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Report

Isolation of a cDNA Representing the Fanconi Anemia Complementation Group E Gene

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Fanconi anemia (FA) is an autosomal recessive chromosomal instability syndrome with at least seven different complementation groups. Four FA genes (*FANCA, FANCC, FANCF,* and *FANCG*) have been identified, and two other FA genes (*FANCD* and *FANCE*) have been mapped. Here we report the identification, by complementation cloning, of the gene mutated in FA complementation group E (*FANCE*). *FANCE* has 10 exons and encodes a novel 536–amino acid protein with two potential nuclear localization signals.

Fanconi anemia (FA) is characterized by bone marrow failure, developmental abnormalities, cancer predisposition, and cellular hypersensitivity to DNA cross-linking agents such as mitomycin C (Auerbach et al. 1998 [MIM 227650]). Complementation analysis has indicated that mutations in at least seven different genes can cause FA (Joenje et al. 1997, 2000). Four FA genes have been identified: *FANCA* (Fanconi Anemia/Breast Cancer Consortium 1996; Lo ten Foe et al. 1996 [MIM 227650]), *FANCC* (Strathdee et al. 1992 [MIM 227645]), *FANCF* (de Winter et al. 2000 [MIM 603467]) and *FANCG* (de Winter et al. 1998 [MIM 602956]). Intriguingly, none of these genes has revealed any decisive clue toward a molecular function of the FA pathway, since they encode novel proteins that lack significant functional domains. The recently described homology between FANCF and the RNA-binding protein ROM (de Winter et al. 2000) appeared to be nonsignificant, because mutations in the FANCF region homologous to ROM did not affect the function of FANCF (J. P. de Winter, unpublished data). Two other FA genes, *FANCD* and *FANCE,* have been mapped to chromosomes 3p25.3 (Whitney et al. 1995; Hejna et al. 2000 [MIM 227646]) and 6p21-22 (Waisfisz et al. 1999 [MIM 600901]), respectively.

Here we report the cloning of a cDNA representing *FANCE,* by complementation of the FA-E lymphoblastoid cell line EUFA410 (Waisfisz et al. 1999) with an episomal expression library (Strathdee et al. 1992). After selection for library uptake in hygromycin-containing medium (100 μg/ml) and subsequent selection for resistance to mitomycin C (15 nM), 4 of the 12 cDNA clones that we recovered from the pool of complemented cells had identical inserts of ~2.5 kb. Secondary transfection of one of these cDNA clones (clone 10 [GenBank accession number AF265210]) into EUFA410 cells again complemented their MMC-hypersensitive phenotype (fig. 1*a*). The cDNA insert has a 1,611-nucleotide open reading frame encoding a 536–amino acid protein (fig. 1*b*). The predicted FANCE protein contains two potential nuclear localization signals but, like the other FA proteins, lacks any significant homology to other proteins.

The Stanford high-resolution TNG3 radiation-hybrid

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panel was used to position *FANCE* between microsatellite markers *D6S439* and *D6S1645*, in agreement with the genetic map location of *FANCE* (Waisfisz et al. 1999). The *FANCE* cDNA appeared identical to a human genomic DNA sequence (clone 109F14 [Genbank accession number AL022721]; Tripodis et al. 2000) on chromosome 6p21.2-21.3. A comparison between this genomic DNA sequence and the *FANCE* cDNA revealed that the *FANCE* gene has 10 exons spanning ~15 kb of genomic sequence. *FANCE* appears to be located between the genes encoding the 60S ribosomal protein RPL10A (Csa-19) and a ZNF127-like protein, a region in which cDNA selection, exon trapping, and exon prediction programs failed to detect a gene (Tripodis et al. 2000).

Mutation screening of the *FANCE* gene revealed a homozygous C→T transition in exon 2 of EUFA410, which changes codon 141 into a stop codon (R141X; table 1). The parents were heterozygous for this mutation. In the FA-E reference cell line EUFA130 (Joenje et al. 1997), a homozygous C→T nonsense mutation was found in codon 119 (Q119X; table 1). The parents and unaffected brother were heterozygous for this mutation. A homozygous mutation IVS5-8G→A was detected in genomic DNA from FA-E cell line EUFA622 (Waisfisz et al. 1999), which creates an alternative splice-acceptor site (table 1). Sequence analysis of cDNA derived from EUFA622 indicated that this mutation results in false splicing and incorporation of six nucleotides from intron 5, including an in-frame stop codon. These findings confirmed the identity of the *FANCE* gene.

Our data show that *FANCE* encodes a novel protein with two nuclear localization signals, which strongly suggests that the pathway defective in FA patients has a nuclear function. Although recent evidence indicates that the FA pathway might be involved in cellular detoxification (Kruyt et al. 1998), transcriptional repression (Hoatlin et al. 1999), or STAT1 activation (Pang et al. 2000), the precise nature of this pathway remains to be elucidated.

Given that *FANCE* is localized in a region containing

Table 1

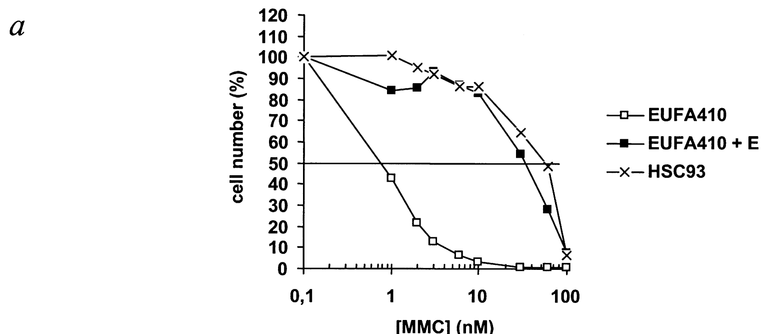
Mutations in Three FA-E Patients

Patient	Ancestry	Mutation	Consequence for Protein
EUFA121	Turkey	355 C→T	Q119X
EUFA410	Bangladesh	421 C→T	R141X
EUFA622	Turkey	IVS5-8G→A[a]	R371_I372insLX

NOTE. Nucleotide numbering starts at the translation initiation site. All these patients are homozygous for the indicated mutations.

[a] Mutation IVS5-8G→A (underlined) ttagctgtag ATC CTC creates an alternative splice-acceptor site that results in false splicing and insertion of six nucleotides from intron 5 (underlined), including an in-frame stop codon GGA CGG ctg tag ATC CTC.

a



b

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1   MATPDAGLPGAEGVEPAPWAQLEAPARLLLQALQAGPEGARRGLGVLRALGSRGWEPFDW  60
61  GRLLEALCREEPVVQGPDGRLELKPLLLRLPRICQRNLMSLLMAVRPSLPESGLLSVLQI 120
121 AQQDLAPDPDAWLRALGELLRRDLGVGTSMEGASPLSERCQRQLQSLCRGLGLGGRRLKS 180
181 PQAPDPEEEENRDSQQPGKRRKDSEEEAASPEGKRVPKRLRCWEEEEDHEKERPEHKSLE 240
241 SLADGGSASPIKDQPVMAVKTGEDGSNLDDAKGLAESLELPKAIQDQLPRLQQLLKTLEE 300
301 GLEGLEDAPPVELQLLHECSPSQMDLLCAQLQLPQLSDLGLLRLCTWLLALSPDLSLSNA 360
361 TVLTRSLFLGRILSLTSSASRLLTTALTSFCAKYTYPVCSALLDPVLQAPGTGPAQTELL 420
421 CCLVKMESLEPDAQVLMLGQILELPWKEETFLVLQSLLERQVEMTPEKFSVLMEKLCKKG 480
481 LAATTSMAYAKLMLTVMTKYQANITETQRLGLAMALEPNTTFLRKSLKAALKHLGP     536
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Figure 1 Complementation of MMC hypersensitivity in FA-E lymphoblastoid cell line EUFA410, and FANCE protein sequence. *a,* MMC hypersensitivity of FA-E cell line EUFA410 is corrected after transfection of FANCE cDNA clone 10. HSC93, wild type control. *b,* Amino acid sequence of the FANCE protein. Nuclear localization signals as predicted by PSORT II (Nakai and Horton, 1999) are shown in bold and underlined.

the HLA class I genes of the major histocompatibility complex (Waisfisz et al. 1999; Tripodis et al. 2000), group E patients are very unlikely to have an HLA-matched unaffected sibling donor for successful bone marrow transplantation. The cloning of *FANCE* now makes this group of patients prime candidates for gene-therapy trials aiming at genetic correction of their bone marrow failure (Liu et al. 1999).

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html (for human *FANCE* cDNA sequence [accession number AF265210] and genomic DNA clone 109F14 [accession number AL022721])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *FANCA* [MIM 227650], *FANCC* [MIM 227645], *FANCD* [MIM 227646], *FANCE* [MIM 600901], *FANCF* [MIM 603467], and *FANCG* [MIM 602956])

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