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# The Fanconi Anemia Group E Gene, *FANCE,* Maps to Chromosome 6p

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## Summary

**Fanconi anemia (FA) is a genetically heterogeneous autosomal recessive disease with bone marrow failure and predisposition to cancer as major features, often accompanied by developmental anomalies. The cells of patients with FA are hypersensitive to DNA cross-linking agents in terms of cell survival and chromosomal breakage. Of the eight complementation groups (FA-A to FA-H) distinguished thus far by cell fusion studies, the genes for three—*FANCA, FANCC,* and *FANCG*—have been identified, and the *FANCD* gene has been localized to chromosome 3p22-26. We report here the use of homozygosity mapping and genetic linkage analysis to map a fifth distinct genetic locus for FA. DNA from three families was assigned to group FA-E by cell fusion and complementation analysis and was then used to localize the *FANCE* gene to chromosome 6p21-22 in an 18.2-cM region flanked by markers *D6S422* and *D6S1610*. This study shows that data from even a small number of families can be successfully used to map a gene for a genetically heterogeneous disorder.**

## Introduction

The autosomal recessive disorder Fanconi anemia (FA; MIM 227650) is clinically characterized by progressive bone marrow failure, sometimes associated with skeletal abnormalities and an increased cancer risk. The spontaneous chromosomal breakage seen in patient cells, and exacerbated by exposure to DNA cross-linking agents, suggests that defective DNA repair is a major consequence of the gene mutation. However, the unusual sensitivity of FA cells to oxygen and their cell-cycle anomalies have led to other hypotheses on the basic defect underlying FA (see Auerbach et al. 1998 for a current review of FA).

Genetically heterogeneous disorders present a challenge to gene localization by linkage analysis, unless patients can be classified into discrete genetic groups on the basis of clinical, biochemical, or cellular phenotype. Patients with FA present with a variable clinical picture, but the hypersensitivity of their cells to DNA cross-linkers has allowed their assignment to eight complementation groups after cell fusion and analysis of cross-linker sensitivity in cell hybrids (Joenje et al. 1997). Localization of the major FA gene, *FANCA,* was facilitated by linkage analysis in families assigned to the FA-A complementation group (Pronk et al. 1995), and the *FANCG* gene was similarly localized in (partially) assigned FA families (Saar et al. 1998). The cloning of the *FANCC* gene (Strathdee et al. 1992*a*) allowed its localization to chromosome 9q by use of FISH (Strathdee et al. 1992*b*), and the *FANCD* gene has been localized to chromosome 3p by use of microcell-mediated chromosome transfer (Whitney et al. 1995). The chromosomal localization of FA genes has provided starting points for molecular cloning of, or has allowed verification of, complementing cDNAs for *FANCA* (The Fanconi Anaemia/Breast Cancer Consortium 1996; Lo Ten Foe et al. 1996) and *FANCG* (de Winter et al. 1998) and is an important goal for the remaining FA complementation groups.

We assigned families to FA complementation group E by use of cell fusion and measurement of the sensitivity of the hybrids to Mitomycin C, a DNA cross-linking agent (Sigma). DNA from three assigned families was then analyzed by use of microsatellite markers for link-

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age to *FANCE*. By use of homozygosity mapping in two families and standard linkage analysis in the third, we identified a chromosomal region on chromosome 6p as the location of the *FANCE* gene.

## Subjects and Methods

### *Subjects*

Families with FA were recruited as part of the European Concerted Action on Fanconi Anemia Research (EUFAR). DNA samples from three families were used for the linkage analyses presented here (pedigrees are shown in fig. 2). Family 1 is of Turkish origin, and the parents are first cousins. Their affected son, patient EUFA130, was the first FA-E patient identified (Joenje et al. 1995), and his lymphoblastoid cell line is the reference for the FA-E group (MIM 600901). Family 2 is from Bangladesh and is not consanguineous. Material from an affected aborted fetus was available for analysis. Family 3 (patient EUFA622) is of Turkish origin and the parents are second cousins. All patients were given their diagnosis on the basis of their chromosomal hypersensitivity to DNA cross-linking agents. Lymphoblastoid B cell lines were established from the blood of all family members by Epstein-Barr virus transformation. Clinical features of these patients have been documented (patient EUFA130, Wegner et al. 1996; patients EUFA409 and EUFA410, Evans et al. 1994; and patient EUFA622, A. Koc, M. Alikasifoglu, H. Joenje, and C. Altay, unpublished data).

### *Cell Fusion and Complementation of Cross-Linker Sensitivity*

After the first patient with FA-E was identified (patient EUFA130; Joenje et al. 1995), the patient's cell line was genetically marked to allow its use as a universal fusion donor. Genetic marking was with G418 resistance and hypoxanthine-guanine phosphoribosyl transferase (HPRT) deficiency, as described earlier for the cell lines of groups FA-B to FA-D (Joenje et al. 1995). The genetic markers allowed hybrids obtained with any unmarked cell line to be selected by use of culturing in the presence of medium containing hypoxanthine, aminopterin, and thymidine in combination with the drug G418, as described elsewhere (Joenje et al. 1995). Cell fusion, selection of hybrids, and assessment of complementation were all performed as described elsewhere (Joenje et al. 1997). To exclude inadvertent mix-ups of cell lines during cell culturing, we authenticated hybrids and transfected cell lines by comparing the alleles we found by use of VNTR minisatellite markers with those present in the original cell lines.

To assess whether chromosome loss was a possible cause of lack of complementation in noncomplemented hybrids, we checked ploidy of the selected cell populations by counting chromosomes in 20 randomly selected metaphases. No evidence for any significant chromosome loss was obtained, since chromosome numbers were in the range of 72-96 per cell (Joenje et al. 1997).

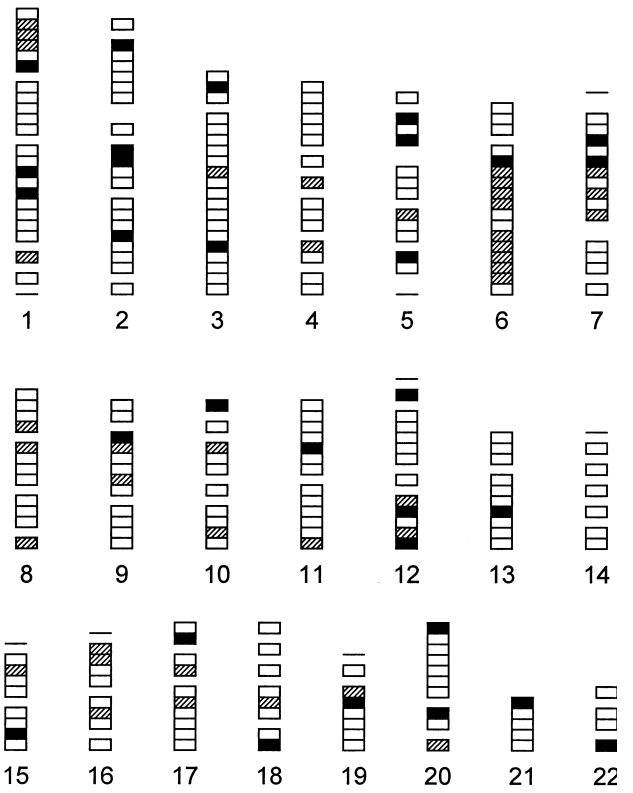


**Figure 1** Homozygosity mapping of microsatellite markers spaced at 11-cM intervals in FA family 1. Each box represents a microsatellite marker located on the 22 autosomes; 274 markers were analyzed. Solid boxes indicate markers that were informative and homozygous in the affected child EUFA130 only; hatched boxes indicate markers that were homozygous in EUFA130 but not fully informative; open boxes indicate markers that were heterozygous in EUFA130; and missing boxes indicate further markers of the MDC-Généthon panel that were not analyzed.

### *Genotyping*

DNA was isolated from lymphoblastoid cell lines by use of standard techniques and analyzed by use of microsatellite markers from the MDC-Généthon mapping panel, with an average spacing of 11 cM, or from the Weber 6B Screening Set, with an average spacing of 10.9 cM. Markers were amplified by PCR by use of fluorescently labeled primers and analyzed on the Pharmacia ALF or ABI 310 sequencers. Length was determined by use of an internal standard and Genescan 1.2 and Genotyper software (Applied Biosystems). The two initial analyses used data from (1) all members of family 1 or (2) patients EUFA130 and EUFA622 together with the

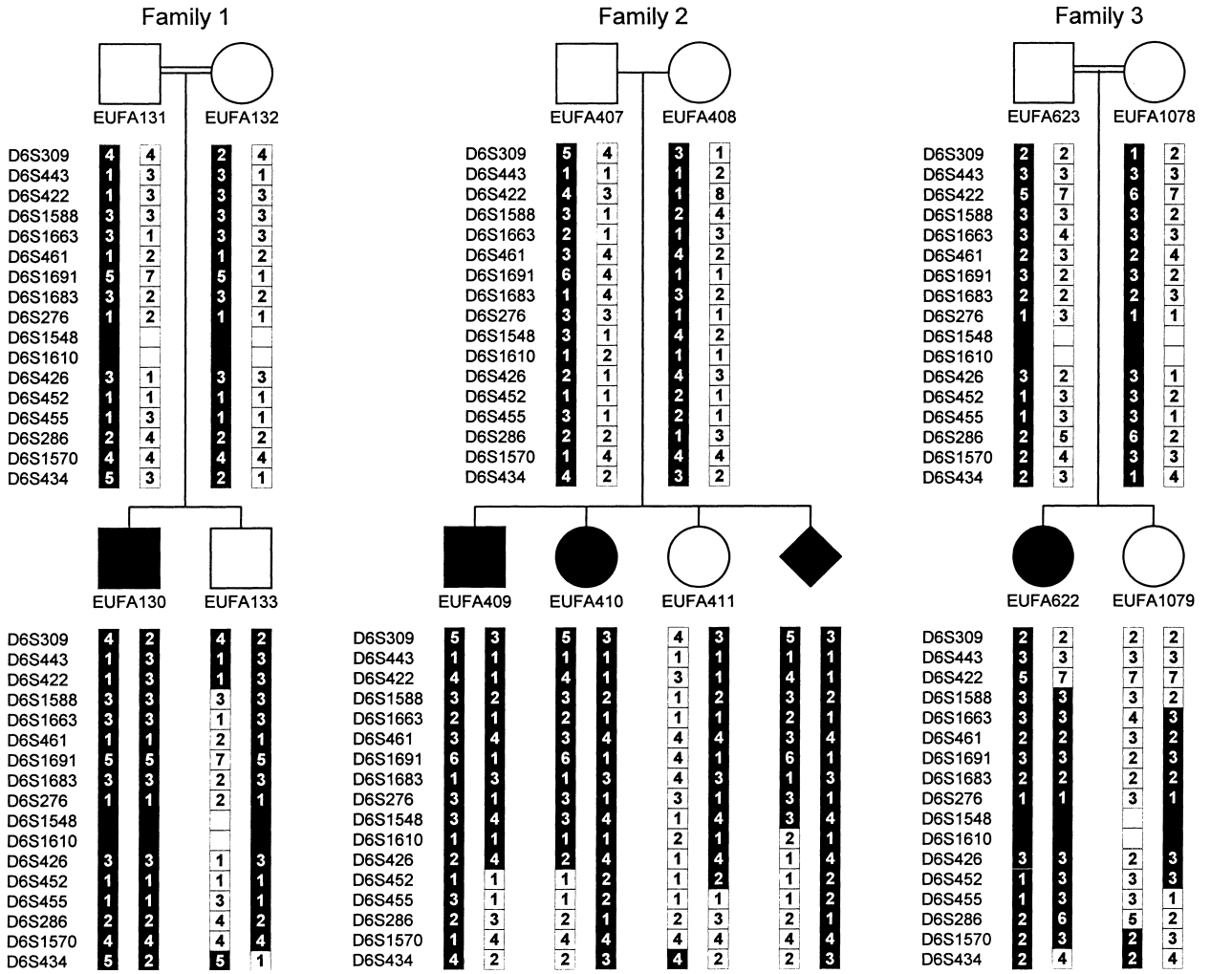


**Figure 2** Most likely haplotypes for microsatellite markers on chromosome 6p. Family 1, with complementation group E reference patient EUFA130, is consanguineous; the parents are first cousins. Family 2 was assigned in this report to complementation group E. Family 3 is consanguineous and was assigned in this report to complementation group E; the parents are second cousins. Symbols for affected individuals are solid.

affected children from family 2, patients EUFA409 and EUFA410. Data from the entire families were then analyzed for candidate *FANCE* regions.

### *Linkage Analysis*

We performed two-point LOD score calculation by use of version 5.1 of LINKAGE software, assuming a fully penetrant autosomal recessive mode of inheritance, a gene frequency of 0.0001, and uniform allele frequencies. Multipoint LOD scores based on the experimentally proven genetic homogeneity were calculated with GENEHUNTER software, version 1.3, again with an assumption of uniform allele frequencies. Recombination frequencies between markers were taken from Dib et al. (1996) and the Cooperative Human Linkage Centre.

## Results

### *Cell Fusion and Assignment to FA Complementation Group E*

Lymphoblastoid cell lines from patients EUFA410 and EUFA622 were fused with reference cell lines representing groups FA-A to FA-E. As summarized in table 1, findings showed that all fusion hybrids were complemented for MMC sensitivity except the hybrids obtained with the reference group E cell line EUFA130NT-L. These results were taken as evidence that the two patients belonged to complementation group E.

### *Candidate* FANCE *Regions*

As shown in figure 1, microsatellite mapping in family 1 excluded 70% of the genome for *FANCE* and iden-

**Table 1**

**Complementation Analysis of Cells from Patients EUFA410 and EUFA622**

| | Cell Line | |
|---|---|---|
| Type of Cell | EUFA410-L | EUFA622-L |
| Unfused cells | 2.1 ±.5 | 2.1 ±1.1 |
| Hybrids after fusion with reference cell line: | | |
| HSC72OT (A) | 92.5 ± 9.6 | 68.3 ± 12.6 |
| HSC230NT (B) | 100 ± 0 | 68.3 ± 10.4 |
| HSC536NT (C) | 57.5 ± 9.6 | 43.3 ± 7.6 |
| HSC62NT (D) | 55 ± 21.2 | 38.5 ± 7.6 |
| EUFA130NT (E) | 2.5 ± .7 | 4.5 ± 1.3 |

Note.—Results are $IC_{50}$ values of the two cell lines and hybrids, given as mean ± SE Mitomycin C concentrations in nmol/l, obtained after fusion with the indicated reference cell lines. Complementation group is indicated in parentheses.

tified several candidate regions, particularly on chromosomes 1, 6, 7, 12, and 19. The second genome scan, using data from the two children of consanguineous parents (patients EUFA130 and EUFA622) and data from the two affected children (patients EUFA409 and EUFA410) in family 2, excluded a further 12% of the genome and left only the candidate regions on chromosomes 6p and 19q, of 44 and 23 cM, respectively. Examination of additional microsatellite markers on chromosome 19 for data from all family members in all three FA-E families gave a maximum LOD score of $Z_{max} = 1.20$ at $\theta = 0$ for marker *D19S226* in families 1 and 3; however, family 2 was not linked at that marker and showed negative LOD scores for all values of $\theta$ between 0 and 0.4. Thus, the candidate region on chromosome 19 could be excluded.

### *Fine Mapping of Chromosome 6p*

Figure 2 shows the most likely haplotypes for 15 markers on chromosome 6p in the three FA-E families. In family 1, patient EUFA130 is homozygous for a large region of 73.5 cM from *D6S422* to *D6S434*. As expected, the homozygous region is smaller in patient EUFA622 in consanguineous family 3, at 36.5 cM between *D6S422* and *D6S452*. Family 3 defines *D6S422* as the most distal flanking marker. The two affected children and the affected aborted fetus in family 2 have identical haplotypes from *D6S309* to *D6S1548*, and the unaffected child has inherited only the maternal haplotype here. The recombination in the paternal chromosome of the aborted fetus between *D6S1548* and *D6S1610* makes the latter marker the current proximal flanking marker. Thus, on the basis of the genetic distances between individual markers in this region (Dib et al. 1996), the current critical region spans 18.2 cM. The statistical significance of this result is illustrated in figure 3, which shows a plot of the multipoint LOD scores along chromosome 6p, with $Z_{max} = 4.55$ for *D6S1691*.

## Discussion

The extreme degree of genetic heterogeneity in FA requires that families be assigned to a genetic complementation group before linkage analysis is possible. This results in some complementation groups with relatively few families, and localization of the causative genes for these is possible only by exploiting highly informative techniques such as homozygosity mapping. In this approach, a region of many centimorgans surrounding a disease gene is expected to be homozygous by descent in the affected child of consanguineous parents (Lander and Botstein 1987). Such chromosomal regions can be rapidly ascertained by analysis of highly polymorphic microsatellite markers. This method has previously been used in the mapping of the *FANCA* and *FANCG* genes (Pronk et al. 1995; Saar et al. 1998).

The initial analysis of data from family 1 was powerful enough to exclude 70% of the genome. A second analysis using just the affected children in families 1, 2, and 3 led to exclusion of 82% of the genome and identified two major candidate regions on chromosomes 6 and 19. Analysis of further markers in all family members excluded the region on chromosome 19, whereas the region on chromosome 6p was substantiated by a multipoint $Z_{max}$ of 4.55 for *D6S1691*. This clearly reflects the power of multipoint linkage analysis, because the maximum

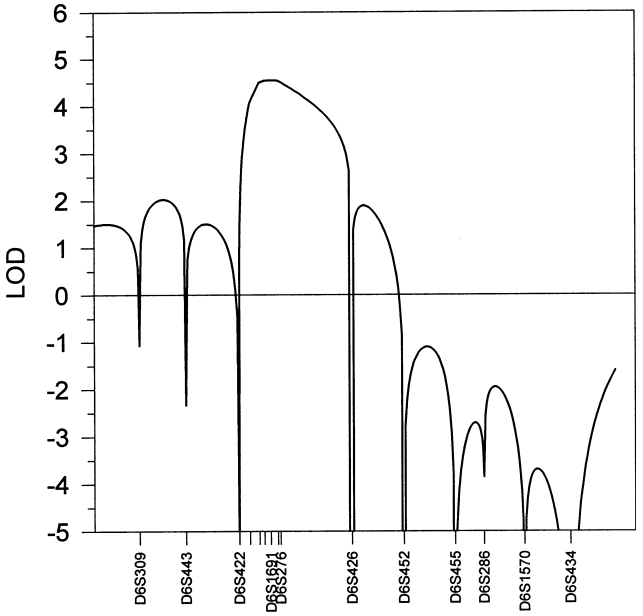


**Figure 3** Multipoint analysis of chromosome 6 markers in FA-E families.

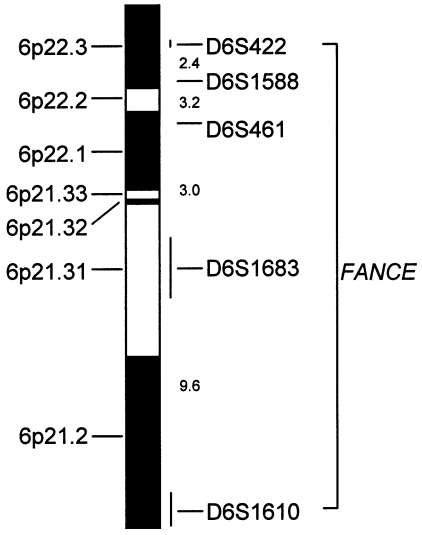


**Figure 4** Map of microsatellite markers on chromosome 6p and the *FANCE* gene. Genetic distances are indicated in centimorgans (cM). Physical locations of makers are taken from the consensus map of chromosome 6p (Genome Data Base).

two-point LOD score obtained was $Z_{max} = 2.68$ at $\theta = 0$ for marker *D6S461*.

As shown in figure 4, *FANCE* is located on chromosome 6p between markers *D6S422* and *D6S1610* in cytogenetic band 6p21.2-22.3. This is an extremely gene-rich region, containing clusters of histone genes, the complement C4 genes, and the human leukocyte antigen (HLA) class I genes of the major histocompatibility complex. This has significant therapeutic consequences for patients with FA assigned to this complementation group. The life-threatening bone marrow insufficiency in patients with FA is treated, when possible, by bone marrow transplantation from an HLA-matched unaffected sibling. The location of *FANCE* among the HLA class I genes, reported in the present article, suggests that patients with FA-E are unlikely to have unaffected siblings who are HLA compatible. For these patients, only unrelated HLA-matched individuals or HLA-matched parents will be suitable donors.

There are several genes in the critical region that could be compatible with the cellular and clinical phenotype of FA and thus represent candidate genes. These include genes involved in cell-cycle regulation and apoptosis (*CCND3, CDKN1,* and *BAK*), an oxygen-regulated protein (ORP150), and the lymphokine LTA (tumor necrosis factor–$\beta$). Mutation analysis of these candidates in FA-E patients is in progress.

The localization of a further FA gene to a discrete chromosomal region reconfirms the genetic heterogeneity of this disease. At this stage, both the proportion of FA cases caused by a mutation of the *FANCE* gene and their geographic distribution are unclear. However, the *FANCE* gene's chromosomal mapping provides a basis for its identification and will have consequences for the diagnosis and treatment of FA.

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## Electronic-Database Information

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

Cooperative Human Linkage Centre, http://www.chlc.org/ (for genetic map and distances)
Généthon, CEPH genotype database, http://www.ceph.fr (for genetic map and distances)
Genome Data Base, http://gdbwww.gdb.org/gdb/ (for chromosome 6 consensus map)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FA [MIM 227650] and the FA-E group [MIM 600901])

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