

ORIGINAL ARTICLE

G6PD deficiency from lyonization after hematopoietic stem cell transplantation from female heterozygous donors

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To determine whether during hematopoietic stem cell transplantation (HSCT), X-chromosome inactivation (lyonization) of donor HSC might change after engraftment in recipients, the glucose-6-phosphate dehydrogenase (*G6PD*) gene of 180 female donors was genotyped by PCR/allele-specific primer extension, and MALDI-TOF mass spectrometry/Sequenom MassARRAY analysis. X-inactivation was determined by semiquantitative PCR for the *HUMARA* gene before/after *HpaII* digestion. X-inactivation was preserved in most cases post-HSCT, although altered skewing of lyonization might occur to either of the X-chromosomes. Among pre-HSCT clinicopathologic parameters analyzed, only recipient gender significantly affected skewing. Seven donors with normal G6PD biochemically but heterozygous for *G6PD* mutants were identified. Owing to lyonization changes, some donor–recipient pairs showed significantly different G6PD levels. In one donor–recipient pair, extreme lyonization affecting the wild-type *G6PD* allele occurred, causing biochemical G6PD deficiency in the recipient. In HSCT from asymptomatic female donors heterozygous for X-linked recessive disorders, altered lyonization might cause clinical diseases in the recipients.

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Introduction

Under physiologic conditions, one of the two X-chromosomes in females is randomly inactivated, a process referred to as lyonization.¹ However, the inactivation may some-

times be skewed, thereby favoring expression of one of the X-chromosome alleles. Skewing of X-chromosome inactivation may increase with age.^{1–4}

Women heterozygous for X-chromosome-linked recessive genetic disorders are usually asymptomatic. However, in heterozygotes with skewed inactivation of the X-chromosome with the wild-type allele, preferential expression of the mutant allele may result in clinical manifestations.^{3–5} This phenomenon occurs occasionally in women heterozygous for mutants of the glucose-6-phosphate dehydrogenase (*G6PD*) gene where, owing to extreme lyonization, biochemical and clinical G6PD deficiencies have been observed.^{6,7}

Allogeneic hematopoietic stem cell transplantation (HSCT) involves the transfer of the donor hematopoiesis to the recipient. We hypothesized that after HSCT from female donors, reconstitution from a limited number of HSC might increase the chance of imbalanced lyonization. Using the *G6PD* gene, we tested whether the potential skewed X-inactivation might manifest phenotypically.

Materials and methods

Patients

Archival blood samples from 180 female donors and their HSCT recipients were studied. All recipients had stable engraftment and complete donor chimerism as documented by analysis of polymorphic microsatellite markers.⁸

G6PD phenotype

G6PD level was assayed by standard methods.⁹ The median *G6PD* level in normal individuals was 11.7 (8.8–7.6) U/g hemoglobin, and in *G6PD*-deficient men 0.38 U/g hemoglobin.^{6,7,9} As the *G6PD* mutants in the Chinese population do not give rise to congenital non-spherocytic hemolytic anemia, the *G6PD* enzyme levels are not expected to be affected by reticulocytosis. For donors carrying *G6PD* mutants, the donor and recipient *G6PD* levels were assayed before and after HSCT. To control for potential changes after HSCT, *G6PD* levels in 25 donor–recipient pairs (10 wild-type male donors, 10 wild-type female donors, 5 *G6PD*-deficient male donors) were also assayed pre- and post-HSCT.

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G6PD genotype

Seven mutants accounting for the majority of Chinese *G6PD* gene mutations (1376G→T, Canton; 1388G→A, Kaiping; 95A→G, Gaozhou; 871G→A, Viangchan; 1024C→T, Chinese 5; 1360C→T, Union; 392 G→T, Chinese 4) were screened for by PCR and allele-specific primer extension, followed by MALDI-TOF mass spectrometry.⁷ The results were analyzed by the Sequenom MassARRAY system (Sequenom, San Diego, CA, USA).

X-chromosome inactivation

A polymorphic site on the *HUMARA* gene was studied by semiquantitative PCR. This was performed on all G6PD heterozygous donors and their recipients, and 50 donor–recipient pairs without *G6PD* mutations as controls. Briefly, DNA was treated overnight with and without the methylation-sensitive enzyme *HpaII*. The *HUMARA* locus was then amplified by PCR with FAM-labeled primers, and analyzed on an automated DNA sequence analyzer (PE Biosystems, Foster City, CA, USA). The percentage of X-chromosome inactivation was estimated from the change in PCR products after the starting DNA was *HpaII*-digested.⁶ An internal control to ensure complete *HpaII* digestion was included.¹⁰ Tests were performed in triplicate and the maximum inter-test variability was 5%. On the basis of skewing, the G6PD activity was calculated with the formula: %skewing × 0.38 (mean activity of deficient males in our population) + (1 – %skewing) × 11.7 (mean activity of 155 individuals with wild-type alleles) as published.^{6,7} As lyonization might occur to any of the two alleles, two results could be calculated depending on which of the alleles is affected.

Statistical analysis

The skewing of X-inactivation in donor cells (D) pre-HSCT and after engraftment in the recipient (R) in 50 donor–recipient pairs with wild-type *G6PD* was correlated by Pearson's test. Possible factors affecting the absolute change in skewing (R-D) and percentage change (R-D/D × 100%), including recipient sex, donor age, recipient age, baseline donor skewing, median colony forming unit (CFU) granulocyte macrophage (GM) infused and time of

study from HSCT, were analyzed by Student's *t*-test or Pearson correlation as appropriate. Finally, donor–recipient pairs with *G6PD* mutants were compared with the control cases for skewing pattern, change in skewing and possible factors affecting skewing (Student's *t*-test). All analyses were performed with SPSS (10.0 Software, Chicago, IL, USA).

Results

Donors and recipients

All donors had normal G6PD levels, but seven were heterozygous for *G6PD* mutants (Table 1). Among 20 donors with wild-type *G6PD* alleles, the pre-HSCT donor G6PD levels (mean: 10.0, 8.3–11.4 U/g hemoglobin) was comparable with the post-HSCT recipient levels (mean: 10.8, 9.0–10.3 U/g hemoglobin), which were all within the normal reference range. For five G6PD-deficient male donors, the pre-HSCT donor G6PD levels (0.02–2.36 U/g hemoglobin) were also comparable with the post-HSCT recipient levels (0.7–1.26 U/g hemoglobin), which were all in the deficient range. However, in three of the recipients of G6PD heterozygous female donors in this study, the G6PD levels were significantly different as compared with their donors (case 1: increased; cases 2 and 6: decreased). Only one recipient (patient 2) had G6PD deficiency. This man was G6PD-deficient pre-HSCT (bearing the same 1376G→T Canton mutant as his heterozygous sister donor). DNA chimerism and red cell phenotyping showed complete donor chimerism, implying that the G6PD deficiency was due to extreme lyonization of the wild-type allele. This was confirmed on analysis of X-chromosome inactivation (Figure 1). Serial analysis in four of seven recipients showed that the degree of lyonization was stable from 1 (cases 1, 3 and 5) to 8 years (case 6) post-HSCT (Table 1).

Correlation between donor cell lyonization before and after HSCT

As a group, donor blood cells pre-HSCT and post-HSCT showed comparable lyonization ($r=0.83$, $P<0.0001$; Figure 1b). However, outliers were found on both sides of the diagonal, implying that significant changes in

Table 1 Clinicopathological features and laboratory results of seven recipients of HSC from donors heterozygous for G6PD gene mutants

Donor pre-HSCT					Recipient post-HSCT		
Sex/age	G6PD gene mutant	G6PD level ^a	Lyonization	Sex/age	G6PD level ^a	Lyonization ^b	Outcome
1 F/4	1388G→A, Kaiping	9.89 (5.1, 7.1)	0.59	M/1	15.65 (2.8, 9.3)	0.21 (0.23)	CR; 18 months+
2 F/41	1376G→T, Canton	7.80 (4.7, 7.4)	0.62	M/43	2.97 (4.1, 7.6)	0.33	CR; 42 months+
3 F/55	1388G→A, Kaiping	7.45 (5.9, 6.2)	0.51	M/53	7.47 (5.9, 6.2)	0.51 (0.52)	CR; 16 months+
4 F/57	1388G→A, Kaiping	7.30 (5.1, 6.9)	0.58	M/43	8.06 (5.0, 7.1)	0.59	CR; 60 months+
5 F/29	1376G→T, Canton	6.12 (5.6, 6.5)	0.54	F/34	6.28 (4.8, 7.3)	0.61 (0.62)	CR; 98 months+
6 F/31	1376G→T, Canton	12.01 (4.1, 8.0)	0.67	F/31	7.77 (4.3, 7.7)	0.65 (0.62/0.66/0.60/0.60/0.65/0.67)	CR; 99 months
7 F/40	1376G→T, Canton	6.80 (4.9, 7.2)	0.60	F/58	7.20 (5.1, 6.9)	0.58	CR; died, 5 months

Abbreviations: G6PD = glucose-6-phosphate dehydrogenase; HSC = hematopoietic stem cells.

G6PD level: (U/g hemoglobin); lyonization: (%).

^aValues in parentheses indicated the two theoretical G6PD levels (depending on the direction of skewing) calculated by the formula: %skewing × 0.38 (mean G6PD activity of deficient males) + (1 – %skewing) × 11.7 (mean G6PD activity).

^bValues in parentheses indicated serial assessments 12 months apart; *n*, number.

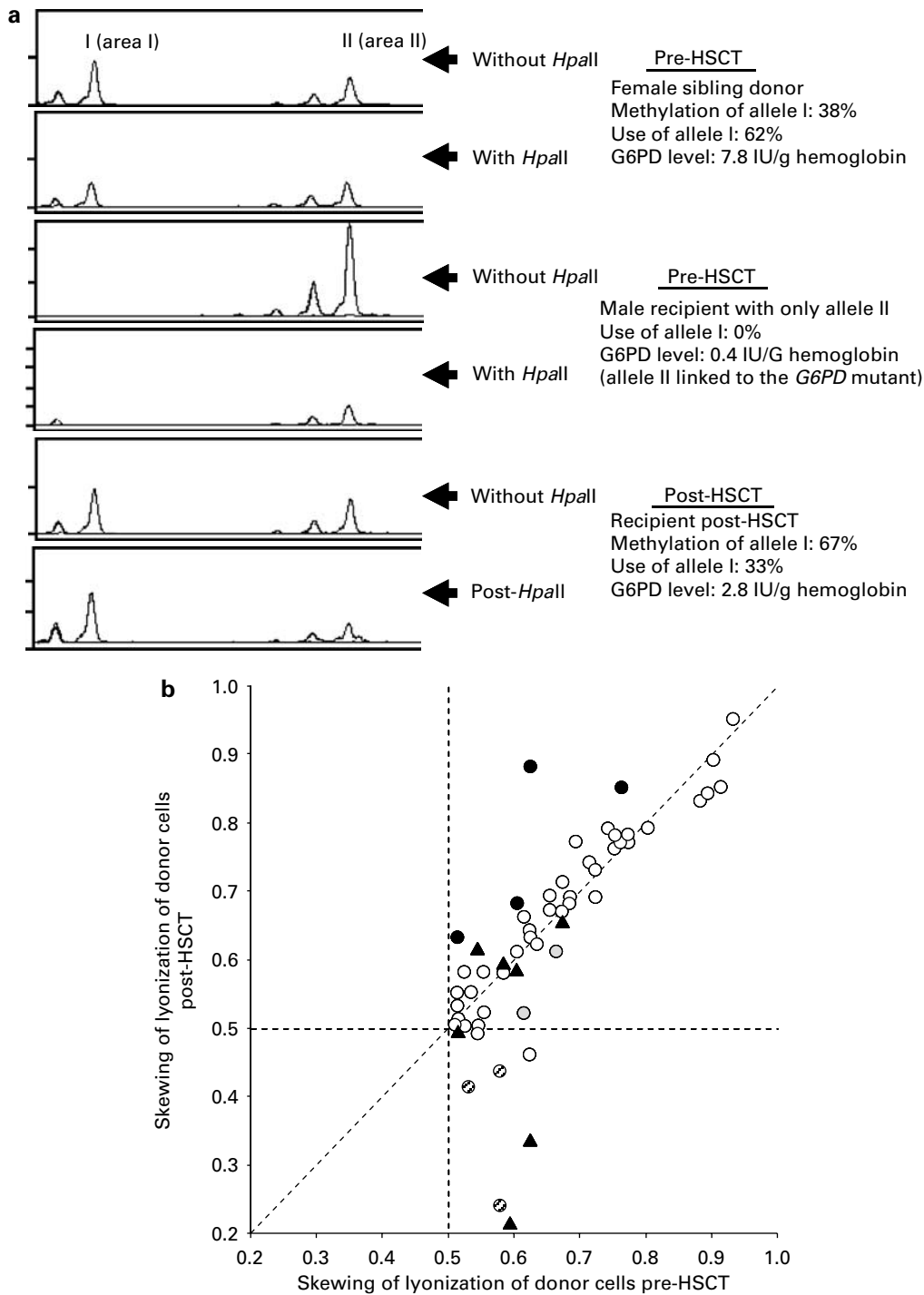


Figure 1 Skewing of X-chromosome inactivation in donor cells before and after allogeneic HSCT. **(a)** PCR for the *HUMARA* gene before and after digestion of the starting DNA with the methylation-sensitive enzyme *HpaII*. The percentage of X-chromosome inactivation was estimated from the change in PCR products with or without previous digestion with *HpaII*, by the formula $1 - (\text{area II} \times E / \text{area I} + \text{area II} \times E)$, where areas I and II referred to the electropherographic areas of amplified alleles I and II, and E the differential amplification efficiency of allele I compared with allele II. In this example, allele II was linked to the *G6PD* mutant (1376G → T, Canton), and allele I the wild-type *G6PD* gene. After HSCT, because of skewing of methylation affecting the chromosome carrying allele I and hence the wild-type *G6PD* gene (as shown by resistance to *HpaII* digestion, indicating methylation of that chromosome), expression of the *G6PD* mutant led to biochemical G6PD deficiency. **(b)** Correlation of skewing of X-chromosome inactivation in donor cells before and after HSCT in 50 donor–recipient pairs with wild-type *G6PD* alleles. Triangle: donor–recipient pairs with *G6PD* mutants; closed circles: more skewing after engraftment; gray circle: less skewing after engraftment; hatched circles: skewing in the opposite direction after engraftment. HSCT = hematopoietic stem cell transplantation.

Table 2 Comparison between donor–recipient pairs for donors with and without *G6PD* gene mutants

	Donors with <i>G6PD</i> gene mutants (n=7)	Donors with wild-type <i>G6PD</i> gene (n=50)	P-value
Recipient sex (male:female)	4:3	27:23	0.95
Recipient age (median)	36.7 years	35.9 years	0.9
Donor age (median)	37 years	35.2 years	0.75
Median time from HSCT	15.7 months	16.8 months	0.87
Median dose of CFU-GM	3.72	8.99	0.18
Median donor lyonization	0.52	0.65	0.01
Median recipient lyonization	0.62	0.65	0.63
Median change in lyonization	0.11	0.04	0.039
Median % change in lyonization	27.7	7.8	0.005

Abbreviations: G6PD = glucose-6-phosphate dehydrogenase; HSCT = hematopoietic stem cell transplantation; n = number of cases.

skewing might occur to either of the alleles. The median change of skewing was 4.8 (0–30)% in magnitude, and 7.8 (0–60)% in relative percentage. Within 50 donor–recipient pairs, the change of skewing was related to recipient sex (7% female versus 3% male, $P=0.023$), but unrelated to time of assessment ($P=0.14$), donor age ($P=0.16$), recipient age ($P=0.25$), original donor skewing ($P=0.091$) and CFU-GM infused ($P=0.75$; Table 2). For seven donor–recipient pairs with *G6PD* mutants, there was a significant change in skewing post-HSCT when compared with the controls (magnitude: 11 versus 4%, $P=0.039$; percentage: 27.7 versus 7.8%, $P=0.005$). However, there was no evidence of preferential skewing towards the normal or abnormal *G6PD* allele.

Discussion

Data on the status of X-chromosome inactivation after HSCT from female donors are scarce. In one study, donor cells pre-HSCT and after engraftment in the recipients post-HSCT showed similar lyonization, suggesting that the pattern of X-chromosome inactivation was unchanged.⁴ Our findings also showed that in most cases lyonization was unaltered in the donor cells before and after HSCT. However, significant changes in lyonization might occur in a minority of cases, implying that the balance of X-chromosome inactivation might be perturbed during the transfer and/or engraftment of HSC. Furthermore, both increases and decreases in skewing were observed. Nevertheless, we were unable to show any relationship between changes in skewing with pre-HSCT clinicopathologic parameters.

The change in skewing might be due to different reasons. During the rapid expansion of HSCs essential for engraftment, the selective proliferation of a subset of HSCs might occur. Whether this process is stochastic,¹¹ or related to

survival advantages conferred by specific alleles remains unclear.¹² The mechanisms controlling lyonization are likely complex, as shown by marked different skewing of X-chromosome in twins,¹³ and in different tissues in the same individual.³ However, once engrafted, lyonization of the donor HSCs remained relatively stable with time.¹⁴

The most important and unequivocal finding was the emergence after HSCT of *G6PD* deficiency in the blood cells of an apparently normal female donor who was in fact a heterozygote. Since most female HSCT donors are young and skewed lyonization is uncommon, heterozygotes are unlikely to show *G6PD* deficiency and hence be detectable by routine tests. Consequently, for recipients of female HSC donors, complacency might have prevented the testing for *G6PD* enzyme level before the prescription of drugs. This applies to both male and female recipients. This finding is especially important for prescription of the pneumocystis prophylaxis sulfamethoxazole-trimethoprim,¹⁵ which might provoke catastrophic and life-threatening hemolysis in *G6PD*-deficient individuals. The problem is particularly relevant in populations with a high prevalence of *G6PD* mutants (4% in Chinese men). In these populations, screening of male donors for *G6PD* deficiency is mandatory. Recipients of marrow from *G6PD*-deficient male donors will develop *G6PD* deficiency, and should therefore be put on pentamidine prophylaxis for pneumocystis, instead of receiving sulfamethoxazole-trimethoprim.¹⁵ Therefore, careful re-documentation of the *G6PD* level would still be necessary after HSCT from female donors in at-risk populations.

Finally, our observations have significant implications on the choice of female sibling donors for X-linked genetic disorders such as Wiskott Aldrich syndrome, chronic granulomatous disease and X-linked hypogammaglobinemia. Since these potential sibling donors may be healthy silent heterozygotes, unpredictable shifts resulting in significant skewing in lyonization after HSCT may lead to clinical manifestations of the disease phenotype, similar to *G6PD* deficiency in our study. Hence, genetic screening for these disorders should be performed for potential female donors, which might affect donor selection if a choice is available.

References

- 1 El Kassar N, Hetet G, Briere J, Grandchamp B. X-chromosome inactivation in healthy females: incidence of excessive lyonization with age and comparison of assays involving DNA methylation and transcript polymorphisms. *Clin Chem* 1998; **44**: 61–67.
- 2 Gale RE, Fielding AK, Harrison CN, Linch DC. Acquired skewing of X-chromosome inactivation patterns in myeloid cells of the elderly suggests stochastic clonal loss with age. *Br J Haematol* 1997; **98**: 512–519.
- 3 Sharp A, Robinson D, Jacobs P. Age- and tissue-specific variation of X chromosome inactivation ratios in normal women. *Hum Genet* 2000; **107**: 343–349.
- 4 Thornley I, Freedman MH. Telomeres, X-inactivation ratios, and hematopoietic stem cell transplantation in humans: a review. *Stem Cells* 2002; **20**: 198–204.
- 5 Vulliamy TJ, Knight SW, Dokal I, Mason PJ. Skewed X-inactivation in carriers of X-linked dyskeratosis congenita. *Blood* 1997; **90**: 2213–2216.

- 6 Au WY, Ma ES, Lam VM, Chan JL, Pang A, Kwong YL. Glucose 6-phosphate dehydrogenase (G6PD) deficiency in elderly Chinese women heterozygous for G6PD variants. *Am J Med Genet A* 2004; **129**: 208–211.
- 7 Au WY, Lam V, Pang A, Chan JL, Lee WM, Song YQ *et al*. Glucose 6 phosphate dehydrogenase deficiency in female octo-, nano- and centenarians. *J Gerontol A Biol Sci Med Sci* 2006; **61**: 1086–1089.
- 8 Au WY, Lie AK, Ma SK, Leung YH, Siu LL, Kwong YL. Therapy-related myelodysplastic syndrome of recipient origin after allogeneic bone marrow transplantation for acute lymphoblastic leukaemia. *Br J Haematol* 2001; **112**: 424–426.
- 9 Beutler E, Blume KG, Kaplan JC, Lohr GW, Ramot B, Valentine WN. International Committee for Standardization in Haematology: recommended screening test for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. *Br J Haematol* 1979; **43**: 465–467.
- 10 van Dijk JP, Heuver LH, van der Reijden BA, Raymakers RA, de Witte T, Jansen JH. A novel, essential control for clonality analysis with human androgen receptor gene polymerase chain reaction. *Am J Pathol* 2002; **161**: 807–812.
- 11 Gale RE, Fielding AK, Harrison CN, Linch DC. Acquired skewing of X-chromosome inactivation patterns in myeloid cells of the elderly suggests stochastic clonal loss with age. *Br J Haematol* 1997; **98**: 512–519.
- 12 Efferth T, Fabry U, Glatte P, Osieka R. Increased induction of apoptosis in mononuclear cells of a glucose-6-phosphate dehydrogenase deficient patient. *J Mol Med* 1995; **73**: 47–49.
- 13 Vickers MA, McLeod E, Spector TD, Wilson IJ. Assessment of mechanism of acquired skewed X inactivation by analysis of twins. *Blood* 2001; **97**: 1274–1281.
- 14 van Dijk JP, Heuver L, Stevens-Linders E, Jansen JH, Mensink EJ, Raymakers RA *et al*. Acquired skewing of lyonization remains stable for a prolonged period in healthy blood donors. *Leukemia* 2002; **16**: 362–367.
- 15 Au WY, Ma SK, Lie AK, Liang R, Cheng T, Kwong YL. Glucose-6-phosphate dehydrogenase deficiency and hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2002; **29**: 399–402.