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ORIGINAL ARTICLE

Associations of complementation group, *ALDH2* genotype, and clonal abnormalities with hematological outcome in Japanese patients with Fanconi anemia

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Abstract

Fanconi anemia (FA) is a genetically and clinically heterogeneous disorder that predisposes patients to bone marrow failure (BMF), myelodysplastic syndromes (MDS), and acute myeloid leukemia (AML). To study which genetic and phenotypic factors predict clinical outcomes for Japanese FA patients, we examined the FA genes, bone marrow karyotype, and aldehyde dehydrogenase-2 (*ALDH2*) genotype; variants of which are associated with accelerated progression of BMF in FA. In 88 patients, we found morphologic MDS/AML in 33 patients, including refractory cytopenia in 16, refractory anemia with excess blasts (RAEB) in 7, and AML in 10. The major mutated FA genes observed in this study were *FANCA* ($n = 52$) and *FANCG* ($n = 23$). The distribution of the *ALDH2* variant alleles did not differ significantly between patients with mutations in *FANCA* and *FANCG*. However, patients with *FANCG* mutations had inferior BMF-free survival and received hematopoietic stem cell transplantation (HSCT) at a younger age than those with *FANCA* mutations. In *FANCA*, patients with the c.2546delC mutation ($n = 24$) related to poorer MDS/AML-free survival and a younger age at HSCT than those without this mutation. All patients with RAEB/AML had an abnormal karyotype and poorer prognosis after HSCT; specifically, the presence of a structurally complex karyotype with a monosomy ($n = 6$) was associated with dismal prognosis. In conclusion, the best practice for a clinician may be to integrate the morphological, cytogenetic, and genetic data to optimize HSCT timing in Japanese FA patients.

Keywords Fanconi anemia · ALDH2 · FA gene · Cytogenetic abnormalities · Hematopoietic stem cell transplantation

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Introduction

Fanconi anemia (FA) is a genetic disorder associated with bone marrow failure (BMF), myelodysplastic syndromes (MDS), and acute myeloid leukemia (AML) [1]. Twenty-two complementation groups associated with FA, designated FA-A to FA-W, have been identified [2] (Online resource Table S1). In the literature, there have been several reports that described factors associated with adverse clinical outcomes among FA patients. For example, mutations in FA genes in group G (FA-G) or group C (FA-C) were reported to be significant and independent risk factors for BMF or MDS/AML [1, 3]. FA patients with MDS/AML frequently exhibit morphologic dysplasia and cytogenetic abnormalities, such as 1q and 3q gains, loss of 7 [4, 5], and *RUNX1* gene abnormalities at 21q [6]. In addition, we previously found that a variant of acetaldehyde dehydrogenase 2 (ALDH2) known as the A allele is associated with accelerated progression of BMF in Japanese patients with FA [7, 8], and the ALDH2 A allele is found in as many as 35–45% of the East Asian population, including Japanese. Compared with individuals homozygous for the *ALDH2*-G allele (*ALDH2*-GG), enzymatic activity is reduced by 60–80% or nearly abolished in individuals with the *ALDH2*-GA or AA genotype, respectively [9]. Of note, FA patients with the *ALDH2*-AA genotype develop severe BMF and MDS extremely early in life and required hematopoietic stem cell transplantation (HSCT). In this context, it is worth mentioning that a normal maternal *ALDH2* G allele is not essential for fetal development of *ALDH2*-deficient patients, in contrast with FA mouse models [8]. This underscores the continuing need to collect clinical data, with the aim of improving risk assessment and management of BMF and MDS in FA patients. We therefore sought to identify which genotypic and cytogenetic factors correlate with adverse outcomes in Japanese FA patients [10]. In this study, we investigated FA gene mutations, *ALDH2* genotype, and the patterns of clonal abnormalities in Japanese patients with FA as well as their associations with hematological outcomes.

Methods

Study design

We analyzed 88 patients with FA from 76 families from 1996 to 2016 with some additional patients who were not in previously reported cases [7, 8]. All patients had a definitive diagnosis of FA based on FA criteria, including tests of chromosomal breakage induced by diepoxybutane or mitomycin C, except for five cases in which the diepoxybutane test was negative owing to *FANCA* reversion mosaicism. We analyzed FA gene mutations as described previously [7, 11] and determined *ALDH2* genotypes with a TaqMan polymerase chain reaction assay [12].

We defined BMF onset according to the International Fanconi Anemia Registry study criteria [13]; BMF onset was not evident in two FA-A patients and one patient with an unknown mutation. The diagnosis of MDS was based on the morphological criteria described by the 2008 World Health Organization classification [14].

We performed cytogenetic studies of BM using routine procedures before chemotherapy or HSCT; however, this was not done for three patients. We described karyotypes according to the International System for Human Cytogenetic Nomenclature 2013. We analyzed at least 10 metaphases (usually 20) in which abnormal mitoses were obtained by G-banding and performed fluorescence in situ hybridization assays as necessary to further characterize structural abnormalities. We defined a monosomal karyotype as two or more monosomies or a single monosomy in the presence of structural abnormalities [15]. Meanwhile, we defined a structurally complex karyotype (SCK) as a complex karyotype characterized by three or more chromosomal aberrations including at least one structural aberration [16].

FA patients with severe aplastic anemia (SAA) or MDS/AML were indicated for HSCT. SAA is defined as hypoplastic marrow with at least two of the following: neutrophil count $< 0.5 \times 10^9$/L, platelet count $< 20 \times 10^9$/L, and reticulocyte count $< 20 \times 10^9$/L. The conditioning regimen was dependent on the era of transplantation and varied according to disease status. We administered low-dose cyclophosphamide in combination with low-dose irradiation or antithymocyte/antilymphocyte globulin or cytarabine or busulfan; 91% received fludarabine.

Statistical analysis

We performed statistical analysis with Prism version 6.0 (GraphPad Software, San Diego, CA, USA). We estimated survival free of BMF or MDS/AML as well as overall survival (OS) by performing Kaplan–Meier analysis. We performed comparisons between two groups by using the Mann–Whitney *U* test. We censored malignant myeloid transformation at final follow-up or at the time of HSCT. The level of statistical significance was set at $p < 0.05$.

Results

Patients' characteristics

The complementation group and ALDH2 genotype of the 88 Japanese patients with FA are shown in Table 1, and hematological characteristics classified according to FA genes are summarized in Table 2. We observed morphologic MDS/

Table 1 Complementation group and *ALDH2* genotype in Japanese Fanconi anemia patients

	Number	Final bone marrow status				
		Normal	Aplastic anemia	RCC or RCMD	RAEB	AML
Number of cases	88	2	53	16	7	10
Mutated FA gene						
FANCA	52	0	28	10	5	9
Homozygous c.2546delC mutation	5	0	4	1	0	0
Heterozygous c.2546delC mutation[a]	19	0	7	5	4	3
c.2546delC mutation negative[b]	28	0	17	4	1	6
FANCB	3	0	2	1	0	0
FANCC	1	0	1	0	0	0
FANCG	23	0	18	4	1	0
FANCI	2	0	2	0	0	0
FANCN	1	1	0	0	0	0
FANCP	3	1	0	1	1	0
FANCT	2	0	1	0	0	1
Unknown	1	0	1	0	0	0
ALDH2 genotype						
AA	6	0	1	5	0	0
GA	39	1	26	8	1	3
GG	43	1	26	3	6	7

AML, acute myeloid leukemia; *RAEB*, refractory anemia with excess blasts; *RCC*, refractory cytopenia of childhood; *RCMD*, refractory cytopenia with multilineage dysplasia; *FA*, Fanconi anemia; *ALDH2*, aldehyde dehydrogenase-2

[a] These patients were compound heterozygous, with a second FANCA mutation that was other than the c.2464delC mutation

[b] These patients were either homozygous or compound heterozygous for FANCA mutations other than the c.2464delC mutation

AML in 33 patients, including 16 males and 17 females aged between 4 months and 32 years at the initial diagnosis of MDS/AML. Fifteen patients had refractory cytopenia of childhood (RCC), one had refractory cytopenia with multilineage dysplasia (RCMD) in adult patients, seven had refractory anemia with excess blasts (RAEB), and ten had AML.

Complementation group and mutations

The major mutated FA genes in our patient cohort were *FANCA* ($n = 52$) and *FANCG* ($n = 23$). Mutation variants in *FANCA* and *FANCG* patients are listed in Online resource Table S2. A small deletion of c.2546delC was most frequently detected in FA-A patients (24/52; 46%); five patients were homozygous for this mutation (Table 1). We detected reversions in five FA-A patients, including two with aplastic anemia (AA), one with RCMD, and two with RAEB. The two RAEB patients with a *FANCA* reversion had an original heterozygous c.2546delC mutation. Detailed information regarding these patients is shown in Online resource Table S3. Most RAEB/AML patients ($n = 14$) had *FANCA* mutations (Table 1).

ALDH2 genotype

We identified the *ALDH2*-GG, GA, and AA genotypes in 43, 39, and 6 patients, respectively. We detected the *ALDH2*-AA genotype in three patients with *FANCA*, two with *FANCG,* and one with *FANCP* (Table 2).

Cytogenetics

Abnormal cytogenetic clones in bone marrow are shown in Table 3. Aberrant chromosomes 1, 3, and 7 were frequently involved in clones. All patients with RAEB/AML had an abnormal karyotype: 12 had an SCK, 6 of whom had a monosomy. We did not detect any monosomies or monosomal karyotype apart from these six cases. Among the 16 patients with RCC or RCMD, only 1 case had an SCK without a monosomy. Table 4 shows the association of complementation groups, ALDH2 phenotype, and clonal abnormalities. Eleven of the thirteen patients with SCK were *FANCA* patients, and four of the eleven patients had monosomal karyotype.

Table 2 Summary of genotypes and hematological characteristics of Japanese Fanconi anemia patients

	Total ($n = 88$)		*FANCA* ($n = 52$)		*FANCG* ($n = 23$)		Other genes ($n = 13$)	
ALDH2 genotype	AA/GA	GG	AA/GA	GG	AA/GA	GG	AA/GA	GG
Number of cases	6/39	43	3/20	29	2/11	10	1/8	4
Final bone marrow status								
Normal	0/1	1	0/0	0	0/0	0	0/1	1
Aplastic anemia	1/26	26	0/13	15	1/9	8	0/4	3
RCC or RCMD	5/8	3	3/5	2	1/2	1	1/1	0
RAEB	0/1	6	0/0	5	0/0	1	0/1	0
AML	0/3	7	0/2	7	0/0	0	0/1	0
Median (range) age at onset (years)								
Bone marrow failure	2.0 (0–7)	6.0 (2–24)	2.3 (0–5)	7.3 (3–24)	2.0 (0–5)	4.6 (2–6)	1.3 (0–7)	4.8 (3–8.0)
Initial diagnosis of MDS or AML	5.0 (0–24)	11.1 (5–32)	9.0 (0–24)	12.6 (5–32)	3.2 (1–5)	8.3 (6–10)	3.9 (0–5)	–
Number of cases with HSCT (%)	38 (84)	37 (86)	17 (74)	25 (86)	13 (100)	9 (90)	8 (89)	3 (75)
Median (range) age at HSCT (years)	7.2 (1–24)	10.7 (4–37)	9.1 (1–24)	13.5 (5–37)	7.0 (1–20)	6.6 (4–11)	6.3 (1–13)	6.0 (5–8)

AML, acute myeloid leukemia; *RAEB*, refractory anemia with excess blasts; *RCC*, refractory cytopenia of childhood; *RCMD*, refractory cytopenia with multilineage dysplasia; *ALDH2*, aldehyde dehydrogenase-2; *MDS*, myelodysplastic syndromes; *HSCT*, hematopoietic stem cell transplantation

Hematological outcomes

There was no death before development of BMF in any of the FA-A and FA-G patients. Compared with FA-A patients, FA-G patients had inferior BMF-free survival (Fig. 1a). The distribution of the *ALDH2* variant alleles did not differ significantly between FA-A and FA-G patients (Table 2). To determine the relationship between FA genes and *ALDH2* genotype, we subdivided 70 patients into the following four groups and compared the survival free of BMF: FA-A patients with *ALDH2*-AA or GA, FA-A patients with *ALDH2*-GG, FA-G patients with *ALDH2*-AA or GA, and FA-G patients with *ALDH2*-GG. FA-G patients with *ALDH2*-GG had inferior BMF-free survival compared with FA-A patients with *ALDH2*-GG, but no significant difference was found between FA-A patients with *ALDH2*-AA or GA and FA-G patients

Table 3 Incidence of abnormal cytogenetic clones in bone marrow

	Number	Final bone marrow status				
		Normal	Aplastic anemia	RCC or RCMD	RAEB	AML
Number of cases	88	2	53	16	7	10
Abnormal cytogenetic clones						
Yes	25	0	2	6	7	10
No	60	0	50	10	0	0
Not done	3	2	1	0	0	0
Abnormal karyotypes						
1q gain	8	0	0	1	4	3
3q gain	1	0	0	0	0	1
del(3q)	2	0	0	1	0	1
− 7	2	0	0	0	1	1
del(7q)	2	0	0	0	0	2
Structurally complex karyotype	13	0	0	1	5	7
With a monosomy	6	0	0	0	2	4
Without a monosomy	7	0	0	1	3	3
Other	7	0	2	3	0	2

AML, acute myeloid leukemia; *RAEB*, refractory anemia with excess blasts; *RCC*, refractory cytopenia of childhood; *RCMD*, refractory cytopenia with multilineage dysplasia

Table 4 Genotypes and abnormal karyotypes of Japanese Fanconi anemia patients

	Total ($n = 88$)		*FANCA* ($n = 52$)		*FANCG* ($n = 23$)		Other genes ($n = 13$)	
ALDH2 genotype	AA/GA	GG	AA/GA	GG	AA/GA	GG	AA/GA	GG
Number of cases	6/39	43	3/20	29	2/11	10	1/8	4
Abnormal cytogenetic clones								
Yes	2/8	15	1/5	13	0/0	2	1/3	0
No	4/29	27	2/14	16	2/11	8	0/4	3
Not done	0/2	1	0/1	0	0/0	0	0/1	1
Abnormal karyotypes								
1q gain	0/2	6	0/2	5	0/0	1	0/0	0
3q gain	0/0	1	0/0	1	0/0	0	0/0	0
del(3q)	1/0	1	0/0	1	0/0	0	1/0	0
− 7	0/1	1	0/0	1	0/0	0	0/1	0
del(7q)	0/1	1	0/1	1	0/0	0	0/0	0
Structurally complex karyotype	0/4	9	0/2	9	0/0	0	0/2	0
With a monosomy	0/3	3	0/1	3	0/0	0	0/2	0
Without a monosomy	0/1	6	0/1	6	0/0	0	0/0	0
Other	1/2	4	1/1	3	0/0	1	0/1	0

ALDH2, aldehyde dehydrogenase-2

with *ALDH2*-AA or GA (Fig. 1b). Although there was no significant difference in MDS/AML-free survival between FA-A and FA-G patients (Fig. 1c), FA-G patients received HSCT at a significantly younger age than FA-A patients (Fig. 1d).

We compared hematological outcome between the FA-A patients with and without the c.2546delC mutation. Although the distribution of the *ALDH2*-GA and GG did not differ significantly between patients with and without the c.2546delC mutation, the *ALDH2*-AA patients were observed only in the c.2546delC-positive group (Table S2). Therefore, we analyzed BMF-free survival and MDS/AML-free survival after exclusion of the *ALDH2*-AA patients. There was no significant difference in BMF-free survival between FA-A patients with and without the c.2546delC mutation (Fig. 2a). However, the c.2546delC mutation was significantly associated with poor MDS/AML-free survival (Fig. 2b). To reduce some of

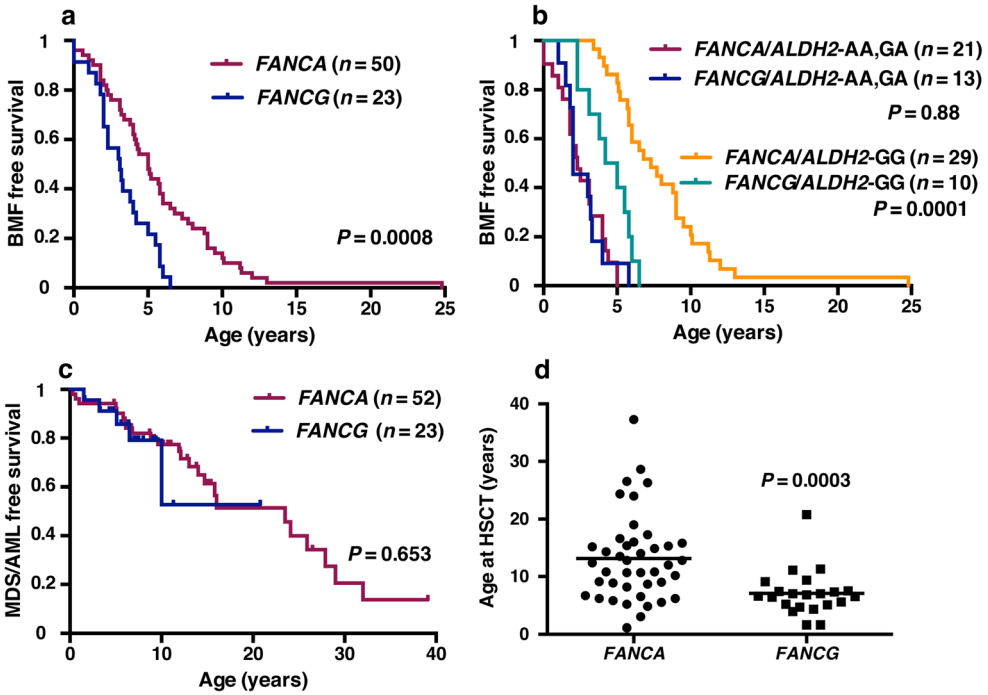

Fig. 1 Comparison of *FANCA* and *FANCG* groups. **a** Bone marrow failure (BMF)-free survival. **b** BMF-free survival according to Fanconi anemia gene + *ALDH2* genotype. **c** Myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML)-free survival. **d** Age at hematopoietic stem cell transplantation (HSCT)

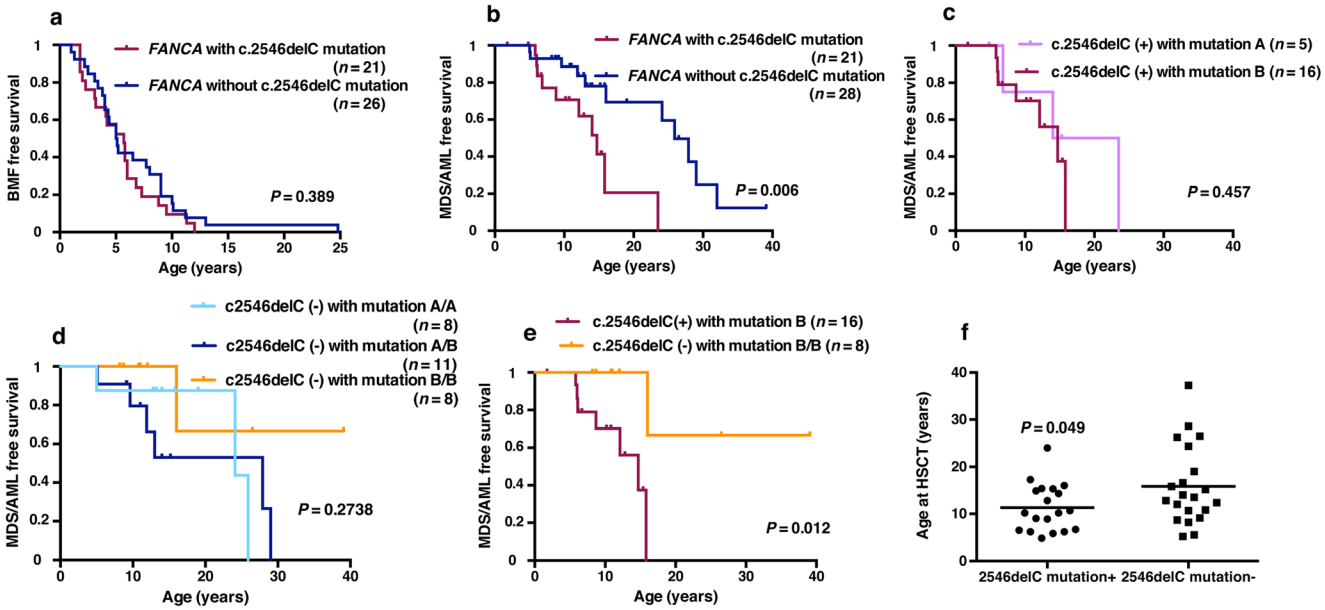

Fig. 2 Comparison of *FANCA* with and without the c.2546delC mutation after exclusion of the *ALDH2*-AA patients. **a** BMF-free survival. **b** MDS/AML-free survival. **c** MDS/AML-free survival according to the second mutational patterns in *FANCA* patients with the c.2546delC mutation. **d** MDS/AML-free survival according to mutational patterns in *FANCA* patients without the c.2546delC mutation. **e** MDS/AML-free survival in patients having mutation B identified at both alleles in the c.2546delC-positive and c.2546delC-negative groups. **f** Age at HSCT

the variability of mutational patterns, we compared MDS/AML-free survival among the different mutational patterns. In this analysis, we defined missense and splicing mutations as mutation A, and mutations that definitely lead to protein truncation, such as nonsense mutation, small insertions/deletions, large deletions, and large duplication, were defined as mutation B. No significant statistical difference was observed in MDS/AML-free survival according to the second mutational patterns (mutation A versus mutation B) in the c.2546delC-positive group (Fig. 2c). There was also no significant difference in the MDS/AML-free survival according to the mutational patterns in the c.2546delC-negative group (Fig. 2d). Furthermore, MDS/AML-free survival was analyzed in patients having mutation B (c.2546delC was regarded as mutation B) identified at both alleles in the c.2546delC-positive and c.2546delC-negative groups. In this subset of patients, a significant difference was reproduced in MDS/AML-free survival (Fig. 2e). FA-A patients with the c.2546delC mutation received HSCT at a younger age than those without the mutation (Fig. 2f).

Additionally, hematological outcome according to mutational patterns in *FANCA* and *FANCG* groups and BMF-free survival according to mutational patterns in the c.2546delC-positive and c.2546delC-negative groups were studied in the way previously described in the above section. There was no significant difference in BMF-free survival and MDS/AML-free survival according to mutational patterns in the *FANCA* and *FANCG* groups (Online resource Fig. S1) and in BMF-free survival according to mutational patterns in the c.2546delC-positive and c.2546delC-negative groups as well (Online resource Fig. S2). Thus, presence of c.2546delC mutation appeared to predispose FA patients to earlier onset of MDS/AML, but not to BMF, compared with other truncating mutations.

ALDH2 deficiency dramatically accelerated BMF onset in Japanese FA patients, corroborating our previous findings (data not shown). The six FA patients who had the *ALDH2*-AA genotype exhibited accelerated BMF (from birth to 7 months), and five of them developed RCC extremely early in life (from 4 to 18 months). These five patients received HSCT at an early age (ranging from 1 to 3 years old) regardless of complementation group, and one patient died at 7 months before he received HSCT. However, there was no significant difference in MDS/AML-free survival between patients with the *ALDH2*-GA and *ALDH2*-GG genotypes ($p = 0.673$). Patients with the *ALDH2*-GA genotype appeared to receive an HSCT at a younger age than those with the *ALDH2*-GG genotype, but the difference did not reach a statistical significance ($p = 0.067$).

Transplant outcomes

HSCT was performed for 75 patients (85%) including 45 in the SAA group and 30 in the MDS/AML group. Seven patients with SAA and four with MDS/AML underwent HSCT from a matched family donor, and the other sixty-four patients received HSCT from an alternative donor. The 5-year OS rates after HSCT were 82% in the total cohort (95% confidence interval (CI), 73–91%) with a median follow-up of 7.5 years (range 0.2–19.8 years), 91% (95% CI, 82–99%) in patients

with SAA, 92% (95% CI, 78–100%) in patients with RCC, and 53% (95% CI, 24–81%) in patients with RAEB/AML (Fig. 3a). There were significant differences in 5-year OS between SAA and RAEB/AML ($p = 0.0001$) and between RCC and RAEB/AML ($p = 0.0075$), whereas there was no significant difference in 5-year OS between SAA and RCC ($p = 0.892$). As most *FANCG* patients did not develop RAEB/AML, the 5-year OS after HSCT was significantly better than *FANCA* or patients with other genes (both $p < 0.05$) (Fig. 3b). In patients with MDS/AML, the 5-year OS after HSCT was 17% in patients with an SCK and a monosomy, compared with 86 and 82%, in patients with an SCK without a monosomy and those without an SCK, respectively (Fig. 3c). The patients with an SCK and a monosomy had dismal prognosis owing to relapse or rejection. The difference in 5-year OS between patients with both an SCK and a monosomy and those with an SCK without a monosomy, as well as those with both an SCK and a monosomy versus those without an SCK, were significant (both $p < 0.01$) (Fig. 3c). There was no significant difference in 5-year OS after HSCT between MDS/AML patients with and without the c.2546delC mutation (Fig. 3d). Detailed information regarding the 33 patients with MDS/AML is shown in Online resource Tables S3 and S4.

Discussion

Most FA patients experience cytopenia and clonal evolution toward MDS or AML. Moreover, their hematological situation may change spontaneously through life. The diagnosis and treatment of FA are complicated by the variability of genetic factors related to ethnic differences or severity of disease status. For example, in contrast with the Japanese patients in the present study, approximately one third of the Italian FA patients improved or maintained mild/moderate cytopenia or normal blood counts [17]. It is critical to monitor disease progression and select major therapeutic interventions such as HSCT for FA patients. We sought to identify hematological risk factors in the Japanese FA population by examining the FA genes, bone marrow karyotype, and aldehyde dehydrogenase-2 (*ALDH2*) genotype.

The major mutated FA genes observed in the current study population were *FANCA* and *FANCG*, unlike the previous report, in which 8 out of 29 Japanese FA patients had homozygous *FANCC* IVS + 4 mutations [18]. Our results indicate that compared with FA-A patients, FA-G patients are a high-risk group for early onset of BMF, and they tend to receive HSCT at a younger age. In particular, BMF developed at a younger age in FA-G patients with *ALDH2*-GG than FA-A patients with the *ALDH2*-GG genotype. These results suggest that FA-G patients may experience rapid disease progression even if they have the *ALDH2*-GG genotype. The European FA Research Group reported that FA-G patients had more severe cytopenia and a higher incidence of leukemia [3]. FANCA and FANCG proteins directly interact in a subcomplex of the FA core E3 ligase complex [19]. However, FANCG protein is reported to have an additional role in DNA repair, which is consistent with the abovementioned observations [20]. Moreover, in the European FA Research Group study, HSCT was performed on only 21% of patients at the time of publication. Meanwhile, in the present study, 96% of FA-G patients received HSCT, which might account for the lower rate of RAEB/AML in Japanese FA-G patients compared with FA-A patients (Table 2). Indeed, most Japanese FA-G patients underwent HSCT before the development of RAEB or AML

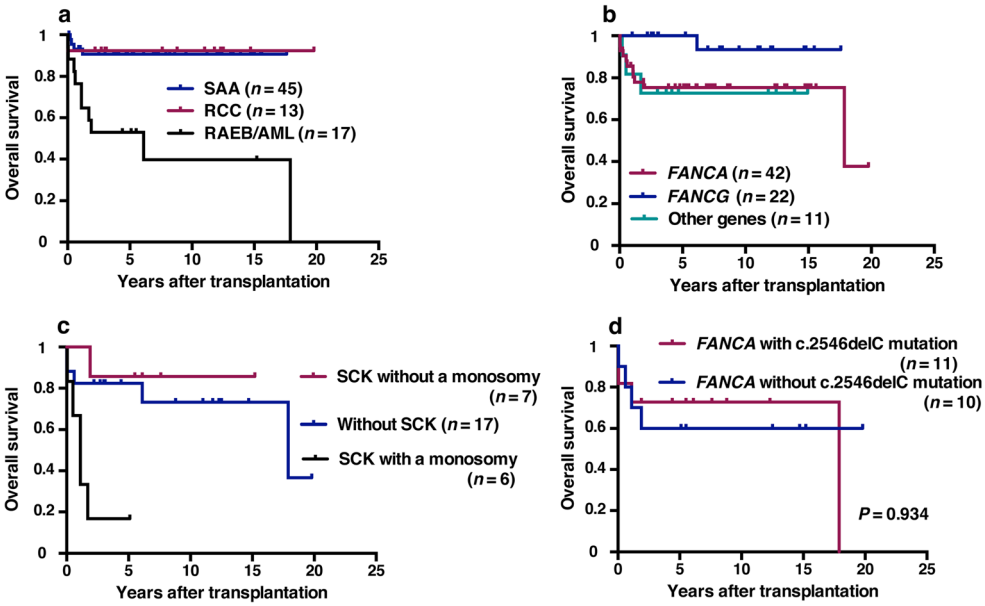

Fig. 3 Overall survival (OS) after hematopoietic stem cell transplantation. **a** OS according to disease status. **b** OS according to FA genes. **c** OS of MDS/AML patients according to cytogenetic characteristics. **d** OS of MDS/AML patients with respect to the *FANCA* c.2546delC mutation. RAEB, refractory anemia with excess blasts; RCC, refractory cytopenia of childhood; SAA, severe aplastic anemia; SCK, structurally complex karyotype

because of the rapid cytopenia progression. Meanwhile, we found *FANCA* mutations in 14 of 17 patients with RAEB/AML and detected a small deletion of c.2546delC in 7 of those 14 FA-A patients. As previously reported, this mutation is particularly common among Japanese FA-A patients [21]. We showed that the c.2546delC mutation was associated with MDS/AML development at a younger age, irrespective of mutational patterns in *FANCA* gene. Loss of FANCA protein due to the c.2546delC mutation may be related to the occurrence of malignant myeloid transformation in Japanese FA patients. Further studies including all FA genotypes are required to more clearly identify which FA patients may have an increased risk of MDS/AML transformation.

The six patients with the *ALDH2*-AA genotype in any FA complementation group developed severe cytopenia or RCC very early in life and received HSCT before the development of RAEB/AML. Further considering the contribution of the *ALDH2* genotype, it is interesting to note that patients with an *ALDH2*-GA genotype displayed earlier onset of BMF but not MDS/AML development compared with patients with the GG genotype. Additionally, *FANCG* mutations appeared to promote the onset of BMF; however, the phenotypic effect was weaker than the detrimental modifying effect exerted by an *ALDH2* GA or AA genotype (Fig. 1b). Thus, it is important to pay attention to both of FA gene mutations and the *ALDH2* genotype in Japanese FA patients.

HSCT remains the only curative treatment for MDS/AML in FA. Morphologic MDS is more important than classical cytogenetics for predicting adverse outcomes in FA [22]. A retrospective analysis published in 2013 by the Center for International Blood and Marrow Transplant Research showed that FA patients with cytogenetic abnormalities only had superior 5-year OS survival after HSCT compared with patients with MDS/acute leukemia [23]. Although MDS (RAEB)/AML FA patients can achieve long-term remission after HSCT, they have significantly poorer survival than FA patients with BMF [24, 25]. Even in the present study, the RAEB/AML group had the lowest 5-year OS rate, whereas the 5-year OS rate was similar between the RCC and SAA groups, regardless of conditioning or transplant year. These results suggest that HSCT should be performed before the development of RAEB or AML. Of note, FA is found with a relatively high prevalence in patients with a morphological picture consistent with RCC, the most common type of MDS in children [26]. SAA and presentation with only morphological dysplasia may also fall into the same category with regard to HSCT treatment.

Cytogenetic data are very informative for the prediction of leukemic transformation as well as prognosis in patients with congenital BMF syndrome [27], and cytogenetic clones—specifically 1q and 3q gains and − 7/7q-—have been frequently observed in FA patients, in contrast with de novo cases of AML [28]. Although a few studies have described the complex karyotypes in FA patients [24, 29], we observed many RAEB/AML patients with complex karyotypes. These results are similar to the findings of the European Working Group studies in children with advanced MDS; specifically, the presence of an SCK was the strongest predictor of poor outcome after HSCT [16]. Similarly, a monosomal karyotype was identified as a highly unfavorable risk factor in adult AML or MDS [30, 31]. In particular, in the present study, patients with an SCK and a monosomy had very short survival periods after HSCT because of relapse or rejection. Thus, these cytogenetic aberrations may play a critical role in the course of HSCT, regardless of pretransplant treatment or conditioning.

This study has some limitations owing to its retrospective design and small study population, which is primarily because FA is a very rare genetic disease. Therefore, further research is required to clarify the value of cytogenetics as a negative prognostic indicator after HSCT in FA patients with MDS/AML.

In conclusion, *FANCG* mutations or an *ALDH2*-AA genotype in Japanese FA patients are associated with earlier onset of BMF and HSCT at a younger age before the development of RAEB or AML. Most RAEB/AML patients have *FANCA* mutations, and the c.2546delC mutation may be related to the occurrence of malignant myeloid transformation. HSCT should be performed before the development of RAEB or AML, and an SCK with a monosomy is a poor prognostic factor after HSCT in Japanese FA patients with RAEB/AML. Our findings highlight the importance of collating morphological, cytogenetic, and genetic data to predict survival, which may inform therapeutic management decisions for Japanese FA patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Research Ethics Committees of Tokai University and Kyoto University, and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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