(RAEB)	-7, add	AML-th, HSCT	TBI-based	UD	BM	Died, infection 11.5 mo after HSCT
D151	F	Missense	c.1113C>A	16	RCC(MDR-AML)	+8, add	AML-th, HSCT	Bu-based	UD	PB	CR (>0.1 y)
D245	M	Missense*	c.1110C>G	8.7	RAEB-t	-7	AML-th, HSCT	n.a.	UD	n.a.	Died, TRM (GVHD/infection, 4 mo after HSCT)
D770	F	FsX†	c.599delG	10.2	RAEB	-7, add	AML-th, HSCT	Bu-based	MSD	PB	Died, relapse 13 mo after HSCT
D907	F	FsX*	c.932_937delinsG	7.5	RAEB(MDR-AML)	-7	AML-th, HSCT	Bu-based	UD	BM	CR (>3.5 y)
D955	F	FsX	c.599dupG	11.4	RAEB-t	-7	AML-th, HSCT	Treo-based	UD	BM	Died, relapse 1 y after HSCT
NL116	F	Missense*	c.1069A>G	15.3	RAEB	De(1;7) add	AML-th, HSCT	Treo-based	MMFD\$	PB	CR (>5.3 y)
CZ057	M	Intron 4	c.1017+572C>T	17.4	RAEB(MDR-AML)	-7	AML-th				Died, disease progress 6 mo after dx
D184	F	Missense*	c.1054T>G	8.8	RAEB(MDR-AML)	-7	AML-th				Died, disease progress 2.2 y after dx
SC021	F	Missense*	c.1243G>A	13.7	RAEB(MDR-AML)	+8	AML-th				Died, infection during AML-th, 2 y after dx
D418	M	Missense	c.1082G>A	7.3	RAEB-t	-7	Supportive				Died, disease progress 1 mo after dx
D506	M	Intron 4*	c.1017+699insT	13.5	RCC	n.a.	w&w				Alive with disease (last FUP 8 y after dx)
D609	M	Intron 4*	c.1017+532T>A	3.1	RCC	Normal	w&w				Alive with disease (last FUP 16 y after dx)
NL134	F	Missense*	c.1069A>G	15.3	RCC	+8	w&w				Alive with disease (last FUP 5 y after dx)

add, one additional cytogenetic aberration (excluding translocations); AML-th, AML-like therapy; Bu-based, busulfan-based conditioning; CR, complete remission (time from last HSCT); dx, diagnosis; fsX, frameshift truncating mutation; FUP, follow-up; Gene Δ, whole gene deletion; GF, graft failure; intron 4, noncoding mutation in intron 4; MDR-AML, MDS-related acute myeloid leukemia; MMFD, mismatched family donor; MSD, matched sibling donor; n.a., data not available; RIC, reduced intensity conditioning; SC source, stem cell source; TBI-based, total body irradiation-based conditioning; trunc, truncating; w&w, watch and wait; -7, monosomy 7; +8, trisomy 8; treo-based, treosulfan-based conditioning; UD, unrelated donor.

*Novel mutation, previously not reported.

†Mutation previously reported only in somatic setting (COSMIC database).

#Haplodidentical transplantation.

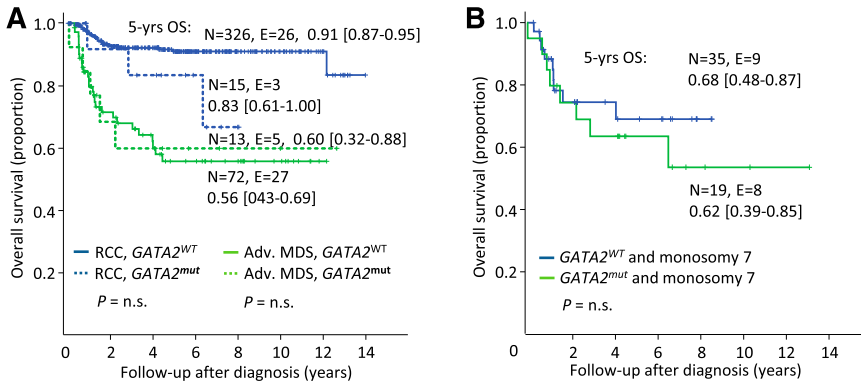


Figure 4. OS according to MDS subtypes and GATA2 mutational status. (A) OS in 426 children and adolescents consecutively diagnosed with primary MDS according to MDS subtypes and GATA2 mutational status. (B) Overall survival in 100 children and adolescents with monosomy 7 according to GATA2 mutational status. Adv. MDS, advanced MDS subtypes; n.s., not significant; N, patients under risk; E, events; GATA2^{mut}, mutation carriers; GATA2^{WT}, patients with wild-type status.

5-year OS (66% vs 69%) and EFS (60% for both) were comparable between the GATA2^{mut} and GATA2^{WT} groups (Figure 5A-B). The relapse rate did not significantly differ between both groups (21% vs 13%; Figure 5C). Of note, TRM was independent of underlying GATA2 deficiency (21% vs 24%; Figure 5D) as was the rate of infectious complications (66% vs 61%, *P* = not significant). Specifically, evaluating viral, bacterial, fungal, parasitic infections, cytomegalovirus reactivation, cytomegalovirus disease, Epstein-Barr virus infections/post-transplant lymphoproliferative disorder, and adenovirus infections, the rate of complication was 66% (33 of 50) in the GATA2^{mut} and 61% (168 of 276) in the GATA2^{WT} groups. Across all karyotypes, AML-like chemotherapy prior to HSCT was administered to 7 patients only; 4 of those died due to TRM or relapse.

predisposition to the etiology of primary MDS. This study shows for the first time that GATA2 germline mutations underlie 7% of consecutively diagnosed pediatric cases with primary MDS, and 15% of advanced MDS. In comparison, the prevalence of germline GATA2 mutations in a cohort of 586 adult patients with MDS and MDS/MPN patients reached only 0.5% (3 of 7 patients with GATA2 mutations; J.P.M., Cleveland Clinic MDS registry, personal communication, January 1, 2015). The absence of GATA2 mutations in secondary MDS of childhood and their rarity in adult MDS reinforces the notion that different etiologies are involved in these conditions. Drawing from histories of our patients and from published findings, it can be concluded that GATA2-related disease generally manifests in adolescence or young adulthood and is unlikely to contribute to MDS in older patients. Median age at diagnosis of GATA2 deficiency in previous reports is estimated at 29 years.¹⁸ In our prospective childhood MDS cohort, GATA2^{mut} patients had a median age at diagnosis of 12 years. In conclusion, the age peak for hemato-immunologic disease manifestation in individuals with GATA2 mutations lies between the second and third decade of life.

Discussion

Outside the setting of known IBMF disorders and familial MDS/AML, little has been known about the contribution of hereditary

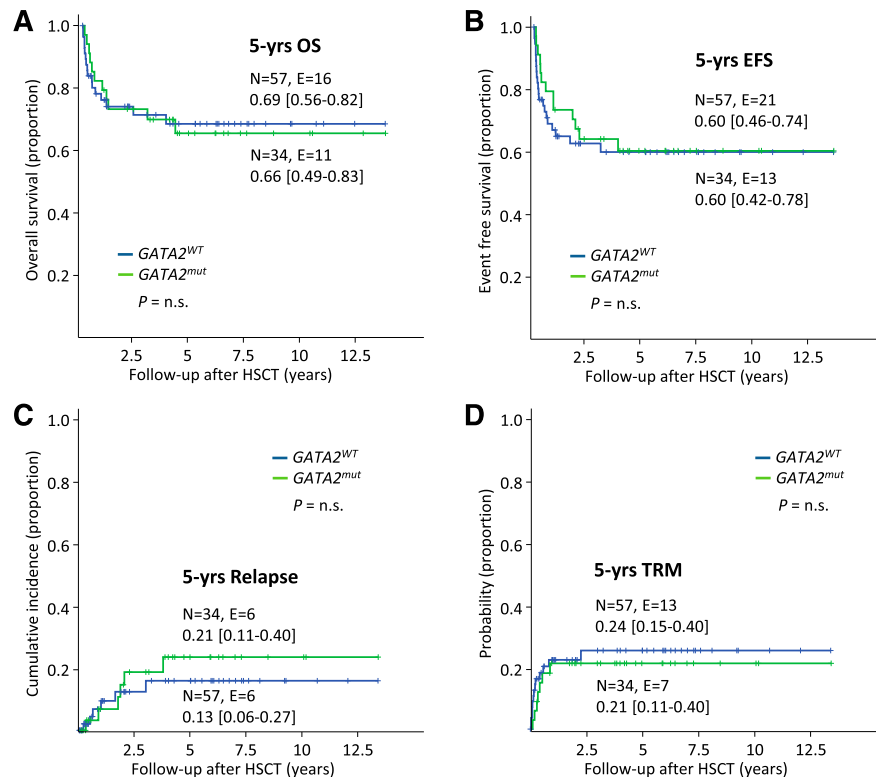


Figure 5. Outcome from HSCT based on GATA2 status in patients with monosomy 7. Kaplan-Meier survival curves and cumulative incidence curves according to GATA2 mutational status in 91 patients with monosomy 7 undergoing HSCT. (A) OS, (B) EFS, (C) incidence of relapse, and (D) probability of TRM. n.s., not significant; N, patients under risk; E, events.

Strikingly, in the majority of GATA2-deficient patients, MDS occurred sporadically without preexisting family history of MDS, and in half of all patients, no known clinical features pointing toward GATA2 deficiency were present. Sporadic disease had previously been reported in few patients with GATA2-related hematologic malignancy without additional accessory phenotypes and in patients with immunodeficiency.^{14,35}

The high rate of de novo GATA2 mutations in patients with MDS and the low number of silent carriers are consistent with a disease exposing high penetrance and expressivity. Although the high incidence of sporadic MDS and the full penetrance for coding (exonic) mutations in our cohort must be interpreted with caution due to referral bias, it is also true that relatively few (to our knowledge, 8 reported) clinically silent carriers with coding mutations were identified among >200 reported GATA2^{mut} cases.¹⁸ Finally, our findings suggestive of reduced penetrance for noncoding mutations are consistent with a previous report of 2 silent carriers with such variants.³⁶

Previous reports suggested that cytopenias and immunodeficiencies result from GATA2 haploinsufficiency. This notion is consistent with the loss of hematopoietic stem cells (HSC) observed in mouse models of GATA2 haploinsufficiency and conditional GATA2 inactivation.^{23,27} On the opposite, GATA2 overexpression inhibits HSC output³⁷ and erythroid differentiation.³⁸ Like others, we found 3 types of monoallelic mutations: truncating mutations, missense mutations in ZF2, and noncoding mutations in the enhancer region of intron 4. Thus far, only a handful of GATA2 mutations have been functionally validated. Loss of function was demonstrated for the ZF2 mutations Thr354Met and Thr355del found in familial MDS/AML,¹³ Arg361Leu and Cys373Arg in Emberger syndrome,¹⁷ and Arg350_Asn351ins8 in de novo AML with unknown germline status.³⁹ Of these, only Thr354Met was present in 3 of our patients. On the other hand, somatic GATA2 mutations identified in myeloid diseases were shown to enhance (Gly320Asp/ZF1 and Leu359Val/ZF2), reduce (Ala318Thr/ZF1 and Leu321Phe/ZF1), or not to alter (Arg308Pro/ZF1) the transactivation activity of GATA2 protein.^{28,31,39} The fact that all truncating mutations identified abolish ZF2 emphasizes its functional significance. Noncoding GATA2 mutations were initially reported by Holland's group⁴⁰ in the +9.5 intronic region shown to act as transcriptional enhancer.⁴¹ Notably, 10.5% of affected patients in our study carried these noncoding variants. For most of them the clinical presentation was similar to patients with coding mutations, pointing toward comparable functional effect. However 2 patients (D506 and D609) with novel intronic variants showed stable hematologic disease and long-term survival between 8 and 16 years, an observation certainly deserving further functional analyses. The variant c.1017+532T>A in D609 falls within the highly conserved E-box region of GATA2 spanning CATCTGC nucleotides starting at position c.1017+530, for which important regulatory/enhancer function has been ascribed; in addition, a family with mutation in this region (c.1017+512del28) presenting with MonoMAC/MDS was previously reported.⁴² The pathogenic significance of the variant c.1017+699insT in D506 is not clear. Although it affects a highly conserved nucleotide and has not been previously reported in control databases, it is also possible that it might represent a nonpathogenic private variant.

The recounted difficulty in identifying genotype-phenotype association was also observed in our study when, for example, hematologic phenotypes of different patients with identical mutations were assessed. Although the same mutation was associated with MDS developing after years of preexisting cytopenias in one child, it caused MDS without prodromal symptoms in another patient. Possibly, GATA2 genotypes can have various functional consequences in

different biological contexts. We therefore propose the category "MDS following GATA2 disease" or "GATA2-related MDS." In this study, we focused on the systematic review of hematologic findings in GATA2^{mut} patients within a large prospective MDS cohort. The known MonoMAC-/Emberger-like phenotypes were also observed in half of our patients, but they did not negatively affect the clinical outcome. Strikingly, we encountered more cases with monocytosis rather than monocytopenia in patients with GATA2-related MDS, which can be attributed to the more advanced disease with monosomy 7. This finding emphasizes the broad heterogeneity of GATA2 disease phenotype. In addition, we report for the first time novel recurrent phenotypes of GATA2 deficiency, namely urogenital tract malformations and behavioral problems.

Advanced MDS and monosomy 7 were highly overrepresented in GATA2-related MDS. By contrast, in the recent National Institutes of Health series of GATA2^{mut} patients evaluated for immunodeficiency, trisomy 8 (24%) was more frequent than monosomy 7 (16%).³⁶ Although the prevalence of monosomy 7 in previous reports on GATA2 deficiency was estimated at 29%,¹⁸ this karyotype was found at least twice as frequent in our pediatric cohort. Moreover, several GATA2^{mut} patients in this study presented with the unbalanced aberration der(1;7)(q10;p10) leading to trisomy 1q and loss of 7q, which is otherwise rare in pediatric MDS⁴ and was not found in GATA2^{WT} patients. Given the high prevalence of germline GATA2 mutations in adolescents with monosomy 7 (72%), an underlying GATA2 deficiency should be excluded during the diagnostic workup in this subgroup.

Accounting for MDS subtypes and cytogenetics, OS was not affected by GATA2 mutations, indicating that MDS subtype rather than GATA2 status was the relevant prognostic factor. HSCT is the only curative option for GATA2-related MDS. The National Institutes of Health reported a survival rate of 54% at 4 years after HSCT in 21 GATA2^{mut} patients that were transplanted for MDS/AML, pulmonary alveolar proteinosis, or recurrent infections.³⁶ The comparably favorable 5-year OS of 66% in our pediatric cohort transplanted for MDS with monosomy 7 can be likely explained by the younger age and the lower prevalence of GATA2-related systemic complications. Our findings indicate that GATA2 deficiency itself does not increase TRM in affected children. We concur with Grossman and colleagues⁴³ that the ideal time window for HSCT in GATA2 deficiency is during the hypocellular MDS phase and before manifestation of severe complications (ie, invasive infections) and support the need for a close monitoring of GATA2^{mut} patients for the occurrence of any of these events. Interestingly, we observed a slightly higher proportion of relapse after HSCT within GATA2^{mut} group with monosomy 7 (21% vs 13%; *P* = not significant), an observation deserving further validation in larger cohorts.

Finally, our findings should be interpreted in the light of some limitations. One is the fact that children with MDS and advanced disease are subjected to HSCT in a very timely manner. In addition, although our results show comparable survival for GATA2^{mut} and GATA2^{WT} children with MDS, this finding might not be translated into the setting of adult patients with other GATA2-related phenotypes (ie, immunodeficiency or pulmonary complications).

In summary, we show that GATA2 deficiency is a predisposing condition for MDS and is responsible for 7% of all primary pediatric MDS cases and prevalent in two third of adolescents with monosomy 7. Our results redefine the paradigm of MDS in children and adolescents and highlight the contribution of de novo germline mutations with a delayed latency oncogenic effect. GATA2

mutational status does not negatively affect the outcome of MDS in children and adolescents. We suggest that decisions on timing and preparative regimen for HSCT in GATA2-related MDS should be guided, as for all children with MDS, by the known risk factors such as cytogenetic evolution, severity of cytopenias, and advanced disease. Ideally, the implementation of early diagnosis of GATA2 deficiency can avoid unnecessary diagnostic procedures, enable tailored surveillance strategy, and limit the use of noncurative therapies specifically avoiding immunosuppression. We propose that GATA2 analysis should be included in the workup of all children and young adults presenting with MDS and monosomy 7, der(1;7), or trisomy 8, irrespective of family history or accessory GATA2 disease phenotype.

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Authorship

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A complete list of the members of the EWOG-MDS appears in "Appendix."

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