PREVALENCE, CLINICAL CHARACTERISTICS AND PROGNOSIS OF GATA2-RELATED MYELODYSPLASTIC SYNDROMES (MDS) IN CHILDREN AND ADOLESCENTS

Marcin W. Wlodarski1,2, Shinsuke Hirabayashi1#, Victor Pastor1, Jan Starý3, Henrik Hasle4, Riccardo Masetti5, Michael Dworzak6, Markus Schmugge6, Marry van den Heuvel-Eibrink8, Marek Ussowicz9, Barbara De Moerloose10, Albert Catala11, Owen P Smith12, Petr Sedlacek3, Arjan C. Lankester13, Marco Zecca14, Victoria Bordon10, Susanne Matthes-Martin6, Jonas Abrahamsson14, Jörn Sven Kühl16, Karl-Walter Sykora17, Michael H. Albert18, Bartłomiej Przychodzien19, Jarosław Maciejewski19, Stephan Schwarz20, Gudrun Göhring21, Brigitte Schlegelberger1, Annamaria Cseh1, Peter Noellke1, Ayami Yoshimi1, Franco Locatelli22, Irith Baumann23, Brigitte Strahm1, and Charlotte M. Niemeyer.1,2

For the European Working Group of MDS in Childhood (EWOG-MDS).

Affiliations:
1) Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, University of Freiburg, Freiburg, Germany
2) German Cancer Consortium (DKTK), Freiburg, Germany, and German Cancer Research Center (DKFZ), Heidelberg, Germany
3) Department of Pediatric Hematology and Oncology, Charles University and University Hospital Motol, Prague, Czech Republic
4) Department of Pediatrics, Aarhus University Hospital Skejby, Aarhus, Denmark
5) Department of Pediatric Oncology and Hematology, University of Bologna, Bologna, Italy
6) St. Anna Children's Hospital and Children's Cancer Research Institute, Medical University of Vienna, Vienna, Austria
7) Department of Hematology and Oncology, University Children's Hospital, Zurich, Switzerland
8) Erasmus Medical Center, Rotterdam, and Dutch Childhood Oncology Group (DCOG), The Hague, The Netherlands
9) Department of Pediatric Hematology, Oncology, and BMT, Medical University of Wroclaw, Wroclaw, Poland
10) Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, Belgium
11) Department of Hematology and Oncology, Hospital Sant Joan de Déu, Barcelona, Spain
12) Paediatric Oncology and Haematology, Our Lady’s Children’s Hospital Crumlin, Dublin, Ireland
13) Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands
14) Pediatric Hematology-Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
15) Pediatric Oncology, Queen Silvia Children’s Hospital, Gothenburg, Sweden
16) Pediatric Oncology, Charité–University Berlin, Germany
17) Pediatric Hematology and Oncology, Hannover Medical School, Germany
18) Department of Pediatric Hematology and Oncology, Dr. v. Hauner Children’s Hospital, Ludwig-Maximilians-University, Munich, Germany
19) Tausig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA
20) Institute of Pathology, University of Erlangen, Erlangen, Germany
21) Institute of Human Genetics, Hannover Medical School, Hannover, Germany
22) Department of Pediatric Hematology and Oncology, Bambino Gesù Children’s Hospital, Rome, University of Pavia, Italy
23) Department of Pathology, Clinical Center, Böblingen, Germany
# Current affiliation: Department of Pediatrics, St. Luke’s International Hospital, Tokyo, Japan

Corresponding author:
Marcin W Wlodarski, MD
Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology
University of Freiburg, Mathildenstr. 1, 79106 Freiburg, Germany
Phone: +49 761270-43000; Fax: -46160; Email: marcin.wlodarski@uniklinik-freiburg.de

Article type: Original report
Running title: GATA2-RELATED MDS
Abstract: 249 words; Manuscript: 3948 words

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

This study has been presented in part at the American Society of Hematology Meeting 2012. (Ref. No. 19).
First author is recipient of EHA-ASH fellowship on Translational Research Training in Hematology.

Copyright © 2015 American Society of Hematology
ABSTRACT

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 two 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 as compared to wildtype 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 hematological 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 towards timely HSCT.
INTRODUCTION

The study of cancer genomes has identified a countless number of somatic gene mutations associated with malignancy. Inherited genetic drivers predisposing to clonal evolution, are, however, less well understood. Myelodysplastic syndromes (MDS) represent a heterogeneous group of clonal hematopoietic disorders occurring with various frequencies in all age groups. Significant differences between primary MDS 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; monosomy 7 is the most frequent aberration in children, while aberrations typically observed in adult MDS such as complex karyotypes or isolated del(5q) are rare. The somatic mutation landscape also differs between children and adults with myeloid diseases. 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 congenita, and the familial MDS/acute myeloid leukemia (AML) syndromes. The latter result from deficiencies of the hematopoietic transcription factors CEBPA, RUNX1, and GATA2. The spectrum of GATA2-related disease additionally involves immunodeficiencies and syndromic features such as congenital deafness and lymphedema originally defining the Emberger syndrome. 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. 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 two zinc fingers (ZF) can occupy GATA-DNA-motifs in several thousand genes. Its major role in early hematopoiesis is attained by cooperating in a complex network of transcription factors in a dose-dependent manner. The *GATA2* germline mutational landscape involves truncating mutations presumably resulting in loss of the 2nd ZF (ZF2). In addition, missense mutations within ZF2 and noncoding variants in the +9.5kb regulatory region of *GATA2* are thought to result in haploinsufficiency. Hence, the term GATA2 deficiency or haploinsufficiency has been widely accepted to describe GATA2-spectrum disorders; and 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 and can have loss-, or gain-of-function effects. 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 WHO 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). We studied a consecutive cohort of 508/544 children and adolescents with material available for genetic testing 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 at least one first-degree relative suffering from hematological neoplasia) and 82 with MDS secondary to chemo- or radiation therapy or to acquired severe aplastic anemia (SAA). Patients with known inherited bone marrow failure (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 towards 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. Hematopoietic stem cell transplantation (HSCT) had been performed in accordance with the EWOG-MDS recommendations (www.ewog-mds.org). All studies have been approved by the institutional ethics committees of the respective institutions. Written informed consent has been obtained from patients or patients guardians.
Genetic studies

Sanger sequencing of exons 2-6 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 (SNP-array based deletion analysis, Affymetrix 6.0, Santa Clara, CA). For germline investigations non-myeloid specimens were used according to the following priority: a) cultured primary skin fibroblasts, b) hair follicles, and c) 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 (unpublished results). Novel GATA2 variants reported here were scored as likely pathogenic and disease-associated according to the following criteria: i) presence of identical mutation in other family member with GATA2-related MDS, ii) presence of known syndromic GATA2-related phenotype (e.g. lymphedema in the affected individuals), iii) mutation previously reported in GATA2-deficiency, iv) novel mutation not present in controls in the EXAC browser (exac.broadinstitute.org), nor in dbSNP database (ncbi.nlm.nih.gov/SNP) and exome variant server (evs.gs.washington.edu), v) high conservation across other species and high pathogenicity score (SIFT, mutation taster).

Statistical analysis

Overall survival (OS) was defined as the time from diagnosis to death or last follow-up, while event-free survival (EFS) 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 two-sided log-rank test was employed 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. Chi-Square-test was used to examine the statistical significance of the association between GATA2 status and categorized factors. Fisher's exact test was calculated for 2x2 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 two-sided and values <0.05 were considered to be statistically significant. Software packages SPSS for Windows 20.0.0 (IBM Corp, NY) and NCSS 2004 (NCSS, Kaysville, UT) were employed.
RESULTS

Prevalence and phenotype of GATA2-related MDS

BM or PB samples of 508 children and adolescents with primary or secondary MDS enrolled in two 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%, 13/85) as compared to RCC (4%, 15/341, p<.01). GATA2 mutated (GATA2\textsuperscript{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) as compared to the GATA2 wildtype (GATA2\textsuperscript{WT}) group. Surprisingly, in the majority (71%) of the GATA2\textsuperscript{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 when compared to GATA2\textsuperscript{WT} (supplemental Table 1). Unexpectedly, there were more GATA2\textsuperscript{mut} cases with monocytosis (>1.0x10\textsuperscript{9}/l) compared to GATA2\textsuperscript{WT} (P<.01).

Because the majority of GATA2\textsuperscript{mut} patients presented with MDS and monosomy 7 we reassessed the above findings in an extended cohort purposely augmented with this cytogenetic category. Within the subgroup of patients with monosomy 7, hematological
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 (Suppl. Figure 1, suppl. Table 2). Thus, the above differences in hematological phenotype between $GATA2^{mut}$ and $GATA^{WT}$ 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 three 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 when compared to the wildtype 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 12-19 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 (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 GATA2mut patients with 44 distinct (31 novel) germline mutations (Figure 3A-B and 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 one patient each a novel 30bp 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 Ser447Arg (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. Finally, no correlation between the type of GATA2 mutation and the hematological phenotype was identified (not shown).

Inheritance and penetrance

Detailed family history was available for 53 GATA2mut patients. Familial MDS/AML had been identified in 12 study patients of 9 pedigrees (Suppl. 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.
While no silent carriers for coding mutations were identified, in 3 families with non-coding mutations found in the index case, the same variant was also present in the respective fathers who were clinically healthy. In 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’s blood.

**Non-hematological phenotype in pediatric MDS cohort with GATA2 deficiency**

Previously reported non-hematologic phenotypes of GATA2-deficiency (syndromic by means of Emberger-like features or signs of immunodeficiency associated with MonoMAC/DCML) were identified in a total of 51% (29/57) of studied patients. In detail, 9% (5/57) of patients presented with deafness, 23% (13/57) with lymphedema/ hydrocele, and 39% (22/57) 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% (7/57) and relevant behavioral problems (e.g. autism and aggressive behavior) in 19% (11/57) of GATA2\textsuperscript{mut} patients.

**GATA2 deficiency is not a poor prognostic factor for overall survival**

Among the 426 consecutively diagnosed children with primary MDS (Figure 1), 5-year OS was, as expected, significantly superior in the RCC subgroup as compared to the advanced MDS subgroup (91% vs. 57%, p<.01, suppl. Figure 3A). When evaluating OS according to mutational status alone, the GATA2\textsuperscript{mut} group had an inferior OS when compared to GATA2\textsuperscript{WT} (73% vs. 84%, p<.05, suppl. 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 from disease progression or infection after AML-like chemotherapy or supportive care. Remaining 3 patients with stable non-transfusion dependent RCC are still alive without severe infectious complications 5-16 years from diagnosis (Table 3). Of note, 2 of these 3 patients had novel, noncoding mutations in \textit{GATA2} intron 4 (D506 and D609).

**Outcome of HSCT is not influenced by the GATA2 mutational status**

Among the cohort of 100 patients with monosomy 7 (Figure 1), 91 including 34 \textit{GATA2} mutation carriers, had been transplanted. The 5-year OS (66\% vs 69\%) and EFS (60\% for both) were comparable between the \textit{GATA2}^{mut} and \textit{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 \textit{GATA2} deficiency (21\% vs. 24\%, Figure 5D) as was the rate of infectious complications (66\% versus 61\%, P=ns). Specifically, evaluating viral, bacterial, fungal, parasitic infections, CMV reactivation, CMV disease, EBV infections/PTLD, and adenovirus infections the rate of complication was 66\% (33/50) in the \textit{GATA2}^{mut} and 61\% (168/276) in the \textit{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.
DISCUSSION

Outside the setting of known IBMF disorders and familial MDS/AML little has been known about the contribution of hereditary 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% (3/7 patients with GATA2 mutations, Dr. Maciejewski, Cleveland Clinic MDS registry, personal communication). 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.\textsuperscript{18} In our prospective childhood MDS cohort, GATA2\textsubscript{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.

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 towards 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.\textsuperscript{14,35}

The high rate of \textit{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 more than 200 reported GATA2 mut cases. Finally, our findings suggestive of reduced penetrance for noncoding mutations are consistent with a previous report of two silent carriers with such variants.

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. Contradicting findings indicate that GATA2 overexpression inhibits HSC output and erythroid differentiation. Like others, we found three types of monoallelic mutations: truncating mutations, missense mutations in ZF2 and noncoding mutations in the enhancer region of intron 4. So far, only a handful of GATA2 mutations had been functionally validated. Loss-of-function was demonstrated for the ZF2 mutations Thr354Met and Thr355del found in familial MDS/AML, Arg361Leu and Cys373Arg in Emberger syndrome, and Arg350_Asn351ins8 in de novo AML with unknown germline status. Of these, only Thr354Met was present in three 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. The fact that all truncating mutations identified abolish ZF2 emphasizes its functional significance. Noncoding GATA2 mutations were initially reported by Holland’s group in the +9.5 intronic region shown to act as transcriptional enhancer. 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 towards comparable functional effect. However two patients (D506; 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.\textsuperscript{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 however also possible that it might represent a non-pathogenic private variant.

The recounted difficulty in identifying genotype-phenotype association was also observed in our study when for example hematological phenotypes of different patients with identical mutations were assessed. While 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 hematological findings in GATA2\textsuperscript{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 NIH series of \( GATA2^{\text{mut}} \) patients evaluated for immunodeficiency, trisomy 8 (24%) was more frequent than monosomy 7 (16%).\textsuperscript{36} Although, the prevalence of monosomy 7 in previous reports on GATA2 deficiency was estimated at 29%,\textsuperscript{18} this karyotype was found at least twice as frequent in our pediatric cohort. Moreover, several \( GATA2^{\text{mut}} \) patients present with the unbalanced aberration \text{der}(1;7)(q10;p10) leading to trisomy 1q and loss of 7q, which is otherwise rare in pediatric MDS\textsuperscript{4} and was not found in \( GATA2^{\text{WT}} \) 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 \( GATA2 \)-status indicating that MDS subtype rather than \( GATA2 \) status was the relevant prognostic factor. HSCT is the only curative option for GATA2-related MDS. The NIH reported a survival rate of 54% at 4 years after HSCT in 21 \( GATA2^{\text{mut}} \) patients that were transplanted for MDS/AML, pulmonary alveolar proteinosis or recurrent infections\textsuperscript{36}. The comparably favorable 5-year OS of 66% in our pediatric cohort transplanted for MDS/-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 colleagues\textsuperscript{43} that the ideal time window for HSCT in GATA2 disease is during the hypocellular MDS phase and before manifestation of severe complications (i.e. invasive infections), and support the need for a close monitoring of
$GATA2^{\text{mut}}$ patients for the occurrence of any of these events. Interestingly, we observed a slightly higher proportion of relapse after HSCT within $GATA2^{\text{mut}}$ group with monosomy 7 (21% vs. 13%; P=n.s.), 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, while our results show comparable survival for $GATA2^{\text{mut}}$ and $GATA2^{\text{WT}}$ children with MDS, this finding might not be translated into the setting of adult patients with other GATA2-related phenotypes i.e. immunodeficiency or pulmonary complications.

In summary, we show that GATA2 deficiency is a predisposing condition for MDS, 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 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 non-curative 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.
ACKNOWLEDGMENTS

Research support. Deutsche Krebshilfe (Max Eder grant #109005 to MWW and the MDS consortium grant #110293 to CMN), German Federal Ministry of Education and Research (DKTK German cancer consortium, molecular diagnostics of pediatric malignancies, to CMN and MWW), and the intramural grant CCIAP4 to MWW. Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic). For excellent technical assistance, data management we would like to acknowledge Sandra Zolles, Gunda Ruzaike, Hideki Makishima, Alexandra Fischer, Wilfried Truckenmüller, and Anne Strauss (Freiburg).

AUTHOR CONTRIBUTIONS


DISCLOSURE OF CONFLICTS OF INTEREST

Authors have nothing to disclose.
REFERENCES

TABLES

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

<table>
<thead>
<tr>
<th></th>
<th>GATA2&lt;sup&gt;mut&lt;/sup&gt; N=28</th>
<th>GATA2&lt;sup&gt;WT&lt;/sup&gt; N=398</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total / distinct</td>
<td>28 / 24</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Median (range), years</td>
<td>12.3 (5.2-17.4)</td>
<td>10.3 (0.2-18.1)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Males, N=248</td>
<td>15 (54%)</td>
<td>233 (58%)</td>
</tr>
<tr>
<td></td>
<td>Females, N=178</td>
<td>13 (46%)</td>
<td>165 (42%)</td>
</tr>
<tr>
<td><strong>Subtype</strong></td>
<td>RCC, N=341</td>
<td>15 (54%)</td>
<td>326 (82%)</td>
</tr>
<tr>
<td></td>
<td>RAEB/RAEB-t, N=85</td>
<td>13 (46%)</td>
<td>72 (18%)</td>
</tr>
<tr>
<td><strong>Karyotype</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Monosomy 7, N=54</td>
<td>19 (70%)</td>
<td>35 (11%)</td>
</tr>
<tr>
<td></td>
<td>Structural complex, N=9</td>
<td>0 (0%)</td>
<td>9 (3%)</td>
</tr>
<tr>
<td></td>
<td>Other, N=30</td>
<td>1 (4%)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>29 (9%)</td>
</tr>
<tr>
<td></td>
<td>Normal, N=253</td>
<td>7 (26%)</td>
<td>246 (77%)</td>
</tr>
<tr>
<td><strong>Familial disease</strong></td>
<td>Index patients (%)</td>
<td>8 (29%)</td>
<td>8 (2%)</td>
</tr>
</tbody>
</table>

*Karyotypes at diagnosis were informative in 346/426 patients (GATA2-mutated: n=27, wildtype: n=319). *Includes monosomy 7 with one or two additional aberrations. §Includes one patient with der(1;7)(q10;p10) and trisomy 8.

**Abbreviations:** MDS, myelodysplastic syndrome; RCC, refractory cytopenia of childhood; RAEB, refractory anemia with excess of blasts; RAEB-t; RAEB in transformation; GATA2<sup>WT</sup>, GATA2-wildtype; GATA2<sup>mut</sup>, GATA2-mutated; n.s., not significant.
Table 2. Cytogenetic abnormalities in GATA2-related childhood MDS

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Patients, N (%) N=56</th>
<th>MDS subtype</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RCC</td>
<td>RAEB/RAEB-t</td>
</tr>
<tr>
<td>Monosomy 7*</td>
<td>38 (68%)</td>
<td>17</td>
<td>16 / 5</td>
</tr>
<tr>
<td>Der(1;7)(q10;p10)§</td>
<td>4 (7%)</td>
<td>1</td>
<td>3 / 0</td>
</tr>
<tr>
<td>Trisomy 8§</td>
<td>5 (9%)</td>
<td>3</td>
<td>1 / 1</td>
</tr>
<tr>
<td>Normal</td>
<td>9 (16%)</td>
<td>9</td>
<td>0 / 0</td>
</tr>
</tbody>
</table>

Karyotypes were available for 56/57 GATA2-deficient patients. *Includes 5 patients with an additional aberration [trisomy 8, trisomy 21, trisomy 22, del(12)(p11p13), del(18q)]. #Includes two patients with additional trisomy 8 and one patient each with trisomy 11 or a marker chromosome. §Includes one patient with a marker chromosome. Abbreviations: see Table 1.
Table 3. Detailed mutational and clinical outcome data of pediatric patients with MDS and GATA2 deficiency

<table>
<thead>
<tr>
<th>Pt#</th>
<th>Sex</th>
<th>GATA2 mutation</th>
<th>Genotype</th>
<th>Age at dx</th>
<th>Initial (highest) MDS subtype</th>
<th>Karyotype at dx</th>
<th>Therapy</th>
<th>Preparative regimen</th>
<th>Donor source</th>
<th>SC source</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>A044</td>
<td>f</td>
<td>FsX*</td>
<td>c.303delC</td>
<td>12.4</td>
<td>RCC</td>
<td>Del1;7</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>MSD</td>
<td>BM</td>
<td>CR (&gt;9.5 y)</td>
</tr>
<tr>
<td>A056</td>
<td>m</td>
<td>Splice site*</td>
<td>c.1018+1_1027del1</td>
<td>p.?</td>
<td>16.1</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>MSD</td>
<td>BM</td>
</tr>
<tr>
<td>B002</td>
<td>f</td>
<td>FsX*</td>
<td>c.965delpA</td>
<td>14.5</td>
<td>RCC (RA EB)</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>MSD</td>
<td>BM</td>
<td>CR (&gt;6.6 y)</td>
</tr>
<tr>
<td>B032</td>
<td>f</td>
<td>Missense*</td>
<td>c.1048G&gt;T</td>
<td>12.7</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>MSD</td>
<td>BM</td>
<td>CR (&gt;4.0 y)</td>
</tr>
<tr>
<td>C2041</td>
<td>m</td>
<td>Inframe deletion*</td>
<td>c.1066_1059delB</td>
<td>15.7</td>
<td>RCC (RA EB)</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;9.0 y)</td>
</tr>
<tr>
<td>C2053</td>
<td>m</td>
<td>FsX*</td>
<td>c.1035_1038dupC</td>
<td>4.4</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;7.2 y)</td>
</tr>
<tr>
<td>C2054</td>
<td>m</td>
<td>Intron 4</td>
<td>c.1017+572del2</td>
<td>17</td>
<td>RCC</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;7.0 y)</td>
</tr>
<tr>
<td>C2061</td>
<td>m</td>
<td>Intron 4</td>
<td>c.1017+572del2</td>
<td>17.5</td>
<td>normal</td>
<td></td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;3.9 y)</td>
</tr>
<tr>
<td>C2078</td>
<td>m</td>
<td>Missense*</td>
<td>c.222_229del1</td>
<td>11.4</td>
<td>RCC</td>
<td>+</td>
<td>HSCT</td>
<td>Tree-based</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;1.0 y)</td>
</tr>
<tr>
<td>D076</td>
<td>m</td>
<td>Missense*</td>
<td>c.227_230del1</td>
<td>12.5</td>
<td>RCC (RA EB)</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;13.4 y)</td>
</tr>
<tr>
<td>D1010</td>
<td>m</td>
<td>Stop gain*</td>
<td>c.1228delG</td>
<td>12.7</td>
<td>RCC</td>
<td>normal</td>
<td>HSCT</td>
<td>Tree-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;0.4 y)</td>
</tr>
<tr>
<td>D314</td>
<td>f</td>
<td>FsX*</td>
<td>c.599delpG</td>
<td>10</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>PB</td>
<td>† TRM (infection 3.5 mo after HSCT)</td>
</tr>
<tr>
<td>D342</td>
<td>m</td>
<td>FsX*</td>
<td>c.416_417delCT</td>
<td>16.6</td>
<td>RCC (RA EB)</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;5.3 y)</td>
</tr>
<tr>
<td>D350</td>
<td>m</td>
<td>Missense*</td>
<td>c.507_508delKG</td>
<td>11</td>
<td>RA EB</td>
<td>Del1;7</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;7.0 y)</td>
</tr>
<tr>
<td>D427</td>
<td>f</td>
<td>Missense*</td>
<td>c.329_330delC</td>
<td>12.8</td>
<td>RCC</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;10.1 y)</td>
</tr>
<tr>
<td>D429</td>
<td>f</td>
<td>Missense*</td>
<td>c.599delG</td>
<td>16.3</td>
<td>RCC</td>
<td>normal</td>
<td>HSCT</td>
<td>RC</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;9.6 y)</td>
</tr>
<tr>
<td>D479</td>
<td>m</td>
<td>Missense*</td>
<td>c.1185delC</td>
<td>16.8</td>
<td>RCC</td>
<td>normal</td>
<td>HSCT</td>
<td>Other</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;4.9 y)</td>
</tr>
<tr>
<td>D569</td>
<td>f</td>
<td>Missense*</td>
<td>c.1031_1036del9</td>
<td>10.3</td>
<td>RCC</td>
<td>normal</td>
<td>HSCT</td>
<td>RC</td>
<td>MSD</td>
<td>BM</td>
<td>CR (&gt;5.0 y)</td>
</tr>
<tr>
<td>D612</td>
<td>m</td>
<td>Missense*</td>
<td>c.685delC</td>
<td>7.5</td>
<td>RCC</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;6.3 y)</td>
</tr>
<tr>
<td>D680</td>
<td>m</td>
<td>Missense*</td>
<td>c.1018-1013del10</td>
<td>12.1</td>
<td>RCC</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;6.5 y)</td>
</tr>
<tr>
<td>D726</td>
<td>f</td>
<td>Missense*</td>
<td>c.303delC</td>
<td>9.6</td>
<td>RCC</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;6.3 y)</td>
</tr>
<tr>
<td>D731</td>
<td>f</td>
<td>Missense*</td>
<td>c.306delA</td>
<td>5.2</td>
<td>RCC</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;4.3 y)</td>
</tr>
<tr>
<td>D794</td>
<td>f</td>
<td>Stop gain*</td>
<td>c.1084delC</td>
<td>6.1</td>
<td>RCC (RA EB)</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;3.8 y)</td>
</tr>
<tr>
<td>D801</td>
<td>m</td>
<td>Missense*</td>
<td>c.1186delT</td>
<td>15.6</td>
<td>RCC</td>
<td>normal</td>
<td>HSCT</td>
<td>Tree-based</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;1.0 y)</td>
</tr>
<tr>
<td>D807</td>
<td>m</td>
<td>Missense*</td>
<td>c.1084-1083del10</td>
<td>13.8</td>
<td>RCC</td>
<td>normal</td>
<td>HSCT</td>
<td>Tree-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;1.4 y)</td>
</tr>
<tr>
<td>D983</td>
<td>m</td>
<td>Intron 4*</td>
<td>c.1017+582del2</td>
<td>13.4</td>
<td>RCC</td>
<td>normal</td>
<td>HSCT</td>
<td>RC</td>
<td>MSD</td>
<td>BM</td>
<td>CR (&gt;1.9 y)</td>
</tr>
<tr>
<td>I112</td>
<td>f</td>
<td>Stop gain*</td>
<td>c.802delA</td>
<td>17.1</td>
<td>RA EB</td>
<td>+</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>† TRM (1.4 y after HSCT)</td>
</tr>
<tr>
<td>I216</td>
<td>f</td>
<td>Missense*</td>
<td>c.597_598del2</td>
<td>18.6</td>
<td>RCC</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>MSD</td>
<td>BM</td>
<td>† TRM (infection 6.5 mo after HSCT)</td>
</tr>
<tr>
<td>I301</td>
<td>m</td>
<td>Missense*</td>
<td>c.1083delT</td>
<td>10.7</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>† TRM (GVHD 1.5 mo after HSCT)</td>
</tr>
<tr>
<td>I305</td>
<td>m</td>
<td>Stop gain*</td>
<td>c.161delC</td>
<td>18</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>Tree-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;3.6 y)</td>
</tr>
<tr>
<td>I306</td>
<td>m</td>
<td>Missense*</td>
<td>c.1187delC</td>
<td>14.3</td>
<td>RCC</td>
<td>normal</td>
<td>HSCT</td>
<td>Tree-based</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;2.8 y)</td>
</tr>
<tr>
<td>NL007</td>
<td>m</td>
<td>Missense*</td>
<td>c.1344delA</td>
<td>12.9</td>
<td>RCC (RA EB)</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>MSD</td>
<td>BM</td>
<td>† TRM (infection 2 mo after HSCT)</td>
</tr>
<tr>
<td>NL013</td>
<td>f</td>
<td>Missense*</td>
<td>c.1166delA</td>
<td>12.8</td>
<td>RA EB</td>
<td>Del1;7</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;6.2 y)</td>
</tr>
<tr>
<td>PL027</td>
<td>f</td>
<td>Missense*</td>
<td>c.1009delC</td>
<td>14.9</td>
<td>RCC</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>PB</td>
<td>CR (&gt;5.9 y)</td>
</tr>
<tr>
<td>SC152</td>
<td>f</td>
<td>FsX*</td>
<td>c.599delpG</td>
<td>12.2</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>BM</td>
<td>CR (&gt;2.0 y)</td>
</tr>
<tr>
<td>D271</td>
<td>m</td>
<td>Missense*</td>
<td>c.1186delT</td>
<td>12.5</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>UD</td>
<td>PB</td>
<td>relapse 1.8 y after 1.HSCT; CR after 2.HSCT (&gt;4.2 y)</td>
</tr>
<tr>
<td>D492</td>
<td>m</td>
<td>Missense*</td>
<td>c.1017+1delK</td>
<td>17.4</td>
<td>RCC (RA EB)</td>
<td>-</td>
<td>HSCT</td>
<td>Bu-based</td>
<td>MMF</td>
<td>BM</td>
<td>relapse 2 y after 1.HSCT; relapse 6 mo after 2.HSCT</td>
</tr>
<tr>
<td>D762</td>
<td>m</td>
<td>Missense*</td>
<td>c.1133delG</td>
<td>9.7</td>
<td>RA EB</td>
<td>-</td>
<td>HSCT</td>
<td>Tree-based</td>
<td>UD</td>
<td>PB</td>
<td>relapse 1.7 y after 1.HSCT; CR after 2.HSCT</td>
</tr>
<tr>
<td>Patient</td>
<td>Sex</td>
<td>Mutation</td>
<td>Gene</td>
<td>Date</td>
<td>Age</td>
<td>Status</td>
<td>karyotype</td>
<td>Treatment</td>
<td>Outcome</td>
<td>Other Details</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
<td>----------</td>
<td>------</td>
<td>------</td>
<td>-----</td>
<td>--------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>D420</td>
<td>f</td>
<td>Gene Δ</td>
<td>del(2q12-21.3)</td>
<td>p. = 15</td>
<td>RCC</td>
<td>RAEB-t</td>
<td>-7</td>
<td>HSCT</td>
<td>TBI-based</td>
<td>MMFD</td>
<td>PB</td>
</tr>
<tr>
<td>I26</td>
<td>f</td>
<td>Splice site</td>
<td>c.1018+2A&gt;T</td>
<td>p. ?</td>
<td>5.1</td>
<td>RCC</td>
<td>RAEB</td>
<td>-7</td>
<td>HSCT</td>
<td>TBI-based</td>
<td>MMFD</td>
</tr>
<tr>
<td>I199</td>
<td>m</td>
<td>FeX*</td>
<td>c.1124delT</td>
<td>p.au375PnX12</td>
<td>7.8</td>
<td>PAEB</td>
<td>RAEB-t</td>
<td>-7</td>
<td>HSCT</td>
<td>TBI-based</td>
<td>MMFD</td>
</tr>
</tbody>
</table>

**Abbreviations:** Pt#, patient identifier; f, female; m, male; *novel mutation, previously not reported; **mutation previously reported only in somatic setting (COSMIC database); fsX, frameshift truncating mutation; intron 4, noncoding mutation in intron 4; Gene Δ, whole gene deletion; dx, diagnosis; SC source, stem cell source; trunc, truncating; MDS, myelodysplastic syndromes; RCC, refractory cytopenia of childhood; RAEB, refractory anemia with excess of blasts; RAEB-t, RAEB in transformation; MDR-AML, myelodysplasia-related acute myeloid leukemia; -7, monosomy 7; +8, trisomy 8; add, one additional cytogenetic aberration (excluding translocations); AMPl, AMPl-like therapy; HSCT, hematopoietic stem cell transplantation; w&w, watch and wait; Bu-based, busulfan-based conditioning; treo-based, treosulfan-based conditioning; RIC, reduced intensity conditioning; TBI-based, total body irradiation – based conditioning; n.a., data not available; UD unrelated donor; MSD, matched sibling donor; MMFD, mismatched family donor; § haploidentical transplantation; BM, bone marrow; PB, peripheral blood; CR complete remission (time from last HSCT); mo, months; y, years; dx, diagnosis; †, died; TRM, transplant-related mortality; GF, graft failure; FUP, follow-up.
FIGURE LEGENDS

Figure 1. Composition of cohorts.

Figure 2. Prevalence of GATA2 deficiency in MDS patients with monosomy 7 and trisomy 8. (A) Proportion of GATA2 mutated and wildtype patients within subgroups of monosomy 7 (top) and trisomy 8 (bottom). (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 grey line are stretched out, i.e. intron 4 that contains the regulatory sites E-box, GATA, and ETS. Italic font depicts splice site variants. Mutations previously reported in the 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.
Figure 4. Overall Survival according to MDS subtypes and GATA2 mutational status (A)
Overall survival 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. GATA2mut, mutation carriers; GATA2WT, patients with wildtype status.

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) Overall survival (OS), (B) event-free survival (EFS), (C) incidence of relapse, and (D), probability of transplant-related mortality (TRM). n.s., not significant; N, patients under risk; E, events.
Figure 1

MDS cohort consecutively diagnosed in Germany, N=508 with material

Secondary MDS N=82
GATA2\textsuperscript{MUT} N=0

Primary MDS N=426
GATA2\textsuperscript{MUT} N=28
Monosomy 7 N=54

EWOG-MDS International N=108
GATA2\textsuperscript{MUT} N=29
Monosomy 7 N=46

Monosomy 7 N=100
GATA2\textsuperscript{MUT} N=57
Mono 7 GATA2\textsuperscript{MUT} N=37
Monosomy 7 (N=100)

- GATA2\textsuperscript{WT} 63%
- GATA2\textsuperscript{mut} 37%

Trisomy 8 (N=32)

- GATA2\textsuperscript{WT} 84%
- GATA2\textsuperscript{mut} 16%

A

B

Age at diagnosis within monosomy 7

- GATA2\textsuperscript{WT} N=63
- GATA2\textsuperscript{mut} N=37

- 4.2 years
- 12.5 years

P < .01

C

Proportion of GATA2\textsuperscript{mut} within monosomy 7

- 0-<6: 7%
- 6-<12: 48%
- 12-19: 72%

Age (years)
Figure 3

A

B

C

TRUNCATING frameshift > stop gain > splice site 53%

MISSENSE 33%

NONCODING 10.5%

OTHER 3.5%

Noncoding variation

Missense variation

Nonsense variation

Frameshift variation

Splicing variation

Inframe deletion

Whole gene deletion

Not previously reported

Reported in COSMIC
Figure 4. Kaplan-Meier survival curves for overall survival (OS) according to (A) MDS subtypes and GATA2 mutational status in 426 children and adolescents consecutively diagnosed with primary MDS, and (B) GATA2 mutational status in 100 children and adolescents with monosomy 7. n.s., not significant; N, Patients under risk; E, events.
Kaplan-Meier survival curves after HSCT in 91 patients with monosomy 7 according to GATA2 mutational status, for (A) overall survival (OS) (B) event free survival (EFS), (C) cumulative relapse incidence, and (D) transplant related mortality (TRM).

For personal use only.on October 31, 2017. by guest
www.bloodjournal.orgFrom
Prevalence, clinical characteristics and prognosis of GATA2-related myelodysplastic syndromes (MDS) in children and adolescents


Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.