Infants with acute lymphoblastic leukemia and a germline MLL gene are highly curable with use of chemotherapy alone: results from the Japan Infant Leukemia Study Group. *Blood*, **107**, 4663–4665.

Pieters, R., Schrappe, M., De Lorenzo, P., Hann, I., De Rossi, G., Felice, M., Hovi, L., LeBlanc, T., Szczepanski, T., Ferster, A., Janka, G., Rubnitz, J., Silverman, L., Stary, J., Campbell, M., Li, C.K., Mann, G., Suppiah, R., Biondi, A., Vora, A. & Valsecchi, M.G. (2007) A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. *Lancet*, **370**, 240–250.

Pui, C.H., Gaynon, P.S., Boyett, J.M., Chessells, J.M., Baruchel, A., Kamps, W., Silverman, L.B., Biondi, A., Harms, D.O., Vilmer, E., Schrappe, M. & Camitta, B. (2002) Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. *Lancet*, **359**, 1909–1915.

Rubnitz, J.E., Camitta, B.M., Mahmoud, H., Raimondi, S.C., Carroll, A.J., Borowitz, M.J., Shuster, J.J., Link, M.P., Pullen, D.J., Downing, J.R., Behm, F.G. & Pui, C.H. (1999) Childhood acute lymphoblastic leukemia with the MLL-ENL fusion and t(11;19)(q23;p13.3) translocation. *Journal of Clinical Oncology*, **17**, 191–196.

Sanders, J.E., Im, H.J., Hoffmeister, P.A., Gooley, T.A., Woolfrey, A.E., Carpenter, P.A., Andrews, R.G., Bryant, E.M. & Appelbaum, F.R. (2005) Allogeneic hematopoietic cell transplantation for infants with acute lymphoblastic leukemia. *Blood*, **105**, 3749–3756.

Sarafoglou, K., Boulad, F., Gillio, A. & Sklar, C. (1997) Gonadal function after bone marrow transplantation for acute leukemia during childhood. *Journal of Pediatrics*, **130**, 210–216.

Schmiegelow, K., Levinsen, M.F., Attarbaschi, A., Baruchel, A., Devidas, M., Escherich, G., Gibson, B., Heydrich, C., Horibe, K., Ishida, Y., Liang, D.C., Locatelli, F., Michel, G., Pieters, R., Piette, C., Pui, C.H., Raimondi, S., Silverman, L., Stanulla, M., Stark, B., Winick, N. & Valsecchi, M.G. (2013) Second malignant neoplasms after treatment of childhood acute lymphoblastic leukemia. *Journal of Clinical Oncology*, **31**, 2469–2476.

Silverman, L.B., McLean, T.W., Gelber, R.D., Donnelly, M.J., Gilliland, D.G., Tarbell, N.J. & Sallan, S.E. (1997) Intensified therapy for infants with acute lymphoblastic leukemia: results from the Dana-Farber Cancer Institute Consortium. *Cancer*, **80**, 2285–2295.

Somali, M., Mpatakoias, V., Avramides, A., Sakellari, I., Kaloyannidis, P., Smias, C., Anagnostopoulos, A., Kourtis, A., Rousso, D., Panidis, D. & Vagenakis, A. (2005) Function of the hypothalamic-pituitary-gonadal axis in long-term survivors of hematopoietic stem cell transplantation for hematological diseases. *Gynecological Endocrinology*, **21**, 18–26.

Tomizawa, D., Koh, K., Sato, T., Kinukawa, N., Morimoto, A., Isoyama, K., Kosaka, Y., Oda, T., Oda, M., Hayashi, Y., Eguchi, M., Horibe, K., Nakahata, T., Mizutani, S. & Ishii, E. (2007) Outcome of risk-based therapy for infant acute lymphoblastic leukemia with or without an MLL gene rearrangement, with emphasis on late effects: a final report of two consecutive studies, MLL96 and MLL98, of the Japan Infant Leukemia Study Group. *Leukemia*, **21**, 2258–2263.

Tomizawa, D., Koh, K., Hirayama, M., Miyamura, T., Hatanaka, M., Saikawa, Y. & Ishii, E. (2009) Outcome of recurrent or refractory acute lymphoblastic leukemia in infants with MLL gene rearrangements: a report from the Japan Infant Leukemia Study Group. *Pediatric Blood & Cancer*, **52**, 808–813.

Willemze, R., Rodrigues, C.A., Labopin, M., Sanz, G., Michel, G., Socie, G., Rio, B., Sirvent, A., Renaud, M., Madero, L., Mohty, M., Ferra, C., Garnier, F., Loiseau, P., Garcia, J., Lecchi, L., Kogler, G., Beguin, Y., Navarrete, C., Devos, T., Ionescu, I., Boudjedir, K., Herr, A.L., Gluckman, E., Rocha, V. & Eurocord, N. & Acute Leukaemia Working Party of the, E. (2009) KIR-ligand incompatibility in the graft-versus-host direction improves outcomes after umbilical cord blood transplantation for acute leukemia. *Leukemia*, **23**, 492–500.

2 Glu487Lys polymorphism: analysis of 2,299 Japanese subjects. *Cancer Epidemiology Biomarkers and Prevention*, **15**, 1009–1013.
Tamakoshi, A., Hamajima, N., Kawase, H., Wakai, K., Katsuda, N., Saito, T., Ito, H., Hirose, K., Takezaki, T. & Tajima, K. (2003) Duplex polymerase chain reaction with confronting two-pair primers (PCR-CTPP) for genotyping alcohol dehydrogenase beta subunit (ADH2) and aldehyde dehydrogenase 2 (ALDH2). *Alcohol and Alcoholism*, **38**, 407–410.
Wang, Y., Yagasaki, H., Hama, A., Nishio, N., Takahashi, Y. & Kojima, S. (2007) Mutation of SBDS and SH2D1A is not associated with aplastic anemia in Japanese children. *Haematologica*, **92**, 1573.

Allogeneic haematopoietic cell transplantation with reduced-intensity conditioning for elderly patients with advanced myelodysplastic syndromes: a nationwide study

The role of allogeneic haematopoietic cell transplantation (Allo-HCT) in the treatment of younger patients with myelodysplastic syndromes (MDS) is well established (de Witte *et al*, 2000; Sierra *et al*, 2002). However, early registry studies showed an adverse association between advanced age and increased non-relapse mortality (NRM) (Arnold *et al*, 1998). The introduction of reduced-intensity conditioning (RIC) has drastically reduced NRM, leading to a significant increase in the number of elderly patients with haematopoietic malignancy being referred for Allo-HCT. We conducted a nationwide retrospective study to clarify whether Allo-HCT with RIC actually improves overall survival (OS) for elderly patients with advanced MDS and sought to identify other variables that significantly influenced the outcome of Allo-HCT for these patients.

Data for patients who fulfilled the following criteria were obtained from the Transplant Registry Unified Management Program (TRUMP) (Atsuta *et al*, 2007): (i) aged 50–69 years, (ii) presence of French-American-British (FAB) class of refractory anaemia (RA) with excess blasts (RAEB) or RAEB in transformation (RAEBt) at any time between diagnosis and HCT, and (iii) received the first allogeneic bone marrow (BM) or peripheral blood stem cell transplantation from a human leucocyte antigen (HLA)-matched-related donor or a HLA-matched or -unmatched unrelated donor (URD) (Weisdorf *et al*, 2008) (Table SI) in Japan between 1 January 1 2001 and 31 December 2010. Patients with RA, RA with ringed sideroblasts or acute myeloid leukaemia evolving from MDS were excluded; such tight restriction of the disease stage meant that the study could deliver practical and useful information to clinical physicians. The conditioning regimen was classified as myeloablative conditioning (MAC) if it included total body irradiation (TBI) >8 Gy, oral busulfan (Bu) ≥9 mg/kg, intravenous Bu ≥7·2 mg/kg, or melphalan (Mel) >140 mg/m^2; otherwise, it was classified as RIC (Giralt *et al*, 2009). Data on the International Prognostic Scoring System (IPSS) components at HCT (Greenberg *et al*, 1997) were missing in TRUMP. Therefore, disease risk stratification used FAB class of RAEBt at any time between diagnosis and HCT, BM blasts ≥5% at HCT, and poor cytogenetics according to IPSS. Further details are provided in the Supplementary Methods.

Patient characteristics are shown in Tables I and SII. Of the 448 patients, 197 (44%) received MAC [cyclophosphamide (Cy)-TBI-based (n = 63), Bu-Cy-based (n = 58), fludarabine (Flu)-Bu-based (n = 42), Flu-Mel-based (n = 22) and other MAC (n = 12)]. The remaining 251 (56%) patients received RIC [Flu-Bu-based (n = 132), Flu-Mel-based (n = 80), Flu-Cy-based (n = 18) and other RIC (n = 21)]. Comparison of the patients who received MAC and RIC revealed that the RIC patients were significantly more likely to be 60–69 years of age (16% vs. 47%; P = 0·001), and less likely to receive a URD transplant (70% vs. 54%; P = 0·001) (Table SII).

The 3-year OS rates of patients receiving MAC and RIC were comparable (42·7% vs. 44·1%; P = 0·330; Fig 1A, Table SIII). The early mortality ratios were also similar between patients receiving MAC and RIC (5·6% vs. 3·6% at 30 days [P = 0·309]; 21·3% vs. 15·4% at 100 days [P = 0·106]). The multivariate analysis (Table I) revealed that the patients receiving MAC and RIC were comparable in terms of OS (RIC: relative risk [RR] 0·85; 95% confidence interval [CI] 0·65–1·13; P = 0·262). Other variables that were significantly and independently associated with OS in the whole cohort were HCT-comorbidity index >2 (RR 1·87; 95% CI 1·15–3·06; P = 0·012) (Sorror *et al*, 2005), BM blasts ≥5% at HCT (RR 1·46; 95% CI 1·02–2·10; P = 0·041), poor cytogenetics (RR 1·78; 95% CI 1·37–2·32; P < 0·001), time from diagnosis to HCT ≥6 months (RR 0·73; 95% CI 0·54–0·99; P = 0·042), partially matched (PM)-URD (RR 1·95; 95% CI 1·31–2·91; P = 0·001) and mismatched (MM)-URD (RR 2·47; 95% CI 1·61–3·79; P < 0·001).

Patients receiving RIC had a significantly lower 3-year cumulative incidence of NRM than patients receiving MAC (25·6% vs. 37·9%; P = 0·002, Fig 1B, Table SIII). The 100-day cumulative incidence of grade II-IV acute graft-versus-host disease (GVHD) and the 1-year cumulative

Table I. Patient characteristics and multivariate analysis for overall survival, non-relapse mortality and relapse.

		OS		NRM		Relapse	
Variables	*n*	RR (95%CI)	*P* value	RR (95%CI)	*P* value	RR (95%CI)	*P* value
Intensity of conditioning regimen							
MAC	197	1·00		1·00		1·00	
RIC	251	0·85 (0·65–1·13)	**0·262**	0·57 (0·39–0·83)	**0·004**	1·59 (1·06–2·39)	**0·027**
Recipient age at HCT							
50–59 years	299	1·00		1·00		1·00	
60–69 years	149	1·07 (0·80–1·43)	**0·671**	1·26 (0·85–1·89)	**0·253**	0·70 (0·45–1·10)	**0·120**
Recipient sex							
Female	128	1·00		1·00		1·00	
Male	320	1·12 (0·84–1·50)	**0·438**	1·28 (0·84–1·94)	**0·254**	0·82 (0·55–1·22)	**0·321**
HCT-CI							
0	120	1·00		1·00		1·00	
1–2	60	1·34 (0·84–2·13)	**0·225**	1·18 (0·60–2·32)	**0·639**	1·36 (0·75–2·49)	**0·312**
> 2	45	1·87 (1·15–3·06)	**0·012**	1·70 (0·84–3·45)	**0·139**	1·52 (0·79–2·94)	**0·214**
Missing	223	1·59 (1·11–2·30)	**0·013**	1·69 (1·00–2·86)	**0·048**	0·96 (0·58–1·58)	**0·868**
FAB class							
RAEB	329	1·00		1·00		1·00	
RAEBt	119	1·26 (0·94–1·68)	**0·120**	1·26 (0·82–1·92)	**0·291**	1·03 (0·67–1·59)	**0·900**
Bone marrow blasts at HCT							
< 5%	78	1·00		1·00		1·00	
≥ 5%	370	1·46 (1·02–2·10)	**0·041**	1·53 (0·94–2·49)	**0·090**	1·27 (0·72–2·23)	**0·409**
Cytogenetics							
Good/standard	270	1·00		1·00		1·00	
Poor	160	1·78 (1·37–2·32)	**<0·001**	1·40 (0·97–2·02)	**0·073**	1·62 (1·10–2·39)	**0·015**
Missing	18	0·93 (0·43–2·01)	**0·847**	0·86 (0·30–2·43)	**0·778**	0·72 (0·21–2·47)	**0·605**
Duration from diagnosis to HCT							
< 6 months	159	1·00		1·00		1·00	
≥ 6 months	289	0·73 (0·54–0·99)	**0·042**	0·88 (0·59–1·31)	**0·532**	0·63 (0·39–0·99)	**0·046**
Donor type							
M-RD	175	1·00		1·00		1·00	
WM-URD	148	1·12 (0·79–1·59)	**0·517**	1·36 (0·85–2·18)	**0·203**	0·94 (0·57–1·53)	**0·791**
PM-URD	77	1·95 (1·31–2·91)	**0·001**	2·63 (1·60–4·33)	**<0·001**	0·81 (0·41–1·59)	**0·545**
MM-URD	48	2·47 (1·61–3·79)	**<0·001**	3·38 (1·93–5·92)	**<0·001**	1·00 (0·49–2·04)	**0·994**
Year at HCT							
2001–2005	164	1·00		1·00		1·00	
2006–2010	284	0·95 (0·69–1·31)	**0·760**	0·75 (0·48–1·19)	**0·222**	1·08 (0·68–1·73)	**0·742**

OS, overall survival; NRM, non-relapse mortality; RR, relative risk; 95%CI, 95% confidence interval; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; HCT, haematopoietic cell transplantation; HCT-CI, HCT-specific comorbidity index; FAB, French-American-British; RAEB, refractory anaemia with excess blasts; RAEBt, RAEB in transformation; M-RD, matched-related donor; URD, unrelated donor; WM-URD, well-matched URD; PM-URD, partially matched URD; MM-URD, mismatched URD.

incidence of chronic GVHD of patients receiving MAC and RIC was 37·6% vs. 36·6% (P = 0·719) and 44·6% vs. 38·6% (P = 0·063), respectively (Fig S1). The multivariate analysis (Table I) showed that patients receiving RIC had a significantly lower NRM than patients receiving MAC (RR 0·57; 95% CI 0·39–0·83; P = 0·004). PM-URD (RR 2·63; 95% CI 1·60–4·33; P < 0·001) and MM-URD (RR 3·38; 95% CI 1·93–5·92; P < 0·001) were also independent variables that associated significantly with NRM. Patients receiving MAC and RIC were comparable in terms of grade II-IV acute GVHD risk (RIC: RR 1·07; 95%CI 0·76–1·49; P = 0·705) and chronic GVHD risk (RIC: RR 0·73; 95%CI 0·51–1·03; P = 0·073).

Patients receiving RIC had a significantly higher 3-year cumulative incidence of relapse than the patients receiving MAC (29·9% vs. 22·8%; P = 0·029, Fig 1c, Table SIII). The multivariate analysis (Table I) showed that patients receiving RIC had a significantly higher relapse risk than patients receiving MAC (RR 1·59; 95% CI 1·06–2·39; P = 0·027). Poor cytogenetics (RR, 1·62; 95% CI, 1·10–2·39; P = 0·015) and time from diagnosis to HCT ≥6 months (RR 0·63; 95% CI 0·39–0·99; P = 0·046) were also independent variables that were significantly associated with relapse risk.

The present study showed that elderly patients with advanced MDS receiving RIC and MAC had comparable OS. Notably, 40% of the patients receiving RIC obtained long-term

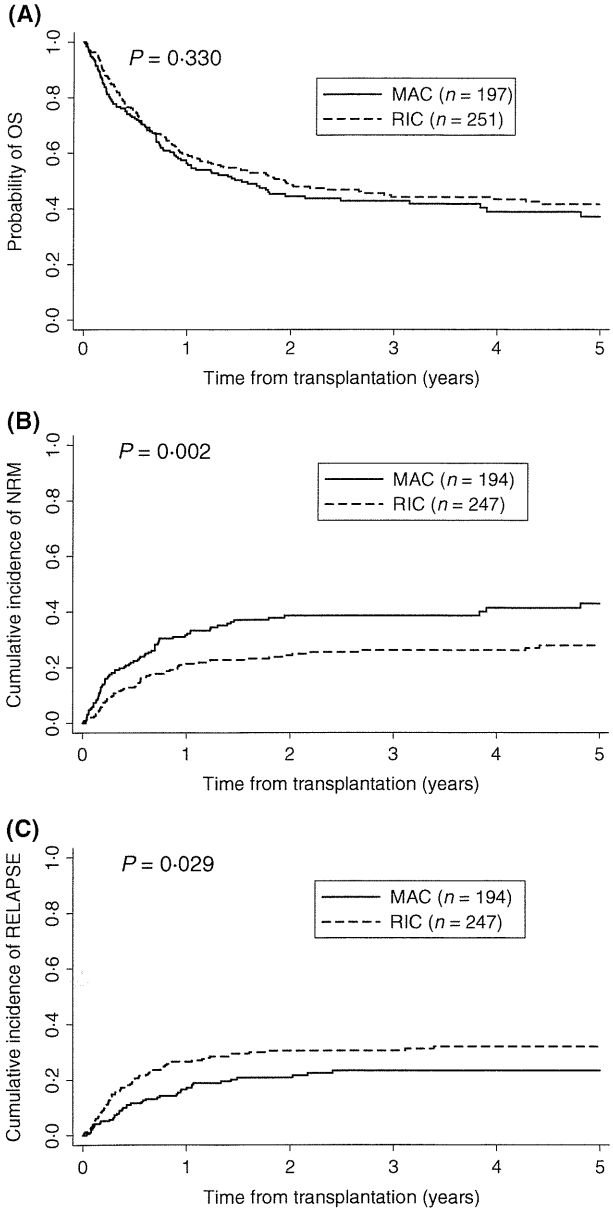

Fig 1. Overall survival, non-relapse mortality and relapse. (A) Unadjusted probability of overall survival of the cohort divided according to the intensity of the conditioning regimen (myeloablative conditioning [MAC] versus reduced-intensity conditioning [RIC]; $P = 0{\cdot}330$). (B) Unadjusted cumulative incidence of non-relapse mortality of the cohort divided according to the intensity of the conditioning regimen (MAC vs. RIC; $P = 0{\cdot}002$). (C) Unadjusted cumulative incidence of relapse of the cohort divided according to the intensity of the conditioning regimen (MAC vs. RIC; $P = 0{\cdot}029$). OS, overall survival; NRM, non-relapse mortality; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

survival. This indicates that, like MAC, RIC followed by Allo-HCT is a curative treatment option for elderly patients with advanced MDS; this effect is probably mediated by the graft-versus-MDS effect.

Compared to MAC, RIC was associated with lower NRM but a higher relapse rate. Other retrospective studies also show that a lower-intensity conditioning regimen associates with a higher relapse rate, especially in patients with advanced disease (de Lima *et al*, 2004; Shimoni *et al*, 2006). These results indicate that both the graft-versus-MDS effect and higher-intensity conditioning regimen are important for curing advanced MDS. The development of a new conditioning regimen that has high anti-MDS activity but does not induce severe organ toxicity may improve the outcome of elderly patients with advanced MDS.

Although randomized trials are needed, we conclude that Allo-HCT with RIC is a potential therapeutic option for those elderly patients with advanced MDS who are not eligible for MAC. The efficacy needs to be explored in prospective study.

Authorship and disclosures

K.A., T.I., K.I., H.I., and Y.M. designed the research and organized the project; K.A. and T.I. wrote the paper; K.A. and T.I. performed statistical analysis; and K.I., J.A., H.I., T.F., K.K., N.U., Y.U., T.E., T.M., T.K., K.I., Y.M., J.T., Y.A., and Y.M. interpreted the data and reviewed and approved the final manuscript. All authors declare no competing financial interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary methods.

Table SI. Available human leukocyte antigen data in the unrelated donors.

Table SII. Patient characteristics.

Table SIII. Unadjusted overall survival (OS), non-relapse mortality (NRM), and relapse.

Fig S1. Grade II-IV acute graft-versus-host disease (GVHD) and chronic GVHD.

References

Arnold, R., de Witte, T., van Biezen, A., Hermans, J., Jacobsen, N., Runde, V., Gratwohl, A. & Apperley, J.F. (1998) Unrelated bone marrow transplantation in patients with myelodysplastic syndromes and secondary acute myeloid leukemia: an EBMT survey. European Blood and Marrow Transplantation Group. *Bone Marrow Transplantation*, **21**, 1213–1216.

Atsuta, Y., Suzuki, R., Yoshimi, A., Gondo, H., Tanaka, J., Hiraoka, A., Kato, K., Tabuchi, K., Tsuchida, M., Morishima, Y., Mitamura, M., Kawa, K., Kato, S., Nagamura, T., Takanashi, M. & Kodera, Y. (2007) Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *International Journal of Hematology*, **86**, 269–274.

Giralt, S., Ballen, K., Rizzo, D., Bacigalupo, A., Horowitz, M., Pasquini, M. & Sandmaier, B. (2009) Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biology of Blood and Marrow Transplantation*, **15**, 367–369.

Greenberg, P., Cox, C., LeBeau, M.M., Fenaux, P., Morel, P., Sanz, G., Sanz, M., Vallespi, T., Hamblin, T., Oscier, D., Ohyashiki, K., Toyama, K., Aul, C., Mufti, G. & Bennett, J. (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*, **89**, 2079–2088.

de Lima, M., Anagnostopoulos, A., Munsell, M., Shahjahan, M., Ueno, N., Ippoliti, C., Andersson, B.S., Gajewski, J., Couriel, D., Cortes, J., Donato, M., Neumann, J., Champlin, R. & Giralt, S. (2004) Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. *Blood*, **104**, 865–872.

Shimoni, A., Hardan, I., Shem-Tov, N., Yeshurun, M., Yerushalmi, R., Avigdor, A., Ben-Bassat, I. & Nagler, A. (2006) Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. *Leukemia*, **20**, 322–328.

Sierra, J., Perez, W.S., Rozman, C., Carreras, E., Klein, J.P., Rizzo, J.D., Davies, S.M., Lazarus, H.M., Bredeson, C.N., Marks, D.I., Canals, C., Boogaerts, M.A., Goldman, J., Champlin, R.E., Keating, A., Weisdorf, D.J., de Witte, T.M. & Horowitz, M.M. (2002) Bone marrow transplantation from HLA-identical siblings as treatment for myelodysplasia. *Blood*, **100**, 1997–2004.

Sorror, M.L., Maris, M.B., Storb, R., Baron, F., Sandmaier, B.M., Maloney, D.G. & Storer, B. (2005) Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*, **106**, 2912–2919.

Weisdorf, D., Spellman, S., Haagenson, M., Horowitz, M., Lee, S., Anasetti, C., Setterholm, M., Drexler, R., Maiers, M., King, R., Confer, D. & Klein, J. (2008) Classification of HLA-matching for retrospective analysis of unrelated donor transplantation: revised definitions to predict survival. *Biology of Blood and Marrow Transplantation*, **14**, 748–758.

de Witte, T., Hermans, J., Vossen, J., Bacigalupo, A., Meloni, G., Jacobsen, N., Ruutu, T., Ljungman, P., Gratwohl, A., Runde, V., Niederwieser, D., van Biezen, A., Devergie, A., Cornelissen, J., Jouet, J.P., Arnold, R. & Apperley, J. (2000) Haematopoietic stem cell transplantation for patients with myelo-dysplastic syndromes and secondary acute myeloid leukaemias: a report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *British Journal of Haematology*, **110**, 620–630.

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ORIGINAL ARTICLE

Pre-transplant diabetes mellitus is a risk factor for non-relapse mortality, especially infection-related mortality, after allogeneic hematopoietic SCT

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Diabetes mellitus (DM) is a factor in the hematopoietic cell transplantation-comorbidity index. However, the impact of pre-transplant DM on morbidity and cause-specific non-relapse mortality (NRM) remains unclear. We performed a retrospective study with registry data that included a total of 7626 patients who underwent their first allogeneic hematopoietic SCT (HSCT) between 2007 and 2010. The median age was 44 years (range 0–88). Compared with patients without pre-transplant DM (non-DM group, $n=7248$), patients with pre-transplant DM (DM group, $n=378$) were older and were more likely to have high-risk disease, a reduced-intensity conditioning regimen and GVHD prophylaxis using tacrolimus. Multivariate analyses showed that pre-transplant DM was associated with increased risks of NRM (hazard ratio (HR)1.46, 95% confidence interval (CI) 1.21–1.76, $P<0.01$) and infection-related NRM (HR 2.08, 95% CI 1.58–2.73, $P<0.01$). The presence of pre-transplant DM was associated with an increased risk of overall mortality in a multivariate analysis (HR 1.55, 95% CI 1.35–1.78, $P<0.01$). In conclusion, pre-transplant DM was a risk factor for NRM, particularly infection-related mortality, after allogeneic HSCT. To improve the clinical outcome in patients with DM, the benefits of strict infection control and appropriate glycemic control should be explored in future trials.

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INTRODUCTION

Allogeneic hematopoietic SCT (HSCT) has become an integral part of treatment for hematological malignancies. The risk of non-relapse mortality (NRM) after allogeneic HSCT has decreased significantly over the past few decades.[1–4] However, the risk of NRM is still high in elderly patients and patients with comorbidities.[4] Sorror *et al.*[5] established a hematopoietic cell transplantation-comorbidity index (HCT-CI) scoring system to predict the risk of NRM using pre-transplant parameters. Previous studies that assessed the impact of HCT-CI only demonstrated its impact on the overall clinical outcome.[5,6] However, detailed information about the risk of each morbidity and mortality in patients with each comorbidity is necessary so that we can intervene efficiently to reduce the risk of complications, which could be expected to improve the overall outcome.

Regarding pre-transplant diabetes mellitus (DM), Derr *et al.*[7] reported that pre-transplant hyperglycemia was associated with an increased risk of infectious diseases. However, they did not assess the impact of pre-transplant hyperglycemia on GVHD because their study mainly included patients who underwent autologous HSCT. In addition, several papers have reported that peritransplant DM was associated with an increased risk of NRM.[8–10] Our group previously showed that preengraftment hyperglycemia could be a risk factor for infectious diseases, acute GVHD and NRM.[9] However, post-transplant hyperglycemia can be caused by the post-transplant complications such as infectious diseases, which clearly increase the risk of subsequent NRM.[11,12] Therefore, the impact of pre-transplant DM on morbidity and cause-specific NRM remains unclear.

The prevalence of DM is increasing worldwide.[13–15] The number of HSCT recipients complicated with DM is also expected to increase. Thus, it is important to explore methods for improving the outcome of patients with DM in allogeneic HSCT. If we could identify morbidities which have a greater risk in patients with DM, we may be able to prevent such morbidities specifically in patients with DM in addition to glucose control, as in our previous report.[16]

In this study, we retrospectively assessed the impact of pre-transplant DM on the clinical outcome after allogeneic HSCT using the registry database of the Japan Society for Hematopoietic Cell Transplantation (JSHCT).[17]

PATIENTS AND METHODS

Patients

The clinical data were obtained from the registry database of the Transplant Registry Unified Management Program provided by the JSHCT.[17] The following patients were included in the study: (i) patients who underwent their first allogeneic HSCT between January 2007 and December 2010, and (ii) patients for whom information was available

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regarding the presence or absence of pre-transplant DM included in the scoring of HCT-CI. Three patients who did not have information about the overall clinical outcome were excluded. Finally, 7626 patients were included in further analyses. Data about the control of DM and the insulin-based protocols were not available owing to the nature of our registry data. This study was approved by the Institutional Review Board of National Cancer Center, Tokyo, Japan.

Clinical outcomes

Endpoints included OS, PFS, relapse/progression, NRM, infectious diseases and acute GVHD. Acute and chronic GVHD were defined based on the standard criteria.[18,19] Regarding cause-specific NRM, NRM was categorized according to the major cause of death including infection, GVHD, organ failure and other.

Statistical analysis

A descriptive statistical analysis was performed to assess the patients' characteristics. Medians and ranges are provided for continuous variables and percentages are given for categorical variables. The probabilities of OS and PFS were calculated by the Kaplan–Meier method. A Cox proportional-hazards regression model was used to analyze OS and PFS. The cumulative incidences of engraftment, NRM, GVHD and infections were evaluated using the Fine and Gray model for univariate and multivariate analyses of cumulative incidence. In the competing risk models for engraftment, GVHD and infectious disease, relapse and death before these events were defined as competing risks. In the competing risk models for NRM, relapse was defined as a competing risk. For each cause-specific NRM, relapse and NRM with other causes were defined as competing risks. Factors that were associated with a two-sided *P*-value of <0.10 in the univariate analysis were included in a multivariate analysis. We used a backward-stepwise selection algorithm and retained only the statistically significant variables in the final model. A two-sided *P*-value of <0.05 was considered statistically significant. The variables that were evaluated in these analyses were as follows: sex mismatch (female to male vs other), patient's age at the time of HSCT (age $\geqslant 40$ years vs age <40), disease risk (standard risk vs high risk), stem cell source (BM vs PB stem cells vs cord blood), HLA disparity assessed by serological typing of HLA A, B and DRB1, performance status (0–1 vs 2–4), intensity of the conditioning regimen (myeloablative conditioning vs reduced-intensity conditioning) and ABO mismatch (match vs minor mismatch vs major mismatch/major and minor mismatch). Standard risk was defined as the first CR of acute leukemia or the first chronic phase of CML or non-malignant diseases. High risk was defined as other diseases. The intensity of the conditioning regimen was defined as described previously.[20,21]

All statistical analyzes were performed with EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Dr Yoshinobu Kanda, Saitama, Japan).[22] More precisely, it is a modified version of R commander (version 1.6-3, Dr Yoshinobu Kanda) that was designed to add statistical functions that are frequently used in biostatistics.

Table 1. Patients' characteristics

	DM (n = *378*) n *(%)*	*Non-DM* (n = *7248*) n *(%)*	P-*value*
Age median (range)	58 (6–73)	43 (0–88)	<0.01
Sex combination			
Female to male	92 (24)	1599 (22)	0.21
Others	249 (66)	5046 (70)	
Missing	37 (10)	608 (8)	
Disease risk			
Standard	115 (30)	2811 (39)	<0.01
High	263 (70)	4437 (61)	
PS			
0–1	319 (84)	6083 (84)	0.26
2–4	32 (9)	763 (11)	
Missing	27 (7)	402 (5)	
Conditioning			
MAC	158 (42)	4480 (62)	<0.01
RIC	211 (56)	2669 (37)	
Missing	9 (2)	99 (1)	
Stem cell source			
Related BM	43 (11)	1261 (17)	<0.01
Related PBSC	53 (14)	1247 (17)	
Unrelated BM	184 (49)	2858 (39)	
CB	98 (26)	1882 (26)	
GVHD prophylaxis			
Cs based	125 (34)	3026 (42)	<0.01
Tacrolimus based	249 (66)	4189 (58)	
HCT-CI score			
0	0 (0)	4978 (69)	<0.01
1–2	259 (69)	1476 (20)	
$\geqslant 3$	119 (31)	784 (11)	
HCT-CI score except DM score			
0	175 (46)	4978 (69)	<0.01
1–2	129 (34)	1476 (20)	
$\geqslant 3$	74 (20)	784 (11)	

Abbreviations: CB = cord blood; DM = diabetes mellitus; HCT-CI = hematopoietic cell transplantation-comorbidity index; MAC = myeloablative conditioning; PBSC = PB stem cell; PS = performance status; RIC = reduced-intensity conditioning.

RESULTS

Patients' characteristics

Table 1 summarizes the patients' characteristics. Among the 7626 patients, 378 (5%) had pre-transplant DM (the DM group). Compared with patients without pre-transplant DM (the non-DM group), the DM group included significantly older patients, more patients with high-risk disease, more patients who received a reduced-intensity conditioning regimen and more patients who received tacrolimus.

In the DM group, 259 (69%) and 119 patients (31%) were classified as HCT-CI score = 1–2 and $\geqslant 3$, respectively. On the other hand, in the non-DM group, 1476 (20%) and 784 patients (11%) were classified as HCT-CI score = 1–2 and $\geqslant 3$, respectively. We also calculated the HCT-CI score without including the DM score to independently assess the impact of DM. When we excluded the DM score, more patients in the DM group were classified as HCT-CI score $\geqslant 1$ and this proportion was higher than that in the non-DM group (54 vs 32%, $P<0.01$). Supplementary Table 1 shows a detailed analysis of the comorbidities other than DM in each group. Patients in the DM group were more likely to be complicated by arrhythmia, cerebrovascular disease, cardiac disease, mild hepatic disease, psychiatric disturbance, obesity and prior solid tumor than those in the non-DM group.

Infections

The cumulative incidence of all documented infections at 1 year after HSCT in the DM group was significantly higher than in the non-DM group (61.5 vs 52.3%, $P<0.01$, Figure 1a). However, in a multivariate analysis, pre-transplant DM was not associated with an increased risk of documented infections. The cumulative incidence of fungal infection at 1 year after HSCT in the DM group was significantly higher than that in the non-DM group (14.9 vs 10.0%, $P=0.02$, Figure 1b). When we focused on the species of fungal infection, there was no significant difference in the cumulative incidence of aspergillus or candida infection between the two groups. Meanwhile, the cumulative incidence of mucor infection at 1 year in the DM group was significantly higher than that in the non-DM group (1.1 vs 0.1%; $P<0.01$). In a multivariate analysis, pre-transplant DM was significantly associated with an

increased risk of mucor infection (hazard ratio (HR) 9.91, 95% confidence interval (CI) 2.99–32.88, $P < 0.01$). There were no significant differences in the cumulative incidences of bacterial and viral infections at 1 year after HSCT between the groups.

Acute GVHD

There was no difference in the cumulative incidence of grade II-IV acute GVHD between the two groups (34.7 vs 34.5%, $P = 0.79$). There was also no significant difference in the cumulative incidence of grade III-IV acute GVHD between the groups (10.6 vs 11.8%, $P = 0.48$). Pre-transplant DM was not a risk factor for acute GVHD in multivariate analyses.

NRM

The median follow-up period of survivors was 583 days (range, 24–1712 days) after HSCT. Patients in the DM group had a significantly higher incidence of 1-year NRM than those in the non-DM group (36.9% vs 20.1%, $P < 0.01$, Figure 2a). In a

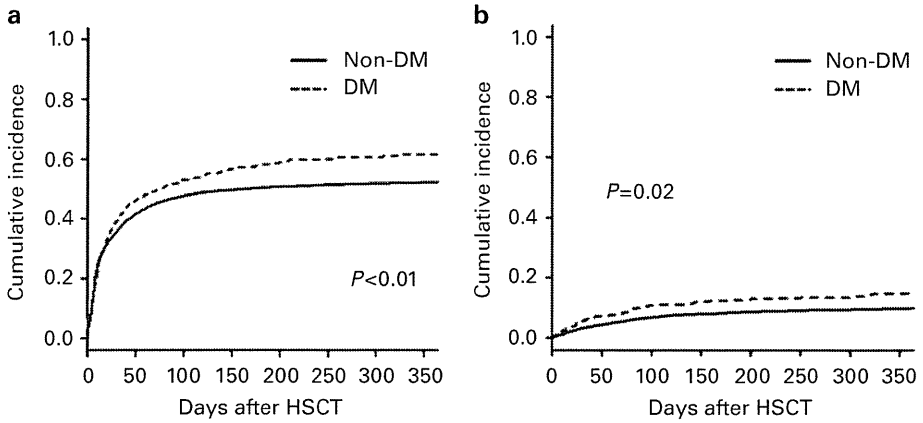

Figure 1. Cumulative incidence curves of all documented infection (**a**) and fungal infection (**b**) grouped according to the presence of pre-transplant DM.

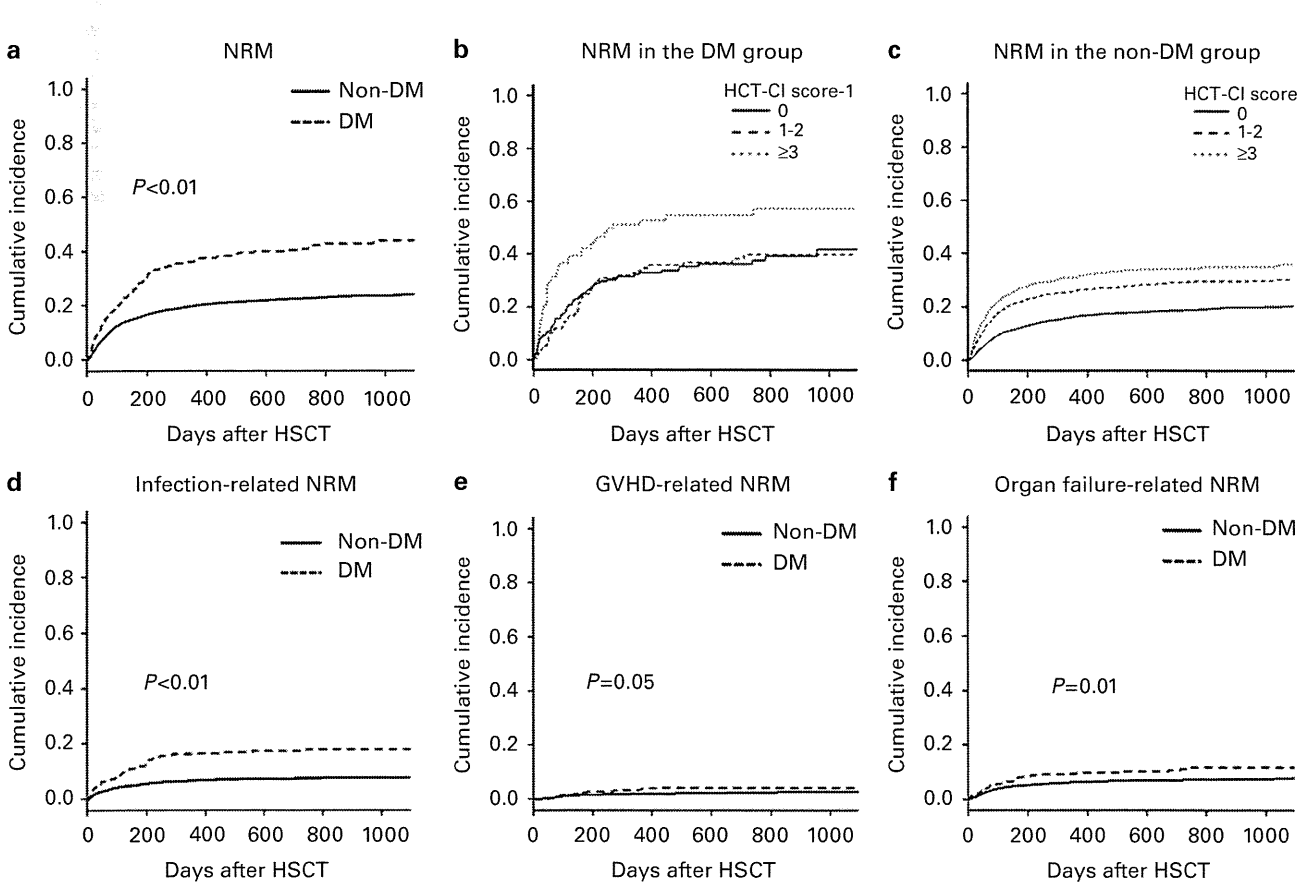

Figure 2. Cumulative incidence curves of NRM (**a**), infection-related NRM (**d**), GVHD-related NRM (**e**) and organ failure-related NRM (**f**) grouped according to the presence of pre-transplant DM. Cumulative incidence curves of NRM in the DM group stratified according to HCT-CI excluding the DM score (**b**), and in the non-DM group stratified according to the HCT-CI score (**c**).

multivariate analysis, pre-transplant DM was significantly associated with an increased risk of NRM (HR 1.46; 95% CI 1.21–1.76; $P < 0.01$, Table 2).

To exclude the impact of other comorbidities, we calculated the HCT-CI score by excluding the DM score and classified patients into three groups: score = 0, 1–2, ⩾3 in the DM group (Figure 2b) and the non-DM group (Figure 2c). The cumulative incidence of NRM at 1 year after HSCT in the DM group was significantly higher than that in the non-DM group for each HCT-CI score group. In the DM group, even in patients without any other comorbidities, the cumulative incidence of NRM at 1 year was 35% (Figure 2b). With respect to the group with score ⩾3, the cumulative incidence of NRM at 1 year increased to 52.6%, which was significantly higher than that in the non-DM group (Figures 2b and c). To further confirm the impact of DM, whilst adjusting for the impact of other HCT-CI factors, we performed a multivariate analysis of NRM that included all of the factors of HCT-CI, and pre-transplant DM was still an independent risk factor for NRM (HR 1.66; 95% CI 1.40–1.97; $P < 0.01$).

Table 2. Multivariate analysis of NRM

Covariates	*Univariate analysis*			*Multivariate analysis*		
	HR	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*
DM						
No	1.00			1.00		
Yes	2.02	1.71–2.38	<0.01	1.46	1.21–1.76	<0.01
Age, years						
<40	1.00			1.00		
⩾40	2.24	1.92–2.62	<0.01	1.96	1.66–2.31	<0.01
Sex combination						
Female to male	1.03	0.92–1.16	0.61			
Others	1.00					
PS						
0–1	1.00			1.00		
2–4	2.41	2.13–2.73	<0.01	2.18	1.91–2.49	<0.01
Disease risk						
Standard	1.00			1.00		
High	1.79	1.61–1.99	<0.01	1.39	1.25–1.56	<0.01
Stem cell source						
Related BM	1.00			1.00		
Related PBSC	1.65	1.37–1.99	<0.01	1.17	0.96–1.42	0.01
Unrelated BM	1.77	1.51–2.09	<0.01	1.53	1.29–1.81	<0.01
CB	2.52	2.13–2.98	<0.01	1.41	1.15–1.73	<0.01
HLA disparity						
Ag match	1.00			1.00		
1 Ag mismatch	1.55	1.37–1.76	<0.01	1.50	1.30–1.72	<0.01
⩾2 Ag mismatch	2.01	1.80–2.25	<0.01	1.72	1.45–2.04	<0.01
Conditioning						
MAC	1.00			1.00		
RIC	1.45	1.32–1.60	<0.01	1.19	1.08–1.32	<0.01
GVHD prophylaxis						
Cs based	1.00					
Tacrolimus based	1.24	1.13–1.37	<0.01			

Abbreviations: CB = cord blood; CI = confidence interval; DM = diabetes mellitus; HR = hazard ratio; MAC = myeloablative conditioning; PBSC = PB stem cell; PS = performance status; RIC = reduced-intensity conditioning.

Cause-specific NRM

The cumulative incidence of infection-related NRM at 1 year after HSCT in the DM group was significantly higher than that in the non-DM group (16.4 vs 6.7%, $P < 0.01$, Figure 2d). In a multivariate analysis, pre-transplant DM was associated with an increased risk of infection-related NRM (HR 2.08, 95%CI 1.58–2.73, $P < 0.01$, Supplementary Table 2). When patients were stratified into three groups according to the pathogen of infection-related NRM (bacterial, fungal and viral), the incidence of each pathogen-related NRM was significantly higher in the DM group. Multivariate analyses showed that pre-transplant DM was associated with an increased risk of each pathogen-related NRM (bacterial, HR 1.53, 95%CI 1.01–2.32, $P = 0.04$; viral, HR 2.66, 95%CI 1.37–5.17, $P < 0.01$; fungal, HR 3.51, 95%CI 2.05–6.03, $P < 0.01$, respectively).

There was no significant difference in the cumulative incidence of GVHD-related NRM between the two groups (3.6 vs 2.0% at 1 year, $P = 0.05$, Figure 2e). In a multivariate analysis, pre-transplant DM was not a risk factor of GVHD-related NRM. The cumulative incidence of organ failure-related NRM at 1 year in the DM group was significantly higher than that in the non-DM group (9.8 vs 6.3%, $P = 0.01$, Figure 2f). In a multivariate analysis, pre-transplant DM was associated with an increased risk of organ failure-related NRM (HR 1.41, 95%CI 1.01–1.96, $P = 0.04$).

OS, PFS and relapse

The probability of OS at 1 year after HSCT in the DM group was significantly worse than that in the non-DM group (44.7 vs 63.9%, $P < 0.01$, Figure 3). A multivariate analysis showed that pre-transplant DM was associated with an inferior OS (HR 1.55, 95%CI 1.35–1.78, $P < 0.001$, Table 3). The probability of PFS at 1 year in the DM group was also significantly worse than in the

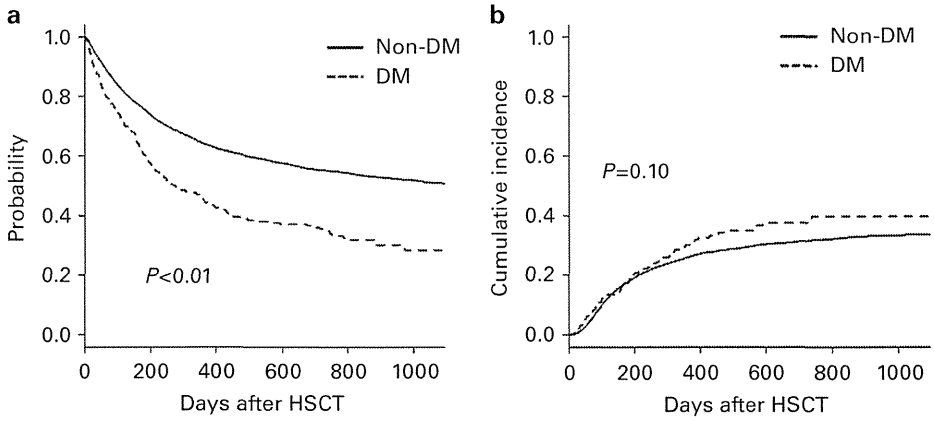

Figure 3. OS grouped according to the presence of pre-transplant DM (**a**) and cumulative incidence curves of relapse (**b**) grouped according to the presence of pre-transplant DM.

Table 3. Multivariate analysis of OS

Covariates	*Univariate analysis*			*Multivariate analysis*		
	HR	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*
DM						
No	1.00			1.00		
Yes	1.80	1.57–2.05	< .001	1.55	1.35–1.78	< 0.01
Age, years						
< 40	1.00			1.00		
⩾40	1.86	1.68–2.06	< 0.01	1.58	1.42–1.76	< 0.01
Sex combination						
Female to male	1.02	0.94–1.11	0.69			
Others	1.00					
PS						
0–1	1.00			1.00		
2–4	2.84	2.60–3.10	< 0.01	2.53	2.31–2.77	< 0.01
Disease risk						
Standard	1.00			1.00		
High	2.66	2.45–2.89	< 0.01	2.24	2.06–2.44	< 0.01
Stem cell source						
Related BM	1.00			1.00		
Related PBSC	1.67	1.47–1.89	< 0.01	1.18	1.04–1.35	0.01
Unrelated BM	1.39	1.25–1.55	< 0.01	1.20	1.07–1.35	< 0.01
CB	2.07	1.85–2.32	< 0.01	1.24	1.08–1.42	< 0.01
HLA disparity						
Ag match	1.00			1.00		
1 Ag mismatch	1.37	1.25–1.50	< 0.01	1.28	1.16–1.42	< 0.01
⩾2 Ag mismatch	1.87	1.72–2.03	< 0.01	1.49	1.33–1.67	< 0.01
Intensity of conditiong						
MAC	1.00			1.00		
RIC	1.32	1.23–1.42	< 0.01	1.10	1.03–1.18	0.01
GVHD prophylaxis						
Cs based	1.00					
Tacrolimus based	1.17	1.09–1.25	< 0.01			

Abbreviations: CB = cord blood; CI = confidence interval; DM = diabetes mellitus; HR = hazard ratio; MAC = myeloablative conditioning; PBSC = PB stem cell; PS = performance status; RIC = reduced-intensity conditioning.

non-DM group (40.6 vs 57.4%, $P < 0.01$). In a multivariate analysis, pre-transplant DM was associated with an inferior PFS (HR 1.44, 95%CI 1.26–1.65, $P < 0.01$). Regarding relapse, there was no significant difference in the relapse rate between the two groups (30.1 vs 26.2% at 1 year, $P = 0.10$). Pre-transplant DM was not associated with an increased risk of relapse in a multivariate analysis.

DISCUSSION

In this study, we clearly demonstrated that pre-transplant DM was associated with an increased risk of NRM that led to an inferior OS. These results were consistent with previous reports by Sorror *et al.*[5,6] The estimated HR for NRM in a multivariate analysis that included all other HCT-CI factors was 1.66, which was similar to the estimated HR (HR 1.6) reported by Sorror *et al.*[6] Our study confirmed the importance of pre-transplant DM as a risk factor of NRM in allogeneic HSCT.

This study is the first to demonstrate that pre-transplant DM is associated with an increased risk of infection-related deaths. The impact of pre-transplant hyperglycemia on the risk of infection during neutropenia has been discussed previously.[7] Derr *et al.*[7] reported that pre-transplant hyperglycemia was associated with an increased risk of post-transplant infectious diseases. Even though pre-transplant DM was not a risk factor of infectious diseases in a multivariate analysis in the current study, the incidence of infection-related NRM was significantly higher in patients with DM. This might reflect the vulnerability of patients with DM to infectious diseases. In particular, in terms of fungal disease, pre-transplant DM was associated with a highly increased risk of death (HR 3.51, 95%CI 2.05–6.03, $P < 0.01$). Therefore, it might be beneficial to intensify the monitoring or the prophylaxis of fungal diseases in patients with DM. In addition, even though the overall incidence was low, the increased risk of mucor infection in patients with DM was significantly higher than in patients without DM (HR 9.91, 95% CI 2.99–32.88, $P < 0.01$), which was consistent with previous observational studies.[23,24] This finding suggests that, when patients with DM develop pneumonia that is suspected to involve *Aspergillus* or *Mucor*, it might be preferable to use antifungal agents that are active against *Mucor*, such as liposomal amphotericin B.

We also analyzed the relationship between pre-transplant DM and other factors of HCT-CI. Patients in the DM group were more likely to be complicated by arrhythmia, cerebrovascular disease, cardiac disease, mild hepatic disease, psychiatric disturbance, obesity and prior solid tumor than those in the non-DM group. This finding was consistent with previous reports.[25–30] However, pre-transplant DM was an independent risk factor of NRM in a multivariate analysis that included all other HCT-CI factors. Furthermore, when patients were stratified according to the HCT-CI score that did not include DM, the incidence of NRM in patients with DM was higher than that in those without DM. In patients with an HCT-CI score of 0, NRM at 1 year in the DM group was two times higher than that in the non-DM group (32.8 vs 16.5%, Figures 2b and c). With respect to patients with an HCT-CI score of 3 or more, 1-year NRM increased to over 52.6%. Thus, these results suggested that pre-transplant DM was associated with a poor clinical outcome independent of such coexisting comorbidities.

One possible intervention for improving the outcome could be intensive glucose control (IGC) after allogeneic HSCT. As reported previously, post-transplant hyperglycemia was common even in patients without pre-transplant DM.[8–10] Previous reports have shown that hyperglycemia is significantly associated with an increased risk of organ dysfunction, grade II–IV acute GVHD and NRM.[8–10] Therefore, we could assume that glucose control is more important in patients with pre-transplant DM. Our group recently published the results of an IGC protocol, and patients with the IGC protocol had a lower incidence of infectious diseases than a matched control cohort without IGC.[16] Even though most patients in that study did not have pre-transplant DM, IGC may offer similar benefits in patients with DM. The benefits of IGC in allogeneic HSCT for patients with DM should be assessed in future trials.

The limitations of this study should be clarified. Although this is the largest study to assess the impact of pre-transplant DM on the clinical outcome after allogeneic HSCT, as it was a retrospective analysis, we were not able to exclude the presence of uncontrolled confounding variables, even if we conducted multivariate analyzes for each clinical outcome. Thus, the present findings should be reevaluated using a different database to reconfirm the importance of pre-transplant DM. Furthermore, the data about the control of DM including the insulin-based protocols used for glycemic control should be also collected in future trials. In addition, even if pre-transplant DM was associated with an increased risk of NRM, this does not necessarily mean that intervention to normalize glucose control, so-called IGC, will

improve the outcome, as demonstrated in the field of intensive care.[31,32] The value of such interventions should be clarified in prospective studies in patients who undergo allogeneic HSCT.

In conclusion, pre-transplant DM was a significant and independent risk factor for NRM, especially infection-related deaths. To further improve the clinical outcome in patients with DM, the benefits of strict infection control and appropriate glycemic control in allogeneic HCT should be explored in future trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

KT participated in research design, data analysis and writing of the paper; SF participated in research design, data analysis and writing of the paper; NU, HO, KO, TE, HS, YM, KK and RS gathered the data; TF participated in research design and writing of the paper. All of the authors approved the submission of this study.

REFERENCES

1 Kurosawa S, Yakushijin K, Yamaguchi T, Atsuta Y, Nagamura-Inoue T, Akiyama H *et al.* Recent decrease in non-relapse mortality due to GVHD and infection after allogeneic hematopoietic cell transplantation in non-remission acute leukemia. *Bone Marrow Transplant* 2013; **48**: 1198–1204.
2 Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M *et al.* Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med* 2010; **363**: 2091–2101.
3 Horan JT, Logan BR, Agovi-Johnson MA, Lazarus HM, Bacigalupo AA, Ballen KK *et al.* Reducing the risk for transplantation-related mortality after allogeneic hematopoietic cell transplantation: how much progress has been made? *J Clin Oncol* 2011; **29**: 805–813.
4 Kurosawa S, Yakushijin K, Yamaguchi T, Atsuta Y, Nagamura-Inoue T, Akiyama H *et al.* Changes in incidence and causes of non-relapse mortality after allogeneic hematopoietic cell transplantation in patients with acute leukemia/myelodysplastic syndrome: an analysis of the Japan transplant outcome registry. *Bone Marrow Transplant* 2013; **48**: 529–536.
5 Sorror ML, Giralt S, Sandmaier BM, De Lima M, Shahjahan M, Maloney DG *et al.* Hematopoietic cell transplantation specific comorbidity index as an outcome predictor for patients with acute myeloid leukemia in first remission: combined FHCRC and MDACC experiences. *Blood* 2007; **110**: 4606–4613.
6 Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG *et al.* Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood* 2005; **106**: 2912–2919.
7 Derr RL, Hsiao VC, Saudek CD. Antecedent hyperglycemia is associated with an increased risk of neutropenic infections during bone marrow transplantation. *Diabetes Care* 2008; **31**: 1972–1977.
8 Hammer MJ, Casper C, Gooley TA, O'Donnell PV, Boeckh M, Hirsch IB. The contribution of malglycemia to mortality among allogeneic hematopoietic cell transplant recipients. *Biol Blood Marrow Transplant* 2009; **15**: 344–351.
9 Fuji S, Kim SW, Mori S, Fukuda T, Kamiya S, Yamasaki S *et al.* Hyperglycemia during the neutropenic period is associated with a poor outcome in patients undergoing myeloablative allogeneic hematopoietic stem cell transplantation. *Transplantation* 2007; **84**: 814–820.
10 Gebremedhin E, Behrendt CE, Nakamura R, Parker P, Salehian B. Severe hyperglycemia immediately after allogeneic hematopoietic stem-cell transplantation is predictive of acute graft-versus-host disease. *Inflammation* 2013; **36**: 177–185.
11 Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M *et al.* Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 2002; **106**: 2067–2072.
12 McCowen KC, Malhotra A, Bistrian BR. Stress-induced hyperglycemia. *Crit Care Clin* 2001; **17**: 107–124.
13 Ginter E, Simko V. Type 2 diabetes mellitus, pandemic in 21st century. *Adv Exp Med Biol* 2012; **771**: 42–50.
14 Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V *et al.* Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095–2128.
15 Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ *et al.* National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet* 2011; **378**: 31–40.
16 Fuji S, Kim SW, Mori S, Kamiya S, Yoshimura K, Yokoyama H *et al.* Intensive glucose control after allogeneic hematopoietic stem cell transplantation: a retrospective matched-cohort study. *Bone Marrow Transplant* 2009; **44**: 105–111.
17 Atsuta Y, Suzuki R, Yoshimi A, Gondo H, Tanaka J, Hiraoka A *et al.* Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol* 2007; **86**: 269–274.
18 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
19 Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA *et al.* Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* 1974; **18**: 295–304.
20 Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V *et al.* Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant* 2009; **15**: 1628–1633.
21 Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M *et al.* Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant* 2009; **15**: 367–369.
22 Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452–458.
23 Rammaert B, Lanternier F, Poirée S, Kania R, Lortholary O. Diabetes and mucormycosis: a complex interplay. *Diabetes Metab* 2012; **38**: 193–204.
24 Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL *et al.* Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin Infect Dis* 2005; **41**: 634–653.
25 Sluik D, Boeing H, Montonen J, Pischon T, Kaaks R, Teucher B *et al.* Associations between general and abdominal adiposity and mortality in individuals with diabetes mellitus. *Am J Epidemiol* 2011; **174**: 22–34.
26 Unick JL, Beavers D, Jakicic JM, Kitabchi AE, Knowler WC, Wadden TA *et al.* Effectiveness of lifestyle interventions for individuals with severe obesity and type 2 diabetes: results from the Look AHEAD trial. *Diabetes Care* 2011; **34**: 2152–2157.
27 Mercer BN, Morais S, Cubbon RM, Kearney MT. Diabetes mellitus and the heart. *Int J Clin Pract* 2012; **66**: 640–647.
28 Shikata K, Ninomiya T, Kiyohara Y. Diabetes mellitus and cancer risk: review of the epidemiological evidence. *Cancer Sci* 2013; **104**: 9–14.
29 Eijgenraam P, Heinen MM, Verhage BA, Keulemans YC, Schouten LJ, van den Brandt PA. Diabetes type II, other medical conditions and pancreatic cancer risk: a prospective study in The Netherlands. *Br J Cancer* 2013; **109**: 2924–2932.
30 Ong JP, Younossi ZM. Epidemiology and natural history of NAFLD and NASH. *Clin Liver Dis* 2007; **11**: 1–16.
31 Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M *et al.* Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**: 1364–1379.
32 Marik PE, Preiser JC. Toward understanding tight glycemic control in the ICU: a systematic review and metaanalysis. *Chest* 2010; **137**: 544–551.

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RESEARCH ARTICLE

Identification of an Interaction between *VWF* rs7965413 and Platelet Count as a Novel Risk Marker for Metabolic Syndrome: An Extensive Search of Candidate Polymorphisms in a Case-Control Study

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Abstract

Although many single nucleotide polymorphisms (SNPs) have been identified to be associated with metabolic syndrome (MetS), there was only a slight improvement in the ability to predict future MetS by the simply addition of SNPs to clinical risk markers. To improve the ability to predict future MetS, combinational effects, such as SNP—SNP interaction, SNP—environment interaction, and SNP—clinical parameter (SNP × CP) interaction should be also considered. We performed a case-control study to explore novel SNP × CP interactions as risk markers for MetS based on health check-up data of Japanese male employees. We selected 99 SNPs that were previously reported to be associated with MetS and components of MetS; subsequently, we genotyped these SNPs from 360 cases and 1983 control subjects. First, we performed logistic regression analyses to assess the association of each SNP with MetS. Of these SNPs, five SNPs were significantly associated with MetS ($P < 0.05$): *LRP2* rs2544390, rs1800592 between *UCP1* and *TBC1D9*, *APOA5* rs662799, *VWF* rs7965413, and rs1411766 between *MYO16* and *IRS2*. Furthermore, we performed multiple logistic regression analyses, including an SNP term, a CP term, and an SNP × CP interaction term for each CP and SNP that was significantly associated with

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MetS. We identified a novel SNP × CP interaction between rs7965413 and platelet count that was significantly associated with MetS [SNP term: odds ratio (OR) = 0.78, $P = 0.004$; SNP × CP interaction term: OR = 1.33, $P = 0.001$]. This association of the SNP × CP interaction with MetS remained nominally significant in multiple logistic regression analysis after adjustment for either the number of MetS components or MetS components excluding obesity. Our results reveal new insight into platelet count as a risk marker for MetS.

Introduction

Metabolic syndrome (MetS) is characterized by a clustering of metabolic abnormalities, including central obesity, insulin resistance, dyslipidemia, and hypertension; moreover, it has been identified as a common precursor to the development of cardiovascular disease (CVD) [1]. The prevalence of MetS has been increasing in Japan during recent decades as a result of changes in diet and physical activity [2]. According to a survey by the Ministry of Health, Labor, and Welfare of Japan in 2007, one out of three males aged 30 to 59 years, who occupy the majority of Japanese employees, were strongly suspected of having or were likely to develop MetS [3]. National health insurers began conducting annual health check-ups of all customers between the ages of 40–74 in April 2008 [4]. There is an urgent need to find appropriate and sensitive risk markers to identify individuals at high risk for developing MetS and thereby prevent further increase in its incidence.

To explore risk markers for predicting MetS development, many studies have been performed utilizing health check-up data among different groups of people, such as company employees [5], people in hospitals [6,7], and members of various communities [8]. In these studies, many commonly measured clinical parameters (CPs) from routine health check-ups were reported to be associated with MetS. For example, Tao et al. reported that hematological parameters, such as white blood cell (WBC) count, low-density lipoprotein (LDL) cholesterol, and glutamic-pyruvic transaminase (GPT) were associated with MetS based on the health check-up data of a Beijing adult population [7]. These clinical parameters are expected to be clinical risk markers for MetS development.

In some studies, several combinations of clinical risk markers have also been explored based on health check-up data. We have previously shown that the combination of the γ-glutamyl transpeptidase (γ-GTP) level and WBC count was the most significant combinatorial risk marker associated with MetS based on the health check-up data of company employees [5]. We have also proposed the ratio of adiponectin to homeostasis model assessment of insulin resistance (A-H ratio) [8] as a combinational risk marker for MetS development based on community-based health check-up data. There is, however, a need to identify new combinational risk markers.

It is also known that genetic factors contribute to the development of MetS and MetS components (e.g., central obesity, insulin resistance, dyslipidemia, and hypertension). Recently, many single nucleotide polymorphisms (SNPs) that are associated with MetS and MetS components have been identified through candidate gene studies [9] and genome-wide association studies (GWAS) [10,11]. Although these SNPs were expected to be genetic risk markers for the development of MetS and MetS components, there are several problems that need to be resolved. First, most of the common variants, such as SNPs, confer relatively small increments in risk (1.1–1.5-fold) with regard to the development of common diseases, such as MetS and MetS components, and explain only a small proportion of heritability, which is the portion of

phenotypic variance in a population that is attributable to additive genetic factors [12]. Additionally, there was only slight improvement in the ability to predict future MetS and MetS components by the simply addition of SNPs to clinical risk markers. For example, for predicting future type 2 diabetes, which is one of MetS components, the simply addition of 11 SNPs to clinical risk markers resulted in a slight increase in the area under the receiver operating-characteristic curve from 0.74 to 0.75 [13]. To improve the ability to predict future MetS and MetS components, combinational effects, such as SNP—SNP interaction, SNP—environment interaction, and SNP—clinical parameter (SNP × CP) interaction, should be also considered. Recently, some SNP × CP interactions have been reported as risk markers. For example, Manning et al. applied a joint meta-analysis approach to test associations with fasting glycemic traits and insulin resistance, which is thought to play a prominent role in MetS [14], on a genome-wide scale. Their results demonstrated that an interaction term between body-mass index (BMI) and an SNP that is located in an intergenic region between the *COBLL1* and *GRB14* is significantly associated with fasting insulin levels [15]. There is, however, a need to identify new SNP × CP interactions as risk markers for MetS development.

In this study, we performed a case-control study to explore novel SNP × CP interactions as risk markers for MetS based on health check-up data of Japanese male employees. We selected 99 candidate SNPs that were previously reported to be associated with MetS, MetS components, and coronary atherosclerosis. Subsequently, we screened SNPs that were significantly associated with MetS and explored SNP × CP interactions for association with MetS development. The explored interaction effect demonstrated in this study is expected to be utilized as a risk marker for MetS development. By combining conventional CP and SNP data, we can estimate the risk of future MetS development.

Materials and Methods

Study subjects

This study is case-control study for MetS, and part of an ongoing cohort, prospective observational study of MetS and chronic kidney disease (CKD). This original study has been following 33776 participants who underwent annual health check-ups for Toyota Motor Co., Ltd in both 2001 and 2009. Of these volunteers, 360 case subjects and 1983 control subjects who satisfied the definitions of cases or controls and attended health check-ups in 2011 or 2012 were randomly enrolled. Health check-up data were collected in 2001 and 2009, and DNA samples were obtained from case and control subjects in 2011 or 2012. This study was performed according to the guidelines of the Declaration of Helsinki. The study protocol was approved by Human Genome, Gene Analysis Research Ethics Committee of Nagoya University School of Medicine, and all participants provided written informed consent.

Definitions of cases and controls

This study is a case-control study, and subjects who satisfied the definitions of cases or controls and attended health check-ups in 2011 or 2012 were randomly enrolled for this study. Health check-up data were collected in 2001 and 2009, and case / control groups were defined post hoc in 2009, while individuals meeting the criteria for MetS in 2001 were excluded. We used the criteria proposed by the Examination Committee of Criteria for the Metabolic Syndrome in Japan [16] to identify case and control subjects: 1) obesity, waist circumference $\geq$ 85 cm in 2009 or BMI $\geq$ 25 kg/m^2 in 2001; 2) raised blood pressure, systolic blood pressure (SBP) $\geq$ 130 mmHg and/or diastolic blood pressure (DBP) $\geq$ 85 mmHg; 3) dyslipidemia, triglyceride $\geq$ 150 mg/dL and/or high-density lipoprotein (HDL)-cholesterol $<$ 40 mg/dL; and 4) raised fasting blood sugar (FBS), FBS $\geq$ 110 mg/dL. Subjects were diagnosed with MetS if they were obese and

showed any two of the other three criteria. Otherwise, subjects were classified as non-MetS. Then, we defined cases and controls according to the following criteria: cases, subjects who were classified as non-MetS in 2001 and were classified as MetS in 2009; controls, subjects who were classified as non-MetS in both 2001 and 2009.

Measurements of CPs

The health examinations performed in 2001 and 2009 included physical measurements and serum biochemical measurements. Physical measurements of height, weight, and BMI were measured in the fasting state. Waist circumference was only measured in 2009. SBP and DBP were measured in the sitting position. Blood samples were obtained from subjects who had fasted for serum biochemical measurements. After the subject had rested for 10 min in the sitting position, 14 mL of blood was collected from the antecubital vein into tubes containing ethylenediaminetetraacetic acid (EDTA). After blood samples were sent to a clinical laboratory testing company, biochemical measurements were determined according to standard laboratory procedures. The study included the biochemical measurements of the following: (1) lipids: total cholesterol, triglyceride, and HDL-cholesterol; (2) carbohydrates: FBS; (3) hematology: red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), and platelet (PLT) count; (4) non-protein nitrogenous compounds: uric acid (UA); and (5) serum enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GTP).

Selection of SNPs

Using public databases, such as PubMed and Online Mendelian Inheritance in Man, we selected 99 candidate SNPs that have been characterized and are associated with coronary atherosclerosis or vasospasm, obesity, hypertension, dyslipidemia, diabetes mellitus, hyperuricemia, or renal disease based on a comprehensive overview of vascular biology, coagulation and fibrinolysis cascades, platelet and leukocyte biology, as well as lipid and glucose metabolism and other metabolic factors (S1 Table).

Genotyping SNPs

All SNPs were genotyped using the DigiTag2 assay [17] as previously described. Briefly, target fragments (including target SNP sites) are prepared by multiplex PCR from genomic DNA. A multiplexed oligonucleotide ligation assay was performed, and a labeling reaction was achieved with two 5′ query probes and one common probe prepared for a single SNP site. The 5′ query probes had a sequence complementary to the 5′-flanking region of the target SNP, and each of the probes had an allele-specific sequence. Two types of end digit (ED), CCGTGTCCACTCTAGAAAAACCT and ACCACCGCTTGAATACAAAACAT, were attached to each of the 5′ query probes. The 3′ query probes had a sequence complementary to the 3′-flanking region of the target SNP, and each of the probes had a first digit (D1) on its 3′ end. Next, a hybridization reaction with D1 probes on a DNA microarray (NGK Insulators, Ltd, Nagoya, Japan) was performed with separated areas. The genotyping success rate was > 99.8%. SNP rs1862513 was excluded from analysis because there was evidence of departure from the Hardy-Weinberg equilibrium ($P < 0.05$). Consequently, 98 SNPs remained for analysis. Primers, probe sequences, and PCR conditions for genotyping are shown in S2 Table and S3 Table.

Study design

This study is a case-control study based on a prospective cohort data. The aim of this study is to identify interactions between SNPs and CPs from the 2001 data that successfully predicted MetS

that was diagnosed in 2009. The explored interaction effect is expected to be utilized as a risk marker for MetS development. From the combined data of conventional CPs from 2001 and the SNPs, the risk of MetS development by 2009 could be estimated for each subject. To reduce false-positive interactions, we applied a two-step approach. We initially performed a screening analysis using the 98 SNPs. In this screening analysis, the cutoff *P* value was defined as less than 0.05 for logistic-regression analysis with adjustment for age. From the screening study, we selected five SNPs that were significantly associated with MetS. We then performed an interaction analysis to assess interactions between the five SNPs and 15 CPs that were measured in 2001 for predicting MetS that was diagnosed in 2009, including BMI, SBP, DBP, total cholesterol, HDL-cholesterol, triglyceride, FBS, RBC, WBC, Hb, PLT, UA, AST, ALT, and γ-GTP.

Statistical analysis

The Hardy-Weinberg equilibrium was assessed using the Fisher's exact test. Simple comparison of characteristics between case and control groups was carried out using the Mann—Whitney U test, Fisher's exact test, and Student's *t*-test. In the screening analysis, the associations between each SNP and MetS diagnosed in 2009 were assessed using logistic regression analysis with adjustment for age. We coded genotypes as 0, 1, or 2, depending on the number of copies of the minor alleles, for the multiple logistic regression analysis. In the interaction analysis, multiple logistic regression analyses, including an SNP term, a CP term, and an SNP × CP interaction term were performed for each combination of 15 clinical parameters and five SNPs that were statistically significant in the screening analysis. In the interaction analysis, the logistic regression models were fit as:

$$\log\left(\frac{p_{case}}{1-p_{case}}\right) = \beta_0 + \beta_{Age} \times Age + \beta_{SNP} \times x_{SNP} + \beta_{CP} \times z_{CP} + \beta_{Interaction} \times (x_{SNP} - \mu_{SNP}) \times z_{CP} \quad Model1$$

$$\log\left(\frac{p_{case}}{1-p_{case}}\right) = \beta_0 + \beta_{Age} \times Age + \beta_{SNP} \times x_{SNP} + \beta_{CP} \times z_{CP} + \beta_{Interaction} \times (x_{SNP} - \mu_{SNP}) \times z_{CP} + \beta_{N_{Mets}} \times N_{Mets} \quad Model2$$

$$\log\left(\frac{p_{case}}{1-p_{case}}\right) = \beta_0 + \beta_{Age} \times Age + \beta_{SNP} \times x_{SNP} + \beta_{CP} \times z_{CP} + \beta_{Interaction} \times (x_{SNP} - \mu_{SNP}) \times z_{CP} + \beta_{N_{Metsex}} \times N_{Metsex} \quad Model3$$

where p_{case} is the probability that the subject is affected by MetS. x_{SNP} is the genotype coded as 0, 1, or 2 for each SNP. μ_{SNP} is the mean value of x_{SNP} for each SNP. z_{CP} is standardized value of each clinical parameter value. N_{MetS} is the number of MetS components. N_{MetSex} is the number of MetS components excluding obesity. Model 1 was used to explore interactions that are significantly associated with MetS. Models 2 and 3 were used to assess that the interaction was independent of the contribution of MetS components to MetS. Given that the distributions of HDL-cholesterol, triglyceride, RBC, WBC, Hb, PLT, UA, AST, ALT, and γ-GTP levels were skewed, these clinical parameter values were logarithmically transformed. To reduce the multi-collinearity, we centered genotypes for each SNP, x_{SNP}, by subtracting the mean value from each genotype value, μ_{SNP}, and

Table 1. Characteristics of the study subjects for age and clinical parameters.

Characteristic	Case (n = 360 males)		Control (n = 1983 males)		*P* value (case vs control)	
	In 2001	In 2009	In 2001	In 2009	In 2001	In 2009
Age (years)	42 (37, 46)	50 (45, 54)	41 (33, 45)	49 (41, 53)	1.93×10^{-9}	1.93×10^{-9}
BMI (kg/m^2)	24.6 (23.1, 26.5)	-	21.6 (20.0, 23.3)	-	1.28×10^{-78}	-
Waist circumference (cm)	-	90.0 (87.0, 95.0)	-	79.0 (74.0, 83.0)	-	1.99×10^{-160}
SBP (mmHg)	123 (114, 130)	133.5 (129, 138)	114 (107, 122)	116 (107, 123)	4.84×10^{-34}	2.64×10^{-113}
DBP(mmHg)	79 (72, 84)	86 (80, 89)	71 (65, 77)	73 (66, 78)	1.33×10^{-37}	2.60×10^{-93}
Total cholesterol (mg/dL)	205 (183, 229)	221 (196, 247)	184 (163, 208)	200 (180, 222)	2.17×10^{-20}	9.43×10^{-24}
HDL-cholesterol (mg/dL)	54 (45, 63)	49 (42, 58)	63 (54, 74)	60 (51, 71)	9.90×10^{-30}	2.34×10^{-40}
Triglyceride (mg/dL)	130 (92, 195)	185 (154, 238)	76 (56, 107)	81 (59, 114)	2.00×10^{-65}	3.74×10^{-128}
FBS (mg/dL)	95 (89, 102)	101 (91, 115)	90 (85, 96)	90 (85, 96)	9.79×10^{-22}	7.72×10^{-52}
RBC ($\times 10^4$/μL)	491 (468, 513.3)	489 (465.8, 513)	476 (455, 499)	468 (446, 491)	4.86×10^{-14}	2.70×10^{-22}
WBC ($\times 10^3$/μL)	7.0 (5.8, 8.2)	6.4 (5.5, 7.7)	6.0 (5.1, 7.2)	5.5 (4.7, 6.6)	2.42×10^{-18}	1.59×10^{-24}
Hb (g/dL)	15.5 (15.0, 16.2)	15.6 (14.9, 16.1)	15.1 (14.6, 15.7)	15 (14.3, 15.5)	5.44×10^{-18}	1.21×10^{-25}
PLT ($\times 10^4$/μL)	25.0 (21.9, 28.9)	24.4 (21.0, 28.5)	23.6 (20.7, 27.1)	22.9 (20.0, 26.4)	2.02×10^{-6}	1.25×10^{-7}
UA (mg/dL)	6.3 (5.5, 7.2)	6.3 (5.5, 7.3)	5.8 (5.1, 6.5)	5.8 (5.0, 6.5)	1.92×10^{-14}	3.11×10^{-16}
AST (IU/L)	23 (19, 28)	24 (20, 31)	20 (17, 24)	20 (17, 23)	3.56×10^{-13}	1.58×10^{-34}
ALT (IU/L)	28 (20.8, 39)	31 (22, 47)	20 (15, 27)	18 (14, 25)	5.71×10^{-33}	3.70×10^{-62}
γ-GTP (IU/L)	42.5 (28, 69)	53 (35, 87)	26 (18, 42)	28 (20, 44)	1.05×10^{-29}	2.40×10^{-51}

Values are medians (1st quartile, 3rd quartile). The abbreviations of the characteristics: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; RBC, red blood cell; WBC, white blood cell; Hb, hemoglobin; PLT, platelet; UA, uric acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase.

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standardized CP values to z_{CP} [18]. We transformed PLT count into a dichotomous value, based on median value of PLT count across all subjects (equal to 23.8×10^4/μL): 1, equal to or greater than 23.8×10^4/μL; and 0, less than 23.8×10^4/μL. We compared the risk of MetS with regard to a combination of rs7965413 and dichotomous PLT count in an age-adjusted logistic regression model using the group with the CC genotype of rs7965413 and PLT count of $< 23.8 \times 10^4$/μL as a reference group. Linear regression analysis with adjustment for age was performed to assess the association of rs7965413 with PLT count in each case and control group. The heterogeneity of the regression coefficient between case and control groups was tested by the χ^2-based Cochrane's Q statistic. Statistical analysis was performed using R (www.r-project.org), PLINK [19], and METAL [20] softwares. In the interaction analysis, the significance level α was determined by dividing 0.05 by the number of CPs for Bonferroni correction ($\alpha = 0.05 / 15 = 0.0033$). Otherwise, $P < 0.05$ was considered statistically significant.

Results

Characteristics of subjects

The characteristics of the study subjects in 2001 and 2009 are shown in Tables 1 and 2. There were significant differences among all characteristics between case and control groups in both 2001 and 2009. Of these characteristics, the number of MetS components and MetS components excluding obesity were the most significantly different between case and control groups in both 2001 and 2009.

Table 2. Characteristics of the study subjects for MetS components.

Characteristic	Case (n = 360 males)		Control (n = 1983 males)		*P* value (case vs control)	
	In 2001	In 2009	In 2001	In 2009	In 2001	In 2009
MetS component						
Obesity, n (%)	157 (43.6)	360 (100)	188 (9.5)	239 (12.1)	4.61×10^{-50}	$< 1.00 \times 10^{-200}$
Raised blood pressure, n (%)	128 (35.6)	326 (90.6)	236 (11.9)	264 (13.3)	3.12×10^{-25}	3.30×10^{-189}
Raised FBS, n (%)	33 (9.2)	131 (36.4)	45 (2.3)	58 (2.9)	5.35×10^{-9}	7.60×10^{-71}
Dyslipidemia, n (%)	151 (41.9)	306 (85.0)	192 (9.7)	197 (9.9)	2.17×10^{-45}	3.45×10^{-186}
Number of MetS components	1.3 ± 0.8	3.1 ± 0.3	0.3 ± 0.6	0.4 ± 0.7	4.10×10^{-130}	$< 1.00 \times 10^{-200}$
Number of MetS components excluding obesity	0.9 ± 0.7	2.1 ± 0.3	0.2 ± 0.5	0.3 ± 0.5	5.10×10^{-85}	$< 1.00 \times 10^{-200}$

Categorical data are n values (%). The numbers of MetS components are mean ± SD.

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Screening analysis

We initially assessed the association between MetS diagnosed in 2009 and 98 genotyped SNPs. Five out of 98 SNPs were found to be significantly associated with MetS (Table 3 and S4 Table), with *VWF* rs7965413 among those with the lowest *P* value [OR = 0.81, 95% confidence interval (CI) = 0.69–0.96; *P* = 0.012].

Interaction analysis

Based on the results of the screening analysis, we focused on the five SNPs listed in Table 3 for further analysis. To explore SNP × CP interactions for MetS, we performed multiple logistic regression analyses including an SNP term, a CP term, and an SNP × CP interaction term. MetS was found to be significantly associated with an interaction between *VWF* rs7965413 and PLT in Model 1 (Table 4 and S5 Table). Furthermore, this association of the SNP × CP interaction

Table 3. Five SNPs that were nominally significantly associated with MetS in the screening study.

SNP	Chr	Position (GRCh37)	Near genes	Minor/major alleles	HWE *P* value	N		MAF		Logistic regression analysis	
						Case	Control	Case	Control	OR (95%CI)	*P* value
rs2544390	2	170,204,846	LRP2	C/T	0.231	360	1983	0.450	0.498	0.84 (0.71–0.98)	0.027
rs1800592	4	141,493,961	UCP1, TBC1D9	G/A	0.772	360	1982	0.456	0.502	0.83 (0.70–0.97)	0.022
rs662799	11	116,663,707	APOA5	G/A	0.515	360	1983	0.368	0.327	1.21 (1.03–1.43)	0.023
rs7965413	12	6,234,889	VWF	T/C	0.770	360	1980	0.400	0.452	0.81 (0.69–0.96)	0.012
rs1411766	13	110,252,160	MYO16, IRS2	T/C	0.515	360	1982	0.131	0.102	1.31 (1.03–1.67)	0.030

HWE, Hardy Weinberg equilibrium; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

HWE *P* values were calculated by Fisher's exact test.

OR and *P* values were calculated by multiple logistic regression analysis with adjustment for age.

OR value represents increased risk of MetS per minor allele copy in each SNP.

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Table 4. Significant interaction effect between SNP rs7965413 and CP PLT in 2001 for MetS.

Model term	Model 1		Model 2		Model 3	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
SNP	0.78 (0.66–0.92)	0.004*	0.84 (0.70–1.02)	0.076	0.82 (0.68–0.98)	0.033
CP	1.35 (1.20–1.52)	1.17×10^{-6}†	1.26 (1.10–1.44)	8.22×10^{-4}†	1.31 (1.15–1.49)	4.14×10^{-5}†
Interaction	1.33 (1.12–1.58)	0.001†	1.32 (1.08–1.60)	0.006*	1.35 (1.12–1.63)	0.002†

PLT, platelet; OR, odds ratio.

OR value for SNP term represents increased risk of MetS per minor allele T copy in rs7965413.

OR value for CP term represents increased risk of MetS per one standard deviation (SD) change in $\log_{10}$(PLT).

OR value for interaction term represents increased risk of MetS per one SD change in $\log_{10}$(PLT) × minor allele T copy in rs7965413.

* $P < 0.05$.

† $P < 0.0033$ (= 0.05/15)

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with MetS remained nominally significant in multiple logistic regression analysis after adjustment for the number of MetS components in Model 2 and significant after adjustment for the number of MetS components excluding obesity in Model 3 (Table 4).

Furthermore, we transformed PLT count into a dichotomous value based on the median value of platelet count across all subjects, which was equal to 23.8×10^4/μL and assessed an interaction effect between SNP rs7965413 and dichotomous PLT for MetS (Fig. 1). Multiple logistic regression analysis showed a significant interaction between SNP and dichotomous PLT for MetS (OR = 1.52, 95% CI = 1.09–2.12; $P = 0.014$). Among rs7965413 genotypes, OR was unchanged in subjects with PLT count $\geq 23.8\times10^4$/μL, which is the median value of PLT count in the study participants. On the other hand, in subjects with PLT count $< 23.8\times10^4$/μL, OR decreased as the number of minor allele T increased.

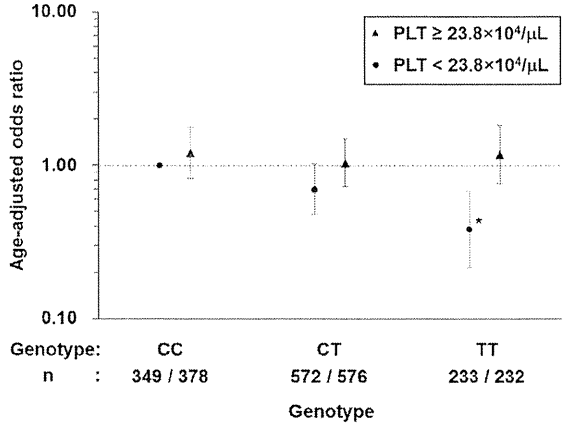

Fig 1. Age-adjusted ORs for MetS in platelet ≥ 23.8×10^4/μL and < 23.8×10^4/μL with different rs7965413 genotypes The vertical bars represent the 95% CIs. The horizontal dashed line indicates the null value (odds ratio (OR) = 1.0). OR represents risk of MetS development in the group with each genotype of rs7965413 and each dichotomous platelet count compared with the group with the CC genotype and PLT count of < 23.8×10^4/μL. *: $P < 0.05$.

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Table 5. Associations of rs7965413 with PLT count in 2001.

Group	N	Linear regression analysis		Heterogeneity	
		$\beta \pm SE$	P value	I^2	*P* value
Case	360	0.19 ± 0.08	0.013*	90.2	0.001*
Control	1980	-0.07 ± 0.03	0.021*		

PLT, platelet; β, partial regression coefficient; SE, standard error.

β value represents standard deviation change in standardized $\log_{10}$(PLT) per minor allele T change in rs7965413.

Multiple linear regression analysis was performed with adjustment for age.

* $P < 0.05$

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VWF rs7965413 and PLT count

Finally, we assessed the association of rs7965413 with PLT count in each group of cases and controls. In the case group, the minor allele T of rs7965413 was significantly positively associated with PLT. In the control group, the minor allele T was significantly negatively associated with PLT (Table 5). There was significant heterogeneity between case and control groups ($I^2 = 90.2$; $P = 0.001$).

Discussion

We performed a case-control study of MetS based on the health check-up data of Japanese male employees and found significant associations of five SNPs with MetS, including *LRP2* rs2544390, rs1800592 between *UCP1* and *TBC1D9*, *APOA5* rs662799, *VWF* rs7965413, and rs1411766 between *MYO16* and *IRS2*. Furthermore, we identified a novel SNP × CP interaction for MetS, which was the interaction between *VWF* rs7965413 and platelet count. These SNPs and associated interaction are expected to be useful as risk markers for MetS development.

As shown in Tables 1 and 2, all characteristics, including CPs directly related to MetS, such as BMI, blood pressures, and cholesterols, as well as characteristics indirectly related to MetS, significantly differed between case and control groups. Of the CPs not directly related to MetS, levels of erythrocyte parameters, including RBC count, WBC count, hemoglobin, and PLT count in the case group were significantly higher than those in the control group. Several cross-sectional and longitudinal cohort studies have demonstrated that elevated erythrocyte parameters were associated with MetS [6,7,21]. Furthermore, Taniguchi et al., in their study on non-obese Japanese type 2 diabetes patients, found platelet count to be an independent predictor of insulin resistance [22]. Insulin resistance is thought to play a prominent role in MetS [14]. Our results were consistent with these reports.

As shown in Table 3, we found that *VWF* rs7965413 was significantly associated with MetS. *VWF* rs7965413 is located in the promoter region of the *VWF* gene. The *VWF* gene encodes von Willebrand factor (*v*WF). *v*WF promotes platelet adhesion and aggregation at sites of vascular injury, so it plays a prominent role in the formation of arterial thrombus [23]. Mutations in the *VWF* gene cause von Willebrand disease because of deficiency of *v*WF. It was also reported that *v*WF was associated with insulin level and insulin resistance [24]. For example, in a cross-sectional study, Meigs et al. reported that *v*WF antigen (*v*WF:Ag) level in men significantly increased across insulin quintiles [25]. Furthermore, *v*WF was reported to be associated with homeostasis model assessment—insulin resistance, which is an index of insulin resistance, and MetS [26]. Thus, an association between *v*WF plasma levels and CVD is expected and has