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Rapid progression to AML in a patient with germline *GATA2* mutation and acquired *NRAS* Q61K mutation

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A B S T R A C T

GATA2 deficiency syndrome is caused by autosomal dominant, heterozygous germline mutations with widespread effects on immune, pulmonary and vascular systems. Patients commonly develop hematological abnormalities including bone marrow failure, myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). We present a patient with *GATA2* mutation and MDS who progressed to AML over four months. Whole exome and targeted deep sequencing identified a new p.Q61K *NRAS* mutation in the bone marrow at the time of AML development. Rapid development of AML is possible in the setting of germline *GATA2* mutation despite stable MDS, supporting close monitoring and consideration of early allogeneic transplantation.

1. Case report

A 25-year-old woman was referred to the National Heart, Lung and Blood Institute, Hematology Branch in March 2014 for a seven year history of pancytopenia. During adolescence she had recurrent pneumonias, oral ulcers, severe varicella infection and arthralgias. She was thought to have Beçhet's disease. She had been treated in the past with immunosuppression with mild improvement in hematological parameters. Prior bone marrow examinations at ages 21 and 23 at outside institutions reported normocellular marrow, tri-lineage hematopoiesis and mild dyspoiesis. Cytogenetics were remarkable for trisomy 8 in 80% (aged 21) and 90% (aged 23) of metaphases.

At our institution, previously unrecognized lymphedema was noted on examination. Peripheral blood counts showed WBC 2.28 K/ul [normal range: 3.98-10.04], HGB 9.9 g/dL [11.2-15.7], PLT: 67 K/ul [173-369], ANC: 1.73 K/ul [1.56-6.13] ALC: 0.36 K/ul [1.18-3.74] and AMC: 0.06 [0.24-0.86]. Peripheral blood flow cytometry demonstrated decreased CD3+/CD4+ (T) cells, CD19+ (B) cells and NK cells. Bone marrow examination showed trilineage hematopoiesis, 50-60% cellularity, mild erythroid predominance and increased, atypical megakaryocytes (Fig. 1). Blasts were less than 5%. Bone marrow flow cytometry confirmed severely decreased B-cells and monocytes, absent B-cell precursors, absent dendritic cells, inverted CD4:CD8 ratio, and an atypical myeloid maturation pattern. Cytogenetics analysis demonstrated trisomy 8 in 90% of metaphases. These findings confirmed the diagnosis of myelodysplastic syndrome (MDS) and were consistent with GATA2 haploinsufficiency and immunodeficiency. At this time her IPSS-R score was 3.5 (intermediate).

Sanger sequencing identified a germline *GATA2* p.L375F (c.1123C>T, chr3:128200682G>A) mutation in the second zinc finger of known pathogenic significance [6,12]. Comprehensive review of the patient's history, physical examination and blood cell counts provided the unifying diagnosis of GATA2 deficiency.

Four months later she returned for a routine clinic visit; she was noted to have increased fatigue and easy bruising with marked new thrombocytopenia (PLT: 10 K/ul). Bone marrow examination showed a striking transformation to markedly hypercellular (90-100%) with diffuse sheets of blasts having fine chromatin, distinct or prominent nucleoli, and visible cytoplasm. The blasts had an immature monocytic phenotype and were positive for CD33, CD56, CD64, CD123, and CD163; and were negative for CD34, CD14, and myeloperoxidase. Cytogenetics showed a new trisomy 20 in 65% of metaphases, in

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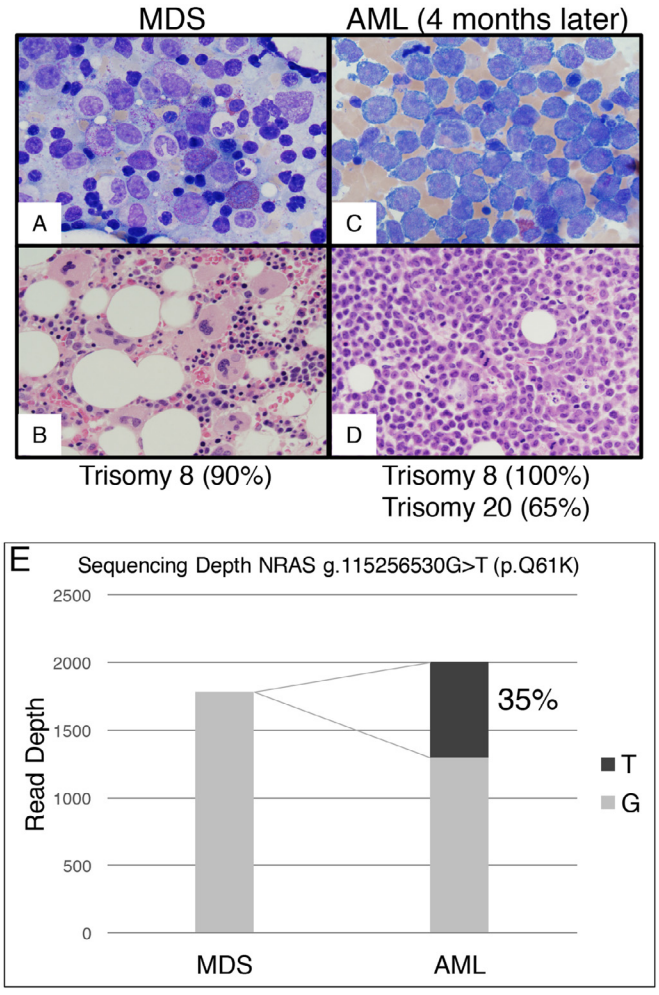

Fig. 1. Histology of bone marrow. (A and B) MDS at the time of presentation to the NIH and identification of *GATA2* mutation (C and D) M5a immature monoblastic AML four months after initial MDS diagnosis. (E) Deep sequencing of targeted AML mutations reveals *NRAS* Q61K mutation at the time of AML progression, not detected at time of initial MDS presentation and representing 35% of reads in the sample from the time of AML progression.

addition to the previously seen trisomy 8 in 100%. Acute monoblastic leukemia (M5a subtype) was diagnosed.

At both clinic visits research samples were collected on IRB approved protocols. Whole exome sequencing of 1ug DNA isolated from bone marrow aspirate was performed using Agilent SureSelect Human All Exon v5 (4Gb) Enrichment Kits on an Illumina HiSeq 2000 sequencer with 100-bp paired-end reads (Macrogen, Rockville, MD). Qualified reads were mapped to human reference genome hg19 (BWA) and processed using an in-house pipeline (Samtools/Picard/GATK/VarScan/Annovar). Mean read depth of target regions was 157 and 149. There was high correlation between both samples with the exception of a *NRAS* p.Q61K mutation (c.181C>A) (57 of 180 reads) seen only in the AML sample. Ultra-deep sequencing for *NRAS* performed using Illumina TruSight Myeloid Sequencing Panel on an Illumina MiSeq confirmed the presence of this known pathogenic mutation at the AML, but not the earlier MDS, timepoint (Fig. 1E).

Multiple rounds of cytotoxic chemotherapy and two allogeneic hematopoietic cell transplants were unsuccessful and she ultimately died of leukemic progression.

2. Discussion

There is increasing recognition of the role of inherited germline predisposition for myeloid disorders such as MDS and AML [7]. However, the additional somatic genetic events required for development of a frank malignancy are less well understood.

GATA2 is an essential transcription factor for hematopoiesis and vasculature development. The level of GATA2 in hematopoietic stem and progenitor cells is tightly regulated, and the balance is critical for normal development and homeostasis [3]. GATA2 is critical for the production, maintenance and function of hematopoietic stem cells (HSCs), and interacts with a complex network of transcription factors including PU.1, FLI1, TAL1, LMO2 and RUNX1 [4,20]. GATA2 can regulate transcription factors including itself, GATA1 and SCL [17,22]. Its expression is tightly regulated by intronic and extragenic enhancers upstream of the start site [3]. Homozygous null *Gata2* mice are embryonic lethal and severely anemic, whereas heterozygous knockout mice have shown that *Gata2* is critical for maintaining adult HSCs [19,24]. Intronic enhancer deletions in mice have identified critical regions for regulation of *Gata2* expression [17,22]. GATA2 is also be regulated by phosphorylation, acetylation, sumolyation and microRNAs [25]. Together these data have shown that the level and cellular context of *Gata2* expression is critical for proper hematopoiesis.

GATA2 deficiency is caused by germline mutations and was previously described as MonoMac, DCML (dendritic cell, monocyte, and lymphocyte deficiency), Emberger syndrome, familial AML and classical NK cell deficiency [23]. Pathogenic mutations have been identified throughout the gene in both coding and non-coding regulatory sequences, and include insertions/deletions, missense, nonsense, frameshift mutations and whole gene deletions. In addition to bone marrow failure, many patients develop non-tuberculous mycobacterial infections, severe HPV infections, pulmonary alveolar proteinosis and lymphedema.

Patients with germline GATA2 deficiency often develop bone marrow abnormalities ranging from the recently described early manifestation GATA2 deficiency related bone marrow and immunodeficiency disorder (G2BMID) to myelodysplasia and frank myeloid malignancies such as AML and chronic myelomonocytic leukemia (CMML) [3,16]. While MDS and AML in GATA2 deficiency patients are very common, the transition between these states remains incompletely defined.

This case demonstrates rapid progression from intermediate risk MDS to frank AML in the setting of GATA2 deficiency. We detected an *NRAS* Q61K mutation at the time of AML diagnosis but not four months earlier during MDS. While ultra-deep targeted confirmatory sequencing was performed it is conceivable that yet even higher sensitivity methodologies such as digital droplet PCR or error-corrected sequencing for measurable residual disease testing in AML could have detected this variant earlier [21]. The *NRAS* Q61K variant allele frequency of 35% at the time of AML diagnosis is consistent with a new heterozygous driver mutation based on data regarding leukemic clone size from both cytogenetic analyses (i.e.: new trisomy 20 in 65% of metaphases) and the 76% blasts on bone marrow differential [27].

The *NRAS* Q61K mutation has been implicated in the pathogenesis of multiple cancers including AML, by constitutive activation of proliferative signaling [13]. Acquisition of this mutation is hypothesized to be the driver of the progression from MDS to AML in this case. Ras/RTK pathway mutations are found in 98% of patients with inv(3)(q21q26) AML [9]. This leukemic inversion results in upregulation of *EVI1* and reciprocal downregulation of *GATA2* [8,9,26]. Haploinsufficiency of *Gata2* accelerates disease progression in a mouse model of Evi1 misexpression leukemia, similar to human disease [14]. Harada et al. used a Gata2 mouse model, in which Gata2 is expressed at 20% of normal wildtype levels, and showed the development of a CMML like leukemia [10]. Bonides et al. also used two mouse AML models and showed that Gata2 is downregulated and suggest that this downregulation may contribute to leukemic transformation [1]. GATA2 deficiency patients have qualitative and/or quantitative (haploinsufficiency) defect in *GATA2* expression and function [2,5,11,15]. This predisposes them to

MDS, as a pre-leukemic state, facilitating the acquisition of a *RAS* activating mutation that can drive AML.

GATA2 deficiency patients frequently develop MDS, and less commonly AML. A unique molecular signature that could predict progression and prognosis would guide timing for transplantation. Rapid development of AML can occur in GATA2 deficiency with apparently stable MDS, supporting both close monitoring and consideration of early allogeneic transplantation in such patients.

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Author contributions

L.J.M., Y.Z., Y.Y., J.T., M.M., A.P.H. and J.Z. performed experiments, performed analysis and made figures; K.R.C. and C.S.H. performed analysis and made figures; L.J.M., R.R.W., D.M.T., D.D.H., S.M.H., K.R.C. and C.S.H. designed the research; L.J.M., K.R.C. and C.S.H. wrote the manuscript. All authors reviewed the manuscript.

Disclosure of conflict of interest

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