# Germ-line *GATA2* p.THR354MET mutation in familial myelodysplastic syndrome with acquired monosomy 7 and *ASXL1* mutation demonstrating rapid onset and poor survival

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## ABSTRACT

While most myelodysplastic syndrome/acute myeloid leukemia cases are sporadic, rare familial cases occur and provide some insight into leukemogenesis. The most clearly defined familial cases result from inherited mutations in *RUNX1* or *CEBPA*. Recently, novel germline mutations in *GATA2* have been reported. We, therefore, investigated individuals from families with one or more first-degree relatives with myelodysplastic syndrome/acute myeloid leukemia with wild-type *RUNX1* and *CEBPA*, for *GATA2* mutations. Screening for other recurrent mutations was also performed. A *GATA2* p.Thr354Met mutation was observed in a pedigree in which 2 first-degree cousins developed high-risk myelodysplastic syndrome with monosomy 7. They were also observed to have acquired identical somatic *ASXL1* mutations and both died despite stem cell transplantation. These findings confirm that germline *GATA2* mutations predispose to familial myelodysplastic syndrome/acute myeloid leukemia, and that monosomy 7 and *ASXL1* mutations may be recurrent secondary genetic abnormalities triggering overt malignancy in these families.

Key words: familial, myelodysplastic syndromes, GATA2, monosomy 7.

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## Introduction

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders characterized by dysplastic changes in the bone marrow, ineffective hematopoiesis and an increased risk of developing acute myeloid leukemia (AML). While the majority of MDS cases are sporadic, rare familial cases have been described. Investigation of these familial cases has identified germline mutations acting as MDS predisposing lesions: *RUNX1*, causing Familial Platelet Disorder with Propensity to Myeloid Malignancy (FPD/AML)[1,2] and *CEBPA*, causing familial AML.[3] Due to an improved understanding of familial MDS and heightened awareness on the part of clinicians, there has been a recent increase in the number of reported cases of familial MDS/AML. Following the reports by Scott *et al.* of mutations in *GATA2* as a predisposing gene in familial MDS/AML,[4,5] mutations have now been detected in dendritic cell, monocyte, B and NK lymphoid (DCML) deficiency, autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome and Emberger syndrome.[6-8] In many of these pedigrees, the *GATA2* mutation acts as a predisposing rather than initiating mutation with many carriers remaining asymptomatic.

We present a novel pedigree with germline *GATA2* mutation in which 2 affected individuals presented with MDS/AML with monosomy 7 and also had identical somatic

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*ASXL1* mutations. We hypothesize that the nature of the secondary genetic events observed in *GATA2*-mutated individuals may explain the clinical heterogeneity observed within and between pedigrees.

## Design and Methods

### Study design and patient selection

Individuals from 5 families with one or more first-degree relatives with MDS/AML were collected for investigation after exclusion of *RUNX1* and *CEBPA* mutations.[9] Ethics approval (06/Q0401/31) was received and informed consent was obtained according to the Declaration of Helsinki.

### Mutation analysis

Genomic DNA was extracted from peripheral blood or bone marrow aspirate mononuclear cells using phenol and chloroform, following standard procedures. DNA was obtained from saliva using a commercially available collection tube (Oragene, Ottawa, Canada) and following product instructions. Primers used for PCR amplification of the entire coding region of *GATA2* (exons 2-6) and the amplified regions and primer sequences for *RUNX1*, *CEBPA*, *NPM1* and *FLT3* are listed in the *Online Supplementary Table S1*. *ASXL1*, *TET2* and *c-CBL* genes were PCR amplified as previously described.[10-12] Sequence analysis was carried out by bidirectional sequencing of the purified PCR products using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA). The obtained sequences were compared to the corresponding germline gene and protein sequences (accession numbers for GATA2: NM_032638.4 and NP_116027.2; for ASXL1: NM_015338.5 and NP_056153.2) available in the National Center for Biotechnology Information (NCBI) GenBank database. All mutations were confirmed from 2 independent PCR amplicons.

## Results and Discussion

Five individuals in a single pedigree (Figure 1) were noted to have *GATA2* mutations with the same *GATA2* p.Thr354Met (c.1061C>T) mutation (Figure 2A) detected in all 5 individuals (III-5, III-7, IV-1, IV-6, IV-10). The mutation was detected in the disease and the available remission sample of IV-6, suggesting a germline mutation. Confirmatory germline DNA was not available from the deceased individuals; however, a germline mutation was confirmed in the salivary DNA of III-7. A somatic *ASXL1* p.Gly646TrpfsX12 (c.1934dupG) frameshift mutation (Figure 2B) was noted in the MDS/AML sample from IV-1 and IV-6 but was lacking from the remission sample of IV-6 suggesting an identical acquired *ASXL1* mutation. No additional mutations were detected in the other screened genes.

### Pedigree

Proband IV-6 presented at the age of 23 years with symptomatic cytopenias and no significant past medical history, specifically no history of recurrent infections. A bone marrow aspirate and biopsy demonstrated trilineage dysplasia with 17% myeloblasts, consistent with a diagnosis of refractory anemia with excess blasts-2 (RAEB-2). His monocyte count was within normal limits at $0.3\times10^9$/L. Cytogenetic analysis demonstrated monosomy 7; no other cytogenetic abnormalities were detected. He underwent treatment with intensive chemotherapy and obtained a complete remission (CR) but with persistent dysplastic features in the marrow. Cytogenetic remission was also achieved with absence of the monosomy 7 clone. A second cycle of chemotherapy was complicated by delayed count recovery with persistent anemia and thrombocytopenia. Seven months following presentation,

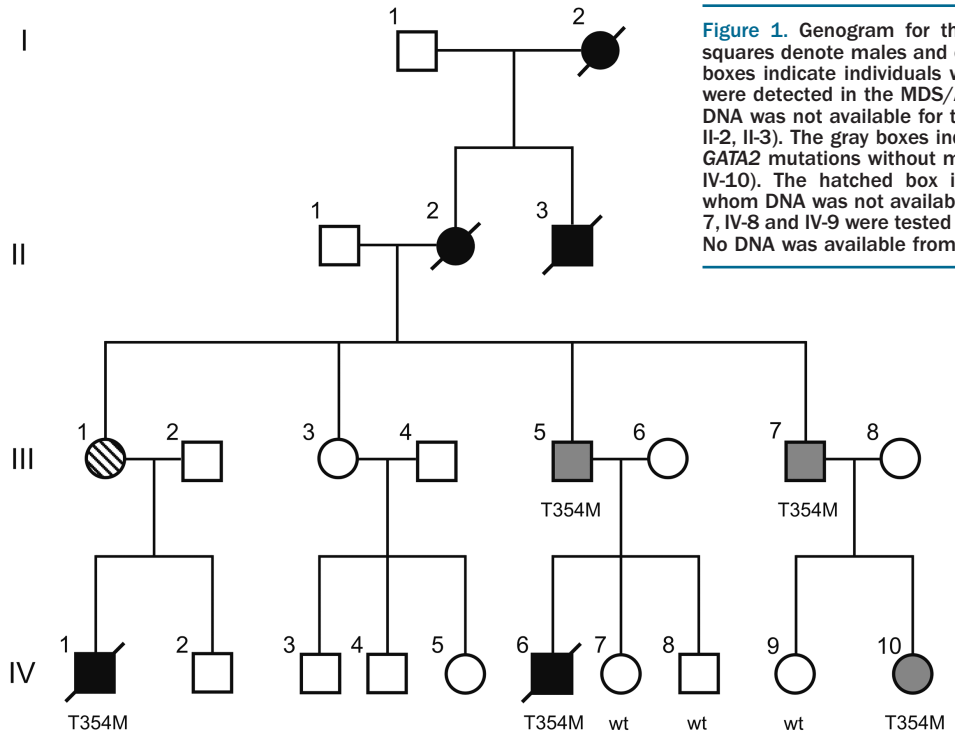


**Figure 1. Genogram for the *GATA2*-mutated pedigree. The squares denote males and circles denote females. The black boxes indicate individuals with MDS/AML. *GATA2* mutations were detected in the MDS/AML sample for IV-1 and IV-6 but DNA was not available for the other affected individuals (I-2, II-2, II-3). The gray boxes indicate individuals with detectable *GATA2* mutations without myeloid malignancy (III-5, III-7 and IV-10). The hatched box indicates an obligate carrier for whom DNA was not available to confirm *GATA2* mutation. IV-7, IV-8 and IV-9 were tested and showed wild-type (wt) *GATA2*. No DNA was available from other family members.**

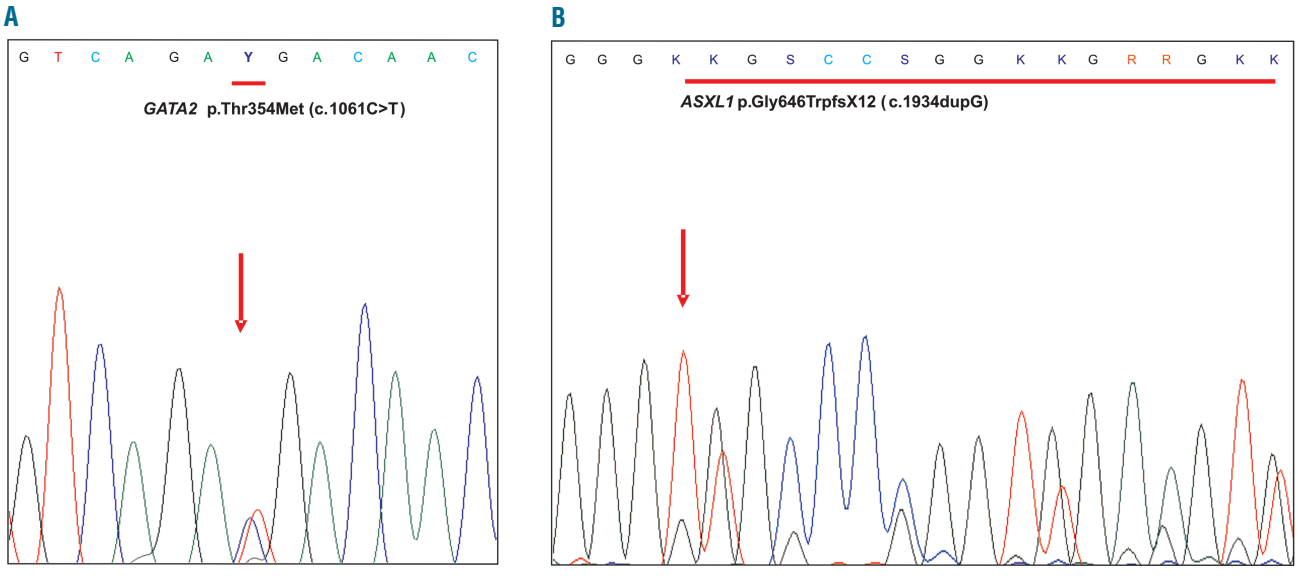


**Figure 2.** (A) The heterozygous germline *GATA2* p.T354M (c.1061C>T) mutation resulting in conversion of a conserved threonine to methionine and (B) the somatic *ASXL1* p.Gly646TrpfsX12 (c.1934dupG) frameshift mutation detected in 2 first-degree cousins.

the patient relapsed with re-emergence of the monosomy 7 clone, 8% myeloblasts and clonal evolution with isochromosome 17. He received re-induction chemotherapy and a haplo-identical hematopoietic stem cell transplant (HSCT) from his mother (III-6) but developed acute graft-*versus*-host disease (GVHD), severe infections and died of pneumonic sepsis at four months post-HSCT.

IV-1, the first cousin of IV-6, presented one week after his cousin at the age of 18 years complaining of fatigue and a history of recurrent infections (though none required hospitalization). He was noted to have multiple plantar warts. His diagnostic bone marrow revealed 7% myeloblasts (RAEB-1) and monosomy 7 with no other cytogenetic abnormalities. No historical blood counts were available to document the pre-MDS monocyte count but he had an absolute monocytopenia (monocytes $0.0\times10^9$/L) at the time of presentation. He underwent a 1 antigen mismatched unrelated donor HSCT but died two years later from relapsed disease.

IV-10 presented in 2010 at the age of 31 years with recurrent minor infections. She was observed to be severely monocytopenic ($0.1\times10^9$/L) and moderately neutropenic ($0.8\times10^9$/L), with mild macrocytosis but normal hemoglobin and platelet count. There was no history of childhood infections. Bone marrow examination was normocellular with no evidence of dysplasia or increase in blasts with a normal karyotype and wild-type *ASXL1*. Probands III-5 and III-7 were confirmed as obligate carriers of *GATA2* mutation. IV-9 was screened as a potential allogeneic sibling donor for IV-10 and had wild-type *GATA2*. IV-7 and IV-8 were screened for the mutation but had wild-type *GATA2*. No DNA was available from other family members; however, genetic counseling and testing is ongoing.

The family history was also notable for the paternal grandmother (II-2) of the proband (IV-6) who died of AML, as well as his paternal great-uncle (II-3) and great-grandmother (I-2), also reported to have died of 'leukemia'. To date, the parental generation has remained in good health with the obligate carriers (III-1, III-5 and III-7) having no history of hematologic illness nor of recurrent infections, despite being 60, 52 and 51 years old, respectively.

Significant progress has been made in delineating the molecular pathophysiology of MDS/AML, with numerous recurrent genetic aberrations identified.[13] While most cases of MDS/AML are sporadic, rare familial cases have provided insight into genetic events that predispose to these diseases. The discovery by Scott *et al.* of novel germline mutations in the hematopoietic stem cell regulator *GATA2* as a cause of familial MDS identifies a new genetic pathway important in MDS and has led to the screening of mutations in related disorders. *GATA2* mutations have now been reported in 13 pedigrees, including cases with DCML deficiency, MonoMAC and Emberger syndrome and pure familial MDS.[4-8] The mutations include single base substitutions as well as insertions and deletions, and are scattered throughout the gene. The missense mutations affecting residues Thr354 and Arg398 appear as recurrent alterations in unrelated families. The p.Thr354Met mutation described here has now been observed in 5 families.[4,14] The Thr354 residue is located in the second zinc finger of the *GATA2* molecule and is involved in DNA binding, heterodimerization and interaction with other transcription factors. Hahn *et al.* demonstrated that the p.Thr354Met mutation results in both loss of function and dominant negative effects on wild-type *GATA2*, causing a disruption of the expression of multiple genes involved in hematopoiesis.[4] While some sporadic cases of MonoMAC and DCML deficiency have been reported, only one mutation in *GATA2* was observed in 268 samples from patients with sporadic MDS/AML and no mutations were detected in 695 samples from healthy controls screened by Hahn *et al.*, nor in 30 sporadic MDS samples analyzed in our laboratory (*data not shown*). This suggests that these mutations are mostly restricted to

familial cases of MDS/AML.[4,5]

Several characteristics of familial *GATA2*-deficiency have been previously reported including: a severe reduction in monocytes, natural killer (NK) cells and B cells, a predisposition in early adulthood to recurrent infections (particularly atypical mycobacterial infections), and a risk of developing MDS/AML.[6,7] However, there is clearly significant clinical heterogeneity in these reported pedigrees. Many affected individuals display no antecedent hematologic abnormalities or infections prior to the development of MDS, and penetrance of the disease is not complete with many obligate carriers remaining healthy into late adulthood.[5] This is also a feature of *RUNX1* and *CEBPA* familial cases where the age of onset can vary considerably within and between pedigrees.[15] *GATA2* is, therefore, an important predisposing mutation but secondary genetic events are required for the development of overt malignant disease.

Of note, both individuals with MDS/AML in our pedigree had an early onset of disease at 18 and 23 years of age with acquired monosomy 7 and *ASXL1* mutation. Monosomy 7 is the most common secondary genetic aberration reported in patients with *GATA2*-deficient MDS and is not reported as a recurrent abnormality in patients with inherited MDS/AML with germline *RUNX1* or *CEBPA* mutations. However, familial cases of MDS/AML associated with complete or partial loss of chromosome 7, (monosomy 7 syndrome) have been previously reported.[16-18] Affected individuals usually present at a young age and have a poor outcome, reminiscent of our pedigree and the cases reported by Hahn *et al.*[4] The etiology for the selective loss of chromosome 7 in these cases is unclear. While monosomy 7 has been reported in other *GATA2* mutated cases, no previous report has screened for recurrent molecular aberrations (not detectable by conventional karyotyping). We screened a range of genes with mutations reported to have prognostic implication in MDS and detected identical somatic *ASXL1* Gly646TrpfsX12 mutations in both of our *GATA2* mutated patients. Mutations in *ASXL1* are among the most common mutations observed in *de novo* MDS patients at 11-20% and are associated with an adverse prognostic outcome.[12,13,19,20] The presence of the identical somatic heterozygous *ASXL1* mutation in both affected members of our pedigree suggests that *ASXL1* mutations may represent an important trigger for the development of overt disease in *GATA2*-mutated patients. Although some authors have proposed that the p.Gly646TrpfsX12 mutation may be a PCR artifact, this mutation was not detected in the remission sample, nor in the obligate carriers in our pedigree, nor previously in a series of healthy adults,[20] confirming that it is an acquired mutation in MDS.

Identifying germline mutations in patients with MDS is not only important for the purpose of delineating pathogenetic mechanisms but also has significant implications for clinical practice, particularly in donor selection for allogeneic HSCT. A recent report described 6 patients with *GATA2* deficiency who underwent allogeneic HSCT for their disease.[21] Two related, 2 unrelated and 2 cord blood HSCTs were described with excellent outcomes in all but the sickest patient (who was transplanted while infected and on a ventilator). Only the patient who received a single umbilical cord blood graft had delayed engraftment. The results from our family are poorer with both individuals dying post allogeneic HSCT; one from early infectious complications and one from relapsed MDS. However, these individuals were transplanted several years ago and underwent a myeloablative conditioning with less than ideal donors, whereas the Cuellar-Rodriguez report used a non-myeloablative regimen with better matched stem cell donors. It is also possible that *ASXL1* mutations predict for poorer outcomes in *GATA2* mutated patients and this would explain the poor results noted in our pedigree. Regardless of this, the recently reported successful HSCT results are encouraging, particularly as this therapy is currently the only potentially curative treatment for MDS.[21] The decision of when to consider allogeneic stem cell transplantation in individuals with *GATA2* mutations remains unclear. This is especially the case of IV-10 who already has significant neutropenia and monocytopenia, and of III-5 and III-7 who have confirmed *GATA2* mutations without hematologic abnormality. Therefore, prognostic factors which help identify *GATA2*-mutated patients at high risk of developing MDS/AML would be particularly helpful.

Given the increased recognition of familial MDS, many modern cases are being discovered as siblings are being investigated as potential HSCT donors in an affected family. Frequently, a comprehensive workup only occurs if the potential donor is discovered to have peripheral blood count abnormalities. This screening may not be sufficient as many patients with inherited *GATA2*, *RUNX1* or *CEBPA* mutations have normal hematologic parameters. Therefore, testing for *GATA2* mutations should be considered prior to sibling-donor allogeneic HSCT in all young patients with MDS.

In conclusion, we present a case of inherited *GATA2* mutation causing early onset familial MDS in which no antecedent hematologic abnormalities or immunodeficiency were noted other than a vague history of infections, suggesting that inherited *GATA2* mutations may be difficult or impossible to detect without genetic screening in some families. This lack of prior hematologic or immune abnormalities is particularly important in the setting of allogeneic HSCT when a sibling donor is being considered. Additionally, the acquisition of *ASXL1* mutations and monosomy 7, detected in both affected individuals, appear to be important secondary events leading to the development of overt MDS/AML.

## Authorship and Disclosures

*The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.*

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