Loss of B cells and their precursors is the most constant feature of GATA-2 deficiency in childhood myelodysplastic syndrome

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Haematologica 2016 [Epub ahead of print]

doi:10.3324/haematol.2015.137711

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Loss of B cells and their precursors is the most constant feature of GATA-2 deficiency in childhood myelodysplastic syndrome

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Running heads: GATA-2 deficiency in childhood MDS

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Word count:
Abstract 200
Main text 3272
Number of figures: 4
Number of tables: 3
Supplemental files: 1
Acknowledgements
This work was a main result of grant NT14534-3. Z.Z. was supported by RVO-VFN64165/2012, J.T was supported by P302/12/G101, M.N. was supported by GAUK 802214 and UNCE 204012. This work was also supported by the project for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic) and Ministry of Education, Youth and Sports NPU I nr. LO1604.
The authors would like to thank Iveta Janotova for data management; Pavel Semerak, Pavla Luknarova and Daniel Thurner for processing flow cytometry samples, Jan Stuchly for consulting statistics and the Czech Pediatric Hematology Group for collaboration (Doctors Sterba, Timr, Mihal, Prochazkova, Blazek and Hak).

Abstract
GATA-2 deficiency was recently described as common cause of overlapping syndromes of immunodeficiency, lymphedema, familiar myelodysplastic syndrome or acute myeloid leukemia. The aim of our study was to analyze bone marrow and peripheral blood samples of children with myelodysplastic syndrome or aplastic anemia to define prevalence of GATA2 mutation and to assess whether mutation in GATA-2 transcription factor exhibit specific immunophenotypic features. The prevalence of a GATA2 mutation in a consecutively diagnosed cohort of children was 14% in advanced forms of myelodysplastic syndrome (refractory anemia with excess blasts, refractory anemia with excess blasts in transformation and myelodysplasia-related acute myeloid leukemia), 17% in refractory cytopenia of childhood and 0% in aplastic anemia. In GATA-2-deficient cases we found the most profound B cell lymphopenia including its progenitors in blood and bone marrow, which correlated with significantly diminished intronRSS-Kde recombination excision circles in comparison to other myelodysplastic syndrome/aplastic anemia cases. The other typical features of GATA-2 deficiency (monocytopenia and natural killer cell lymphopenia) were less discriminative. In conclusion, we suggest screening for GATA2 mutations in pediatric myelodysplastic syndrome, preferentially in patients with impaired B cell homeostasis in bone marrow and peripheral blood (low number of progenitors, intronRSS-Kde recombination excision circles and naive cells).
Introduction
Myelodysplastic syndrome (MDS) is a rare disease of childhood with an approximate frequency of 0.8 to 1.8 per million children\(^1\). The most common subtype of MDS is refractory cytopenia of childhood (RCC), which represents a distinct category that was introduced as a provisional entity in a WHO classification 2008\(^2\). Aplastic anemia (AA) shares several clinical and laboratory features with RCC, and histopathological assessment is nowadays a key method for the separation of the two diseases\(^3\). Advanced MDS in children can be separated into three categories: refractory anemia with excess blasts (RAEB), RAEB in transformation (RAEB-t) or myelodysplasia related acute myeloid leukemia (MDR-AML)\(^4\). In some children, MDS or hypoplastic bone marrow failure is associated with an underlying genetic predisposition (e.g., Fanconi anemia, dyskeratosis congenita or Shwachman-Diamond syndrome)\(^5\).

A mutation in the \textit{GATA2} gene, which encodes the transcription factor GATA-2, was recently found by whole genome sequencing\(^6\)\(^7\) or by candidate approaches\(^8\)\(^9\) as a common cause of several overlapping syndromes: familial MDS/acute myeloid leukemia (AML); dendritic cell, monocyte, B and NK lymphoid (DCML) deficiency; mycobacterial infections and monocytopenia (MonoMAC); and hereditary lymphedema (Emberger syndrome)\(^6\)\(^7\). Several abnormalities identifiable by flow cytometry (FC) in peripheral blood (PB) are known to be present in patients with \textit{GATA2} mutations: a decreased number of B cells, NK cells, monocytes and dendritic cells\(^10\)\(^11\); plasma cells with an aberrant immunophenotype in bone marrow (BM); clonal T large granular lymphocyte (LGL) proliferation; and aberrant maturation patterns of granulocytic lineage\(^10\)\(^12\). MDS manifests in GATA-2-deficient patients earlier than in the general population\(^11\). A \textit{GATA2} mutation in pediatric non-familial MDS patients was found in 16\% of patients with aberrant karyotype (monosomy 7)\(^13\).

FC is recognized as an important diagnostic method especially in adult forms of MDS\(^14\)\(^15\). In children the amount of FC abnormalities in comparison to adults especially in RCC is often limited\(^17\). Myeloid compartment is severely reduced in both RCC and AA in comparison to healthy controls, but in AA the reduction is more pronounced\(^18\). All Czech patients with suspected MDS and AA have undergone trephine biopsy analysis by one of two expert pathologists since 2005, and BM aspirates were always analyzed in parallel using FC when material is available. We also analyzed level of intronRSS-Kde recombination excision circles (KRECs) in PB and BM to assess B cell production in children with MDS and AA.

The aims of our study were two-fold. First aim was to define prevalence of \textit{GATA2} mutation in nation-wide pediatric cohort of MDS/AA patients. Second aim was to identify FC profile characteristic for GATA-2-deficient patients.

Methods

Patients
Patients entered the study after their parents or guardians signed informed consent and the institutional ethics committee approved the study. Patients with RCC and AA were analyzed between 2005 and 2014, and all patients were analyzed histopathologically. Non-RCC (12 RAEB, 5 RAEB-t, 3 MDR-AML) patients were analyzed in period 1998 – 2014. Only the patients with available material for screening of GATA2 mutation entered the study (three additional AA patients were analyzed using FC during the study period, but no material was available for GATA2 mutation screening, and neither FC nor GATA2 screening was performed in one RCC patient and no material for GATA2 mutation screening was available in 3 non-RCC patients).

The prevalence of GATA2 mutation was analyzed among Czech pediatric primary MDS/AA patients (RCC (n=30), AA (n=38), non-RCC (n=22)). The flow chart describing cohort of patients is in Online Supplementary Appendix.

We used residual material from grafts for stem cell transplantation and samples taken for infiltration assessments of non-hematopoietic tumors as control BM samples (n=35). All control samples were obtained from individuals under 20 years of age (median 4.6y, range 0.01 - 19.3). Only control samples with no tumor cell infiltration as assessed by morphology entered the study.

**Diagnostic criteria**

Diagnosis was established according WHO classification (2008)\(^2\).

The distinction between RCC and AA was based on histopathological criteria and cytogenetic findings (both summarized in Online Supplementary Appendix). Patients with cytogenetic aberration before start of the treatment were classified as RCC regardless of the histopathological picture. Two patients were classified as RCC without histopathological dysplasia: one RCC GATA2 wild type patient with familiar history of MDS had simultaneous monosomy 7 and trisomy 8, and one GATA-2-deficient patient had monosomy 7.

Further details on diagnostics including flow cytometry, cytogenetics and DNA isolation may be found in the Online Supplementary Appendix.

**GATA2 sequencing**

GATA2 mutation status was investigated in all MDS/AA patients with available material. Genomic DNA was extracted from BM or PB samples. The entire coding region of GATA2 and an intronic enhancer region 3’ to exon 6 were amplified using genomic PCR. Further details may be found in the Online Supplementary Appendix.

**KREC/TREC detection**

Albumin gene level was quantitatively detected in isolated samples using qPCR, and a standard dilution series was derived from human genomic DNA with a known starting concentration (Roche, Basel, Switzerland). The levels of T cell receptor excision circle (TREC) and KREC signal joints were
assessed separately using serial dilutions of cloned plasmid standards as previously described\textsuperscript{19–21}. The results were subsequently recalculated to albumin gene levels and expressed as the number of TREC (KREC) copies per 1 µg of DNA. Thus, the final KREC (TREC) levels in unsorted populations serve as surrogate marker of developing B (T) lymphocytes, irrespective of their proliferation history\textsuperscript{22}. The detection limit was 25 copies/µg DNA. The controls for KREC and TREC analyses in PB consisted of 87 samples (median age, 8 y; range, 0-18 y) and 5 samples from BM (median age, 10.1 y; range, 5.2-14.2 y). The control groups for PB and dried blood spot were included in a previous study\textsuperscript{21}.

Statistics
Details may be found in the Online Supplementary Appendix.

Results

Prevalence of GATA-2 deficiency in pediatric MDS/AA
We investigated the prevalence of GATA-2 deficiency in samples taken from Czech pediatric patients under 18 years of age who developed MDS or AA. Eight of 90 pediatric MDS/AA patients had a GATA2 mutation. Three of these patients were diagnosed with non-RCC and 5 patients were diagnosed with RCC (Supplementary figure 1). The prevalence of a GATA2 mutation was 17%, 14% and 0% within RCC, non-RCC, and AA groups, respectively. The prevalence of a GATA2 mutation in patients with cytogenetic aberrations was 41% and 17% in patients with monosomy 7 and trisomy 8, respectively.

Bone marrow histology of GATA-2 deficient patients
GATA-2-deficient RCC patients did not differ in histopathology from other RCC patients, similarly no difference between GATA2 mutated patients with advanced MDS in comparison to other advanced patients was observed. All bone marrow samples from RCC patients including GATA-2-deficient in our study were hypocellular. There was only one patient (UPN3 with Emberger syndrome) within GATA-2-deficient group with higher degree of fibrosis (MF-2).

B cell compartment composition and production of B cells exhibit distinct features, especially in GATA-2-deficient patients
The proportion of B cells in BM in our control group inversely correlated with age (Supplementary Figure 2), which is in line with previously published data\textsuperscript{23,24}. The lowest proportion of B cells was present in GATA-2-deficient patients (Figure 1, Table 3). The highest proportion of B cells was present in the AA group, which may be explained by a severe reduction in the myeloid compartment and relative lymphocytosis. The B cell compartment in AA is composed primarily of mature B cells. B cell progenitors (defined as CD19\textsuperscript{pos}10\textsuperscript{pos}34\textsuperscript{pos}) out of all cells were significantly lower in all disease groups compared with controls, and the proportion was lowest in GATA-2-deficient patients (Figure 1, Table 3). There was no significant difference between AA and RCC in the percentage of B cell progenitors out of all cells. The highest proportion of plasma cells (CD19\textsuperscript{pos}10\textsuperscript{neg}20\textsuperscript{neg}) in B cells was observed in GATA-2-deficient patients (Figure 1, Table 3).
B cell subsets were analyzed in PB using the lineage-defining marker CD19 in combination with CD27, IgM and IgD. A decrease in naïve B cells was observed in 7 of 9 patients with GATA2 mutations (range, 15-64%; median, 32%; normal range, 47.3-82.5% for age >5 years\(^2\); Figure 3A). A decreased percentage of PB B cells was present in 10 of 12 patients (Figure 3A). A normal percentage of B cells was observed in one RCC patient (UPN3), and one value out of three was below the normal range in another RCC patient (UPN6; Figure 3A).

The level of KREC signal joints, which correlates with de-novo production of B cells, was examined in BM and PB\(^2\). The lowest KREC levels were observed in GATA-2-deficient patients in BM and PB (range BM, 25-118, median, 25; range PB, 25-146, median, 25; Figures 1 and 2; Table 3). Very low levels of KRECs together with a decrease in B cell progenitors and proportional increase in plasma cells in BM indicate a defect in B cell production in GATA-2-deficient patients. We identified three RCC patients with almost no KRECs in BM and PB and no GATA2 mutation (Figures 1 and 2). Notably, one of these patients had a family history of MDS; her mother underwent SCT for MDS RAEB. We also analyzed available newborn blood spots (Guthrie cards), which were used for the neonatal screening of metabolic disorders, from four GATA-2-deficient patients. We observed normal KREC levels at birth in three patients (UPN1, UPN6, UPN8), but there were no KRECs at birth in another patient (UPN11) who exhibited the earliest MDS RAEB manifestation in our GATA-2-deficient cohort (Figure 2, Table 2).

Patient UPN9 with a GATA2 intronic mutation progressed rapidly to AML within three months after initial MDS RAEB diagnosis. AML blasts were immunophenotypically characterized by the coexpression of progenitor markers CD34 and CD117 and myeloid marker CD33. Simultaneously, a subpopulation of CD34\(^{pos}\) blasts demonstrated clear B cell differentiation by CD19, CD10 and CD20 markers (Supplemental Figure 3). This uncommon simultaneous presence of AML and BCP ALL blasts was so far not reported in GATA-2-deficient patients.

**T cells in GATA-2-deficient patients are proportionally increased in bone marrow and peripheral blood**

There was no correlation between the percentage of CD3\(^{pos}\) T cells in BM and age (Supplementary Figure 2). We found a significantly higher percentage of CD3\(^{pos}\), CD4\(^{pos}\) and CD8\(^{pos}\) T cells in BM of GATA-2-deficient patients compared with healthy controls (Table 3). Activation of T cells measured by HLA DR expression was significantly lower in GATA-2-deficient patients compared to healthy controls. All disease groups, including GATA-2-deficient patients, exhibited significantly higher amounts of CD3\(^{pos}\)16,56\(^{pos}\) cells compared with healthy controls (Table 3).

We observed an increased percentage of T cells also in PB in GATA-2-deficient patients.

**The progenitor compartment is severely reduced in AA**
The percentage of CD34\(^{pos}\), CD117\(^{pos}\) and CD34\(^{pos}\)19\(^{pos}\)10\(^{pos}\) progenitors in healthy pediatric controls inversely correlated with age (p<0.05; Supplementary Figure 2), as previously published\(^{23,24}\). As expected, AA presented with a lower total progenitor (assessed as CD34\(^{pos}\) or CD117\(^{pos}\)) frequency than RCC or GATA-2 deficiency. In contrast, immature B cell progenitors CD34\(^{pos}\)19\(^{pos}\)10\(^{pos}\) were lower in GATA-2 deficiency compared to AA or RCC subsets. All progenitors (assessed as CD34\(^{pos}\), CD117\(^{pos}\) or CD34\(^{pos}\)19\(^{pos}\)10\(^{pos}\) cells) were decreased in all three conditions (AA, RCC and GATA-2 deficiency) compared with controls. However, neither CD34 nor CD117 alone can be used for the diagnostic discrimination between RCC and AA because of the substantial overlap.

**Myeloid populations and NK cells in GATA-2 deficiency**

Unexpectedly, the analysis of BM monocytes revealed that the only group different from controls was AA presenting with BM monocytopenia (Figure 1, Table 3). In PB, monocytopenia is often regarded as one of the hallmark of GATA-2 deficiency\(^{10}\). Although we did observe absolute monocytopenia at least in some specimens of 10 of 11 GATA-2 deficient patients, majority of the patients show monocytopenia only in less than half of the investigated periods (Supplementary figure 4). A stable decrease in the percentage and absolute count of monocytes was present in only 2 patients, who both suffered from severe lung disease (Figure 3, Supplementary figure 4). One patient (UPN7) was recently described as exhibiting diffuse parenchymal lung disease as the first manifestation of GATA-2 deficiency\(^{26}\).

The granulocytic compartment in BM generally contains neutrophils; eosinophils and basophils are only minor subpopulations in normal BM. We focused on the evaluation of the total percentage of granulocytes in BM. The percentage of granulocytes in GATA-2-deficient patients did not differ from controls (Table 3), but we frequently found aberrancies in maturation as detected by CD16 and CD13 expression in this group. We observed a complete absence of CD16 on all forms of granulocytes in one of six patients with GATA-2 deficiency analyzed. We observed a disturbed maturation profile with an accumulation of CD16\(^{neg}\)13\(^{neg}\) granulocytes (43\%) and a reduction of the mature forms CD16\(^{pos}\)13\(^{pos}\) (16\%) in one patient. This result is consistent with an earlier report\(^{10}\). Non-RCC and AA patients presented with fewer granulocytes than the remaining cohorts, including controls (Table 3). In PB, neutropenia was frequently present in GATA-2-deficient patients (Figure 3B), and at least some of the absolute neutrophil count (ANC) values were below 1x10^9/L in all but one patient (UPN7).

NK lymphopenia was present in half of the GATA-2-deficient cases (Figure 3B).

**B lymphopenia is more specific and sensitive parameter for discriminating pediatric patients with GATA-2 deficiency**

To determine which parameters are the best for identifying GATA-2-deficient patients among patients with MDS and AA, we performed ROC curves analysis comparing peripheral blood monocytes, B cells and natural killer (NK) cells (absolute and relative counts). Higher sensitivity and specificity can
be reached using either relative or absolute counts of B cells compared to both monocytes and NK cells (Figure 4). Lower-than-physiological counts of B cells were found in 10 of 12 GATA-2-deficient patients (Figure 3A), and decreased KREC levels were found in all GATA-2-deficient patients (Figure 1A and 2A).

**Discussion**

Mutations in the transcription factor GATA-2 leading to haploinsufficiency is a frequent germline genetic aberration found in pediatric MDS. We compared flow cytometry results, KREC and TREC levels in pediatric patients with MDS and AA to pediatric control samples with focus on GATA2 mutation. The most typical feature in GATA-2-deficient patients is the profound reduction of B cells and their progenitors in BM and PB. A decreased production of B cells was also documented by the low levels of KRECs in BM and PB. KREC levels on Guthrie cards taken for the neonatal screening of inherited disorders of metabolism revealed normal levels in some of the patients, which indicates the normal production of immature B cells prenatally. Three RCC patients without GATA2 mutation and mostly absent KRECs were highly suspected of having an unknown underlying genetic aberration that was responsible for MDS development, but so far we could not identify common genetic aberration. Nevertheless, significant decrease of KRECs among GATA-2-deficient patients in BM and in PB (Figures 1A and 2A) indicates usefulness of KREC in the diagnostic workup, possibly as a genetic prescreening. Peripheral B cell subpopulations in GATA-2-deficient patients shift towards mature memory subsets. The production of B cells was defective in GATA-2-deficient patients, but immunoglobulin levels were largely normal in most of our patients (data not shown). Immunoglobulin substitution is rarely required in GATA-2-deficient patients. B cell progenitors defined as CD19pos10pos34pos were also significantly reduced in all other disease groups (RCC, non-RCC and AA without GATA2 mutations) compared with controls, but the reduction of these cells in GATA-2 deficiency was even more profound (p<0.05).

The other features known to be associated with GATA-2 deficiency, i.e. monocytopenia and NK lymphopenia were less discriminative in our cohort. The most profound BM monocytopenia in the AA group contrasts a recently published study by Ganapathi et al., who found the lowest amount of monocytes in GATA-2-deficient MDS patients, lower than AA. In our GATA-2-deficient cohort, the most profound monocytopenia in BM and PB was found in two patients with immunodeficiency and severe lung problems. Two patients with advanced form of MDS presented with monocytosis. This result is consistent with previous observations that GATA-2-deficient patients whose disease progresses into advanced MDS may exhibit monocytosis. Recently published study by Wlodarski et al. also found tendency to higher monocytes in pediatric MDS cases with GATA2 mutation (the patients partially overlap with our study). Difference in results between our cohort and cohort published by Ganapathi et al. might be explained by lower incidence of advanced MDS cases (RAEB/RAEB-t) (3 out of 52 versus 3 out of 12 in our study, chi square p=0.04) and by lower
incidence of monosomy 7 (4 out of 48 versus 4 out of 12, chi square p=0.01). Study by Pasqet et al. found significant monocytopenia and analyzed blood counts before MDS/AML phase. Study by Spinner et al. also identified significant monocytopenia in GATA-2 deficient patients and included predominantly immunodeficient cases (study is overlapping with the study by Ganapathi et al., which selected patients with MDS/AML). Median age of our study was lower in comparison to Ganapathi et al. In our pediatric cohort, both PB relative and absolute B cell lymphopenia were more specific and sensitive parameters in comparison with absolute and relative monocytopenia (Figure 4).

Neutropenia is a frequent finding in GATA-2-deficient patients, and it contributes to their immunodeficiency symptoms\textsuperscript{29,30}. Study by Pasquet et al. identified GATA-2-deficient patients in the cohort based on neutropenia\textsuperscript{30}. Nearly normal ANC values were present only in UPN5 and UPN7, which was likely related to long-term corticosteroids use due to lung disease.

Relative T cell counts were increased in GATA-2-deficient patients in PB and BM. Low levels of TREC\textsuperscript{s} in GATA-2 may be explained by the decreased production and/or by expansion of mature T cells during infections. In contrast to previously published GATA-2-deficient cohorts, we did not observe an expansion of T large granular lymphocytes in BM in any of our patients. T cell deficiency, namely CD4 lymphopenia contributes to immunodeficiency in GATA2-mutated patients\textsuperscript{28}. We observed CD4 lymphopenia below 0.4.10\textsuperscript{9}/L in 3 patients, 2 of them were followed for severe lung disease.

We assessed the prevalence of GATA-2 deficiency in Czech children with MDS or AA. A GATA2 mutation was exclusively identified in patients with RCC (17\%) or advanced MDS (non-RCC; 14\%). AA and RCC generally exhibit similar clinical and laboratory features. Some of the flow cytometric differences between RCC and AA that we observed in the past were driven by the GATA-2-deficient group (namely B cell lymphopenia)\textsuperscript{31,32}. Differences between the overlapping categories RCC and AA are frequently discussed, especially in patients with hypocellular BM and without adverse cytogenetics. The separation of patients into two categories seems to be less relevant because immunosuppressive therapy is indicated for both disease groups\textsuperscript{33–35}, and there is no difference in prognosis nor in probability of progression into advanced MDS\textsuperscript{35}. We observed significant differences between RCC and AA in a limited number of parameters in BM (decreased in AA: CD34\textsuperscript{pos}, CD117\textsuperscript{pos}, granulocytes and erythroid precursors, increased in AA: CD19\textsuperscript{pos} and lymphocytes).

In summary, we found that the disturbances in the B cell compartment were the strongest distinguishing biological feature of GATA-2 deficiency in childhood MDS, in contrast to other recently published factors, such as monocytopenia, which were less common and unspecific in our study. The finding of decreased B cell numbers in BM and PB, and most specifically, low levels of B cell progenitors in BM together with very low or absent KRECs in BM and PB can identify
appropriate candidates for GATA2 mutation testing in pediatric MDS patients. Information on mutational status in the family is of importance not only when matched family donor is considered for the transplantation. GATA-2 deficiency does not only predispose to cancer but also an immunodeficient condition in which close immunological monitoring with careful treatment of infections might prevent organ damage as we observed in UPN7.

Conflicts of Interest
All authors have nothing to disclose.

Supplemental data
The online version of this article contains the Supplementary Appendix.
References


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*Table 1. Disease group characteristics.* Monosomy 7 or trisomy 8 was categorized as positive if present at any time point during follow up. One patient (UPN2) with trisomy 8 and one patient (UPN7) developed cytogenetic abnormality and RCC in adulthood. M, male; F, female.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>M/F ratio</th>
<th>age years (median, range)</th>
<th>follow up since first symptoms to therapy weeks (median, range)</th>
<th>monosomy 7</th>
<th>trisomy 8</th>
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<tr>
<td>GATA-2</td>
<td>12</td>
<td>5.0</td>
<td>16.3 (4.3 - 21.3)</td>
<td>125.4 (3.1 -1046.1)</td>
<td>8/12</td>
<td>2/12</td>
</tr>
<tr>
<td>non RCC</td>
<td>20</td>
<td>2.3</td>
<td>8.3 (0.79 - 17.8)</td>
<td>7.1 (0.43-66.1)</td>
<td>4/20</td>
<td>1/20</td>
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<tr>
<td>RCC</td>
<td>27</td>
<td>1.1</td>
<td>7.4 (3.7-19)</td>
<td>9.9 (0.71-422.9)</td>
<td>6/27</td>
<td>4/27</td>
</tr>
<tr>
<td>AA</td>
<td>39</td>
<td>1.3</td>
<td>9.9 (1.1 - 17.9)</td>
<td>3.9 (0.71-74)</td>
<td>0/39</td>
<td>0/39</td>
</tr>
<tr>
<td>controls flow cytometry</td>
<td>35</td>
<td>1.2</td>
<td>4.6 (0.01 - 19.3)</td>
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</table>
### Table 2. Overview of clinical and laboratory findings in GATA-2-deficient patients.

Exons of GATA2 are numbered according to their 5' to 3' order within the GATA2 gene (NCBI RefSeqGene NG_029334.1). FS, family search; ID, immunodeficiency; SCT, stem cell transplantation; MUD, matched unrelated donor; MSD, matched sibling donor.

<table>
<thead>
<tr>
<th>UPN</th>
<th>Familial/Sporadic</th>
<th>Primary manifestation</th>
<th>Age (MDS dg)</th>
<th>Mutation Exon/Intron</th>
<th>Mutation cDNA</th>
<th>Mutation consequence</th>
<th>clinical symptoms</th>
<th>Cytogenetics (any aberrancy identified during follow up)</th>
<th>treatment</th>
<th>follow-up</th>
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<tbody>
<tr>
<td>UPN1</td>
<td>RCC Sporadic</td>
<td>MDS-RCC</td>
<td>11y</td>
<td>Exon 4</td>
<td>c.222_229+6</td>
<td>frameshift</td>
<td>Cafe-au-lait, hydrops</td>
<td>monosomy 7, trisomy 8</td>
<td>MUD SCT</td>
<td>15 years, healthy</td>
</tr>
<tr>
<td>UPN2</td>
<td>ID/RCC in adulthood</td>
<td>Sporadic Imunodeficiency</td>
<td>21y</td>
<td>Exon 8</td>
<td>c.1187G&gt;A</td>
<td>substitution</td>
<td>recurrent urinary tract infections, skin herpetic infections, bronchial asthma, aphthous stomatitis, cholecytolithiasis</td>
<td>trisomy 8</td>
<td>MUD SCT</td>
<td>28 years, healthy</td>
</tr>
<tr>
<td>UPN3</td>
<td>Sporadic</td>
<td>MDS RCC</td>
<td>12y</td>
<td>Exon 5</td>
<td>c.391_395del17</td>
<td>frameshift</td>
<td>vesicourethral reflux, congenital hydronephrosis, hearing disorder, lymphedema</td>
<td>monosomy 7</td>
<td>MUD SCT</td>
<td>14 years, 2 relapses, died of progressive disease [age 14y]</td>
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<td>UPN4</td>
<td>RCC Sporadic</td>
<td>MDS RCC</td>
<td>17y</td>
<td>Intron 6</td>
<td>c.1017+572C&gt;T</td>
<td>intronic mutation</td>
<td>chronic active EBV infection</td>
<td>monosomy 7</td>
<td>MUD SCT</td>
<td>26 years, healthy</td>
</tr>
<tr>
<td>UPN5</td>
<td>ID/RCC</td>
<td>Sporadic Immunodeficiency</td>
<td>17y</td>
<td>Exon 7</td>
<td>c.1084C&gt;T</td>
<td>stop gain</td>
<td>chronic bronchitis, sinusitis, interstitial lung disease, multiple warts</td>
<td>not found</td>
<td>corticosteroids</td>
<td>died of lung insufficiency, CMV pneumonitis [age 21y]</td>
</tr>
<tr>
<td>UPN6</td>
<td>RCC Sporadic</td>
<td>MDS RCC</td>
<td>17y</td>
<td>Intron 6</td>
<td>c.1017+572C&gt;T</td>
<td>intronic mutation</td>
<td>recurrent infections</td>
<td>monosomy 7</td>
<td>MUD SCT</td>
<td>25 years, healthy</td>
</tr>
<tr>
<td>UPN7</td>
<td>ID/RCMD in adulthood</td>
<td>Sporadic Immunodeficiency</td>
<td>17y</td>
<td>Exon 7</td>
<td>c.1081C&gt;T</td>
<td>substitution</td>
<td>Decreased physical performance, 17y weight loss, decreased physical performance, attention deficit and hyperactive disorder</td>
<td>monosomy 7</td>
<td>recently ongoing MUD SCT</td>
<td>20 years, alive</td>
</tr>
<tr>
<td>UPN8</td>
<td>FS Sporadic</td>
<td>Identified through family search</td>
<td>17y</td>
<td>Exon 7</td>
<td>c.1081C&gt;T</td>
<td>substitution</td>
<td>asymptomatic carrier, brother of UPN7, leukopenia and thrombocytopenia</td>
<td>not found</td>
<td>watch and wait</td>
<td>17 years, healthy</td>
</tr>
<tr>
<td>UPN9</td>
<td>RAEB/AML Sporadic</td>
<td>MDS RAEB/AML</td>
<td>17y</td>
<td>Intron 6</td>
<td>c.1017+572C&gt;T</td>
<td>intronic mutation</td>
<td>orthostatic collapse</td>
<td>monosomy 7</td>
<td>AML therapy</td>
<td>died of MDS progression, mycotic lung infection [age 18y]</td>
</tr>
<tr>
<td>UPN10</td>
<td>RCC/RAEB-t Sporadic</td>
<td>MDS RCC/RAEB-t</td>
<td>16y</td>
<td>Exon 7</td>
<td>c.1066_1065+9delB0</td>
<td>deletion</td>
<td>recurrent urinary tract infections, 15y weight loss, decreased physical performance, attention deficit and hyperactive disorder</td>
<td>monosomy 7</td>
<td>MUD SCT</td>
<td>25 years, healthy</td>
</tr>
<tr>
<td>UPN11</td>
<td>RAEB Sporadic</td>
<td>MDS RAEB</td>
<td>4.4y</td>
<td>Exon 7</td>
<td>c.1035_1038dupGGC</td>
<td>frameshift</td>
<td>recurrent UTI, bronchitis, aphthous stomatitis, bronchial asthma, hypoaesthesia, vesicourethral reflux, speech disorder, Asperger syndrome, nail dysplasia</td>
<td>monosomy 7</td>
<td>MUD SCT</td>
<td>13 years, healthy</td>
</tr>
<tr>
<td>UPN12</td>
<td>ID/RCC Sporadic</td>
<td>MDS RCC</td>
<td>13y</td>
<td>Exon 7</td>
<td>c.1128C&gt;G</td>
<td>stop gain</td>
<td>lymphedema, deafness, recurrent infections</td>
<td>not found</td>
<td>MUD SCT</td>
<td>14 years, healthy</td>
</tr>
</tbody>
</table>
Table 3. Summary of the analyzed BM parameters.
The values are defined as medians with range in brackets. * indicates significantly different level (p<0.05, Mann-Whitney test) of the respective parameter compared to control samples, o in non RCC, RCC and AA means significantly different from GATA-2 by Mann-Whitney test (p<0.05).

<table>
<thead>
<tr>
<th>BONE MARROW POPULATIONS</th>
<th>GATA-2</th>
<th>non RCC</th>
<th>RCC</th>
<th>AA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell populations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulocytes (%)</td>
<td>49 (26 - 72)</td>
<td>35 (7 - 79)*</td>
<td>48 (1.3 - 82)</td>
<td>26 (1.3 - 85)**</td>
<td>51 (19 - 83)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>26 (7.3 - 48)</td>
<td>14 (2.8 - 76)</td>
<td>33 (9.1 - 78)</td>
<td>51 (6.5 - 90)**</td>
<td>27 (8.5 - 66)</td>
</tr>
<tr>
<td>Monocytes CD14pos (%)</td>
<td>3.3 (0.27 - 23)</td>
<td>3.6 (0.03 - 26)</td>
<td>4.7 (0.05 - 9.6)</td>
<td>3.5 (0.3 - 11)*</td>
<td>4.8 (2.9 - 12)</td>
</tr>
<tr>
<td>Erythroid cells CD45**7<em>7</em> (%)</td>
<td>4.5 (1.5 - 9.5)</td>
<td>3.6 (0.58 - 22)</td>
<td>2.4 (0.39 - 15)</td>
<td>1.3 (0.1 - 9.5)**</td>
<td>3.6 (0.93 - 11)</td>
</tr>
<tr>
<td><strong>Progenitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progenitors CD34pos (%)</td>
<td>0.49 (0.1 - 19)</td>
<td>4.9 (0.13 - 32)</td>
<td>0.49 (0.08 - 2.9)*</td>
<td>0.2 (0.03 - 1.3)**</td>
<td>2.1 (1 - 8.6)</td>
</tr>
<tr>
<td>Progenitors CD117pos (%)</td>
<td>1.4 (0.12 - 19)</td>
<td>8.3 (1.1 - 25)**</td>
<td>0.55 (0.13 - 2.3)**</td>
<td>0.23 (0.02 - 1.6)**</td>
<td>1.4 (0.77 - 4.1)</td>
</tr>
<tr>
<td><strong>B lineage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD19pos total B cells (%)</td>
<td>1.4 (0.19 - 5.2)*</td>
<td>3.7 (0.1 - 21)**</td>
<td>6.6 (1.4 - 17)**</td>
<td>10 (0.9 - 40)</td>
<td>11 (3 - 47)</td>
</tr>
<tr>
<td>Progenitors CD34pos10pos19pos (%)</td>
<td>0.01 (0 - 0.04)*</td>
<td>0.04 (0 - 1.7)**</td>
<td>0.07 (0 - 1.7)**</td>
<td>0.03 (0 - 0.99)**</td>
<td>1 (0.22 - 5.3)</td>
</tr>
<tr>
<td>CD10pos out of CD19pos (%)</td>
<td>4.7 (1.2 - 46)*</td>
<td>13 (1.3 - 70)**</td>
<td>16 (1.1 - 97)**</td>
<td>10 (0.58 - 96)*</td>
<td>60 (30 - 95)</td>
</tr>
<tr>
<td>CD20pos10pos out of CD19pos (%)</td>
<td>75 (40 - 93)*</td>
<td>69 (8.5 - 90)*</td>
<td>79 (1.5 - 96)*</td>
<td>80 (3.1 - 97)*</td>
<td>36 (4.7 - 65)</td>
</tr>
<tr>
<td>Plasma cells CD10pos20pos out of B cells (%)</td>
<td>11 (3.1 - 48)*</td>
<td>8.4 (0.76 - 69)*</td>
<td>3.6 (0.02 - 22)**</td>
<td>2.3 (0.13 - 48)**</td>
<td>1.6 (0.24 - 4.6)</td>
</tr>
<tr>
<td>Plasma cells CD10pos20pos out of all cells (%)</td>
<td>0.11 (0.02 - 1.4)</td>
<td>0.34 (0.03 - 1.9)</td>
<td>0.19 (0.04 - 1.3)</td>
<td>0.35 (0.02 - 5)*</td>
<td>0.17 (0.03 - 0.45)</td>
</tr>
<tr>
<td>KREC (copies/ug DNA)</td>
<td>25 (25 - 118)*</td>
<td>371 (25 - 26071)**</td>
<td>7149 (23 - 123732)**</td>
<td>6655 (107 - 157298)</td>
<td>15875 (10226 - 81858)</td>
</tr>
<tr>
<td><strong>T lineage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3pos total T cells (%)</td>
<td>23 (7.3 - 42)*</td>
<td>10 (1.9 - 54)</td>
<td>23 (2.9 - 60)*</td>
<td>32 (2 - 70)*</td>
<td>11 (4.4 - 20)</td>
</tr>
<tr>
<td>CD3pos4pos T cells (%)</td>
<td>9.8 (1.1 - 20)*</td>
<td>5.4 (1.3 - 35)</td>
<td>9.4 (0.31 - 35)*</td>
<td>12 (0.22 - 38)*</td>
<td>5.4 (1.4 - 13)</td>
</tr>
<tr>
<td>CD3pos8pos T cells (%)</td>
<td>10 (3.9 - 24)*</td>
<td>4.3 (0.7 - 19)</td>
<td>9.7 (1 - 37)*</td>
<td>14 (1.4 - 37)*</td>
<td>4.2 (1.2 - 10)</td>
</tr>
<tr>
<td>CD4/8 ratio</td>
<td>0.75 (0.2 - 1.5)*</td>
<td>1.5 (0.44 - 2.4)**</td>
<td>0.97 (0.11 - 2.1)*</td>
<td>1 (0.12 - 2.3)</td>
<td>1.2 (0.52 - 3.4)</td>
</tr>
<tr>
<td>CD3pos16,56pos cells (%)</td>
<td>2.5 (0.5 - 8.4)*</td>
<td>1.3 (0.17 - 3.7)*</td>
<td>0.8 (0.06 - 5.1)**</td>
<td>0.81 (0.01 - 4.4)**</td>
<td>0.14 (0.03 - 2)</td>
</tr>
<tr>
<td>HLA DRpos out of CD3pos (%)</td>
<td>3.2 (1.4 - 8.9)*</td>
<td>5.3 (2.8 - 40)*</td>
<td>8.1 (0.79 - 87)*</td>
<td>11 (1.1 - 67)**</td>
<td>8.1 (2.1 - 20)</td>
</tr>
<tr>
<td>HLA DRpos out of CD3pos4pos (%)</td>
<td>2.6 (1.5 - 4.1)*</td>
<td>13 (2.4 - 35)*</td>
<td>5.1 (2 - 77)*</td>
<td>7.5 (1.7 - 41)*</td>
<td>6.2 (1.7 - 14)</td>
</tr>
<tr>
<td>HLA DRpos out of CD3pos8pos (%)</td>
<td>3.5 (1.5 - 9.2)*</td>
<td>30 (3.4 - 43)</td>
<td>29 (4.5 - 89)**</td>
<td>30 (5.8 - 82)**</td>
<td>12 (1.7 - 28)</td>
</tr>
<tr>
<td>TREC (copies/ug DNA)</td>
<td>2248 (241 - 15385)</td>
<td>4083 (230 - 11108)</td>
<td>6257 (25 - 61522)</td>
<td>12736 (278 - 75757)**</td>
<td>3123 (1599 - 8779)</td>
</tr>
<tr>
<td><strong>Other lymphoid populations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK cells (%)</td>
<td>1 (0.1 - 7.9)</td>
<td>3 (0.78 - 6.9)</td>
<td>1.3 (0.24 - 9.1)</td>
<td>2.4 (0.18 - 10)*</td>
<td>1.5 (0.34 - 6.8)</td>
</tr>
<tr>
<td><strong>DNA analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA G01 (%)</td>
<td>85 (74 - 94)</td>
<td>89 (67 - 98)</td>
<td>91 (77 - 96)**</td>
<td>93 (70 - 98)**</td>
<td>89 (75 - 92)</td>
</tr>
<tr>
<td>DNA SG2M (%)</td>
<td>9 (4.5 - 13)</td>
<td>9.7 (0.5 - 18)</td>
<td>6.7 (2.7 - 19)**</td>
<td>4.2 (1 - 15)**</td>
<td>8.1 (5.1 - 13)</td>
</tr>
</tbody>
</table>
Figure legends

**Figure 1.** Cell populations in bone marrow.
(A) B cell subpopulations and KRECs. (B) T cell subpopulations and TRECs. (C) NK cells. (D) Monocytes. (E) Progenitors. Braces indicate significant difference between the parameters using the non-parametric Mann-Whitney test (p<0.05). Black lines represent medians. A grey area indicates the range of control samples.

**Figure 2.** Cell populations in peripheral blood.
(A) B cells and KRECs. (B) T cells and TRECs. (C) NK cells. (D) Monocytes. Braces indicate significant differences between parameters using the non-parametric Mann-Whitney test (p<0.05). A gray area indicates the range of control samples. Absolute counts are shown. In relative counts, similar results were found except for relative T cells, which were increased in GATA-2-deficient compared to controls.

**Figure 3.** Follow-up peripheral blood samples of GATA-2-deficient patients.
Each column represents one patient. Black lines represent medians. A grey area indicates a normal range for the age category of most of the patients (either 12-18y for B cells, NK cell; >15y for monocytes and neutrophils; or >16y for class switched memory B cells and naive B cells). (A) B cells and subpopulations. (B) Monocytes, NK cells, neutrophils.

**Figure 4.** ROC curves for PB monocytes, B cells and NK cells in GATA-2-deficient patients in comparison with all other patients with AA and MDS.
Light green, relative B cell count; dark green, absolute B cell count; light red, relative monocyte count; dark red, absolute monocyte count, light blue, relative NK cell count; dark blue, absolute NK cell count.
Figure 1 Bone marrow

A Total B cells (%)

B Total T cells CD3pos (BM)

C NK cells CD3neg 19neg 16,56pos

D Monocytes CD14pos

E Progenitors (BM)
Figure 2 Peripheral blood

**A** B cells (PB)

- **Absolute count B cells (PB)**: Graph showing the absolute count of B cells in peripheral blood with different conditions.
- **KREC (PB)**: Graph showing the KREC levels in peripheral blood.
- **KREC (Guthrie)**: Graph showing the KREC levels in Guthrie samples.

**B** T cells (PB)

- **Absolute count T cells (PB)**: Graph showing the absolute count of T cells in peripheral blood with different conditions.
- **TREC (PB)**: Graph showing the TREC levels in peripheral blood.
- **TREC (Guthrie)**: Graph showing the TREC levels in Guthrie samples.

**C** NK cells (PB)

- **Absolute count NK cells (PB)**: Graph showing the absolute count of NK cells in peripheral blood with different conditions.

**D** Monocytes (PB)

- **Absolute count monocytes (PB)**: Graph showing the absolute count of monocytes in peripheral blood with different conditions.
Figure 3

**A B cells in GATA-2-deficient cases (PB)**

- Relative count GATA-2 B cells (PB)
- Absolute count GATA-2 B cells (PB)
- Naive B cells (PB) $CD19^{pos}27^{neg}IgD^{pos}$
- Class switched memory B cells (PB) $CD19^{pos}27^{pos}IgM^{neg}IgD^{neg}$

**A Monocytes, NK cells and neutrophils in GATA-2-deficient cases (PB)**

- Relative monocyte count
- Absolute monocyte count
- Relative count NK cells
- Absolute count NK cells
- Relative neutrophil count
- Absolute neutrophil count
Figure 4

ROC curve: monocytes, NK and B cells in PB

Sensitivity% vs. 100% - Specificity%

- NK abs
- NK rel
- Mono abs
- Mono rel
- B cells abs
- B cells rel
Summary of clinical findings in GATA2 non mutated patients

Clinical findings of GATA-2-deficient cases are summarized in table 2. We carefully reviewed clinical data of remaining patients during the study. Generally pediatric patients with primary MDS are suspect of having primary germline aberrancy associated with bone marrow failure/MDS. Among GATA-2-deficient patients 7 out of 12 were suspect of having primary genetic cause based on abnormalities outside hematopoietic system or clinical behavior (table 2).

Among patients with advanced MDS GATA2 wildtype we found 4 out of 20 patients with suspect germline aberrancy:

pt1: family history – father with thrombocytopenia, very young child – first symptoms already at age of 6 months, manifestation of advanced MDS at age of 10 months, stigmatization
pt2: skin lesions present at 6 months, xanthogranuloma at age of 1 year, MDS manifested at age of 4 years
pt3: young child diagnosed at age of 1 year, complex karyotype, manifested with unexpectedly severe acute GVHD after stem cell transplantation
pt4: urogenital abnormalities

Among RCC patients we found 5 patients out of 27 patients with suspect germline abnormality:

pt1: immunodeficiency
pt2: family history (mother after SCT for MDS RAEB)
pt3: stigmatization, nail dystrophy
pt4: cardiac abnormalities, stigmatization
pt5: suspect family history (cousin suffered from ALL)

No AA patient was suspect to have primary genetic abnormality based on reviewing of clinical data.

Diagnostic criteria

RCC is a provisional entity that is characterized by persistent cytopenia with dysplasia and <5% blasts in BM. All patients with suspected MDS or AA underwent trephine biopsy, and two pathologists (VC or GK) performed all analyses. The distinction between RCC and AA was based on the presence or absence of patchy left-shifted erythropoiesis as the primary differentiating parameter\(^1\). Patients with cytogenetic aberration were classified as MDS even if the dysplasia in histopathology was absent. The “Non-RCC” group consisted of patients with advanced forms of MDS (RAEB, RAEB-t and
myelodysplasia related (MDR)-AML). Classification of advanced forms of MDS was performed according Hasle et al. and the 2008 WHO classification as follows. RAEB was defined by the presence of 2-19% of blasts in PB and/or 5-19% of blasts in BM; RAEB-t had 20-30% of blasts in PB or BM and AML1/ETO and CBFb/MYH11 fusion genes were absent. MDR-AML was defined by MDS related cytogenetic abnormality or when multilineage dysplasia was morphologically evident and more than 30% of blasts was present by morphology.

Flow cytometry and definition of subpopulations

BM and PB samples were collected in EDTA-containing tubes and analyzed within 24 hours. Sample cellularity was assessed using flow cytometry via calculation from the sample flow rate (5 µl of sample diluted in 250 µl of ammonium chloride solution). Selected sample amounts corresponded to 100,000-200,000 acquired events per tube. Sample preparation consisted of a 15-min incubation with monoclonal antibodies (mAb) used at the sample-to-mAb volume ratios recommended by the manufacturers. Red blood cells were lysed during a 15-min incubation in ammonium chloride, followed by a 5-min centrifugation (500 g). The supernatant was discarded, phosphate-buffered saline (PBS) was added, and data acquisition was immediately begun. Cells were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA), DAKO Cyan (Dak, Glostrup, Denmark) or BD LSR II (BD Biosciences, San Jose, CA, USA), and the data were analyzed using FlowJo software (TreeStar, Ashland, Oregon, USA). The mAb combinations used in the study are described in Supplementary table 1. All populations were estimated as a percentage of nucleated cells defined using SYTO-16 or SYTO-41 nucleated dye (Thermo Fisher Scientific Inc., Waltham, MA, USA). The following populations were analyzed: monocytes (CD14pos45posSSCmed); granulocytes (CD45posSSChigh); lymphocytes (CD45posSSClow); red cells (CD45neg71brightSSClow); CD34pos and CD117pos precursors; B cells (CD19pos); T cells (CD3pos); and NK cells (CD16 or CD56pos3neg).

Supplemental table 1 describes the detailed composition of the panel. Analysis of monocyte count in peripheral blood was performed by hematological analyzer and/or microscope.

Cytogenetics

Cytogenetic and FISH analyses were performed prior to therapy according to standard protocols. BM cells were cultivated for 24h in RPMI 1640 medium with 10% fetal calf serum without mitogenic stimulation. Chromosomal preparations were made according to conventional techniques using colcemide, hypotonic treatment, fixation in methanol/acetic acid and Wright stain (G-banding). The karyotypes were characterized according to the International System of Human Cytogenetic Nomenclature. All patients were screened for monosomy 7 or deletion 7q and trisomy 8, using interphase fluorescence in-situ hybridization (I-FISH). All FISH assays were performed according to the manufacturer’s recommendations.
DNA isolation

DNA was isolated from 200 µl of fresh PB and BM or frozen cell pellets using a QIAamp DNA Blood Mini Kit or QIAamp DNA Blood Micro Kit (Qiagen GmbH, Hilden, Germany). A retrospective search for dried newborn blood spots (DBS, Guthrie cards) was performed after obtaining written informed consent from patients or their guardians in 4 GATA2-mutated patients (UPN1, UPN6, UPN8, and UPN11).

A circle with a diameter of 3.2 µm was cut from each DBS, and DNA was eluted at 99°C for 1 hour in a shaker (500 rpm) using 100 µl of Generation DNA Elution Solution (Qiagen GmbH, Hilden, Germany) supplemented with 100 µg/ml of yeast tRNA (Life Technologies, Carlsbad, CA, USA).

GATA2 sequencing

GATA2 mutation status was investigated in all MDS/AA patients with available material. Genomic DNA was extracted from BM or PB samples. The entire coding region of GATA2 and an intronic enhancer region 3’ to exon 6 were amplified using genomic PCR and directly sequenced using primers specified in Supplementary table 2. Sequences were visualized, aligned to the reference sequence and analyzed using BioEdit software. Available parents of all GATA2-mutated patients who agreed to the analysis were investigated to assess the familiar origin of the particular GATA2 mutations.

Statistics

Statistical analyses were performed using Statistica (StatSoft, Inc., Tulsa, OK, USA) and GraphPad (GraphPad Software, Inc., La Jolla, CA, USA). The results were considered significant when p values were less than 0.05.

Supplementary figures and tables

Supplementary figure 1.
Flow chart describing number of analyzed patients. Three patients assigned as “others” are GATA-2-deficient patients followed for immunodeficiency and changes in blood count (UNP2, UPN7 and UPN8). Two of these patients developed MDS in early adulthood (UPN2 and UPN7).

Supplementary figure 2.
Correlation of age and levels of assessed cell types in bone marrow and KREC and TREC in both BM and PB in patient groups and in controls.
Statistical dependence of age and each parameter was tested using the Spearman rank correlation coefficient. The R in the graph indicates a correlation coefficient in samples, which is shown only where p values are lower than 0.05. No significant correlation of age and respective parameter was found in GATA-2-deficient patients. The dots represent controls, and the larger shapes represent patient categories: triangle, GATA-2; square, RCC; rhombus, non RCC; inversed triangle, AA. The gray lines represent semi-log trends and are shown only when the Spearman rank correlation p value is lower than 0.05.

Supplementary figure 3.
Consecutive samples of UPN9 are shown in dot plots CD117 against side scatter (Ssc). In lower line B cell phenotype of blasts is shown by CD10 and CD20 expression. Graph shows percentage of different phenotype of blasts in bone marrow in consecutive samples.

Supplementary figure 4.
Absolute and relative monocyte counts in PB are shown in follow up samples of GATA-2-deficient patients. Grey lines represent normal values. Day 0 represents day of hematopoietic transplantation or death or last follow up.

Supplementary table 1.
Antibody panels and antibody clones used in our study are shown in Supplementary table 1.

Supplementary table 2.
Primer sequences used for PCR and sequencing and genomic coordinates of regions analyzed for mutational status.
Supplementary figure 1

Flow cytometry analysis
- 26 non RCC
  - 3 no material
  - 1 no material

34 RCC
- 3 no material

42 AA
- 3 no material

3 others
- 3 available (3GATA-2)

Prevalence analysis
- 22 national (19wt/3GATA-2)
- 30 national (25wt/5GATA-2)
- 38 national (wt)
Supplementary figure 3

*Development of B cell phenotype in blasts in GATA2-mutated patient. (UPN9)*

- **suspect aplastic anemia (08/2006)**: CD19 atypical blasts 10%
- **RAEB (10/2006)**: CD19 atypical blasts 18%
- **AML (12/2006)**: CD19 atypical blasts 38%
- **increase of BCP ALL blasts, decrease of CD117pos blasts (03/2007)**: CD19 atypical blasts 2.3%

- **Gate CD19**
- **basophils**
- **CD117+ blasts**

- **Month (dg=0)**
  - CD34%
  - CD117%
  - blasts total
  - CD19 atypical blasts

- **BCP blasts 4%**
- **BCP blasts 21%**

- **CD10**
- **CD20**
Supplementary figure 4

*Relative and absolute monocyte counts during the follow up of GATA-2-deficient patients*

Absolute monocyte count

<table>
<thead>
<tr>
<th>UPN1</th>
<th>Relative monocyte count</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPN2</td>
<td>Absolute monocyte count</td>
</tr>
<tr>
<td>UPN3</td>
<td>Relative monocyte count</td>
</tr>
<tr>
<td>UPN4</td>
<td>Absolute monocyte count</td>
</tr>
</tbody>
</table>
Absolute monocyte count

Relative monocyte count

UPN5

UPN6

UPN7

UPN8
### Supplementary table 1. Antibody panel and used antibody clones

<table>
<thead>
<tr>
<th>Tube</th>
<th>FITC/Syto 16</th>
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Supplementary table 2. Primer sequences used for PCR and sequencing and genomic coordinates of regions analysed for mutational status

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* exons are numbered according to NG029334.1 NCBI Reference sequence
* genomic coordinates are according to GRCh37/hg19; analysed regions include coding sequences of individual exons and 5' part of intron 6 adjacent to exon 6