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Monozygotic twins with GATA2 deficiency: same haploidentical-related donor, different severity of GvHD

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Twin studies, especially those involving monozygotic twins, are one of the oldest and most powerful methods for separating heritable from non-heritable traits. Recently, monozygotic twin studies have been used to study the frequencies of major immune cell populations.[1,2] However, even monozygotic twins can diverge somatically, genetically, epigenetically and environmentally. Here we describe monozygotic twins with GATA2 deficiency[3–5] who both had nearly complete absence of NK cells and B-cells, monocytopenia (20 K/μL) and CD3 counts < 300/μL prior to transplant, who underwent allogeneic hematopoietic stem cell transplant (HSCT) from the same haploidentical 8/10 HLA-matched sibling donor using the identical conditioning regimen and identical GvHD prophylaxis. The first twin developed acute grade II GvHD (skin and gastrointestinal involvement) requiring systemic corticosteroids for 6 months, oral budesonide for 2 months and tacrolimus for 1 and 1.5 years post transplant. In contrast, the second twin developed only grade I aGvHD (stage 1 skin), never required systemic steroids, and stopped tacrolimus 6 months following HSCT. This difference in GvHD highlights the complex role of host genetic and other factors in determining the incidence and severity of GvHD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Twin 1: A 27-year-old female monozygotic twin with a heterozygous (c.988 C>T, p.R330X) mutation in GATA2 in the blood and skin fibroblasts presented for allogeneic HSCT. The GATA2 mutation was not present in either parent or an older sister, consistent with a *de novo* germline mutation. Her pre-transplant clinical course, HSCT regimen, and post-transplant course are described (Table 1). Her day 28 donor chimerisms were all 100% including myeloid, CD3 and natural killer (NK) subsets. On day +28, she developed a skin rash comprising 25–50% of her body surface area, nausea, vomiting and diarrhea. She underwent upper and lower gastrointestinal endoscopy for evaluation of GvHD. Biopsies from the upper GI tract were unremarkable; however, lower GI tract biopsies from the rectum were consistent with acute GvHD, which in conjunction with the clinical manifestations was considered grade II. Although the maximum aGvHD grade was limited to grade II, in addition to tacrolimus she required both systemic and topical steroids to control her skin and GI symptoms (oral budesonide). Steroids were required for 6 months and tacrolimus for 18 months post transplant. Bone marrow studies on day +100 demonstrated 96% donor chimerism with complete eradication of the trisomy eight cytogenetic abnormality present prior to transplant. Day 100 immune reconstitution studies (performed while on prednisone 20 mg daily and tacrolimus) demonstrated $CD3^+$ cells of 285/μL, NK cells of 139/μL, $CD19^+$ cells of 21/μL and resolution of monocytopenia to a count of 440 K/μL

Twin 2: The 27-year-old female monozygotic twin sister of twin 1 had the identical (c.988 C>T (p.R330X) mutation in GATA2 and presented for allogeneic HSCT 6 months after her sister (Table 1). She underwent a haploidentical-related donor bone marrow HSCT from the same GATA2 wild-type 29-year-old sister. Conditioning and GvHD prophylaxis were identical to those used in her twin. Her post-transplant clinical course was uneventful. She mild skin GvHD (stage 1, grade 1) on day +28, which responded topical steroids and she did not require any additional immunosuppression. Tacrolimus was stopped by 6 months transplant. Day 30 chimerism studies demonstrated 100% in the whole blood and 100% donor cells in the subcompartments, including myeloid, CD3 and NK cells. Bone marrow studies performed on day +35 demonstrated 96% donor chimerism with complete eradication of trisomy 8 on cytogenetics. Day 100 immune reconstitution studies (performed while on alone) demonstrated $CD3^+$ cells of 375/μL, NK cells of 10^6/μL, $CD19^+$ cells of 262/mμL and resolution of monocytopenia to a count of 700 K/μL

Heritable conditions in monozygotic twins provide the opportunity to study the interplay between genetic and environmental factors that determine both the disease manifestations and outcomes of therapy. This is the first report of haploidentical transplantation of twins using the same conditioning regimen and the same donor. Although cryptic and minor histocompatibility antigens may contribute to different outcomes in transplant,[6] presumably these factors were identical in this set of monozygotic twins, allowing for an isolated study of the impact of non-heritable factors. Previous case reports of monozygotic twins using the same allogeneic source are variable and provide limited information regarding the heterogeneity of transplant outcomes,[7–9] and other studies of second transplants using the same donor and similar conditioning regimens provide some data regarding disparate GvHD, with potential confounders of change in conditioning regimen, donor source (marrow vs peripheral blood) or GvHD prophylaxis.[10–12] Our twins experienced disparate GvHD, despite using the same donor, similar cell product parameters, the same conditioning

regimen and the same GvHD prophylaxis. Despite these commonalities, we observed different strengths of alloresponsiveness from the same haploidentical graft.

Brodin *et* al.[13] recently examined the variation in the human immune system using both mono- and dizygotic twins. They studied 95 different immune cell subsets and demonstrated that heritable factors strongly influenced naive, $CD27^+$ and central memory T cells. However, heritable factors were undetectable in 61% of all cell populations, including adaptive T and B cells and innate cell types (monocytes, neutrophils and NK cells). Their findings demonstrated that the immune system varied between monozygotic twins presumably as a consequence of non-heritable factors and with limited influence of heritable ones. Some parameters became more variable with age, suggesting the cumulative influence of environmental exposure. These findings were especially notable in monozygotic twins discordant for CMV infection, confirming that the immune system in healthy individuals is largely reactive and adaptive. Given the apparent role of non-heritable factors, they speculated that the many different microbes that an individual may have encountered over their lifetime may play an even more significant role in shaping the immune system of healthy individuals, particularly as it relates to adaptive immunity.[13,14]

The different previous exposures in our twins, particularly infections, likely contributed to their differences in alloreactivity and post-transplant GvHD outcomes. The first twin had a significant history of *Salmonella* gastroenteritis causing significant clinical disease years prior to transplant. She also had *Clostridium difficile* infection in the stool 2 months prior to transplant. She remained on oral vancomycin for prophylaxis during the transplant, although she was PCR negative at the time of transplant. She had also received 2 weeks of amoxicillinclavulanate prior to transplant for a recent pneumonia. Post transplant, she developed grade II GvHD requiring 6 months of systemic corticosteroids, as well as oral budesonide for 3 months post-HSCT and continuation of tacrolimus for 1.5 years post-HSCT. The second twin had no antecedent history of gastrointestinal infection. She developed only grade I skin involvement, which did not require systemic steroids; tacrolimus was stopped 6 months post HSCT without complications. Of unclear importance is that during neutropenia, twin 1 received ceftazidime while twin 2 received ceftazidime plus daptomycin (added for a transient PICC line infection with *Staphylococcus hominis* was identified in twin 2 on day +1, which led to removal of the line and no further positive cultures). These regimens are of low intensity with regard to an effect on the fecal microbiota, which has a major influence on the incidence of gastrointestinal GvHD.[15] Additionally, neither twin had significant infectious complications during the transplant course.

The study of monozygotic twins has a long history in the endeavor to separate heritable from non-heritable traits. With growing recognition of familial genetic diseases in which HSCT is the primary therapeutic option, it is likely that the use of a single family donor for more than one sibling will become more common. Accordingly, our report demonstrates that variability in outcomes may be expected, and that these outcomes may depend on prior infectious exposures.

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REFERENCES

1. Clementi M, Forabosco P, Amadori A, Zamarchi R, De Silvestro G, Di Gianantonio E et al. CD4 and CD8 T lymphocyte inheritance. Evidence for major autosomal recessive genes. Hum Genet 1999; 105: 337–342. [PubMed: 10543402]
2. Evans DM, Zhu G, Duffy DL, Frazer IH, Montgomery GW, Martin NG. A major quantitative trait locus for CD4-CD8 ratio is located on chromosome 11. Genes Immun 2004; 5: 548–552. [PubMed: 15306848]
3. 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: 2653–2655. [PubMed: 21670465]
4. 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: 2656–2658. [PubMed: 21765025]
5. 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: 1012–1017. [PubMed: 21892162]
6. McCarroll SA, Bradner JE, Turpeinen H, Volin L, Martin PJ, Chilewski SD et al. Donor-recipient mismatch for common gene deletion polymorphisms in graft-versus-host disease. Nat Genet 2009; 41: 1341–1344. [PubMed: 19935662]
7. Rao AA, Gourde JA, Marri P, Galardy PJ, Khan SP, Rodriguez V. Congenital amegakaryocytic thrombocytopenia: a case report of pediatric twins undergoing matched unrelated bone marrow transplantation. J Pediatr Hematol Oncol 2015; 37: 304–306. [PubMed: 25171451]
8. Vo PT, Pantin J, Ramos C, Cook L, Cho E, Kurlander R et al. Conditioning with rabbit versus horse ATG dramatically alters clinical outcomes in identical twins with severe aplastic anemia transplanted with the same allogeneic donor. J Hematol Oncol 2015; 8: 78. [PubMed: 26113077]
9. Shinkoda Y, Ijichi O, Tanabe T, Ishikawa S, Kamitamari A, Nishikawa T et al. Identical reconstitution after bone marrow transplantation in twins who received fresh and cryopreserved grafts harvested at the same time from their older brother. Clin Transplant 2004; 18: 743–747. [PubMed: 15516255]
10. Orti G, Sanz J, Bermudez A, Caballero D, Martinez C, Sierra J et al. Outcome of second allogeneic hematopoietic cell transplantation after relapse of myeloid malignancies following allogeneic hematopoietic cell transplantation: a retrospective cohort on behalf of the grupo espanol de trasplante hematopoyetico. Biol Blood M arrow Transplant 2016; 22: 584–588.
11. Ruutu T, de Wreede LC, van Biezen A, Brand R, Mohty M, Dreger P et al. Second allogeneic transplantation for relapse of malignant disease: retrospective analysis of outcome and predictive factors by the EBMT. Bone M arrow Transplant 2015; 50: 1542–1550.
12. Weisdorf D The role of second transplants for leukemia. Best Pract Res Clin Haematol 2016; 29: 359–364. [PubMed: 27890260]
13. Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJ, Furman D et al. Variation in the human immune system is largely driven by non-heritable influences. Cell 2015; 160: 37–47. [PubMed: 25594173]
14. Casanova JL, Abel L. Disentangling inborn and acquired immunity in human twins. Cell 2015; 160: 13–15. [PubMed: 25594170]

15. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl M ed 2016; 8: 339ra371.

Table 1.

Patient, transplant and GvHD characteristics

	Twin 1	Twin 2
Age at HSCT (years)	27	27
Pre-HSCT comorbidities	• Submandibular abscesses (history) • EBV mononucleosis with persistent EBV viremia • Salmonella gastroenteritis (severe) • Meningococcal meningitides • HPV-related genital and skin warts • Disseminated *Mycobacterium avium* complex • Hypoplastic MDS, trisomy 8 in 50% of metaphases	• EBV mononucleosis with persistent EBV viremia • HPV-related genital and skin warts • Infectious mononucleosis • Streptococcal pharyngitis • Herpes varicella Zoster infection • Hypoplastic MDS, trisomy 8 in 50% of metaphases
Transplant conditioning regimen	• Cyclophosphamide 14.5 mg/kg (days −6 and −5) • Fludarabine 30 mg/m2/d (days −6 to −2) • Busulfan* 3.2 mg/kg/day (days −4, −3, −2): AUC target 4800 • TBI: 200 cGy on day −1	• Cyclophosphamide 14.5 mg/kg (days −6 and −5) • Fludarabine 30 mg/m2/d (days −6 to −2) • Busulfan* 3.2 mg/kg/day (days −4, −3,−2): AUC target 4700 • TBI: 200 cGy on day −1
Donor	• 8/0 HLA-matched haploidentical sister, blood type: AB^+	• 8/0 HLA-matched haploidentical sister, blood type: AB^+
Recipient ABO	A^+	A^+
Recipient/donor CMV	Matched (−/−)	Matched (−/−)
Graft (bone marrow)	• TNC: 4.6 $\times 10^8$ cells/kg • $CD34^+$: 5.3 $\times$ 10^6 cells/kg • $CD3^+$: 5.37$\times 10^7$cells/kg	• TNC: 5.5 $X10^8$ cells/kg • $CD34^+$: 6.13 $\times$ 10^6 cells/kg • $CD3^+$:5.92$\times 10^7$cells/kg
GvHD prophylaxis	• Post-HSCT cyclophosphamide (50 mg/kg) on days +3 and +4 • Tacrolimus: days +5 to day+180 (goal level 5–10) • MMF: days +5 to+35	• Post-HSCT cyclophosphamide (50 mg/kg) on days +3 and +4 • Tacrolimus: days +5 to day+180 (goal level 5–10) • MMF: days +5 to+35
First day of GvHD manifestations	Day +28 (skin rash and diarrhea)	Day +28 (skin rash)
Maximum grade GvHD	Grade II	Grade 1
Skin stage	2	1
Gl stage	1 (biopsy proven)	0

	Twin 1	Twin 2
Did GvHD require systemic immunosuppression	Yes	No
When systemic immunosuppression was stopped (months post-HSCT)	18 months	6 months

Abbreviations: EBV = Epstein-Barr virus; HPV = human papilloma virus; HSCT = hematopoietic stem cell transplantation; MDS = myelodysplastic syndrome; MMF = Mycophenolate mofetil.

* Identical conditioning regimen with exception that the targeted AUC for busulfan for twin 2 was 4700 (vs 4800 for twin 1).

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