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SHORT COMMUNICATION

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Four novel mutations of the Fanconi anemia group A gene (*FAA*) in Japanese patients

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Abstract Fanconi anemia (FA) is an autosomal recessive disorder characterized by pancytopenia, predisposition to cancers, and a diverse variety of congenital malformations. At least eight complementation groups, A through H, have been described. Recently, the FA-A gene (*FAA*) has been isolated, and a large number of distinct mutations reported in ethnically diverse FA-A patients. Here, we report on the mutation analysis of five FA patients by single-strand conformation polymorphism. Out of five patients, at least three were found to have mutations in the *FAA* gene. The first patient was a compound heterozygote with a 1-bp deletion and a single-base substitution. The second patient had a heterozygous 2-bp deletion, which introduces a premature termination codon, and the third patient had a heterozygous splice donor site mutation in intron 27.

Key words Fanconi anemia · *FAA* gene · Mutation · Polymorphism · SSCP · Direct sequencing

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Introduction

Fanconi anemia (FA) is an autosomal recessive disorder characterized by pancytopenia, skin pigmentation, a high incidence of lymphoid and other cancers, and a diverse variety of congenital malformations (Alter BP 1993; Andrea et al. 1997). Cells from FA patients display chromosome instability (Liu et al. 1994), spontaneous delay and arrest in the G_2 cell cycle checkpoint (Dutrillaux et al. 1982), and increased sensitivity to DNA cross-linking agents (Strathdee et al. 1992a). At least eight complementation groups (FA-A to FA-H) have been described (Joenje et al. 1997). Among these groups, FA-A accounted for two-thirds of 47 FA patients from European and US/Canadian populations (Buchwald 1995). The underlying gene for FA-C (*FAC*) has been isolated and mapped to 9q22.3 (Strathdee et al. 1992b,c). The gene responsible for FA-A (*FAA*) was isolated and mapped to 16q24.3 (Fanconi Anemia/Breast Cancer Consortium 1996; Lo Ten Foe et al. 1996). The *FAA* gene showed no significant homology to any known genes; however, recent studies have demonstrated that (1) FAA protein binds to FAC protein (Kupfer et al. 1997a) and (2) FAC protein is involved in cell cycle regulation via cyclin-dependent kinase cdc-2 (Kupfer et al. 1997b; Kruyt et al. 1997). A large number of distinct mutations and polymorphisms have been reported in ethnically diverse FA-A patients (Savino et al. 1997; Levran et al. 1997), suggesting the presence of genetic heterogeneity in FA-A patients. To search for mutations in the *FAA* gene in five Japanese FA patients, we screened the *FAA* cDNAs by single-strand conformation polymorphism (SSCP) analysis. Four novel mutations and two common polymorphisms were identified in three patients.

Materials and methods

Patient cell lines

A total of five FA cell lines were collected for this study: cell lines FA1CH (from Chiba prefecture), FA2CH (Chiba),

and FA3IC (Chiba) are primary fibroblasts, while cell lines KCMC319 (Kanagawa) and SGL2952 (Saitama) are Epstein-Barr-(EB) transformed lymphoblastoid cells. All the patients, aged from 3 to 12 years old, had symptoms consistent with a diagnosis of FA, including pancytopenia, skin pigmentation, short stature, and congenital malformations. All families were unrelated. Hypersensitivity to mitomycin C (MMC) in the lymphoblastoid cell lines was confirmed by the MMC assay as described previously (Joenje et al. 1995). The complementation groups to which the five patients belonged were unknown.

RT–PCR and SSCP analysis

FAA cDNA that included an open reading frame (ORF) was amplified by polymerase chain reaction (PCR) into 10 overlapping segments, and analyzed by SSCP. PCR primers were designed according to the published *FAA* cDNA sequence data (Table 1). In brief, total RNAs were extracted from FA cell lines using a TRIZOL Reagent (Gibco BRL, Gaithersburg, MD, USA). cDNA was synthesized from 10 μg of total RNA with 10 units of Moloney murine leukemia virus reverse transcriptase and 5 ng of random hexamer primers per microliter. One-tenth of the sample was amplified by PCR using primers end-labeled with [γ-^{32}P]ATP (Amersham U.K.). PCR conditions were 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with a final extension time of 5 min at 72°C. The amplified products were digested with a restriction enzyme to give a size of 0.3 kb (Table 1), and were electrophoresed on to a 5% nondenaturing polyacrylamide gel containing 5% glycerol at a constant temperature of 4°C or 15°C for 4 h. Heteroduplex bands were analyzed by autoradiography. The results were confirmed by two independent experiments.

Nucleotide sequencing

The SSCP variants were subjected to direct sequencing using fluorescent-labeled primers, SequiTherm EXCEL II Long Read DNA Sequencing Kits-LC (Epicentre Technologies WI, U.S.A.), and DNA sequencer 4000L (LI-COR NE, U.S.A.).

Results and discussion

To screen for mutations in five Japanese patients, *FAA* cDNA that included the 4365-bp ORF was amplified by PCR into 10 overlapping segments, and SSCP analysis followed by direct sequencing was carried out. Of five patients, three were found to have mutations in the *FAA* gene. Patient KCMC319 had a heterozygous 1-bp deletion and three kinds of heterozygous single-base substitutions. A 1-bp deletion, 2546delC, introduces a premature termination signal at codon 888 (Fig. 1a). A C to T transition at nt position 3163 results in an Arg to Trp substitution at the 1055th residue (Fig. 1b). No amino acid substitutions were observed in 50 normal Japanese individuals, suggesting that this is the missense mutation which could abolish the FAA protein function. The other two kinds of single-base substitutions were probably polymorphisms. A G to T transversion at nt position 1143 does not cause an amino acid substitution. Out of 100 normal chromosomes, 11 were found to have the transversion. A G to A transition at nt position 1501 introduces a Gln to Ser substitution at the 501st residue. We detected the transition in six of 100 normal Japanese chromosomes (Savino et al. 1997; Levran et al. 1997). Taking the results together, we concluded that patient KCMC319 was a compound heterozygote with the deletion 2546delC and the substitution 3163C > T in the *FAA* gene. Patient FA3IC had a heterozygous 2-bp deletion, 978-979delGA. The deletion introduces a premature termination signal at codon 337 (Fig. 1c). Patient FA1CH was found to have a heterozygous 6-bp deletion at nt position 2602–2607, which results in an in-frame two-amino acid deletion, 868–869delFQ. Nucleotide sequencing of intron 27 of the patient revealed that the deletion was derived from a splice acceptor site mutation, IVS27-2A > T (Fig. 1d). No substitutions were detected in 50 Japanese controls, suggesting that the two-amino acid deletion could cause the FA phenotype, although we cannot exclude completely the

Table 1 Primers used to amplify *FAA* cDNA

Names	Forward	Reverse	Amplified product (bp)	Restriction enzyme	SSCP size (bp)
FAA1F-1R (−6/636)[a]	AAGGCCATGTCCGACTCGTGG	TTCACAAAGGCACAGAATTC	641	*Bgl*II	373/268
FAA2F-2R (479/1050)	TGTTCTCCCGTCTTTCCTTCTG	TGAGGTGAGCAGAGGGTGTGTC	571	*Pst*I	329/242
FAA3F-3R (893/1474)	GGTTCGGAGTGTTCAGTGGACA	CCAGGGGTGGGTGGAAGAATGTG	581	*Taq*I	363/218
FAA4F-4R (1404/1923)	GGCCCTGGTCTTCCTGTTTACG	CTCCTCAGCAGAGTTGGGTTCT	519	*Pvu*II	203/316
FAA5F-5R (1792/2327)	GACTCCCGTGTGGCGTTTATAG	CCCAGCACATGTGGGGCACTCA	535	*Pvu*II	169/366
FAA6F-6R (2169/2748)	GACGTGTTTCTGTCAGAACCTG	CCCAGCACATGTGGGGCACTCA	579	*Hin*fI	313/266
FAA7F-7R (2616/3144)	GTTCAGATTGTTCTCAGAGGCC	TTCCTCTTTCAACACCTCTCGG	528	*Hin*fI	280/248
FAA8F-8R (3029/3613)	GCCGCACAGGAAATGAGGATAT	CCGTCTGCGGAAAATCTCAAAG	584	*Bam*HI	210/374
FAA9F-9R (3497/4015)	TTTTGACCTCTGCTCTGGTGTG	AGAGGAAATCGCTGGCAAACTG	518	*Hin*fI	202/316
FAA10F-10R (3897/4472)	GATGTCCTGGCTGGCACTCTTT	TCCTTTCCCCACTAAAGCAGTC	575	*Pvu*II	239/336

SSCP, single-strand conformation polymorphism

[a] The numbers in parentheses indicate locations in the *FAA* cDNA. The A of an ATG initiation codon is represented as nucleotide +1. Primers are presented in the 5′–3′ direction

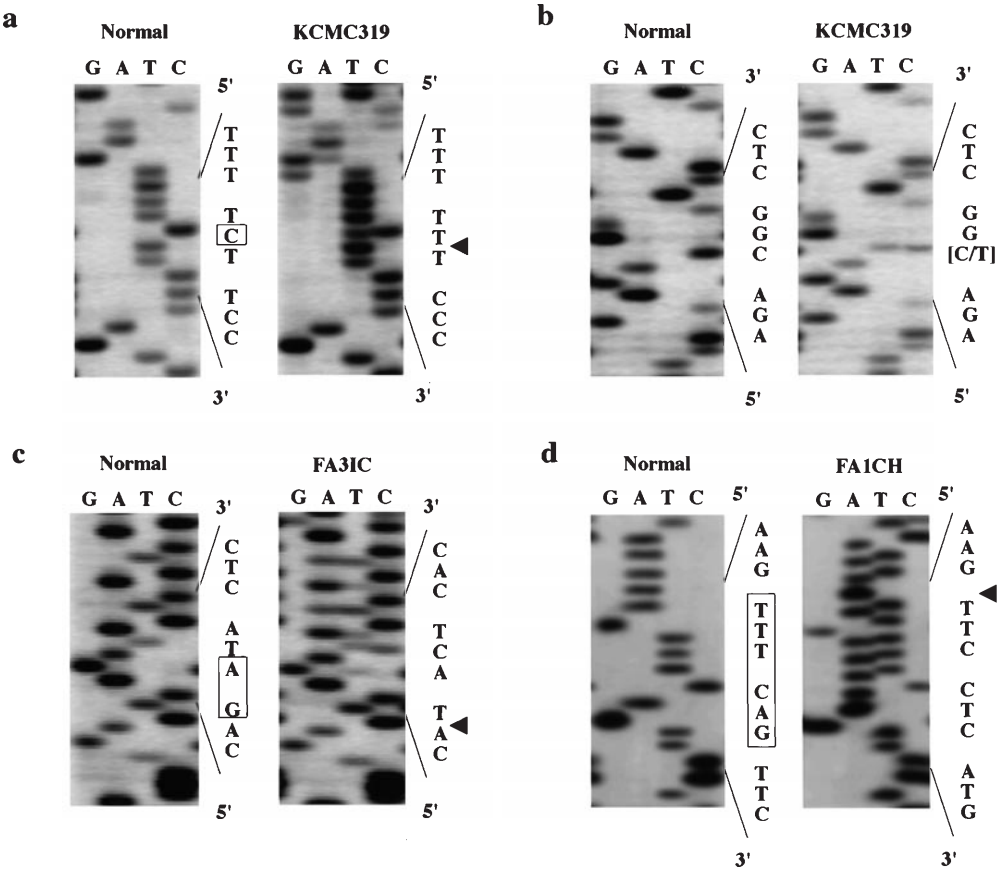

Fig. 1a–d Direct sequence analysis of the *FAA* cDNAs from three Japanese patients. **a** Nucleotide sequence of the region around position 2546 of the *FAA* cDNA from a normal control and patient KCMC319. A heterozygous 1-bp deletion, 2546delC, is shown. **b** Nucleotide sequence of patient KCMC319. A heterozygous single-base substitution, 3163C > T, is indicated. **c** Sequence of the *FAA* cDNA from patient FA3IC. A heterozygous 2-bp deletion, 978-979delGA, is shown. **d** Nucleotide sequence of patient FA1CH. A heterozygous 6-bp deletion, which is derived from a splice acceptor site mutation, is shown

possibility that it may represent a rare polymorphism. Functional analysis of the truncated FAA protein will resolve this problem. We could not detect any SSCP variants in other alleles of the patients (FA3IC and FA1CH), possibly due to the limitations of the technical procedure. Further studies including direct sequencing of the genomic DNAs will be needed.

Three patients were found to have distinct mutations, suggesting the presence of genetic heterogeneity in Japanese FA-A patients. When we searched for mutations in the *FAC* cDNA of five Japanese patients; no SSCP variants were detected (data not shown). These results suggested that the relative prevalence of FA-A might also be high among Japanese FA patients. Continued screening for the *FAA* gene may provide a better understanding of the molecular basis of FA-A patients.

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References

Alter BP (1993) Fanconi's anemia and its variability. Br J Haematol 85: 9–14

Andrea ADD, Grompe M (1997) Molecular biology of Fanconi anemia: implications for Diagnosis and therapy. Blood 90: 1725–1736

Buchwald M (1995) Complementation groups: one or more per gene? Nat Genet 11: 228–230

Dutrillaux B, Aurias A, Dutrillaux AM, Briot D, Prieur M (1982) The cell cycle of lymphocytes in Fanconi anemia. Hum Genet 62: 327–332

Fanconi Anemia/Breast Cancer Consortium (1996) Positional cloning of the Fanconi anemia group A gene. Nat Genet 14: 324–328

Joenje H, Lo Ten Foe JR, Oostra AB, Van Berkel GM, Rooimans MA, Schroeder-Kurth T, Wegner RD, Gille JJP, Buchwald M, Arwert F (1995) Classification of Fanconi anemia patients by complementation analysis: evidence for a fifth genetic subtype. Blood 86: 2156–2160

Joenje H, Oostra AB, Wijker M, Di Summa FM, Van Berkel CGM, Rooimans MA, Ebell W, Van Weel M, Pronk JC, Buchwald M, Arwert F (1997) Evidence for at least eight Fanconi anemia genes. Am J Hum Genet 61: 940–944

Kruyt FAE, Dijkmans LM, Arwert F, Joenje H (1997) Involvement of the Fanconi anemia protein FAC in a pathway that signals to the cyclin B/cdc2 kinase. Cancer Res 57: 2244–2251

Kupfer GM, Naf D, Suliman A, Pulsipher M, D'Andrea AD (1997a) The Fanconi anemia proteins, FAA and FAC, interact to form a nuclear complex. Nat Genet 17: 487–490

Kupfer GM, Yamashita T, Naf D, Suliman A, asano S, D'Andrea AD (1997b) The Fanconi anemia polypeptide, FAC, binds to the cyclin-dependent kinase, cdc2. Blood 90: 1047–1054

Levran O, Erlich T, Magdalena N, Gregory JJ, Batish SD, Verlander PC, Auuuerbach AD (1997) Sequence variation in the Fanconi anemia gene *FAA*. Proc Natl Acad Sci USA 94: 13051–13056

Liu JM, Buchwald M, Walsh CE, Young NS (1994) Fanconi anemia and novel strategies for therapy. Blood 84: 3995–4007

Lo Ten Foe JR, Rooimans MA, Bosnoyan-Collins L, Alon N, Wijker M, Parker L, Lightfoot J, Carreau M, Callen DF, Savoia A, Cheng NC, Van Berkel CGM, Strunk MHP, Gille JJP, Pals G, Kruyt FAE, Pronk JC, Arwert F, Buchwald M, Joenje H (1996) Expression

cloning of a cDNA for the major Fanconi anemia gene, FAA. Nat Genet 14: 320–323

Savino M, Ianzano L, Strippoli P, Ramenghi U, Arslanian A, Bagnara GP, Joenje H, Zelante L, Savoia A (1997) Mutation of the Fanconi anemia group A gene (*FAA*) in Italian patients. Am J Hum Genet 61: 1246–1253

Savoia A, Zatterale A, Principe DD, Joenje H (1996) Fanconi anemia in Italy: high prevalence of complementation group A in two geographic clusters. Hum Genet 97: 599–603

Strathdee CA, Buchwald M (1992a) Molecular and cellular biology of Fanconi anemia. Am J Ped Hematol Oncol 14: 177–185

Strathdee CA, Gavish H, Shannon WR, Buchwald M (1992b) Cloning of cDNAs for Fanconi's anemia by functional complementation. Nature 356: 763–767

Strathdee CA, Duncan AMU, Buchwald M (1992c) Evidence for at least four Fanconi anemia genes including FACC on chromosome 9. Nat Genet 1: 196–198