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Phenotypic heterogeneity associated with germline *GATA2* haploinsufficiency; a comprehensive kindred study

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Letter to the Editor

GATA2 is a zinc finger transcription factor that plays a critical role in hematopoiesis, lymphangiogenesis, and leukemogenesis [1]. *GATA2* haploinsufficiency can occur with somatic or germline aberrations of a single allele. *GATA2* somatic variants in myeloid progenitor cells are associated with distinct acute myeloid leukemia (AML) subtypes, including acute erythroid leukemia and AML with normal cytogenetics harboring bi-allelic *CEBPA* variants [2], blast phase chronic myelogenous leukemia [3], high risk myelodysplastic syndromes (MDS), and MDS-EB (MDS with excess blasts) [4]. Germline *GATA2* haploinsufficiency leads to a range of variably penetrant autosomal dominant disorders, including B/NK/dendritic cell immunodeficiency, familial MDS/AML, monocytopenia and mycobacterial infection (DCML deficiency, MonoMac) syndrome, and primary lymphedema or Emberger Syndrome (ES) [1]. Severe morbidity or mortality arises from opportunistic infections such as atypical mycobacterial, disseminated viral or fungal infections, HPV-driven warts and malignancy, and pulmonary alveolar proteinosis (PAP) from macrophage dysfunction. Hematological manifestations develop in approximately 80% of individuals with *GATA2* haploinsufficiency, and can present as clonal cytopenias, aplastic anemia, MDS, or AML [5]. Myeloid clonal evolution in individuals with germline *GATA2* haploinsufficiency is commonly associated with acquisition of additional somatic mutation(s) such as *ASXL1*, *RUNX1*, *SETBP1*, and *NRAS* [6].

In this paper we expand on our previously described *GATA2* haploinsufficiency cohort focusing on a seven-member kindred harboring an atypical missense mutation in the C-terminal domain (c.1339A>C, p.S447R) (Figure 1A, Table 1) [1]. This study was approved

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by the IRB at Mayo Clinic in Rochester, MN. Patient data were extracted from our institutional database of individuals with germline *GATA2* haploinsufficiency. Sanger and next generation sequencing (NGS) were used to characterize pathogenic variants in *GATA2*, as previously described [1]. The timing of patients' earliest clinical manifestations were estimated based on patient history, and onset of hematological manifestations was defined by blood and bone marrow histology showing clonal cytogenetic abnormalities, clonal cytopenias, marrow failure, MDS, or AML. In 3 of 7 patients a myeloid-specific NGS panel (single base substitutions and insertion/deletion events: accuracy>99%; reproducibility 100% and sensitivity 5–10% variant allele fraction with a minimum depth coverage of 250X) was obtained to identify somatic variants. Relevant transplant outcome measure was GVHD relapse-free survival (GRFS). Overall survival was calculated from the time of *GATA2* haploinsufficiency diagnosis. Standard statistical methods were performed using JMP Software (SAS Institute Inc., Cary, NC).

The seven-member kindred of interest illustrates incomplete penetrance and variable expressivity of *GATA2* germline variants (Figure 1A). Two asymptomatic patients (#1 and 2), followed annually with a complete blood count (CBC) and a comprehensive physical examination, have no clinical features associated with *GATA2* haploinsufficiency to date. Multiple bone marrow assessments in both patients have been normocellular without morphological or cytogenetic evidence for myeloid transformation. Furthermore, as B cell lymphopenia has proven very common in *GATA2* deficiency, even in patients with normal CBCs, serial quantitative B and NK cell assessments in both these patients were surprisingly normal [7]. Patient #3 has suboptimal NK cell cytotoxic function with bone marrow assessments revealing mild hypercellularity for age, without dysplasia or somatic variants. Four patients developed myeloid clonal evolution including aplastic anemia (#6), MDS-MLD (multi lineage dysplasia) (#4 and 7) and AML (#5). Aplastic anemia in patient #6 was associated with somatic *ASXL1* mutation acquisition with variant allele frequency (VAF) of 12%. Patient #6 developed MDS-MLD 13 years after diagnosis though NGS was not performed. Similarly, patient #7 developed MDS-MLD with normal cytogenetics and *ASXL1* mutation (VAF 23%). Only patient #5 developed AML with somatic mutations involving *ASXL1*, *DNMT3A* and *ETV6*. Interestingly, we demonstrate a significant variability in the non-hematologic manifestations in these kindred. Only patients #4 and 6 demonstrated recurrent HPV driven-warts and only patient #6 developed lymphedema (ES).

Since first described, *GATA2* haploinsufficiency remains a challenge to treat given varied clinical presentations and syndrome-related limitations to the only potential cure, allogenic HCT. As in our cohort, although myeloablative regimens are more likely to eradicate aggressive clones when hematologic malignancies are present, they may be less tolerated in patients with disseminated opportunistic infections and comorbidities. Patient #5 relapsed 10 months following HSCT with extramedullary disease, presenting with right eye proptosis. An orbital mass biopsy demonstrated a myeloid sarcoma with a PET-CT revealing involvement of the ninth right rib, left lateral thigh and right buccal space. After treatment with external beam radiation therapy to the orbit and high dose cytarabine, the patient remains in remission 48 months later. He was the only kindred who underwent non-myeloablative conditioning due to comorbidities. Of the other 3 patients who underwent HSCT, all received myeloablative conditioning with transplant soon after detection of clonal

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myeloid evolution. Currently, timing of HCT and choice of conditioning regimen depends on severity of the syndrome and anticipated tolerability.

Management difficulties of *GATA2* haploinsufficiency are rooted in challenges delineating the genotype-phenotype relationship. If prediction of clinical consequences from the specific variant was possible, more personalized management and monitoring frequency for clonal evolution would be possible. To this end, we largely look to protein structure to define function, and by extension, we may determine structural consequences of the variant to predict functional corollaries. GATA2 has several well-characterized functional domains including two transactivation domains, negative regulatory domain, nuclear localization signal, and N-terminal and C-terminal zinc fingers (N-finger and C-finger respectively) (Figure 1B). Most commonly germline *GATA2* variants are truncating (typically frameshift) followed closely by missense variants. Truncating variants almost exclusively occur upstream of the C-finger while most missense variants are located either within the C-finger or immediate vicinity. Missense variants in the N-finger are exceedingly uncommon [8–11]. Taken together, this suggests C-finger disruption is critical for the *GATA2* haploinsufficiency phenotype. However, the S447R variant in our cohort is unique given its significant distance from the C-finger (Figure 1B) raising the question how this mutation confers similar clinical manifestations.

Given most variants affect the GATA2 C-finger, hypothesized mechanistic consequences revolve around disruption of GATA2-DNA interaction. Testing recurrent C-finger variants demonstrated impaired transcription, transactivation and reduced binding of GATA2 to intronic *cis*-elements important for autoregulation [5,12,13]. This led to the hypothesis that C-finger variants confer a dominant-negative effect causing haploinsufficiency. However, not all missense variants are likely interchangeable as function and regulatory mechanisms of GATA2 are diverse and there is a paucity of mechanistic studies testing the variety of *GATA2* mutations observed. Given GATA2 has two zinc finger domains with slightly different sequence preference, each can bind DNA independently and both domains can bind cooperatively in the context of two proximal GATA sites. Long-range regulation of gene expression can be mediated by distinct zinc finger domains binding separate DNA molecules. Furthermore, each domain can independently or cooperatively interact with other proteins forming homodimers or heterodimers [14]. Post-translational modifications also augment GATA2 activity with putative phosphorylation, acetylation and SUMOylation sites. In our cohort, given transition of a serine residue, one may initially hypothesize this represents disruption of a phosphorylation site given GATA2 is a downstream target of MAPK and AKT [14]. However, *in silico* analysis (NetPhos 3.1) does not support this hypothesis. More likely consequences of S447R include augmentation of the C-terminal transactivation domain in which it is located or disruption of a region important for ubiquitination (amino acids 412–480) altering steady-state GATA2 levels [14]. Taken together, these complexities illustrate how different germline variants may result in heterogeneous phenotypes.

Recent studies question the haploinsufficiency paradigm entirely. Katsumura et al. showed common *GATA2* variants disrupt DNA binding. While mutant GATA2 had impaired erythroid differentiation promotion, relative to wildtype GATA2 it had enhanced promotion

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of myeloid differentiation and proliferation [13]. Similarly, common variants were found to enhance binding to hematopoietic differentiation factor PU.1 relative to wildtype GATA2 [5]. Therefore, missense variants likely do not simply abrogate GATA2 function, and gain-of-function consequences must be considered contributing to varied clinical presentations.

However, this does not explain differential expressivity, incomplete penetrance, and variable symptom onset in our kindred. Familial cohorts could prove invaluable to understand these differences. Broadly, such variability is likely to be epistatic and/or epigenetic. While susceptibility/protective loci have not been identified, there are some studies characterizing epigenetic regulation of GATA2 activity [15]. Nonetheless, penetrance is typically high based on one cohort with diverse variants having probability of 8% remaining symptom-free at 40 years [10]. To our knowledge, only one other study has identified a germline variant (c. 1341C>A) resulting in the same protein change as this kindred, p.S447R. This patient was diagnosed at age 12.9 years with MDS-RCC (refractory cytopenia of childhood) and karyotype with monosomy 7 [11]. If included in our cohort, three individuals (37.5%) remain asymptomatic without clonal hematopoiesis at age > 40 years. Though there is an order of magnitude difference in patients between cohorts, our kindred may harbor epistatic or epigenetic mechanisms limiting penetrance, and epigenomic and transcriptomic studies may be useful in these cohorts.

Our cohort shares recurrent somatic mutations (*ASXL1, DNMT3A*) and karyotype abnormalities (monosomy 7) with other patients harboring different germline *GATA2* variants, and who have undergone myeloid clonal evolution [16]. Such recurrent findings suggest *GATA2* variants may result in common evolutionary trajectories. Ultimately, additional targeted mechanistic studies comparing germline variants and their effects on the transcriptome and epigenome are necessary to elucidate not only the complex genotype-phenotype relationship but also events in evolution to MDS/AML. With systematic, mechanistic and longitudinal studies of *GATA2* haploinsufficiency, we may more precisely manage this complex disease.

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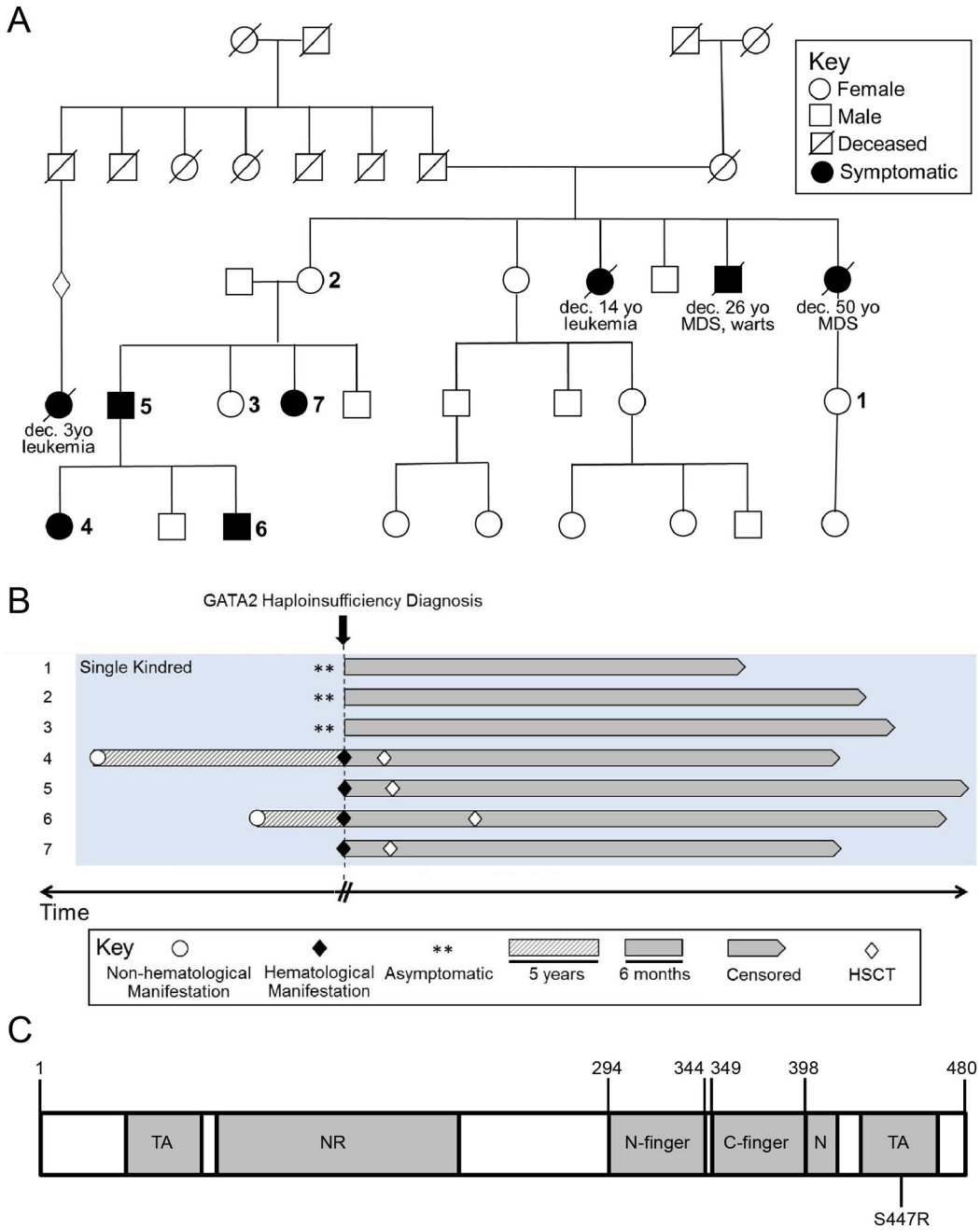

Figure 1.

A) Family tree of kindred of interest. Large bold numbers correspond to number indicated in the Swimmer plot below. B) Swimmer plot demonstrating clinical evolution of the seven-member kindred over time. C) Diagram depicting functional domains of GATA2 protein. Above the diagram key amino acid residues are numbered. Below the diagram the kindred's S447R relative location is annotated. TA, transactivation domain; NR, negative regulatory domain; N-finger, N-terminal zinc finger; C-finger, C-terminal zinc finger; N, nuclear localization signal

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Table 1

Clinical presentation, treatment, and outcomes of 7-member *GATA2* (c.1339A>C, p.S447R) kindred

Kindred	Age (Dx)	Sex	Earliest Clinical Manifestation	Bone Marrow Findings (Dx)	Cytogenetics at Evolution	Time to Clonal Evolution (mo.)	Somatic Mutations at Evolution	Allele Burden of Somatic Mutations	HSCT Donor & Conditioning	GRFS (mo.)	Overall Survival	Status
1	35	F	Asymptomatic	N/A	N/A	N/A	N/A	N/A	N/A	N/A	41.7	Alive, Observation
2	56	F	Asymptomatic	N/A	N/A	N/A	N/A	N/A	N/A	N/A	54.3	Alive, Observation
3	30	F	Suboptimal NK cell function	Mildly hypercellular	N/A	N/A	N/A	N/A	N/A	N/A	57.2	Alive, Observation
4	16	F	Warts	MDS-MLD	46,XX[20]	156	Not Done	N/A	M-MUD, MA	47.6	51.9	Alive, Remission
5	37	M	AML	60% blasts with dyserythropoiesis, dysgranulopoiesis	46,XY,+1,der(1;21)(q10;q10)[9] 47,XY,+8[4] 46,XY[7]	*	ASXL1, DNMT3A, ETV6	43%	M-MUD, NMA	10.1	64.9	Alive, Relapse, Remission
6	9	M	Warts, Lymphedema	Aplastic Anemia	46,XY[20]	72	ASXL1	12%	MUD, MA	48.9	62.6	Alive, Lymphedema
7	31	F	MDS-MLD	MDS-MLD	46,XX[20]	*	ASXL1	23%	MUD, MA	46.9	51.7	Alive, Remission

Dx, at diagnosis. Genomic variant information including evidence of and somatic status in cancer, significant protein domains, and known pathogenicity was further annotated using public databases: the Catalogue of Somatic Mutations in Cancer – COSMIC. M-MUD, mismatched unrelated donor. MUD, matched unrelated donor. MA, myeloablative conditioning. NMA, nonmyeloablative conditioning. GRFS, GVHD-free relapse-free survival. Time to clonal evolution was calculated from earliest GATA2 syndrome symptoms. “*” denotes cases where myeloid transformation was the earliest symptom.