Supplemental Methods

Patients. Diagnosis of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) is based on morphological criteria described by the FAB committee. Aplastic anemia (AA) is defined as hypoplastic marrow with two of the following: neutrophil count <1.5 x 10^9/l, platelet count <100 x 10^9/l, and hemoglobin level <10g/dl. Severe AA (SAA) is defined as hypoplastic marrow with two of the following: neutrophil count <0.5 x 10^9/l, platelet count <20 x 10^9/l, and reticulocyte count <20 x 10^9/l. The onset of BMF was defined as the time when one of the following laboratory parameter values used in the International Fanconi Anemia Registry study[1] was observed: a platelet count <100 X 10^9/L, a hemoglobin level < 10 g/dl, or an absolute neutrophil count <1 X 10^9/L[1].

PCR, sequencing, and Taqman PCR. Genomic DNA or total RNA was isolated from either PHA-stimulated lymphocytes or cultured fibroblasts using Puregene (Qiagen) or RNAeasy (Qiagen) kits, respectively. cDNA was synthesized with a Primescript II cDNA synthesis kit (Life Technologies). Mutation analyses of cDNA and genomic DNA samples regarding *FANCA, FANCC,* or *FANCG* were carried out by PCR and direct sequencing as previously described[2]. In addition, 45 patients were examined by the Multiplex Ligation-mediated Probe Amplification (MLPA) test for *FANCA* (Falco Biosystems). Additional patients (n=29) were screened by whole exome sequencing for mutations in the known 16 FA genes as described below. The *ALDH2* genotype was determined by a previously established Taqman-PCR assay[3].

Whole exome sequencing. For exome sequencing, genomic DNA from each patient was enriched for protein-coding sequences with a SureSelect Human All Exon V4 or V5 kit

(Agilent Technologies, Santa Clara, CA, USA). This was followed by massively parallel sequencing with the HiSeq 2000 platform with 100 bp paired-end reads (Illumina, San Diego, CA, USA). Candidate germline variants were detected through our in-house pipeline for exome-sequencing analysis with minor modifications for the detection of germline variants[4]. The obtained sequences were aligned to the human reference genome (hg19) with the Burrows-Wheeler Aligner (BWA). After removal of duplicate artifacts caused by PCR, the single nucleotide variants with an allele frequency > 0.25 and insertion-deletions with an allele frequency > 0.1 were called. All of the identified variants in the FA genes were verified by PCR and Sanger sequencing.

References

1. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. Blood. 1994;84(5):1650-1655.
2. Tachibana A, Kato T, Ejima Y, et al. The FANCA gene in Japanese Fanconi anemia: reports of eight novel mutations and analysis of sequence variability. Hum Mutat. 1999;13(3):237-244.
3. Matsuo K, Wakai K, Hirose K, Ito H, Saito T, Tajima K. Alcohol dehydrogenase 2 His47Arg polymorphism influences drinking habit independently of aldehyde dehydrogenase 2 Glu487Lys polymorphism: analysis of 2,299 Japanese subjects. Cancer Epidemiol Biomarkers Prev. 2006;15(5):1009-1013.
4. Kunishima S, Okuno Y, Yoshida K, et al. ACTN1 mutations cause congenital macrothrombocytopenia. Am J Hum Genet. 2013;92(3):431-438.
5. Guardiola P, Pasquini R, Dokal I, et al. Outcome of 69 allogeneic stem cell transplantations for Fanconi anemia using HLA-matched unrelated donors: a study on behalf of the European Group for Blood and Marrow Transplantation. Blood. 2000;95(2):422-429.

Legend for Supplemental Table 1. Clinical characteristics of the patients studied.

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; SAA, severe aplastic anemia; AA, non-severe aplastic anemia; SCT, hematopoietic stem cell transplantation; NI, not identified; WES, whole exome sequencing; MLPA, Multiplex Ligation-mediated Probe Amplification method.

[A] These mutations underwent reversion to wild type in lymphocytes.

[B] These mutations were presumptive, since their functional significance has not been determined.

[C] FA-A cases in which nonsense mutations, frame-shifts, or large deletions have been identified in both alleles. Progression of BMF in these cases was analyzed and presented in Figure 1C.

[D] This synonymous mutation is predicted to likely disturb normal splicing by Mutation Taster (http://www.mutationtaster.org).

[E] Physical abnormalities included skin abnormalities (hyperpigmentation and *café au lait* spots), low birth weight, growth defects (short stature), and malformations (affecting thumb, radius, skeleton, head/face, eyes, ears, kidneys, gastrointestinal tract, urogenital tract and cardiovascular system). The sum of the number of physical abnormalities and the number of anatomical sites involved in malformations is presented.

[F] Extensive malformation was defined as the involvement of at least three sites including at least one deep organ.

[G] Genomic PCR and sequencing confirmed that the exon27 deletion detected by MLPA was caused by the mutation c.2546delC. This mutation probably abolishes the exon 27 signal because one of the MLPA probes set on exon 27 overlaps with FANCA C2546. In cases #43 and #59, we have not yet determined whether the Exon 27 deletion is associated with a c.2546delC mutation or not.

Supplemental Table 1. Clinical characteristics of the patients studied.

Patient No.	ALDH2 allele	Disease	Defective FA genes	Allele1	Allele2	DNA origin	WES	FANCA-MLPA	Onset of BMF(months)	MDS or AML (months)	SCT (months)	The last observation (months)	Number of physical abnormalities[E]	Extensive malformation[F]	% birth weight
1	AA	MDS	A[C]	c.2546delC (p.S849FfsX40)	c.3781_3785delTTCTT (p.P1261LfsX15)	Blood			7	7	36		3		78.9
2	AA	MDS	A	c.2546delC (p.S849FfsX40)	NI	Blood			0	12	13		5	Yes	79.2
3	AA	MDS	P	c.343delA (p.S115AfsX11)	c.343delA (p.S115AfsX11)	Blood	Yes	Normal	0	4		6	6	Yes	73.8
4	GA	SAA	A	c.2546delC (p.S849FfsX40)	NI	Blood			24		58		5		85.2
5	GA	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.3931-3932delAG (p.S1311X)	Skin-fibroblast		ex27[G]/-	21		69		6	Yes	61.2
6	GA	SAA	A	c.1023G>C (p.Q341H)[B]	c.1639G>T (p.A547S)[B]	Skin-fibroblast		Normal	24		249		3		91.2
7	GA	MDS	A	c.4168-2A>G	c.2546delC (p.S849FfsX40)	Skin-fibroblast		ex27[G]/-	28	168	171		2		69.8
8	GA	SAA/tongue cancer	A[C]	c.2593delA (p.I879LfsX24)	ex18-21 del	Blood		ex18-21/-	53			469	0		71.8
9	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood			38		56		3		66.5
10	GA	SAA	A[C]	c.2546delC (p.S849FfsX40)	ex3 del	Blood		ex27[G]/ex1-3	22		122		5	Yes	76.4
11	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood			36		90		2		76.1
12	GA	SAA	A[C]	c.2546delC (p.S849FfsX40)	ex30 del	Blood		ex27[G]/ex30	37		74		1		97.4
13	GA	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.2546delC (p.S849FfsX40)	BM-fibroblast		ex27[G]/ex27[G]	38		80		5		78.9
14	GA	MDS	P	c.2629G>A (p.A877T)[B]	NI	Blood	Yes	Normal	12	108	135		2		98.2
15	GA	MDS	G	c.1066C>T (p.Q356X)	c.307+1G>C	Blood	Yes		12	61	62		5	Yes	82.9
16	GA	SAA	I	c.158-2A>G	c.288G>A (p.E96E)[D]	Blood	Yes	Normal	7		45		11	Yes	52.4
17	GA	SAA	A	c.1303C>T (p.R435C)	c.4168-1G>C	Blood	Yes		26		168		2		53.5
18	GA	MDS	M	c.2330A>G (p.Y777C)[B]	NI	Blood	Yes	Normal	24	51	51		12	Yes	66.2
19	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood	Yes	Normal	48		88		7	Yes	90.6
20	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood	Yes		39		84		5		100.6
21	GA	SAA	I	c.3346_3347insT (p.S1116FfsX16)	c.2826+3 A>G[B]	Blood	Yes	Normal	15		28		9	Yes	78.6
22	GA	SAA	A	c.2602-2A>T	c.4198C>T (p.R1400C)	Blood	Yes	Normal	48		154		3		94.9
23	GA	SAA	G	c.907_908delCT (p.L303GfsX5)	c.307+1G>C	Blood	Yes		21		78		1		66.9
24	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood	Yes		69		135		4	Yes	74

25	GA	SAA	G	c.307+1G>C	c.307+1G>C	Blood	Yes	Normal	18			72	2		85.8
26	GA	MDS	A	ex 30 del	NI	Blood		ex30/-	60	192	199		4	Yes	102.1
27	GG	SAA	A	c.2602-2A>T	c.2527T>G(p.Y843D)	Skin-fibroblast		Normal	78		228		3		83
28	GG	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.2546delC (p.S849FfsX40)	Blood			114		128		3		87.7
29	GG	AML	A	c.2602-2A>T	c.2602-2A>T	Blood		Normal	62	311	316		3		81.5
30	GG	AML	A	c.4124_4125delCA (p.T1375SfsX49)	c.2290C>T (p.R764W)	Blood		Normal	156	156	162		3		90.1
31	GG	SAA	G	c.307+1G>C	c.1066C>T (p.Q356X)	Blood			50		52		4		66.2
32	GG	MDS	A reversion(loss of allele2 mutation)	c.2546delC (p.S849FfsX40)	c.3295C>T[A] (p.Q1099X)	BM-fibroblast		ex27[G]/ex33	49	189	192		6	Yes	72.4
33	GG	MDS/tongue cancer	A reversion(loss of allele1 mutation)	c.44_69delA (p.P15RfsX13)	c.2170A>C (p.T724P)	BM-fibroblast		Normal	108	348		384	2		62.4
34	GG	AML	A	c.2602-1G>A	NI	Blood			108	384	448		3		82.2
35	GG	SAA	A[C]	c.2546delC (p.S849FfsX40)	ex38 del	Blood			72		122		1		92.3
36	GG	SAA	A	ex24-28 skipping	NI	Blood			60		144		4		74.8
37	GG	AML	A	c.1470+?_1626+?del	NI	Blood		ex16-17/-	61	61	66		1		70.6
38	GG	SAA	A	c.2546delC (p.S849FfsX40)	c.4168-2A>G	Blood		ex27[G]/-	106		184		2		70.4
39	GG	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.2546delC (p.S849FfsX40)	Blood		ex27[G]/ex27[G]	70		153		5	Yes	76.6
40	GG	SAA	G	c.307+1G>C	c.307+1G>C	Blood			28		67		0		84.9
41	GG	MDS	A	c.2546delC (p.S849FfsX40)	c.1567-1G>A	Blood		ex27[G]/ex32	82	82	108		3		67.9
42	GG	SAA	A[C]	c.2546delC (p.S849FfsX40)	c.3720_3724del (p.E1240DfsX36)	Blood		ex27[G]/ex37	88		185		2		98.7
43	GG	MDS	A[C]	ex27 del	ex1-28 del	Blood	Yes	ex27/ex1-28	72	72	78		1		84
44	GG	SAA	A	ex30 del	NI	Blood		ex30/-	45		130		4		84
45	GG	SAA	A	ex11-15 duplication	NI	Blood		ex11-15/-	297		318		2		Unknown
46	GG	SAA	A	ex30 del	NI	BM-fibroblast		ex30/-	96		182		2		97
47	GG	MDS	A	c.2870G>A (p.W957X)	c.2723_2725TCT>GCC (p.LS908_909RP	Blood	Yes		121	335	343		3		Unknown
48	GG	MDS	G	c.1386delC (p.A463GfsX55)	c.1637-15 G>A[B]	Blood	Yes	Normal	69	120	133		2		88.1
49	GG	SAA	G	c.1066C>T (p.Q356X)	c.1066C>T (p.Q356X)	Blood	Yes	Normal	66		77		4		70.5
50	GG	SAA	G	c.1066C>T (p.Q356X)	c.1066C>T (p.Q356X)	Blood	Yes	Normal	72		79		1		68.6
51	GG	SAA	G	c.91C>T (p.Q31RfsX5)	c.307+1G>C	Blood	Yes		27		109		4		77

52	GG	SAA	G	c.91C>T (p.Q31RfsX5)	c.307+1G>C	Blood	Yes		60		113		4		66.7
53	GG	AML	A	c.4240_4241delAG (p.S1414LfsX10)	c.2602-1G>A	Blood	Yes	Normal	41	115	128		6	Yes	67
54	GG	SAA	A	c.2602-2A>T	c.4198C>T (p.R1400C)	Blood	Yes	Normal	120		190		3		86.4
55	GG	MDS	A^C (reversion suspected)	c.2546delC (p.S849FfsX40)	c.3919_3920insT (p.Q1307LfsX6)	Blood	Yes	$ex27^G$/-	144	145	208		2		89.4
56	GG	AA	A	c.2602-2A>T	c.2602-1G>A	Blood	Yes	Normal	134			147	2		67.4
57	GG	AA	A	ex30 del	NI	Blood		ex30/-	51			65	3		89.5
58	GG	SAA	A	ex30 del	NI	Blood		ex30/-	92		104		3		81.2
59	GG	AML	A^C	ex27 del	ex37 skipping	Blood		ex27/-	136	176	179		2		Unknown
60	GA	SAA	Not detected	NI	NI	Blood	Yes		87		162		3		67.7
61	GA	AML	Not detected	NI	NI	Blood	Yes	Normal	41	41	99		3		84
62	GG	SAA	Not detected	NI	NI	Blood	Yes	Normal	58		72		10	Yes	57.4
63	GG	SAA	Not detected	NI	NI	Blood	Yes	Normal	40		61		6	Yes	75.7
64	GG	SAA	Not detected	NI	NI	Blood	Yes	Normal	96		106		2		73.1

Legend for Supplemental Table 2. DEB test results of the patients and control subjects.

Achromatic areas less than a chromatid in width were scored as gaps. Exchange configurations, and dicentric and ring chromosomes were scored as rearrangements. Gaps were excluded from the calculation of chromosome aberrations per cell, and rearrangements were scored as 2 breaks.

Supplemental Table 2. DEB test results of the patients and control subjects.

Patient and control number	Spontaneous breakage rate (breaks/cell)	Spontaneous breakage rate (% aberrant cells)	DEB-induced breakage rate (breaks/cell)	DEB-induced breakage rate (% aberrant cells)
Patient 1	0.08	10	6.45	95
2	0.08	6	0.44	37
3	0.02	3	0.91	38
4	0.02	2	1.21	48
5	0.04	10	1.3	59
6	0.06	8	8.32	100
7	0	4	0.36	39
8	0.04	6	2.28	38
9	0.02	2	0.79	46
10	0.02	14	0.82	59
11	0.09	9	3.16	72
12	0.02	2	2.66	83
13	0.02	4	0.64	30
14	0.05	11.5	0.3	22.9
15	0.12	16	8.54	100
16	0	0	0.52	17
17	0.06	8	0.56	38
18	0.06	6	4.2	92
19	0.07	10	3.49	92
20	0	2	2.36	68
21	0.04	7	0.96	52.4
22	0.01	4	4.07	99
23	0.08	14	6.45	100
24	0.01	1	6.14	99
25	0	2	2.68	73

26	0	3	1.17	67
27	0.06	16	0.45	43
28	0.08	12	1.96	60
29	0	0	4.97	84
30	0.02	4	5.16	95
31	0.02	2	4.77	97
32	0	0	0.12	9
33	0	0	0.03	2
34	0.04	4	5.92	97.9
35	0	0	6.15	81
36	0.1	8	9	100
37	0.04	4	7.67	99
38	0.02	6	1.59	72
39	0.05	6	2.06	76
40	0.06	9	1.93	73
41	0	0	1.92	70
42	0.02	2	5.39	97
43	0.04	6	6.38	97
44	0	2	1.72	68
45	0.1	10	6.12	97.3
46	0.02	6	4.92	96
47	0.1	3	4.39	90
48	0.01	5	2.56	82
49	0	6	3.1	91
50	0.04	4	2.54	80
51	0.06	6	0.92	54.4
52	0.04	6	0.93	49.5
53	0.02	3.2	6.3	97.3
54	0.01	2	3.58	92
55	0.01	1	0.01	2
56	0.01	2	1.4	57

	57	0	0	4.85	93
	58	0.01	4	0.59	46
	59	0.02	8	0.67	36.9
	60	0	0	0.48	28
	61	0.01	2	0.89	35
	62	0.49	52	3.8	95.7
	63	0	0	7.8	100
	64	0.09	9	2.38	54
Control	1	0	0	0.02	2
	2	0	0	0.01	1
	3	0	0	0.01	1
	4	0.01	1	0.05	5
	5	0.01	1	0.05	5
	6	0	1	0.01	1
	7	0	2	0.04	4
	8	0	1	0.04	4
	9	0	0	0.02	2
	10	0	0	0.03	3
	11	0	0	0.02	3
	12	0	1	0	2
	13	0	2	0.02	4
	14	0	0	0.02	2
	15	0	1	0	1
	16	0	0	0	1
	17	0	0	0.04	4

18	0	0	0	2
19	0	1	0.05	5
20	0	0	0	0
21	0	2	0.04	6
22	0.02	2	0.04	4
23	0	0	0	1
24	0	0	0.04	5
25	0	0	0	4
26	0	0	0.02	3
27	0.02	2	0	1
28	0	0	0.02	4
29	0	0	0	0
30	0	0	0.03	2
31	0	0	0.07	10
32	0.02	3	0.04	5