

## Collaborating constitutive and somatic genetic events in myeloid malignancies: *ASXL1* mutations in patients with germline *GATA2* mutations

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Recently, major advances in our understanding of the pathogenesis of sporadic myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) have been made through unbiased gene discovery approaches. Similar advances have been made in understanding the genetic basis of rare cases of familial MDS and AML. Currently, germline mutations in genes encoding transcription factors, including *RUNX1*, *CEBPA*, and more recently *GATA2*,<sup>1-8</sup> have been identified in patients with familial MDS/AML. In patients with familial MDS/AML, however, there is great heterogeneity in the age of disease onset as well as the clinical characteristics of the myeloid malignancy which develops in affected members of such families. For instance, in the largest single survey of disease phenotypes in individuals with germline *GATA2* mutations, 50% of patients were without symptoms at the age of 20 and 16% continued to remain without symptoms by the age of 40.<sup>7</sup> In this issue of *Haematologica*, West *et al.* begin to unravel the genetic alterations that frequently occur together and collaborate with germline *GATA2* mutations to promote the development of MDS and AML.<sup>8</sup>

In 2011, four papers were published identifying heterozygous germline *GATA2* mutations as the cause of four previously described clinical syndromes: primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome),<sup>1</sup> *RUNX1/CEBPA* wild-type familial AML/MDS,<sup>2</sup> monocytopenia and mycobacterial infections (MonoMAC syndrome),<sup>9</sup> and the dendritic cell, monocyte, B and NK lymphoid deficiency syndrome (DCML deficiency).<sup>10</sup> Since then, approximately 200 patients with germline *GATA2* mutations have been described (Table 1), each presenting with a variety of clinical presentations but all with a high risk of developing MDS/AML. In a summary of these studies, approximately 70% of *GATA2*-deficient individuals appear to develop MDS or AML in their lifetime.

Interestingly, attempts have been made to correlate the risk and outcomes of myeloid malignancy in patients with germline *GATA2* mutations with the genotype of the *GATA2* mutation present, but this has been limited by the number of patients.<sup>7</sup> Although monosomy 7 clearly appears to be enriched in *GATA2*-deficient individuals who develop MDS and AML (30% of individuals; Table 1), the first clue to a specific molecular abnormality which might be an important collaborating genetic event for the development of overt myeloid malignancy in *GATA2* mutant families came from recent work by Bodor *et al.*<sup>3</sup> In this prior study of a germline *GATA2*-mutant kindred, somatic *ASXL1* mutations were present exclusively in the two members of the family who developed MDS/AML. This finding strongly suggested that *ASXL1* mutations might be an important trigger for the development of overt disease in *GATA2*-mutated patients.

West *et al.* performed targeted sequencing of *ASXL1* in 48 patients with germline *GATA2* mutations and identified heterozygous *ASXL1* mutations in 14 of them (29%). Given the

rarity of *ASXL1* mutations in individuals with myeloid malignancies less than 60 years old, the high frequency of *ASXL1* mutations in *GATA2*-deficient individuals developing MDS/AML is remarkable. Eight different *ASXL1* mutations were seen in ten different *GATA2*-mutant backgrounds. Similar to the pedigree studied by Bodor *et al.*, in this study one pair of sisters had the same *GATA2* mutation but only the sister who had an *ASXL1* mutation actually developed clinically evident MDS. This finding further underscores the likely collaboration between *GATA2* and *ASXL1* mutations in promoting the development of MDS. In another informative pedigree here, two sisters with the same *GATA2* mutation had discordant *ASXL1* genotypes but both developed chronic myelomonocytic leukemia (CMML). This finding further validates the already strong link between the presence of *ASXL1* mutations and the clinical phenotype of CMML.<sup>11</sup>

Although this study had a relatively limited number of patients due to the rarity of germline *GATA2* mutant patients, the authors were able to determine that overall survival in *ASXL1*-mutant/*GATA2*-mutant patients was worse than that in patients with an *ASXL1* wild-type/*GATA2* mutant genotype.<sup>8</sup> This finding is quite consistent with those of larger studies in MDS patients revealing an unwavering association between the presence of *ASXL1* mutations and adverse outcome.<sup>11,12</sup>

In contrast to *de novo* MDS, MDS occurring in patients with *GATA2* germline mutations are usually hypocellular for age with increased reticulin fibrosis.<sup>7</sup> Interestingly, results from the study by West *et al.* suggest that the development of *ASXL1* mutations coincides with progression from the hypoplastic MDS characteristic of *GATA2* deficiency to a more proliferative disease.<sup>8</sup> More sensitive quantitative sequencing, comparing samples during the hypoplastic MDS phase of disease and during acute transformation will be needed to understand how early the *ASXL1* mutations occur in the pathogenesis of myeloid disease in these individuals. This is especially relevant since most individuals with co-occurring *GATA2* and *ASXL1* mutations in this study had additional cytogenetic abnormalities such as monosomy 7.

Further unbiased genome-wide sequencing studies, currently being undertaken by this group and others, are needed to understand the full spectrum of somatic mutations in hematopoietic cells in individuals with disease evolution. For instance, recent whole exome sequencing of serial samples over a 17-year period from a single patient with severe congenital neutropenia progressing to AML showed a number of early and late genetic defects associated with leukemic progression.<sup>13</sup> A nonsense mutation in the gene encoding for granulocyte colony-stimulating factor receptor, *CSF3R*, appeared to be a clear, early event in the severe congenital neutropenia phase of the disease. In contrast, mutations arising later in the development of AML included mutations in *ASXL1*, *SUZ12*, *EP300*, *RUNX1*, and an additional mutation in *CSF3R*. Based on the work by West *et al.*, it is quite plausible that the development

**Table 1.** Development and characteristics of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) in individuals with germline *GATA2* mutations.<sup>1</sup>

Study	Clinical syndrome at disease presentation	N. of <i>GATA2</i> mutated patients	N. of patients developing AML or MDS <sup>2</sup>	Median age of patients at AML/MDS diagnosis <sup>2</sup>	N. of patients with monosomy 7	Death due to AML/MDS <sup>2</sup>
Hahn <i>et al.</i> <sup>2</sup>	Familial MDS/AML	28	14	20.5 (10-53)	6/9	7
Ostergaard <i>et al.</i> <sup>1</sup>	Emberger syndrome	14	8	12 (9-53)	6	5
Bodor <i>et al.</i> <sup>3</sup>	Familial MDS/AML	5	2	20.5 (18-23)	2	2
Holme <i>et al.</i> <sup>4</sup>	Familial MDS/AML	6	6	20 (12-48)	1/3	0
Pasquet <i>et al.</i> <sup>6</sup>	Congenital neutropenia	14	10	15 (6-35)	4	4
Hsu <i>et al.</i> <sup>5</sup>	MonoMAC syndrome	20 <sup>3</sup>	11/16	21 (1.5-65)	ND <sup>4</sup>	ND
Spinner <i>et al.</i> <sup>7</sup>	<i>GATA2</i> deficiency	57	42/50	19 (0.4-78)	8/42	13
West <i>et al.</i> <sup>8</sup>	<i>GATA2</i> deficiency	48	42	32.5 (12-78)	8/41	10
Dickinson <i>et al.</i> <sup>20</sup>	<i>GATA2</i> deficiency	30	11/30	ND	2	3
<b>Total</b>		<b>222</b>	<b>146/211 (69%)</b>	<b>29 (0.4-78)</b>	<b>37/126 (29%)</b>	<b>43/128 (34%)</b>

<sup>1</sup>Only studies with five or more cases are included here. <sup>2</sup>Chronic myelomonocytic leukemia (CMML) included in MDS. <sup>3</sup>Fourteen patients from Hsu *et al.* who are also described in Spinner *et al.* study were excluded. <sup>4</sup>ND: not described.

of MDS and AML in *GATA2*-deficient individuals might be similarly driven by a stepwise accumulation of genetic mutations with clonal expansion and selection, with *ASXL1* seeming to play a central role.

The strong genetic link between *GATA2* and *ASXL1* mutations in patients with this rare germline disorder raises the question of the frequency of *ASXL1* mutations in diseases marked by somatic *GATA2* mutations. For instance, somatic *GATA2* mutations have been described in Philadelphia chromosome-positive chronic myeloid leukemia patients at transformation to myeloid blast crisis.<sup>14</sup> Most of these cases were associated with a gain-of-function mutation in *GATA2* (Leu359Val) in contrast to *GATA2* mutations seen in patients with *GATA2*-mutant germline syndromes. Somatic *GATA2* mutations have also been identified in *de novo* AML. In such cases, *GATA2* mutations tend to be enriched in normal karyotype AML patients with biallelic *CEBPA* mutations. From the limited data published on such patients, *GATA2* mutant *de novo* AML does not appear to be significantly associated with *ASXL1* mutations. Moreover, *GATA2* mutations in *de novo* AML appear to be associated with a relatively favorable prognosis.<sup>15</sup>

The significant co-occurrence of *GATA2* deficiency with *ASXL1* mutations at development of MDS/AML strongly suggests a cooperative interaction of these genetic events in promoting hematopoietic transformation. *ASXL1* is a Polycomb associated protein which has been shown to affect transcription through effects on the ability of the Polycomb repressive complex 2 to perform histone H3 lysine 27 methylation<sup>16</sup> and also potentially by interacting with the histone H2A lysine 119 deubiquitinase enzyme BAP1.<sup>17</sup> Genome-wide localization studies of *ASXL1* by chromatin immunoprecipitation followed by next-generation sequencing recently showed that *ASXL1* localizes strongly to promoter regions of the genome.<sup>18</sup> Moreover, *ASXL1* binding strongly overlaps with that of ETS transcription factors.<sup>18</sup> It is now well understood that deletion of key ETS transcription factors, such as PU.1, promotes aggressive myeloid malignancies *in vivo*. Interestingly even lowering levels of PU.1 to 20% of normal levels promotes leukemogenesis. This observation highlights the impor-

tance of transcriptional regulation of ETS target genes in the pathogenesis of myeloid malignancy. These facts, taken together with the knowledge that *GATA2* interacts with and represses PU.1,<sup>19</sup> possibly suggest that the explanation for the genetic interaction between *ASXL1* and *GATA2* may lie in the intersection of these factors in transcriptional regulation of key PU.1 target genes. Given the importance of *ASXL1* mutations in myeloid malignancies and the development of molecular knowledge regarding *GATA2* function in hematopoiesis, future functional work dissecting the interaction of *ASXL1* mutations and *GATA2* haploinsufficiency may address this hypothesis. It is hoped that further *in vitro* and *in vivo* work will elucidate this fascinating genetic interaction identified by West *et al.*<sup>8</sup>

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## References

- Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ, *et al.* Mutations in *GATA2* cause primary lymphedema

- associated with a predisposition to acute myeloid leukemia (Emberger syndrome). *Nat Genet.* 2011;43(10):929-31.
2. Hahn CN, Chong CE, Carmichael CL, Wilkins EJ, Brautigan PJ, Li XC, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet.* 2011;43(10):1012-7.
  3. Bodor C, Renneville A, Smith M, Charazac A, Iqbal S, Etancelin P, et al. Germ-line GATA2 p.THR354MET mutation in familial myelodysplastic syndrome with acquired monosomy 7 and ASXL1 mutation demonstrating rapid onset and poor survival. *Haematologica.* 2012;97(6):890-4.
  4. Holme H, Hossain U, Kirwan M, Walne A, Vulliamy T, Dokal I. Marked genetic heterogeneity in familial myelodysplasia/acute myeloid leukaemia. *Br J Haematol.* 2012;158(2):242-8.
  5. Hsu AP, Johnson KD, Falcone EL, Sanalkumar R, Sanchez L, Hickstein DD, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. *Blood.* 2013;121(19):3830-7, S1-7.
  6. Pasquet M, Bellanne-Chantelot C, Tavitian S, Prade N, Beaupain B, Larochelle O, et al. High frequency of GATA2 mutations in patients with mild chronic neutropenia evolving to MonoMac syndrome, myelodysplasia, and acute myeloid leukemia. *Blood.* 2013;121(5):822-9.
  7. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics and immunity. *Blood.* 2013 Nov 13. [Epub ahead of print]
  8. West RR, Hsu AP, Holland SM, Cuellar-Rodriguez J, Hickstein DD. Acquired ASXL1 mutations are common in patients with inherited GATA2 mutations and correlate with myeloid transformation. *Haematologica.* 2014;99(2):276-81.
  9. Hsu AP, Sampaio EP, Khan J, Calvo KR, Lemieux JE, Patel SY, et al. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood.* 2011;118(10):2653-5.
  10. Dickinson RE, Griffin H, Bigley V, Reynard LN, Hussain R, Haniffa M, et al. Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. *Blood.* 2011;118(10):2656-8.
  11. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. *J Clin Oncol.* 2013;31(19):2428-36.
  12. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med.* 2011;364(26):2496-506.
  13. Beekman R, Valkhof MG, Sanders MA, van Strien PM, Haanstra JR, Broeders L, et al. Sequential gain of mutations in severe congenital neutropenia progressing to acute myeloid leukemia. *Blood.* 2012;119(22):5071-7.
  14. Zhang SJ, Ma LY, Huang QH, Li G, Gu BW, Gao XD, et al. Gain-of-function mutation of GATA-2 in acute myeloid transformation of chronic myeloid leukemia. *Proc Natl Acad Sci USA.* 2008;105(6):2076-81.
  15. Fasan A, Haferlach C, Alpermann T, Jeromin S, Grossmann V, Eder C, et al. The role of different genetic subtypes of CEBPA mutated AML. *Leukemia.* 2013 Sep 13. [Epub ahead of print]
  16. Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer Cell.* 2012;22(2):180-93.
  17. Scheuermann JC, de Ayala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Frateman S, et al. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature.* 2010;465(7295):243-7.
  18. Abdel-Wahab O, Gao J, Adli M, Dey A, Trimarchi T, Chung YR, et al. Deletion of *Asxl1* results in myelodysplasia and severe developmental defects in vivo. *J Exp Med.* 2013;210(12):2641-59.
  19. May G, Soneji S, Tipping AJ, Teles J, McGowan SJ, Wu M, et al. Dynamic analysis of gene expression and genome-wide transcription factor binding during lineage specification of multipotent progenitors. *Cell Stem Cell.* 2013;13(6):754-68.
  20. Dickinson RE, Milne P, Jardine L, Zandi S, Swierczek SI, McGovern N, et al. The evolution of cellular deficiency in GATA2 mutation. *Blood.* 2013 Dec 17. [Epub ahead of print]

## Allogeneic T cells: maestro in the co-ordination of the immune response after hematopoietic stem cell transplantation

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Allogeneic hematopoietic stem cell transplantation (HSCT) is currently used to treat different bone marrow disorders and hematologic malignancies.<sup>1</sup> In this context, allogeneic donor T cells play key roles in the post-transplant immunity as they can attack the patient's malignant cells in a phenomenon referred to as graft-versus-leukemia (GvL), which is the beneficial aspect of tissue disparity. However, donor T cells also react against the tissues of the patient contributing to the development of one of the main complications after allogeneic HSCT, graft-versus-host disease (GvHD).

Moreover, the description of the GvL effect in hematologic malignancies led to the development of a cell therapy approach using donor lymphocytes or donor lymphocyte infusion (DLI) in order to treat patients with hematologic relapse following allogeneic HSCT. DLI is currently an effective treatment to restore remission in patients with relapsed chronic myeloid leukemia (CML).<sup>2</sup>

In terms of allogeneic immune response post transplant, it has been demonstrated that allogeneic cytotoxic CD8<sup>+</sup> T cells are the main mediators of the GvL effect as well as GvHD, while CD4<sup>+</sup> T cells are mainly 'helpers' in the

immune response by inducing maturation of dendritic cells (DC) and activation of other immune cells such as CD8<sup>+</sup> T cells and B cells. It is currently known that CD4<sup>+</sup> T cells can stimulate the production of auto-reactive as well as allo-reactive antibodies in the context of allogeneic HSCT,<sup>3,4</sup> but the importance of the co-operation between CD4<sup>+</sup> T cells and B cells in the immunity after HSCT has so far only been reported against DDX3Y, a male specific antigen,<sup>5,6</sup> and needs further investigation.

Different HLA class II restricted polymorphic antigens have previously been characterized as targets for allogeneic CD4<sup>+</sup> T cells in a patient suffering from CML who received DLI after allogeneic HSCT.<sup>7,8</sup> One of the identified antigens was derived from PTK2B, a protein belonging to the focal adhesion kinase family. As reported in this edition of the Journal, in their study, Kremer *et al.*<sup>9</sup> chose to focus their attention on the response towards this specific antigen, as it has been documented that PTK2B can be an antibody target in certain transplanted patients treated with DLI for CML relapse.<sup>10</sup> However, it is still unclear whether the antibody response activated in these patients is of an allogeneic or an autologous nature and whether there is a specific T-cell