



# Long-term follow-up of Fanconi anemia: clinical manifestation and treatment outcome

Byung Gyu Yoon, MD<sup>1</sup>, Hee Na Kim, MD<sup>1</sup>, Ui Joung Han, MD<sup>1</sup>, Hae In Jang, MD<sup>1</sup>, Dong Kyun Han, MD<sup>1</sup>, Hee Jo Baek, MD<sup>1,2</sup>, Tai Ju Hwang, MD<sup>1</sup>, Hoon Kook, MD<sup>1,2</sup>

<sup>1</sup>Department of Pediatrics, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, Gwangju, <sup>2</sup>Environmental Health Center for Childhood Leukemia and Cancer, Hwasun, Korea

**Purpose:** The aim of this study was to characterize Korean patients with Fanconi anemia (FA), which is a rare but very challenging genetic disease.

**Methods:** The medical records of 12 FA patients diagnosed at Chonnam National University Hospital from 1991 to 2012 were retrospectively reviewed.

**Results:** The median age at diagnosis was 6.2 years. All patients showed evidence of marrow failure and one or more physical stigmata. Chromosome breakage tests were positive in 9 out of 11 available patients. The median follow-up duration was 69.5 months. The Kaplan-Meier (KM) survival of all patients was 83.3% at 10 years and 34.7% at 20 years, respectively. Seven patients underwent 9 stem cell transplantations (SCTs). Among them, 5 were alive by the end of the study. Ten-year KM survival after SCT was 71.4% with a median follow-up of 3.4 years. All 5 patients treated with supportive treatment alone died of infection or progression at the median age of 13.5 years, except for one with short follow-up duration. Acute leukemia developed in 2 patients at 15.4 and 18.1 years of age. Among 6 patients who are still alive, 3 had short stature and 1 developed insulin-dependent diabetes mellitus.

**Conclusion:** We provide information on the long-term outcomes of FA patients in Korea. A nation-wide FA registry that includes information of the genotypes of Korean patients is required to further characterize ethnic differences and provide the best standard of care for FA patients.

**Key words:** Fanconi anemia, Korean, Stem cell transplantation, Long-term outcome

**Corresponding author:** Hoon Kook, MD

Department of Pediatrics, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, 322 Seoyang-ro, Hwasun 519-763, Korea

Tel: +82-61-379-7693

Fax: +82-61-379-7697

E-mail: [hoonkook@chonnam.ac.kr](mailto:hoonkook@chonnam.ac.kr)

Received: 16 August, 2013

Revised: 7 October, 2013

Accepted: 17 October, 2013

## Introduction

Fanconi anemia (FA) is a rare autosomal and X-linked recessive disorder, characterized by physical abnormalities, progressive bone marrow failure (BMF), hypersensitivity to DNA cross-linking agents and predisposition to malignancy<sup>1</sup>. It was first described in 1927 by Fanconi<sup>2</sup>, who described a family in which three children had pancytopenia and birth defects, such as short stature, hypogonadism and skin disorder. Since then, 1,075 patients were registered in the International Fanconi Anemia Registry (IFAR) between May 1982 and August 2008<sup>3</sup>.

FA is considered the most common inherited cause of BMF, which is usually apparent between 6 and 8 years of age. Most patients with FA have birth defects including skin hyperpigmentation and/or cafe au lait spots, skeletal malformations, short stature and urogenital abnormalities<sup>3</sup>. However, one-third of the patients have few or none of these features, which makes it difficult to diagnose<sup>4</sup>. FA patients may develop myelodysplastic syndrome (MDS), or acute myeloid leukemia (AML), with relative risk of developing AML being 800-fold higher than that of the general population. There is also a strong

Copyright © 2014 by The Korean Pediatric Society

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

predisposition to specific solid tumors, including head, neck, gynecological squamous cell carcinoma (SCC) and liver tumors<sup>4,5</sup>.

Hematopoietic stem cell transplantation (SCT) is currently the only treatment to restore normal hematopoiesis because of nonresponse to immunosuppressive therapy, such as antilymphocyte globulin, antithymocyte globulin (ATG) and cyclosporine, which is usually given to treat idiopathic aplastic anemia (AA)<sup>6</sup>. Moreover, because of hypersensitivity to chemotherapeutic agents or radiation, higher risk of developing malignancies, and potential recurrence in the siblings, the approach to SCT should be different from that of idiopathic AA in terms of the donor selection, optimal timing of SCT, and the choice of conditioning regimen. Also, the prognosis after SCT is quite different<sup>7,8</sup>. Therefore, FA should be sought carefully in any patients with AA or BMF syndromes in children and young adults.

A screening study with DNA cross-linking agents in patients with BMF and clinical manifestation of 6 FA patients was published from our institution in 1997 and 1998, respectively<sup>9,10</sup>. The present study represents an update and long-term follow-up study of 12 patients diagnosed with FA at the Chonnam National University Hospital over the last 20 years. Clinical presentations, laboratory findings, diagnostic methods, treatment modalities, outcomes and long-term sequelae of patients were retrospectively analyzed to increase awareness of this rare but very challenging genetic disease.

## Materials and methods

### 1. Subjects

This study included 12 FA patients seen at the Department of Pediatrics, Chonnam National University Hospital and Chonnam National University Hwasun Hospital between January 1991 and December 2012. The patients were diagnosed on the basis of family history, physical findings in addition to hematologic abnormalities, and chromosomal breakage tests induced by diepoxybutane (DEB) or mitomycin-C (MMC)<sup>3,4</sup>.

### 2. Chromosome breakage tests

The screening for the diagnosis of FA chromosome breakage tests was performed on 104 subjects including 78 AA, 6 MDS, 6 acute leukemia, 2 idiopathic thrombocytopenic purpura, 5 Diamond-Blackfan anemia, and 7 sibling of FA between January 1996 to December 2012.

Chromosome breakage tests with DEB and MMC were carried out according to the technique described by Auerbach et al.<sup>11</sup> with minor modifications<sup>10</sup>. Two patients (cases 11, 12) with negative blood results underwent chromosomal breakage tests on cultured skin fibroblasts. Gene analysis for FANCA was per-

formed in case 12 using a multiplex ligation-dependent probe amplification (MLPA). The gene dosage assay for FANCA to detect deletion/duplications was carried out using a SALSA MLPA Kit P031-P032 FANCA (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's instructions.

### 3. Retrospective analysis

A retrospective analysis of the patients' clinical manifestations, diagnostic methods, treatment modalities, outcomes and long-term sequelae was performed. Height and weight were standardized for age and gender using data from the Korea Center for Disease Control and Prevention (2007)<sup>12</sup>. Short stature was designated when height was lower than 2 standard deviation score.

The following were the criteria for the response to the treatment. Complete response was defined when hemoglobin level  $\geq$  age-adjusted level, and absolute neutrophil count (ANC)  $\geq 1.5 \times 10^9/L$ , and platelet count  $\geq 100 \times 10^9/L$ . Partial response was defined when there is no requirement for red cell transfusion or reticulocyte count increase  $\geq 30 \times 10^9/L$ , ANC increase  $\geq 0.3 \times 10^9/L$ , and no platelet transfusion and platelet increase  $\geq 20 \times 10^9/L$ <sup>13</sup>.

Survival was assessed on the date of last patient contact and analyzed as of December 2012. Statistical analysis was performed using SPSS ver. 19.0 (SAS Institute Inc., Chicago, IL, USA). The probability of overall survival (OS) was estimated according to the Kaplan-Meier (K-M) survival method. Failure-free survival (FFS) was defined as survival with response. Death, transfusion-dependency, disease progression to MDS or AML, a second course of SCT, and relapse were considered treatment failures<sup>14</sup>. Statistical significance was defined as  $P < 0.05$ .

### 4. IRB Approval

The study was approved by the Ethics Committee of the Chonnam National University Hospital (CNUH-2013-107).

## Results

### 1. Clinical manifestations

Of 12 patients with a diagnosis of FA, 9 were males and 3 were females. Their age at first evaluation ranged from 5 months to 14.3 years (median, 6.2 years). Characteristics of the patients are summarized in Table 1.

One patient (case 2) had a family history in which his elder brother died of AA. Low birth weight was observed in 7 patients (63.6%), with the median body weight of 2.8 kg. All patients had at least one of the following associated anomalies: skin pigmentation including *cafe au lait* spots, hyperpigmentation or hypopigmentation (100%), skeletal abnormality (50.0%), microphthalmia (33.3%), cardiac anomaly (16.7%), microcephaly (16.7%), and

**Table 1.** Clinical characteristics of study participants at the initial hospital visit

Case	Sex	Age (yr)	BW (kg)	F/H	Associated anomaly
1	M	0.5	2.8	(-)	Hyperpigmentation, <i>café au lait</i> spots, peculiar face, microcephaly, small eye
2	M	6	2.3	(+)	<i>Café au lait</i> spots
3	F	5.3	2.2	(-)	Congenital dislocation of hip, polydactyly, <i>café au lait</i> spots
4	M	6.2	ND	(-)	Hyperpigmentation, short stature, microcephaly, peculiar face, small eye
5	M	6.7	3.2	(-)	<i>Café au lait</i> spots
6	M	13.3	3.0	(-)	Hyperpigmentation, <i>café au lait</i> spots, microcephaly, peculiar face, polydactyly, developmental delay, MR, small eye
7	M	0.3	3.1	(-)	<i>Café au lait</i> spots, hypopigmentation, peculiar face, right thumb hypoplasia
8	M	14.3	2.8	(-)	<i>Café au lait</i> spots
9	F	6.7	2.1	(-)	Right thumb duplication, dark skin color
10	M	3	2.3	(-)	<i>Café au lait</i> spots, hypopigmentation, small eye, low nasal bridge
11	F	4.8	1.78	(-)	<i>Café au lait</i> spots, right thumb hypoplasia, left thumb polydactyly, PDA, ASD
12	M	8.6	3.18	(-)	<i>Café au lait</i> spots, both thumb hypoplasia, small-eye, ASD, VSD, GERD
Median	M:F (9:3)	6.2	2.8		

BW, birth weight; F/H, family history; ND, not done; MR, mental retardation; PDA, patent ductus arteriosus; ASD, atrial septal defect; GERD, gastroesophageal reflux disease.

short stature (8.3%). Other findings included developmental delay, congenital dislocation of hip, and polydactyly (Table 2).

## 2. Hematologic findings

Laboratory findings of the patients at diagnosis are summarized in Table 3. All patients had evidence of BMF at the time of their first visit. The median blood counts were: white blood cell, 3,420/mm<sup>3</sup>; hemoglobin, 6.0 g/dL; and platelets, 22,500/mm<sup>3</sup>. Most patients had macrocytosis with median mean corpuscular volume of 95.8 fL. A bone marrow exam showed hypocellular marrow with loss of hematopoietic cells, and fatty replacement in all patients except one. The 3-month-old patient (case 7) had a normocellular marrow with macrocytosis, erythroid hyperplasia and significant dyserythropoiesis which was compatible with MDS<sup>15</sup>. According to the classification of AA by severity<sup>16</sup>, 7 patients (63.6%; cases 1, 2, 4, 5, 8, 10, and 12) among 11 patients diagnosed as AA had severe AA including 2 very severe diseases (cases 1, 2).

## 3. Chromosomal breakage test

Chromosomal breakage tests with DEB and MMC have successfully discriminated the two groups among 104 subjects: FA patients with hypersensitivity to both DEB and MMC, and non-FA patients with no hypersensitivity. The FA patients showed increased chromosomal breaks/cell of 1.51±1.45 (mean±standard deviation) (range, 0 to 4.25) to DEB ( $P<0.01$ ), and 1.11±0.85 (range, 0 to 2.5) to MMC ( $P<0.01$ ) (Table 4). Nine cases were found to have increased chromosomal breakage to DEB and MMC, confirming the diagnosis of FA. The remaining 3 patients (cases 4, 11, and 12) were diagnosed on the basis of characteristic physical stigma in addition to hematologic abnormalities. Case 4 died before the

**Table 2.** Frequency of physical abnormalities at diagnosis

Abnormality	No/total (%)
Skin pigmentation ( <i>café au lait</i> , hyperpigmentation and hypopigmentation)	12/12 (100)
Low birth weight	7/12 (58.3)
Skeletal (radial ray, hip, vertebral scoliosis, rib)	6/12 (50.0)
Eyes (microphthalmia)	4/12 (33.3)
Cardiac	2/12 (16.7)
Microcephaly	2/12 (16.7)
Short stature	1/12 (8.3)
Gastrointestinal (e.g., anorectal, duodenal atresia)	1/12 (8.3)
No abnormality	0/12 (0)

availability of the tests. Breakage tests on skin fibroblasts failed unfortunately in case 11 and were negative in case 12. FANCA mutation was not detected in case 12.

Ninety-two subjects with diseases other than FA including 67 patients with idiopathic AA showed no increased hypersensitivity: chromosomal breakages/cell of 0.01±0.02 (range, 0 to 0.1) to DEB and 0.02±0.05 (range, 0 to 0.4) to MMC, respectively. No false positivity was found among subjects other than FA. Increased spontaneous breakage was found in 6 among 11 FA patients, and thus was not sensitive as a diagnostic method.

## 4. Treatment

Even though the treatment modalities varied with time, FA patients received a combination of supportive treatment, oxymetholone, prednisolone, immunosuppressive treatment with ATG, and SCT (Table 5). In earlier years, 8 patients were treated with oxymetholone and prednisolone initially for 3 to 76 months. Oxymetholone and prednisolone treatment was partially bene-

**Table 3.** Laboratory profiles of cases

Case	WBC (/mm <sup>3</sup> )	ANC (/mm <sup>3</sup> )	Hemoglobin (g/dL)	MCV (fl)	RDW (%)	Platelet ( $\times 1,000/\text{mm}^3$ )	C-Reti (%)
1	3,400	100	5.4	93.3	13.4	5	1.10
2	2,400	200	4.0	99.3	25.6	18	0.10
3	4,400	1,300	8.4	98.8	14.8	44	1.00
4	2,300	400	5.0	90.5	15.8	22	0.40
5	3,700	300	3.3	91.9	16.2	12	0.90
6	2,900	300	7.4	102.0	18.5	43	2.20
7	4,300	450	4.6	104.1	-	23	0.65
8	1,000	200	3.4	94.3	14.1	3	0.01
9	3,440	620	11.2	93.0	12.9	75	0.89
10	7,700	1,925	6.6	97.3	17.8	15	0.47
11	4,700	590	8.6	100	16.1	37	0.67
12	1,300	310	12.9	82.5	12.7	71	0.08
Median (range)	3,420 (1,000–7,700)	355 (100–1,925)	6.0 (3.3–12.9)	95.8 (82.5–104.1)	15.8 (12.7–22.6)	22.5 (3–75)	0.66 (0.01–2.2)

WBC, white blood cell; ANC, absolute neutrophil count; MCV, mean corpuscular volume; RDW, red cell distribution width; C-Reti, corrected reticulocyte count.

**Table 4.** Results of chromosomal breakage tests in patients with Fanconi anemia and other hematologic diseases and in controls

Diagnosis	No.	Spontaneous (breaks/cell)	DEB (breaks/cell)	MMC (breaks/cell)	Interpretation*
Aplastic anemia					
FA1		0.000	1.100	1.150	Positive
FA2		0.050	1.950	1.150	Positive
FA3		0.050	4.250	2.00	Positive
FA4		ND	ND	ND	ND
FA5		0.250	3.700	1.300	Positive
FA6		0.050	1.150	2.500	Positive
FA8		0.050	0.300	1.450	Positive
FA9		0.000	1.500	ND	Positive
FA10		0.100	1.150	0.44	Positive
FA11		0.000	0.000	0.000	Negative
FA12		0.000	0.000	0.000	Negative
Idiopathic	67	0.00±0.00	0.01±0.02	0.02±0.06	Negative
MDS					
FA7		0.000	Positive	Positive	Positive
Idiopathic	5	0.00±0.00	0.00±0.00	0.01±0.02	Negative
Acute Leukemia	6	0.00±0.00	0.00±0.00	0.04±0.06	Negative
ITP	2	0.00±0.00	0.00±0.00	0.03±0.04	Negative
DBA	5	0.00±0.00	0.05±0.07	0.07±0.04	Negative
Sibling of FA	7	0.00±0.00	0.02±0.03	0.03±0.03	Negative
FA	12	0.05±0.07	1.51±1.45	1.11±0.85	Positive
Non-FA	92	0.00±0.00	0.01±0.02	0.02±0.05	Negative
<i>P</i>		<0.05	<0.01	<0.01	

Values are presented as mean±standard deviation. Statistical significance was defined as  $P < 0.05$ .

DEB, diepoxybutane; MMC, mitomycin-C; ND, not done; FA, Fanconi anemia; MDS, myelodysplastic syndrome; ITP, idiopathic thrombocytopenic purpura; DBA, Diamond-Blackfan anemia.

\*A breakage level  $\geq 1.0$  aberrations/cell was considered positive for increased breakage.

**Table 5.** Treatment modalities and outcome

Case	Supportive treatment (mo)	Response	Stem cell transplantation ( )*	F/U (mo)	Age at last F/U (mo)	Malignancy	Survival
1	Oxy/PRD (3) IST (12)	NR PR	PMRD-BMT	19	25		Died of GVHD (Gr IV) & sepsis
2	Oxy/PRD (76)	PR	No	79	219	AML (80 mo after Dx)	Died of AML
3	Oxy/PRD (53)	NR	No	76	140		Died of sepsis
4	Oxy/PRD (3)	NR	No	63	187		Died of disseminated fungal infection
5	IST (24) Oxy/PRD (34)	NR NR	MS-BMT	247+	328+		Alive
6	Oxy/PRD (5)	PR	MS-BMT	185+	356+		Alive
7	Oxy/PRD (17)	NR	No	17	20		Died of pneumonia, chronic diarrhea
8			MS-BMT MS-BMT (13 mo later)	19	192	AML-M2 (13 mo after Dx, 12 mo after BMT)	Died of AML, pneumonia
9			UCBT	102+	195+		Transfer, Alive
10	Oxy/PRD (65)	PR	UCBT UBMT (24 mo later)	162+	201+		Alive
11			PMRD-BMT	38+	96+		Alive
12			No	32+	125+		Alive
Median (range)				69.5 (17-247)	189.5 (20-356)		

F/U, follow-up; Oxy, oxymetholone; PRD, prednisolone; IST, immunosuppressive therapy; NR, no response; PR, partial response; PMRD-BMT, partially matched related donor bone marrow transplantation; GVHD, graft versus host disease; Gr, grade; AML, acute myeloid leukemia; Dx, diagnosis; MS-BMT, matched sibling BMT; MS-PBSCT, MS peripheral blood stem cell transplantation; UCBT, unrelated cord blood transplantation; UBMT, unrelated BMT.

( )\*, interval between transplants.

ficial in 3 patients (37.5%) (cases 2, 6, and 10), although peliosis hepatis (case 10) and masculinization (case 3) developed as side effects. All 4 patients (cases 2, 3, 4, and 7) treated with oxymetholone and prednisolone alone eventually died of invasive fungal infection, pneumonia, sepsis and development of AML, respectively.

Two patients received immunosuppressive treatment. Case 5 was initially diagnosed with idiopathic AA before the availability of chromosomal breakage test as he showed minimal physical stigma. He was later diagnosed with FA and transplanted. Case 1 with severe disease had received oxymetholone, prednisolone and ATG treatment to no avail before he underwent a mismatched SCT.

Seven patients underwent 9 hematopoietic SCTs. The median age at transplant was 11.7 years. The stem cell sources were: matched siblings (n=4), partially matched related donors (n=2), unrelated cord blood (n=2), and unrelated bone marrow (n=1). Case 8 showed initial graft rejection after matched sibling transplant, and underwent a second transplant from the same donor when he developed AML 1 year later. Case 10 suffered from engraftment failure after initial cord blood transplant (CBT), but was rescued by unrelated bone marrow. Another patient (case 9) with CBT also rejected initial graft, but she showed autologous recovery. None was T cell depleted.

The conditioning regimens varied over 2 decades. In earlier years, low-dose cyclophosphamide (Cy), ATG with or without

low-dose irradiation were mainly used (n=5; Table 6). Recently, fludarabine (Flu)-based containing regimen without irradiation was used (n=4).

## 5. Survival

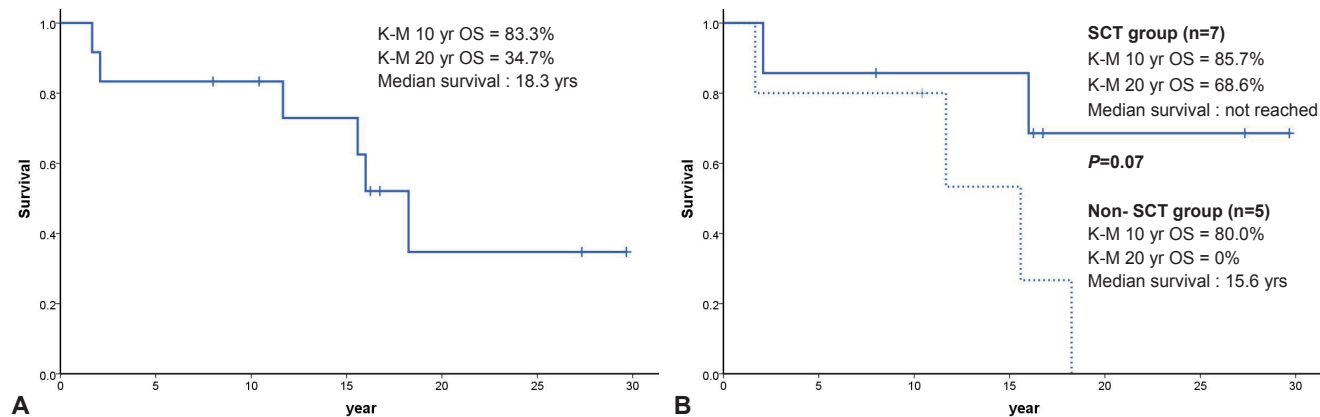
Among 12 FA patients with the median age of 189.5 months (range, 20 to 356 months) at last follow-up, six patients (50%) are alive at a median follow-up of 69.5 months (range, 17 to 247 months) with the median age of 198 months (range, 96 to 356 months) at last follow-up. The K-M probability of OS was 83.3% at 10 years and 34.7% at 20 years for the entire group. Median survival was 18.3 years (range, 14.9 to 21.6 years) after diagnosis (Fig. 1A). The median survival of non-SCT group was 15.6 years (range, 4.0 to 27.1 years), while that of the SCT group was not reached ( $P=0.07$ ). For the SCT group, the K-M probability of survival after diagnosis was 85.7% at 10 years and 68.6% at 20 years, respectively (Fig. 1B).

Among 7 who underwent SCTs, 5 are alive with the K-M 10-year OS of 71.4% after SCT with the median follow-up of 3.4 years. The K-M probability of FFS at 10 years was 57.1% after SCT (Fig. 2). Case 1 who underwent mismatched related donor transplant died of grade IV acute graft-versus-host disease (GVHD) at 40 days after transplant, while another patient (case 8) who developed AML (M2) died at 12 months after initial transplant. Because of the small number of cases, the survival was not different by the conditioning method no matter whether the patients received

**Table 6.** Hematopoietic stem cell transplantation outcomes

Case	Prior therapy	Conditioning	Donor ( )*	GVHD prophylaxis	GVHD grading		Hemorrhagic cystitis or VOD	Survival	F/U (mo)
					Acute	Chronic			
1	Oxy/PRD IST	Cy 25 mg/kg×4 ATG 40 mg/kg×3	PM brother	CyA+PRD	IV	0	No	Died of GVHD & sepsis	19
5	Oxy/PRD IST	Cy 5 mg/kg×4 ATG 40 mg/kg×3 TAI 400 cGy	Sister	CyA+PRD	0	0	VOD	Alive	242
6	Oxy/PRD	Cy 20 mg/kg×4 ATG 1.2 mg/kg×3 TNI 5 Gy	Sister	CyA+MTX	0	0	No	Alive	180
8		Cy 5 mg/kg×4 ATG 40 mg/kg×3 TAI 400 cGy	Brother	CyA+PRD	0	0	No	Died of pneumonia	19
		FLU 30 mg/m <sup>2</sup> ×6 Cy 10 mg/kg×4 hATG 1.5 vial/10kg×3	Brother (13 mo later)	CyA	I	0	VOD hemorrhagic cystitis		
9		FLU 30 mg/m <sup>2</sup> ×5 Cy 5 mg/kg×4 ATG 2.5 mg/kg×3	Cord	CyA	0	0	No	Alive	50
10	Oxy/PRD	Cy 10 mg/kg×4 rATG 2.5 mg×4 TAI 400 cGy	Cord	CyA	0	0	VOD hemorrhagic cystitis	Alive	157
		FLU 30 mg/m <sup>2</sup> ×6 Cy 10 mg/kg×4 hATG 1.5 vial/10kg×3	UBM (24 mo later)	Tacrolimus+MTX	I	0	No		
11		FLU 30 mg/m <sup>2</sup> ×6 Cy 10 mg/kg×4 rATG 2.5 mg/kg×3	PM father	Tacrolimus+MMF	0	0	No	Alive	33

GVHD, graft versus host disease; VOD, veno-occlusive disease; F/U, follow-up; Oxy, oxymetholone; PRD, prednisolone; IST, immunosuppressive therapy; Cy, cyclophosphamide; ATG, antithymocyte globulin; PM, partially matched; CyA, cyclosporine; TAI, thoraco-abdominal irradiation; TNI, total nodal irradiation; MTX, methotrexate; hATG, horse anti-thymocyte globulin; FLU, fludarabine; rATG, rabbit anti-thymocyte globulin; MMF, mycophenolate mofetil. ( )\*, Interval between transplants.



**Fig. 1.** Kaplan-Meier (K-M) survival statistics for patients with Fanconi anemia. (A) Overall survival (OS) after diagnosis for all cases (n=12). (B) OS after diagnosis of the stem cell transplantation (SCT) group (n= 7) versus the non-SCT group (n=5).

Cy-based, Flu-based, or radiation containing regimens. Also, the use of oxymetholone and prednisolone prior to SCT was not associated with poor outcome as 3 of 4 patients (cases 1, 5, 6, and 10) are surviving.

Infection was the main cause of deaths, as seen in 5 patients (83.3%). Acute leukemia developed in 2 patients (cases 2 and 8),

while solid tumors have thus far not been identified.

### 6. Late sequelae

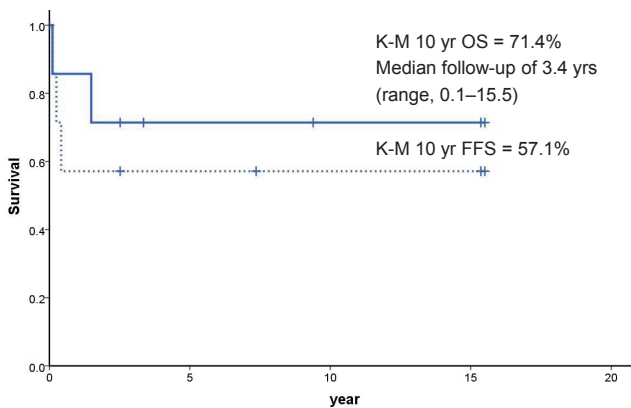
Among the 6 patients who are alive, 3 patients are suffering from short stature with less than 2 standard deviation score in height (Table 7). The median standard deviation score was -2.03



**Table 7.** Endocrine dysfunction among the 6 patients surviving by the end of the study

Case	Age at last F/U (yr)	Height (cm), (SDS)	Body weight (kg), (SDS)	Thyroid function test	DM
5	27.4	162 (-2.05)	55 (-1.37)	ND	No
6	25.0	162.6 (-1.94)	64 (-0.2)	ND	No
9	16.2	149.8 (-2.0)	37 (-3.01)	ND	No
10	16.8	138.6 (-5.57)	33.2 (-5.48)	(at 15.7 yr) Free T4 1.12 ng/dL TSH 3.47 $\mu$ IU/mL	Yes, Dx at 9.7 yr
11	8.2	115.3 (-2.09)	20.3 (-1.61)	(at 8.2 yr) Free T4 1.23 ng/dL TSH 5.61 $\mu$ IU/mL	No
12	10.6	141.1 (0.26)	30.5 (-0.75)	ND	No
Median SDS		-2.03	-1.49		

F/U, follow-up; SDS, standard deviation score; DM, diabetes mellitus; ND, not done; TSH, thyroid stimulating hormone; Dx, diagnosis.



**Fig. 2.** Kaplan-Meier (K-M) survival statistics for the 7 patients with Fanconi anemia who underwent 9 stem cell transplantations (SCT) in total. The solid line represents overall survival (OS) after SCT (n=7), while the dotted line represents failure-free survival (FFS) after SCT (n=9).

in height and -1.49 in body weight, respectively. No surviving patient showed symptoms of hypothyroidism and 2 patients evaluated for thyroid function revealed normal values. Case 10, who received huge amounts of transfusions after the initial graft rejection, developed an insulin-dependent diabetes mellitus secondary to iron accumulation in the pancreas with elevated ferritin level of 5,961 ng/mL, requiring insulin treatment.

## Discussion

FA is a genetic disorder characterized by multiple congenital anomalies, hematological abnormalities and predisposition to a variety of cancers. Approximately two-thirds of FA patients present with a combination of various congenital abnormalities such as short stature, Fanconi facies with microphthalmia, skeletal deformities, skin hyperpigmentation, and cardiac, renal, genitourinary, and/or other malformations<sup>3,17</sup>. Skin abnormalities have been described in the literature in 55% of cases of FA<sup>4</sup>.

However, all patients in this study had skin hyperpigmentation and/or *café-au-lait* spots. Skeletal abnormalities, found in 43% of cases of FA, were hypoplasia or absence of the thumbs, bifid or supernumerary thumbs, and hypoplasia or absence of the radius<sup>4</sup>. One third in this study had skeletal abnormalities. Other less common abnormalities were 2 cardiac anomalies and 1 gastrointestinal defect. Physical abnormalities can be subtle or absent as 3 patients (cases 2, 5, and 8) in the present study showed *café-au-lait* spots only.

Hematologic abnormalities are the most important clinical features of FA. BMF develops at a median age of 7 years (range, birth to 41 years)<sup>5,18</sup>. For the majority of patients, the suspicion of FA is made only after the onset of pancytopenia. Initial hematologic findings are diverse. At birth, blood count is usually normal and macrocytosis is commonly the first detected abnormality followed by thrombocytopenia and anemia. During the first decade of life, about 75%-90% of FA patients develop BMF<sup>18</sup>.

In some patients, the underlying diagnosis of FA is not known until MDS or AML occurs. The relative risk of developing AML is approximately 800 fold higher than that of the general population, with the median onset of 14 years<sup>19</sup>. In this study, 2 patients (cases 2 and 8) developed AML at 15.4 and 18.1 years of age, respectively. MDS was detected in 23 cases among 119 FA patients cohort at a median of 12 years (range, 2 to 44 years) in a recent study<sup>20</sup>. The presentation of MDS at the age of 3 months (case 3) in this study should be extremely rare<sup>15</sup>.

In addition to hematologic malignancy, solid tumors develop at markedly higher rates in FA patients. Most of the solid tumors in FA patients are SCC, particularly of the head and neck, esophagus and anogenital regions. Hepatic tumors may also be found at higher rates, which may be related to androgen use. Approximately one-third of FA patients were known to develop a solid tumor by the fourth decade of life<sup>21</sup>. However, not a single case of solid tumor has thus far been identified in this study. With wider application of SCT and improved survival, long-term

survivors will have a particularly high risk of developing solid tumors.

Because of the heterogeneity on clinical features and recessive mode of inheritance FA can not be diagnosed with certainty by careful history and physical examination in patients with BMF. The biologic diagnosis of FA is primarily based on the exquisite sensitivity of FA cells to the cytotoxic and clastogenic effect of DNA cross-linking agents such as DEB or MMC. The chromosomal breakage test with these agents is the technique of reference for diagnosing FA<sup>3,10,11</sup>. The incidence of FA has been reported to represent 25% to 30% of pediatric AA. In Korea, however, the frequency of FA among patients with AA has not been systematically evaluated and has probably been underestimated as DEB and MMC tests have not been routinely incorporated in the evaluation of patients with AA in every institution. In accordance with the previous study<sup>10</sup>, the chromosomal breakage of FA cells to DEB and MMC was distinguishable from non-FA group in this study. Spontaneous chromosomal breakage was not effective in distinguishing FA from non-FA patients because of overlap of breakage ranges<sup>11</sup>. In the current study, 5 FA patients did not show spontaneous chromosomal breakages.

Other blood tests such as cell-cycle analysis and evaluation of FANCD2 mono-ubiquitination may also be used to diagnose FA. However, all of these tests can be ambiguous or even falsely negative in patients who develop somatic mosaicism and hematopoietic reversion<sup>22</sup>. Because these phenomena have not been demonstrated in fibroblasts, primary fibroblast cells can be tested for chromosomal breakage tests or other tests for FA diagnosis<sup>23</sup>. In the current study, 2 patients (cases 11 and 12) with peculiar physical characteristics along with BMF showed negative results for chromosomal breakage tests on blood. Attempts on skin fibroblasts were unfortunately not successful in case 11 and negative in case 12. The possibility of somatic mosaicism or hematopoietic reversion should be contemplated in those cases.

Since the first FA gene was demonstrated in 1992<sup>24</sup>, 16 FA complementation groups (FANCs) have been identified (FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M, -N, -O, -P, and -Q) so far. The majority of FA genes are located on autosomes except FANCB, which is on the X chromosome. Among the 15 complementation groups, FA-A (60%–70%), FA-C (up to 14%), and FA-G (up to 10%) collectively accounts for more than 90% of the FA groups in the western population<sup>17</sup>. However, considerable variation in the frequency of each complementation group has been reported according to ethnicity. FA-A and FA-G mutations were detected in 70%–80% and 10%–22% in Japanese population<sup>25</sup>. A recent study from Korea identified 6 FA-A (46%), 7 FA-G (54%) and no FA-C among 13 FA patients using MLPA and direct sequencing<sup>26</sup>. Unfortunately, 1 patient (case 12) who underwent gene analysis was not able to be subtyped in the present study.

It is crucial to identify patients with FA in the management of BMF patients. Patients with BMF who happen to have underlying undiagnosed FA will not respond to the immunosuppressive therapy that is used to treat patients with idiopathic AA. Moreover, patients with FA will often die of toxicity due to hypersensitivity to chemoradiotherapy when given conventional conditioning for SCT, and therefore reduced intensity conditioning regimens should be used for FA patients<sup>8</sup>. Also, human leukocyte antigen (HLA)-matched sibling donors should also be screened for FA as the recurrence rate for FA in a sibling is 25% for autosomal recessive disease.

In FA patients, hematological complications are the most life-threatening event. According to the IFAR report, the risk of developing BMF and hematological malignancies by 40 years is 90% and 33%, respectively<sup>5</sup>. Hematopoietic SCT remains the only treatment to correct the hematologic manifestations in FA patients. Because of high sensitivity to radiation and conditioning agents (alkylating agent), alternative conditioning regimens using lower dose Cy, using Flu and avoiding irradiation have shown hopeful results so far<sup>27</sup>.

Recently, a promising outcome using Flu (120–180 mg/m<sup>2</sup>), low-dose Cy (40 mg/kg) and ATG based regimens was reported in 8 FA patients. Donors were either related (n=4), or unrelated (n=4). All patients achieved hematopoietic recovery and were alive at median follow up of 72 months (range, 4 to 117 months)<sup>28</sup>. In the United Kingdom, a similar nonradiation conditioning regimen consisting of Flu 125–150 mg/m<sup>2</sup>, Cy 20–30 mg/kg and ATG was used for 7 FA patients. They were transplanted from unrelated umbilical cord blood (UCB) (n=4), HLA matched unrelated (n=2) and haploidentical (n=1) peripheral blood grafts. All patients were alive at median follow-up of 37 months (range, 13 to 54 months), although 2 rejected their grafts initially, but were rescued by a second transplant<sup>7</sup>. In the present study, however, the survival was not different according to the conditioning method possibly secondary to the small number of cases.

SCT from HLA-matched sibling donor generally gives the best outcome, if performed early prior to the development of MDS or leukemia<sup>27</sup>. The data from the International Bone Marrow Transplant Registry showed that, among 209 patients transplanted from matched siblings between 1994 and 1999, the 3-year survival was 81% in patients <10 years of age (n=109) and 69% in older patients (n=100)<sup>29</sup>.

However, the majority of FA patients do not have a HLA matched sibling donor. Until recently, SCT from an alternative donor (i.e., unrelated or HLA mismatched related) has been markedly less successful due to the high rate of graft failure, regimen related toxicity, GVHD and opportunistic infections<sup>27</sup>. In the present study, among 2 transplanted from partially matched related donors case 1 died of grade IV acute GVHD and sepsis.

Since the first successful transplant in 1989 for a FA patient<sup>30</sup>,



UCB has been increasingly used as a donor source. Although the engraftment after UCB transplant (UCBT) was slower, engraftment and survival have been comparable to bone marrow transplant and the incidence of GVHD was reduced<sup>4)</sup>. In the present study, 2 patients received unrelated UCBT, but both of them rejected initial grafts. Thus, caution should be taken to perform UCBT, especially if the cell number of the UCB unit is marginal or if the patient had received lots of transfusions (case 9).

There are no nation-wide data on FA incidence in Korea, but a recent retrospective study from 9 centers of Korea reported preliminary data on 41 FA patients who underwent SCT between 1996 and 2009<sup>31)</sup>. Thirty patients (73.2%) were alive with a median of 35 months after transplantation. Flu-based regimens were used in 28 patients (68.3%), and radiation in 21 patients (51.2%). Event-free survival differed by donor types with the best outcome of HLA-matched unrelated donor (79%, n=20), followed by matched sibling donor (67%, n=7), haploidentical parental donor (50%, n=2), and UCB (25%, n=12). The incidence of acute grades II-IV and chronic GVHD was 39% (n=16) and 19% (n=7), respectively.

Many patients who develop BMF initially respond to supportive care such as blood transfusions, androgens, and cytokines (e.g., G-CSF and GM-CSF)<sup>4)</sup>. Synthetic androgens, such as oxymetholon and danazol, are often considered to be effective for treating BMF in FA patients, although long-term androgen use has side effects that include masculinization, acne, hyperactivity and increased liver tumor incidence<sup>32)</sup>. In this study, a female patient (case 3) treated with androgen suffered from masculinization and one (case 10) developed peliosis hepatis. Three out of 8 patients treated with androgen showed partial responses. However, all 4 patients who received androgen therapy alone because of the lack of a suitable donor or before the availability of SCT at the institution eventually died. Among 4 patients who finally underwent a SCT, 3 are alive with a median follow up of 173.5 months.

Growth and endocrine abnormalities are clinically important aspects of FA as well. They are often either secondary to hormonal deficiencies, including pituitary hypofunction with hypogonadism, growth hormone deficiency, hypothyroidism and abnormal glucose or insulin metabolism, or treatment-related, such as hemochromatosis with repeated transfusions, or transplant-related complications<sup>3)</sup>. A thorough baseline and annual endocrine evaluation should be performed in every FA patient to reduce morbidity and improve quality of life.

Despite the small sample sizes and without complementation grouping, the current study provides information on clinical manifestation and long-term outcome of Korean FA patients from a single institution. A nation-wide screening and registry for FA along with genetic subtyping should be initiated (1) to understand the possible ethnic differences in the frequency of

subtypes, (2) to unravel genotype-phenotype correlation, (3) to further refine techniques of HSCT, (4) to develop potential future therapies, such as improved gene therapy, or antioxidant compounds, and (5) to establish guidelines for long-term follow-up.

## Conflict of interest

No potential conflict of interest relevant to this article was reported.

## References

1. Joenje H, Patel KJ. The emerging genetic and molecular basis of Fanconi anaemia. *Nat Rev Genet* 2001;2:446-57.
2. Fanconi G. Familiare infantile perniziosaartige Anämie (pernizioses Blutbild und Konstitution). *Jahrb Kinderh* 1927;117:257-80.
3. Auerbach AD. Fanconi anemia and its diagnosis. *Mutat Res* 2009; 668:4-10.
4. Kook H. Fanconi anemia: current management. *Hematology* 2005; 10 Suppl 1:108-10.
5. Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, et al. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood* 2003;101:1249-56.
6. Locasciulli A, Oneto R, Bacigalupo A, Socie G, Korthof E, Bekassy A, et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica* 2007; 92:11-8.
7. Motwani J, Lawson SE, Darbyshire PJ. Successful HSCT using non-radiotherapy-based conditioning regimens and alternative donors in patients with Fanconi anaemia: experience in a single UK centre. *Bone Marrow Transplant* 2005;36:405-10.
8. Gluckman E, Wagner JE. Hematopoietic stem cell transplantation in childhood inherited bone marrow failure syndrome. *Bone Marrow Transplant* 2008;41:127-32.
9. Cho SH, Kook H, Kim GM, Yoon WS, Cho TH, Hwang TJ. A clinical study of Fanconi's anemia. *Korean J Pediatr Hematol Oncol* 1997; 4:70-7.
10. Kook H, Cho D, Cho SH, Hong WP, Kim CJ, Park JY, et al. Fanconi anemia screening by diepoxybutane and mitomicin C tests in Korean children with bone marrow failure syndromes. *J Korean Med Sci* 1998;13:623-8.
11. Auerbach AD, Rogatko A, Schroeder-Kurth TM. International Fanconi Anemia Registry: relation of clinical symptoms to diepoxybutane sensitivity. *Blood* 1989;73:391-6.
12. Korea Centers for Disease Control and Prevention, Division of Chronic Disease Surveillance, Committee for the Development of Growth Standard for Korean Children and Adolescents; Korean Pediatric Society, Committee for School Health and Public Health Statistics. 2007 Korean children and adolescents growth standard (commentary for the development of 2007 growth chart). Cheongwon: Korea Centers for Disease Control and Prevention, Division of Chronic Disease Surveillance, 2007.
13. Yoshida N, Yagasaki H, Hama A, Takahashi Y, Kosaka Y, Kobayashi R, et al. Predicting response to immunosuppressive therapy in

- childhood aplastic anemia. *Haematologica* 2011;96:771-4.
14. Marsh J, Schrezenmeier H, Marin P, Ilhan O, Ljungman P, McCann S, et al. Prospective randomized multicenter study comparing cyclosporin alone versus the combination of antithymocyte globulin and cyclosporin for treatment of patients with nonsevere aplastic anemia: a report from the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anaemia Working Party. *Blood* 1999;93:2191-5.
  15. Cho TH, Kook H, Ryu NE, Kim CJ, Lee JH, Hwang TJ. Infant with Fanconi anemia presenting with myelodysplastic syndrome. *Korean J Pediatr Hematol Oncol* 1998;5:322-7.
  16. Camitta BM, Thomas ED, Nathan DG, Santos G, Gordon-Smith EC, Gale RP, et al. Severe aplastic anemia: a prospective study of the effect of early marrow transplantation on acute mortality. *Blood* 1976;48:63-70.
  17. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. *Blood Rev* 2010;24:101-22.
  18. 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;84:1650-5.
  19. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. *Blood* 2003;101:822-6.
  20. Cioc AM, Wagner JE, MacMillan ML, DeFor T, Hirsch B. Diagnosis of myelodysplastic syndrome among a cohort of 119 patients with Fanconi anemia: morphologic and cytogenetic characteristics. *Am J Clin Pathol* 2010;133:92-100.
  21. Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG, et al. High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. *Arch Otolaryngol Head Neck Surg* 2003;129:106-12.
  22. Lo Ten Foe JR, Kwee ML, Rooimans MA, Oostra AB, Veerman AJ, van Weel M, et al. Somatic mosaicism in Fanconi anemia: molecular basis and clinical significance. *Eur J Hum Genet* 1997;5:137-48.
  23. Pinto FO, Leblanc T, Chamoussat D, Le Roux G, Brethon B, Cassinat B, et al. Diagnosis of Fanconi anemia in patients with bone marrow failure. *Haematologica* 2009;94:487-95.
  24. Strathdee CA, Duncan AM, Buchwald M. Evidence for at least four Fanconi anaemia genes including FACC on chromosome 9. *Nat Genet* 1992;1:196-8.
  25. Yagasaki H, Hamanoue S, Oda T, Nakahata T, Asano S, Yamashita T. Identification and characterization of novel mutations of the major Fanconi anemia gene FANCA in the Japanese population. *Hum Mutat* 2004;24:481-90.
  26. Park J, Chung NG, Chae H, Kim M, Lee S, Kim Y, et al. FANCA and FANCG are the major Fanconi anemia genes in the Korean population. *Clin Genet* 2013;84:271-5.
  27. MacMillan ML, Wagner JE. Haematopoietic cell transplantation for Fanconi anaemia: when and how? *Br J Haematol* 2010;149:14-21.
  28. Shimada A, Takahashi Y, Muramatsu H, Hama A, Ismael O, Narita A, et al. Excellent outcome of allogeneic bone marrow transplantation for Fanconi anemia using fludarabine-based reduced-intensity conditioning regimen. *Int J Hematol* 2012;95:675-9.
  29. IBMTR/ABMTR. Report on state of the art in blood and marrow transplantation: the IBMTR/ABMTR summary slides with guide. IBMTR/ABMTR Newsletter [Internet]. 2002 Feb [cited 2013 Jun 9];9(1). Available from: [http://www.cibmtr.org/ReferenceCenter/Newsletters/Documents/Newsletter\\_Feb2002.pdf](http://www.cibmtr.org/ReferenceCenter/Newsletters/Documents/Newsletter_Feb2002.pdf).
  30. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med* 1989;321:1174-8.
  31. Han YJ, Kang HJ, Shin HY, Chung HJ, Park JE, Lim YT, et al. Results of hematopoietic stem cell transplantation in Fanconi anemia: Retrospective multi-center study in Korea [abstract]. Proceedings of the 59th Annual Fall Meeting of the Korean Pediatric Society; 2009 Oct 23; Seoul, Korea. *Korean Pediatric Society*; 2009. p.252.
  32. Scheckenbach K, Morgan M, Filger-Brillinger J, Sandmann M, Strimling B, Scheurlen W, et al. Treatment of the bone marrow failure in Fanconi anemia patients with danazol. *Blood Cells Mol Dis* 2012; 48:128-31.