Research Article

Diagnosis of Fanconi Anemia: Mutation Analysis by Multiplex Ligation-Dependent Probe Amplification and PCR-Based Sanger Sequencing

Johan J. P. Gille, Karijn Floor, Lianne Kerkhoven, Najim Ameziane, Hans Joenje, and Johan P. de Winter

Department of Clinical Genetics, VU University Medical Center, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands

Correspondence should be addressed to Johan J. P. Gille, jjp.gille@vumc.nl

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Fanconi anemia (FA) is a rare inherited disease characterized by developmental defects, short stature, bone marrow failure, and a high risk of malignancies. FA is heterogeneous: 15 genetic subtypes have been distinguished so far. A clinical diagnosis of FA needs to be confirmed by testing cells for sensitivity to cross-linking agents in a chromosomal breakage test. As a second step, DNA testing can be employed to elucidate the genetic subtype of the patient and to identify the familial mutations. This knowledge allows preimplantation genetic diagnosis (PGD) and enables prenatal DNA testing in future pregnancies. Although simultaneous testing of all FA genes by next generation sequencing will be possible in the near future, this technique will not be available immediately for all laboratories. In addition, in populations with strong founder mutations, a limited test using Sanger sequencing and MLPA will be a cost-effective alternative. We describe a strategy and optimized conditions for the screening of FANCA, FANCB, FANCC, FANCE, FANCF, and FANCG and present the results obtained in a cohort of 54 patients referred to our diagnostic service since 2008. In addition, the follow up with respect to genetic counseling and carrier screening in the families is discussed.

1. Introduction

Fanconi anemia (FA) is a rare inherited syndrome with diverse clinical symptoms including developmental defects, short stature, bone marrow failure, and a high risk of malignancies. Fifteen genetic subtypes have been distinguished: FA-A, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M, -N, -O, and -P [1–4]. The majority of patients (~85%) belong to the subtypes A (~60%), C (~10–15%), or G (~10%), while a minority (~15%) is distributed over the remaining 12 subtypes, with relative prevalences between <1 and 5%. These percentages may differ considerably within certain ethnic groups, due to founder effects. All subtypes seem to fit within a “classical” FA phenotype, except for D1 and N (mutated in BRCA2/FANCD1 and PALB2/FANCN), which are associated with a distinct, more severe, syndromic association. The mode of inheritance for all subtypes is autosomal recessive, except for FA-B, which is X-linked. These two different modes of inheritance have important consequences for the counseling of FA families. The relative prevalence of FA-B amongst unselected FA patients is estimated at 1.6% [5]. For all genetic subtypes disease genes have been identified (Table 1). Many mutations found in the various subtypes are private, but recurrent mutations are known, particularly in specific ethnic backgrounds (Table 2).

Most FA genes encode orphan proteins with no known molecular function. At least eight FA proteins (FANCA, -B, -C, -E, -F, -G, -L, and -M) assemble into a nuclear multiprotein core complex, which is required to activate FANCD2 and FANCI by monoubiquitination [6]. FANCL, which carries a RING finger domain, is supposed to represent the ubiquitin E3 ligase in this activation [7]. FANCM probably acts as a sensor of DNA damage and recruits the FA core complex to the site of damage, but FANCM also interacts with other proteins including Blm [6]. Monoubiquitination of FANCD2 and FANCI directs these proteins to areas of damaged chromatin where they interact with other proteins, resulting in repair of the damage [6]. The remaining FA
Table 1: Fanconi anemia complementation groups, genes, and proteins.

| Group | Gene symbol(s) | Cytogenetic location | Protein (amino acids) | Domain structure (references) |
|-------|----------------|----------------------|-----------------------|-------------------------------|
| A     | FANCA          | 16q24.3              | 1455                  | HEAT repeats [8]              |
| B     | FANCB          | Xp22.31              | 859                   |                               |
| C     | FANCC          | 9q22.3               | 558                   | HEAT repeats [8]              |
| D1    | BRCA2          | 13q12.3              | 3418                  | RAD51- and DNA-binding motifs [9] |
| D2    | FANCD2         | 3p25.3               | 1451                  |                               |
| E     | FANCE          | 6p21.3               | 536                   |                               |
| F     | FANCF          | 11p15                | 374                   |                               |
| G     | FANCG          | 9p13                 | 622                   | Tetra-tricopeptide repeats (TPR) [10] |
| I     | FANCI          | 15q26.1              | 1328                  |                               |
| J     | BRIP1          | 17q22                | 1249                  | DNA helicase [11, 12]         |
| L     | FANCL          | 2p16.1               | 375                   | RING finger motif (E3 ligase) [7, 8] |
| M     | FANCM          | 14q21.3              | 2014                  | DNA helicase, nuclease [13]   |
| N     | PALB2          | 16p12.1              | 1186                  |                               |
| O     | RAD51C         | 17q25.1              | 376                   |                               |
| P     | SLX4           | 16p13.3              | 1834                  | Endonuclease scaffold [3, 4]  |

aFor gene nomenclature see http://www.genenames.org/.

bThe proteins defective in groups D1, J, N, O, and P (boldface) act downstream or independent of the monoubiquitination of FANCD2; all other FA proteins act upstream of this process.

Table 2: Major recurrent mutations in FA.

| Gene  | Mutation*               | Geographic/ethnic background | Comment                                      | References          |
|-------|-------------------------|------------------------------|----------------------------------------------|---------------------|
| FANCA | c.3788_3790del (p.Phe1263del) | European, Brazilian          | Relatively mild                              | [14, 15]           |
|       | c.1115_1118delTTGG (p.Val372fs) | European                      | Relatively mild                              | [16]                |
|       | Exon 12–17del           | South-African                 | Relatively common in Afrikaners              | [17]                |
|       | Exon 12–31del           | Spanish Gypsy population     | Worldwide highest prevalence of mutant FANCA allele | [18]                |
|       | c.295C>T (p.Gln99X)     | Homozygous in 80% of Ashkenazi Jewish FA; relatively common in Japan. | Severe phenotype in Jews, milder in Japanese. | [19–22]            |
| FANCC | c.711+4A>T (originally reported as IVS4+4A>T) | European                      | Like other exon 1 mutations, relatively mild phenotype. | [19, 23–25]        |
|       | c.67delG (originally reported as 322delG) | Homozygous in approx. 50% of Dutch FA patients |                               | [19, 23–25]        |
| FANCD2| c.1948-16T>G            | Turkish                       | Founder mutation                             | [26]                |
|       | c.313G>T (p.Glu105X)    | European                      | 44% of mutated FANCG alleles in Germany.     | [27]                |
| FANCG | c.1077-2A>G            | Portuguese/Brazilian          | Founder mutation                             | [27, 28]           |
|       | c.1480+1G>C            | French-Canadian               | Founder mutation                             | [28]                |
|       | c.307+1G>C             | Japanese                      | Founder mutation                             | [28, 29]           |
|       | c.1794_1803del (p.Trp599fs) | European                      | Founder mutation                             | [28]                |
|       | c.637_643del (p.Tyr213fs) | Sub-Saharan Africa            | 82% of all black FA patients Found in ca. 50% of FA-J patients of diverse ancestry; ancient mutation or hot spot. | [30]                |
| FANCI | c.2392G>T (p.Arg798X)  |                               |                                              |                     |

Nucleotide numbering based on ΔTG = +1.

Published sequence variations in FA genes, with their descriptions conforming to the current nomenclature rules, are listed at http://www.rockefeller.edu/fanconi/.
proteins function downstream of or parallel to the FANCD2 activation step [6]. The exact nature of the DNA damage response, which when defective causes FA, remains to be defined. FANCJ/BRIP1 and FANCM possess DNA helicase motifs, which strongly suggests that the FA pathway acts through a direct interaction with DNA, presumably to resolve or remodel blocked DNA replication forks resulting from DNA interstrand cross-link damage [6]. This idea is strengthened by the recent extension of the FA pathway with SLX4, a scaffold protein for structure-specific endonucleases involved in unhooking the DNA cross-link [3, 4].

2. Laboratory Diagnostics in FA

Cells derived from FA patients are—by definition—hypersensitive to chromosomal breakage induced by DNA cross-linking agents such as mitomycin C (MMC) or diepoxybutane (DEB) [31]. This cellular phenotype is ascertained using stimulated blood T lymphocytes. The indications for FA laboratory testing are rather broad [32]. As a consequence, in only a small proportion of patients (about 10%) the chromosomal breakage test is positive, and an FA diagnosis is established. Since mutation testing by Sanger sequencing and MLPA is rather laborious, time consuming and therefore expensive, a positive chromosomal breakage test is a prerequisite for starting mutation screening. Confirmation of the FA diagnosis at the DNA level is important in patients in whom the chromosomal breakage test was inconclusive. Furthermore, knowledge about the FA subtype is relevant for the treatment and prognosis of the patients. In addition, identification of mutations allows carrier testing in the family and will enable prenatal DNA testing and preimplantation genetic diagnosis (PGD) in future pregnancies. Finally, this information can be used to rule out FA in potential donors for bone marrow transplantation.

Although simultaneous testing of all FA genes by next generation sequencing will be possible in the near future, this technique will not be available immediately for all laboratories worldwide. In addition, in populations with strong founder mutations, a limited test using Sanger sequencing and MLPA will be a cost-effective alternative [33]. The strategy outlined below has been developed at our DNA diagnostics laboratory to provide a molecular diagnosis of FA. It is recognized that mutations in FANCA account for 60–70% of all FA cases and that about 15–20% of the mutations in this gene are large deletions [33, 34]. Therefore, DNA testing usually starts with a screen for deletions in FANCA. However, depending on the circumstances strategies may differ from case to case.

2.1. Materials. Genomic DNA (from e.g., leukocytes or fibroblasts derived from the proband or the parents) is adequate for most mutation screening assays. Screening on cDNA is more efficient but has several drawbacks: for high-quality cDNA, growing cells (stimulated leukocytes, lymphoblastoid cell lines, or fibroblasts) are necessary. In addition, common alternative splice variants will hamper the evaluation of DNA sequences. Therefore, screening on gDNA is the preferred method for mutation screening. However, during the diagnostic process, growing cells from the proband will be helpful in a couple of situations. Growing cells are indispensable for studying the effect of unclassified variants on splicing or to verify the disease gene by functional complementation of the cellular phenotype with a construct expressing a wild type copy of the suspected gene [35–37]. Finally, if no mutations can be detected, growing cells can be used to reconfirm the diagnosis FA by checking MMC sensitivity in cell growth or G2-arrest assays [38, 39].

2.2. Mutation Screening Strategy

2.2.1. Hints from Ethnic Background or Phenotype. Information on the ethnic background of the proband may provide a clue for a specific pathogenic mutation that most likely causes the disease, such as c.711 + 4A>T (IVS4 + 4A>T) in FANCC, a mutation present in homozygous state in 80% of all FA cases of Ashkenazi Jewish ancestry, and c.295C >T in FANCA, which was present homozygously in all 40 FA cases of Spanish Gypsy ancestry so far investigated. More examples of recurrent mutations are shown in Table 3. The distinct clinical phenotype of D1 and N patients (severely affected, often combined with leukemia or solid tumors below the age of 5 years) may provide a clue to favor BRCA2/FANCD1 and PALB2/FANCN as the first gene to be screened [40–44]. This is especially worthwhile if confirmed by the cellular phenotype: in contrast to cells from all other known FA subtypes, cells from D1, N and O patients are unable to form RAD51 foci upon exposure to X rays or MMC [43–45].

2.2.2. No Clues Available

(1) In the absence of any clue to the disease gene, mutation screening starts with a search for deletions in FANCA, as this type of mutation accounts for 40% of all mutant FANCA alleles. The quantitative multiplex ligation-dependent probe amplification (MLPA) method [46] is used for this initial screen, which identifies FANCA as the most likely disease gene in 1 out of 4 patients by the detection of a—usually hemizygous-deletion. In parallel, the FANCA gene is completely sequenced. The combination of these two approaches identifies 60–70% of all FA patients as FA-A.

(2) Next, FANCC, -E, -F, and -G are screened by DNA sequencing.

(3) Only if the proband is a male, FANCB is screened by MLPA and DNA sequencing.

In Table 4, optimized conditions are provided for the PCR amplification of FANCA, -C, -E, -F, -G, and -B. Most PCRs can be performed under standard conditions. The PCR primers have M13 extensions which allow sequencing of all fragments with universal sequencing primers. MLPA was performed according to the instructions of the supplier. Detailed information about the sequences of the MLPA probes is available from the website of the
### Table 3: Mutations detected in a cohort of 54 patients by screening FANCA, FANCC, FANCE, FANCF and FANCG.

| Country of origin | Gene | DNA change | Effect | Number of database entries | DNA change | Effect | Number of database entries |
|-------------------|------|------------|--------|-----------------------------|------------|--------|-----------------------------|
| ES                | FANCA | ex16_ex17del | del    | 12x                          | c.1115_1118del | p.Val372fs | 62x                          |
| PT                | FANCA | c.718C>T    | p.Glu240X | 2x                          | c.2870G>A   | W957X  | 1x                           |
| NL                | FANCA | ex15del     | del    | 3x                          | ex15del    | del    | 3x                           |
| NL                | FANCA | c.3788_3790del | p.Phe1263del | 215x                      | c.3788_3790del | p.Phe1263del | 215x                      |
| CA                | FANCA | c.718C>T    | p.Glu240X | 2x                          | c.1085T>C   | p.Leu362Pro | novel                      |
| PT                | FANCA | c.3788_3790del | p.Phe1263del | 215x                      | c.4130C>G   | p.Ser1377X | 1x                           |
| IE                | FANCA | c.2812_2830dup | p.Asp944fs | 3x                          | c.2812_2830dup | p.Asp944fs | 3x                           |
| AU                | FANCA | c.2303T>C    | p.Leu768Pro | 5x                          | c.2303T>C   | p.Leu768Pro | 5x                           |
| NL                | FANCA | c.862G>T     | p.Glu288X | 1x                          | c.862G>T    | p.Asn707fs | novel                      |
| NL                | FANCA | ex11_ex12del | del    | 1x                          | c.2121delC  | del    | 1x                           |
| DK                | FANCA | ex18del      | del    | 1x                          | c.3788_3790del | p.Phe1263del | 215x                      |
| UK                | FANCA | c.337_338del | p.Ala114fs | 1x                          | p.Arg117Gly | 2x                              |
| UK                | FANCA | c.3568C>T    | p.Gln190X | novel                      | c.3568C>T   | p.Gln190X | novel                      |
| NL                | FANCA | c.487delC    | p.Arg163fs | 1x                          | p.Arg951Trp | 11x                             |
| SE                | FANCA | c.88delG     | p.Val30fs | novel                      | p.100A>T    | p.Lys34X | 2x                           |
| NL                | FANCA | c.862G>T     | p.Glu288X | 9x                          | c.1771C>T   | p.Arg591X | 9x                           |
| PT                | FANCA | c.1709_1715+4del | p.Glu570fs | novel                      | c.3430C>T   | p.Arg144Trp | novel                      |
| NO                | FANCA | c.100A>T     | p.Lys34X | 2x                          | c.1378C>T   | p.Arg460X | novel                      |
| PT                | FANCA | ex15_ex16del | del    | 2x                          | ex15_16del  | del    | 2x                           |
| NL                | FANCA | c.2982_2983A>G | splice | novel                      | ex7_31del   | del    |                           |
| AU                | FANCA | c.427_427+3del | splice | novel                      | c.1771C>T   | p.Arg591X | 9x                           |
| AU                | FANCA | c.3491C>T    | p.Pro1164Leu | novel                      | c.3491C>T   | p.Pro1164Leu | novel                      |
| CA                | FANCA | ex4_ex5del   | del    | 2x                          | ex5_6del    | del    | 2x                           |
| NL                | FANCA | c.3391A>G    | p.Met1? | 1x                          | c.3788_3790del | p.Phe1263del | 215x                      |
| IE                | FANCA | c.851dup     | p.Val285fs | novel                      | c.2534T>C   | p.Leu845Pro | 4x                           |
| NL                | FANCA | c.2852G>A    | p.Arg951Gln | 6x                          | c.3624C>T   | p. (splice) | 2x                           |
| AU                | FANCA | c.331_332dup | p.Leu112fs | novel                      | ex22_29del  | del    | novel                      |
| NL                | FANCA | c.862G>T     | p.Glu288X | 9x                          | c.3920delA  | p.Gln1307fs | 2x                           |
| IR                | FANCA | ex21del      | del    | novel                      | c.3391A>G   | p.Met1? | 1x                           |
| SE                | FANCA | ex1_12del    | del    | novel                      | ex22_29del  | del    | novel                      |
| NL                | FANCA | c.755_767del | p.Leu252fs | novel                      | —           | —      | —                           |
| NL                | FANCA | c.67delG     | p.Asp23fs | 50x                          | p.Arg185X   | 14x                              |
| NL                | FANCA | c.67delG     | p.Asp23fs | 50x                          | c.67delG    | p.Asp23fs | 50x                          |
| CA                | FANCA | c.67delG     | p.Asp23fs | 50x                          | c.553C>T    | p.Arg185X | 14x                          |
| NL                | FANCA | c.67delG     | p.Asp23fs | 50x                          | c.1115_1118del | p.Val372fs | 62x                          |
| NL                | FANCA | c.67delG     | p.Asp23fs | 50x                          | c.67delG    | p.Asp23fs | 50x                          |
| NL                | FANCA | c.67delG     | p.Asp23fs | 50x                          | c.67delG    | p.Asp23fs | 50x                          |
| PT                | FANCE | c.1111C>T    | p.Arg371Trp | 6x                          | c.1111C>T   | p.Arg371Trp | 6x                           |
| UK                | FANCF | c.496C>T     | p.Gln166X | 4x                          | c.496C>T    | p.Gln166X | 4x                           |
| UK                | FANCG | c.307+2delT  | splice | novel                      | c.307+2delT | splice | novel                      |
| UK                | FANCG | c.1471_1473delinsG | p.Lys491fs | novel                      | c.1471_1473delinsG | p.Lys491fs | novel                      |
| NL                | FANCG | c.65G>C      | p.Arg22Pro | 6x                          | c.65G>C    | p.Arg22Pro | 6x                           |
| IR                | FANCG | c.307+1G>C   | splice | 21x                          | c.307+1G>C | splice | 21x                          |
| NL                | FANCG | c.85_1G>A    | splice | novel                      | c.85_1G>A  | splice | novel                      |

1 Country of origins: AU: Australia; CA: Canada; DK: Denmark; ES: Spain; GR: Greece; IE: Ireland; IR: Iran; NL: Netherlands; PT: Portugal; SE: Sweden; UK: United Kingdom

2 Effect c.2982_2983A>G: by studying cDNA it was shown that the mutation created a new splice donor site resulting in an aberrant mRNA.
Table 4: Primers and conditions for PCR on genomic DNA of the coding sequence plus intron/exon boundaries of **FANCA**, **FANCC**, **FANCE**, **FANCF**, **FANCG**, and **FANCB**.

(a)  

| **FANCA** Primer | **Sequence** (5′ > 3′) | **Product length (bp)** |
|-----------------|-------------------------|-------------------------|
| **FANCA** ex1F  | gtaaaacgcggccag GC'CCTCCCCACGGACCAACA | 362 |
| **FANCA** ex1R  | caggaaacagctatga AGGCTCTGGGAGGGAAGGGATCGG | |
| **FANCA** ex2F  | gtaaaacgcggccag CTCTTCGGGAGGGTGTCGCTGGT | 328 |
| **FANCA** ex2R  | caggaaacagctatga CTCTTCGGGAGGGTGTCGCTGGT | |
| **FANCA** ex3F  | gtaaaacgcggccag GCCTGGCCTGGAGCTTGAAT | 392 |
| **FANCA** ex3R  | caggaaacagctatga CGCAGGTTGAATCAGACGCTGTT | |
| **FANCA** ex4F  | gtaaaacgcggccag TAAGGCATTTTAAACAGCAAGTC | 430 |
| **FANCA** ex4R  | caggaaacagctatga TGCCAATAAATACATGAGCAAGCT | |
| **FANCA** ex5F  | gtaaaacgcggccag GCCTGGCCTGGAGCTTGAAT | 392 |
| **FANCA** ex5R  | caggaaacagctatga CGCAGGTTGAATCAGACGCTGTT | |
| **FANCA** ex6F  | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
| **FANCA** ex6R  | caggaaacagctatga TGAAGGTACTTCTTTCCAATCCA | |
| **FANCA** ex7F  | gtaaaacgcggccag TAAGGGCCTGGGGCTGGTCCTTAACAAA | 401 |
| **FANCA** ex7R  | caggaaacagctatga AGGCAGCATGGAGAATTTTACAAAG | |
| **FANCA** ex8F  | gtaaaacgcggccag GTGGTCTCAGGTGGAATTAGAATTTGG | 406 |
| **FANCA** ex8R  | caggaaacagctatga GGCTTCTGCCTGGTGATATTGA | |
| **FANCA** ex9F  | gtaaaacgcggccag TGCTCTTGTTGTTGTTAAGTGACGATTC | 332 |
| **FANCA** ex9R  | caggaaacagctatga TGCTCTTGTTGTTGTTAAGTGACGATTC | |
| **FANCA** ex10F | gtaaaacgcggccag TGCTCTTGTTGTTGTTAAGTGACGATTC | 332 |
| **FANCA** ex10R | caggaaacagctatga TGCTCTTGTTGTTGTTAAGTGACGATTC | |
| **FANCA** ex11F | gtaaaacgcggccag TTCTTCTGCTGCTGCTGCTGCT | 410 |
| **FANCA** ex11R | caggaaacagctatga TACTGCTGCTGCTGCTGCTGCTGCT | |
| **FANCA** ex12F | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
| **FANCA** ex12R | caggaaacagctatga TGAAGGTACTTCTTTCCAATCCA | |
| **FANCA** ex13F | gtaaaacgcggccag TCCTTCCTGCTGCTGCTGCTGCT | 377 |
| **FANCA** ex13R | caggaaacagctatga CTGCTGCTGCTGCTGCTGCTGCT | |
| **FANCA** ex14F | gtaaaacgcggccag TCTGCTGCTGCTGCTGCTGCTGCT | 411 |
| **FANCA** ex14R | caggaaacagctatga ACTGCTGCTGCTGCTGCTGCTGCT | |
| **FANCA** ex15F | gtaaaacgcggccag ACTGCTGCTGCTGCTGCTGCTGCT | 430 |
| **FANCA** ex15R | caggaaacagctatga ACTGCTGCTGCTGCTGCTGCTGCT | |
| **FANCA** ex16F | gtaaaacgcggccag TCTGCTGCTGCTGCTGCTGCTGCT | 312 |
| **FANCA** ex16R | caggaaacagctatga ACTGCTGCTGCTGCTGCTGCTGCT | |
| **FANCA** ex17F | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
| **FANCA** ex17R | caggaaacagctatga TGAAGGTACTTCTTTCCAATCCA | |
| **FANCA** ex18F | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
| **FANCA** ex18R | caggaaacagctatga TGAAGGTACTTCTTTCCAATCCA | |
| **FANCA** ex19F | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
| **FANCA** ex19R | caggaaacagctatga TGAAGGTACTTCTTTCCAATCCA | |
| **FANCA** ex20F | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
| **FANCA** ex20R | caggaaacagctatga TGAAGGTACTTCTTTCCAATCCA | |
| **FANCA** ex21F | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
| **FANCA** ex21R | caggaaacagctatga TGAAGGTACTTCTTTCCAATCCA | |
| **FANCA** ex22F | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
| **FANCA** ex22R | caggaaacagctatga TGAAGGTACTTCTTTCCAATCCA | |
| **FANCA** ex23F | gtaaaacgcggccag GAGTATTGTTTTCAGGTAATTTGTT | 356 |
### FANCA

| Primer name  | Sequence (5’ > 3’)                              | Product length (bp) |
|--------------|-------------------------------------------------|---------------------|
| FANCA_ex23R  | cagaaacagctatga GGCCTTGGAAACATCTGATACGAC        |                     |
| FANCA_ex24F  | gtaaaacgcggccag CCTTCCTGCAGTCGGTCCGTC           | 229                 |
| FANCA_ex24R  | cagaaacagctatga CAGACTTGGCCAGCAAGAG             |                     |
| FANCA_ex25F  | gtaaaacgcggccag CGGCTTGATGTTGGATAGCTGTG         | 296                 |
| FANCA_ex25R  | cagaaacagctatga TTTCCAAGGCGACTGAAGCAAAAT       |                     |
| FANCA_ex26F  | gtaaaacgcggccag AGCTTGGAAAGGCGACTGCTG          | 347                 |
| FANCA_ex26R  | cagaaacagctatga CTCTTCTAATATTTTACAAAGG         |                     |
| FANCA_ex27F  | gtaaaacgcggccag AGACTGCTCTACAAACAAACGAG        | 356                 |
| FANCA_ex27R  | cagaaacagctatga CGGTCGGAAAGGCGGTAAAC           |                     |
| FANCA_ex28F  | gtaaaacgcggccag GTTGATGGTC GTTTCACCATCTG       | 401                 |
| FANCA_ex28R  | cagaaacagctatga GAGGGAAAGGCTAGCTCTGGT          |                     |
| FANCA_ex29F  | gtaaaacgcggccag GACATGGAGAGCAGCAGTAAAGG        | 411                 |
| FANCA_ex29R  | cagaaacagctatga GTGCGTGTGATGACGGGAAAGATG       |                     |
| FANCA_ex30F  | gtaaaacgcggccag CCCGACGCCCTGGCTCTCAACCA        | 411                 |
| FANCA_ex30R  | cagaaacagctatga AAAGGCGACCCCTGGCTGTAAGCT       |                     |
| FANCA_ex31F  | gtaaaacgcggccag GATAGCCTGCTTGATGTAAGCT         | 406                 |
| FANCA_ex31R  | cagaaacagctatga TGCCATAAATAATCTTTAAGCA         |                     |
| FANCA_ex32F  | gtaaaacgcggccag TTTCCAGCCGGATGATGCTCT          | 395                 |
| FANCA_ex32R  | cagaaacagctatga GGGCGGCGACCCCTGGCTAAGCT        |                     |
| FANCA_ex33F  | gtaaaacgcggccag TTCCCTGACTCTACTAGGGTGTG        | 311                 |
| FANCA_ex33R  | cagaaacagctatga CGTAAAGGCGCTATCTAGGGTGTG       |                     |
| FANCA_ex34F  | gtaaaacgcggccag ACCATTTCCTTCAGTGCTGGACA        | 378                 |
| FANCA_ex34R  | cagaaacagctatga TTTACATGTGCACATGATTGGT         |                     |
| FANCA_ex35F  | gtaaaacgcggccag TTCCCTGACTCTACTAGGGTGTG        | 311                 |
| FANCA_ex35R  | cagaaacagctatga CGTAAAGGCGCTATCTAGGGTGTG       |                     |
| FANCA_ex36F  | gtaaaacgcggccag ACCATTTCCTTCAGTGCTGGACA        | 378                 |
| FANCA_ex36R  | cagaaacagctatga TTTACATGTGCACATGATTGGT         |                     |
| FANCA_ex37F  | gtaaaacgcggccag ACCATTTCCTTCAGTGCTGGACA        | 378                 |
| FANCA_ex37R  | cagaaacagctatga TTTACATGTGCACATGATTGGT         |                     |
| FANCA_ex38F  | gtaaaacgcggccag TTTCTCAAGATGACCACTTAAAGG       | 362                 |
| FANCA_ex38R  | cagaaacagctatga CTGAAAGGCGCTATCTAGGGTGTG       |                     |
| FANCA_ex39F  | gtaaaacgcggccag TTTCTCAAGATGACCACTTAAAGG       | 362                 |
| FANCA_ex39R  | cagaaacagctatga CTGAAAGGCGCTATCTAGGGTGTG       |                     |
| FANCA_ex40F  | gtaaaacgcggccag ACCAGGCGCTTGGTCCTACACATTT      | 353                 |
| FANCA_ex40R  | cagaaacagctatga ACCAGGCGCTTGGTCCTACACATTT      |                     |
| FANCA_ex41F  | gtaaaacgcggccag ACCAGGCGCTTGGTCCTACACATTT      | 353                 |
| FANCA_ex41R  | cagaaacagctatga ACCAGGCGCTTGGTCCTACACATTT      |                     |
| FANCA_ex42F  | gtaaaacgcggccag ACCAGGCGCTTGGTCCTACACATTT      | 353                 |
| FANCA_ex42R  | cagaaacagctatga ACCAGGCGCTTGGTCCTACACATTT      |                     |
| FANCA_ex43F  | gtaaaacgcggccag ACCAGGCGCTTGGTCCTACACATTT      | 353                 |
| FANCA_ex43R  | cagaaacagctatga ACCAGGCGCTTGGTCCTACACATTT      |                     |

### FANCC

| Primer name  | Sequence (5’ > 3’)                              | Product length (bp) |
|--------------|-------------------------------------------------|---------------------|
| FANCC_ex1F   | gtaaaacgcggccag ACCAGGCGCTTGGTCCTACACATTT      | 378                 |
| FANCC_ex1R   | cagaaacagctatga ACCAGGCGCTTGGTCCTACACATTT      |                     |
### FANCC

| Primer name | Sequence (5′ > 3′) | Product length (bp) |
|-------------|---------------------|---------------------|
| FANCC_ex2F  | gtaaaacgcggccag CTAAACAAGAAGCATTCAGTTC | 303 |
| FANCC_ex2R  | caggaaacagctga GAGAAGAGGTTCTAAATGTAAGC | |
| FANCC_ex3F  | gtaaaacgcggccag TCAGCAGAAGAGATGTCGAAA | 405 |
| FANCC_ex3R  | caggaaacagctga AACATCATAGAATTGGATCCAC | |
| FANCC_ex4F  | gtaaaacgcggccag TGTAATAAAAGGCACTGACTT | 380 |
| FANCC_ex4R  | caggaaacagctga TCCCATCTCAATTCTCTCCGTA | |
| FANCC_ex5F  | gtaaaacgcggccag AGAAGTCTGATGTAATCCTGTTT | 367 |
| FANCC_ex5R  | caggaaacagctga TTACTGCTCTGAGAGTCCA | |
| FANCC_ex6F  | gtaaaacgcggccag GCTTTTGGACTTTTAATGCA | 387 |
| FANCC_ex6R  | caggaaacagctga AACATCATAGAATTGGATCCAC | |
| FANCC_ex7F  | gtaaaacgcggccag ATTAGTGTAGCTTTTGAATT | 422 |
| FANCC_ex7R  | caggaaacagctga CAAAATAAAAAATGTAATACACG | |
| FANCC_ex8F  | gtaaaacgcggccag CTCCTTTGGCTGATAATAGCA | 336 |
| FANCC_ex8R  | caggaaacagctga CTGCTCTCCCTATGCTAGATA | |
| FANCC_ex9F  | gtaaaacgcggccag CTCCTTTGGCTGATAATAGCA | 416 |
| FANCC_ex9R  | caggaaacagctga CGCCTCCCTCCTTCCCTTTC | |
| FANCC_ex10F | gtaaaacgcggccag CAATTACATTTAAGCCAAACGG | 451 |
| FANCC_ex10R | caggaaacagctga AGGTTGCCATGACATATGCCATC | |
| FANCC_ex11F | gtaaaacgcggccag CGTCCTCCCTCCTTCCCTTTC | 425 |
| FANCC_ex11R | caggaaacagctga ATACTGCTGAAGCTTATGGCAC | |
| FANCC_ex12F | gtaaaacgcggccag CTCCTCTCAGGGGCCAGTGCTTA | 435 |
| FANCC_ex12R | caggaaacagctga GTCTTTTGGACACTGCTGTCGTA | |
| FANCE_ex13F | gtaaaacgcggccag CTCCTCTCAGGGGCCAGTGCTTA | 387 |
| FANCE_ex13R | caggaaacagctga AGAAGTCTGATGTAATCCTGTTT | |

### FANCE

| Primer name | Sequence (5′ > 3′) | Product length (bp) |
|-------------|---------------------|---------------------|
| FANCE_ex1F  | gtaaaacgcggccag CGCCCTCCCTCCTCTCTTCTC | 540 |
| FANCE_ex1R  | caggaaacagctga CCCGCCCTCCCATACCTGCTAA | |
| FANCE_ex2F  | gtaaaacgcggccag GCTCTGCCCATCTGCTCTGTCG | 469 |
| FANCE_ex2R  | caggaaacagctga CTCTGAGTTCTTCTGCTGTT | |
| FANCE_ex3F  | gtaaaacgcggccag GCCAGAGACAGCTCCAAAGTCTA | 479 |
| FANCE_ex3R  | caggaaacagctga GAGGGGATACAGCCATGCTCTA | |
| FANCE_ex4F  | gtaaaacgcggccag TGGAGCAACTGTAATGCCTA | 352 |
| FANCE_ex4R  | caggaaacagctga ATCTCTCTCGCATGCATGCTACTC | |
| FANCE_ex5F  | gtaaaacgcggccag TATGAGGTTATTGGGAGCTTATT | 436 |
| FANCE_ex5R  | caggaaacagctga AGGTTGCCATGACATATGCCATC | |
| FANCE_ex6F  | gtaaaacgcggccag TTGGAGCAGCAGATAGATACTCA | 380 |
| FANCE_ex6R  | caggaaacagctga GAGGGGATACAGCCATGCTCTA | |
| FANCE_ex7F  | gtaaaacgcggccag TTGGAGCAGCAGATAGATACTCA | 388 |
| FANCE_ex7R  | caggaaacagctga GTGCGGTGCTGCTGCTGCTGCT | |
| FANCE_ex8F  | gtaaaacgcggccag TTGGAGCAGCAGATAGATACTCA | 380 |
| FANCE_ex8R  | caggaaacagctga GAGGGGATACAGCCATGCTCTA | |
| FANCE_ex9F  | gtaaaacgcggccag GTGCGGTGCTGCTGCTGCTGCT | 388 |
| FANCE_ex9R  | caggaaacagctga GTGCGGTGCTGCTGCTGCTGCT | |
Anemia

(c) Continued.

**FANCE**

| Primer name | Sequence (5′ > 3′) | Product length (bp) |
|-------------|---------------------|---------------------|
| FANCE_ex10F | gtaaaaacagccgccaTGGCCTCCTCCTCTCTCAATAGA | 369 |
| FANCE_ex10R | caggaaacagctatgAACAGGGAGGCAGTTGAATCTG | |

(d)

| Primer name | Sequence (5′ > 3′) | Product length (bp) |
|-------------|---------------------|---------------------|
| FANCF_ex1aF | gtaaaaacagccgcaaTTTCGCGGATGTTCCAATCAGTA | 449 |
| FANCF_ex1aR | caggaaacagctatgTTTCGCGGATGTTCCAATCAGTA | |
| FANCF_ex1bF | gtaaaaacagccgcaaAGTGGAGGCAAGGAGGCGGCTTTT | 456 |
| FANCF_ex1bR | caggaaacagctatgAGTGGAGGCAAGGAGGCGGCTTTT | |
| FANCF_ex1cF | gtaaaaacagccgcaaCCCACATCTCAGAGGACTCTC | 444 |
| FANCF_ex1cR | caggaaacagctatgCCCACATCTCAGAGGACTCTC | |
| FANCF_ex1dF | gtaaaaacagccgcaaGCTTTTGACTTTGTAGACTAGCC | 456 |
| FANCF_ex1dR | caggaaacagctatgGCTTTTGACTTTGTAGACTAGCC | |

(e)

| Primer name | Sequence (5′ > 3′) | Product length (bp) |
|-------------|---------------------|---------------------|
| FANCG_ex1F  | gtaaaaacagccgcaaAGCCTGGGCGGGTGATGGAATGGAAC | 369 |
| FANCG_ex1R  | caggaaacagctatgAGCCTGGGCGGGTGATGGAATGGAAC | |
| FANCG_ex2F  | gtaaaaacagccgcaaCAGGCCAAGGTAACACGGTTGCT | 460 |
| FANCG_ex2R  | caggaaacagctatgCAGGCCAAGGTAACACGGTTGCT | |
| FANCG_ex3F  | gtaaaaacagccgcaaTATTGTAGCTGTTTTGGGTGGAG | 362 |
| FANCG_ex3R  | caggaaacagctatgTATTGTAGCTGTTTTGGGTGGAG | |
| FANCG_ex4F  | gtaaaaacagccgcaaAGGATGGAGGATGAGGTGCAC | 411 |
| FANCG_ex4R  | caggaaacagctatgAGGATGGAGGATGAGGTGCAC | |
| FANCG_ex5F  | gtaaaaacagccgcaaAGATGGAGATAGGAGAAGCAGAG | 454 |
| FANCG_ex5R  | caggaaacagctatgAGATGGAGATAGGAGAAGCAGAG | |
| FANCG_ex6F  | gtaaaaacagccgcaaGATATGGTTATTGGAATTCTTAGCA | 487 |
| FANCG_ex6R  | caggaaacagctatgGATATGGTTATTGGAATTCTTAGCA | |
| FANCG_ex7F  | gtaaaaacagccgcaaGCACCCCAAGGTAACACCGGTAA | 460 |
| FANCG_ex7R  | caggaaacagctatgGCACCCCAAGGTAACACCGGTAA | |
| FANCG_ex8F  | gtaaaaacagccgcaaCAGGCCAAGGTAACACGGTTGCT | 438 |
| FANCG_ex8R  | caggaaacagctatgCAGGCCAAGGTAACACGGTTGCT | |
| FANCG_ex9F  | gtaaaaacagccgcaaGTTGTAGCTGTTTTGGGTGGAG | 400 |
| FANCG_ex9R  | caggaaacagctatgGTTGTAGCTGTTTTGGGTGGAG | |
| FANCG_ex10F | gtaaaaacagccgcaaCAGACTCTGCAATGGTACCAG | 460 |
| FANCG_ex10R | caggaaacagctatgCAGACTCTGCAATGGTACCAG | |
| FANCG_ex11F | gtaaaaacagccgcaaAGCTCCATGTTCACCTCTTCCTC | 397 |
| FANCG_ex11R | caggaaacagctatgAGCTCCATGTTCACCTCTTCCTC | |
| FANCG_ex12F | gtaaaaacagccgcaaAGGATGGAGGATGAGGTGCTAC | 405 |
| FANCG_ex12R | caggaaacagctatgAGGATGGAGGATGAGGTGCTAC | |
| FANCG_ex13F | gtaaaaacagccgcaaCTCGCTTCCATATGTGAGTGTAGGCCCTTCTC | 340 |
| FANCG_ex13R | caggaaacagctatgCTCGCTTCCATATGTGAGTGTAGGCCCTTCTC | |
| FANCG_ex14F | gtaaaaacagccgcaaCAGTTCCATGGGCTTCTTAGACC | 400 |
| FANCG_ex14R | caggaaacagctatgCAGTTCCATGGGCTTCTTAGACC | |
| FANCG_ex15F | gtaaaaacagccgcaaTTTGTGAAGATAGGAGAAGCAGAG | 454 |
| FANCG_ex15R | caggaaacagctatgTTTGTGAAGATAGGAGAAGCAGAG | |
| FANCG_ex16F | gtaaaaacagccgcaaAGCTCCATGTTCACCTCTTCCTC | 397 |
| FANCG_ex16R | caggaaacagctatgAGCTCCATGTTCACCTCTTCCTC | |

(f)

| Primer name | Sequence (5′ > 3′) | Product length (bp) |
|-------------|---------------------|---------------------|
| FANCB_ex3AF | gtaaaaacagccgcaaGATATGGTTATTGGAATTCTTAGCA | 721 |
| FANCB_ex3AR | caggaaacagctatgGCCATCCTTCCATCTCATAGGCTTAGT | |

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Anemia
supplier (www.mlpa.com). In a well-equipped laboratory with sufficient dedicated personal, testing of FANCA, -C, -E, -F, -G and -B can be completed within 1-2 weeks.

After screening FANCA, -C, -E, -F, -G, and -B, a molecular diagnosis is obtained for ~85% of the patients [34]. In our cohort of 54 patients, referred to our diagnostic service since 2008, mutations were detected in 45 patients (83%). FANCA mutations were found in 31 of the patients (57%), FANCC mutations in 6 patients (11%), and FANCG mutations in 5 patients (9%). FANCB, FANCE, and FANCF mutations were found in single families (Table 3). In the small group of patients without mutations no complementation analysis or FANCD2 western blotting was performed. Therefore, we do not know if we missed FANCA, -C, -E, -F, -G, and -B mutations in these patients or that these patients have mutations in other FA genes. Table 3 does not include prenatal cases, because prenatal testing is only offered in couples in which the FA-causing mutations are already established. Testing was offered as a diagnostic service for which a fee was charged.

For the patients negative for FANCA, -C, -E, -F, -G, and -B mutations, next generation sequencing can be used to analyze all other FA genes. If this technique is not available, further analysis will depend on the availability of growing cells from the proband. In that case a western blot should reveal whether both FANCD2 isoforms are present at normal levels.

(1) If both FANCD2 bands are absent or very weak, FANCD2 is sequenced. Because of the presence of FANCD2 pseudogene sequences in the genome, this testing must be performed on cDNA or gDNA using specially designed primers [26].

(2) If only the short isoform of FANCD2 is present, FANCL and FANCN are sequenced. If no mutations are found, the patient may be mutated in FANCJ or in another unidentified FA gene acting upstream of FANCD2.

(3) If both isoforms are present, and if the clinical phenotype is compatible with FA-D1 or FA-N, BRCA2/FANCD1 and PALB2/FANCN are screened by MLPA and DNA sequencing.

(4) If negative, BRIP1/FANCJ, PALB2/FANCN, RAD51C/ FANCO, and SLX4/FANCN are sequenced.

(5) If negative again, the patient should be screened for mutations in NBS1, ESCO2 and DDX11 to test for Nijmegen breakage syndrome, Roberts syndrome and Warsaw Breakage syndrome, respectively [47, 48]. The latter two syndromes can also be
excluded by analyzing metaphase spreads for sister chromatid cohesion defects. If again negative, the patient is likely to be mutated in a novel FA gene acting downstream of FANCD2 ubiquitination.

3. Notes

3.1. Mutation Screening in Mosaic Patients. If an available lymphoblastoid cell line from an FA patient is phenotypically normal due to genetic reversion at the disease locus, mutation screening is still possible in the reverted cell line, since at least one mutation will be present [49–51]. The second mutation may be identified through investigating the parents.

3.2. Unclassified Variants. Missense mutations or in-frame deletions or insertions should be judged using in silico prediction algorithms (SIFT, POLYPHEN2, Align GVGD). Alternatively, they can be tested for pathogenicity in a cellular transfection assay to check the ability of the variant gene product to complement the cellular FA defect in a deficient cell line (see e.g., [10, 35, 52]). Generally, these tests are only feasible in a setting where a diagnostic laboratory is equipped with a research laboratory with all necessary technology.

3.3. Functional Assignment to Genetic Subtypes. Retroviral constructs have been used to identify the FA subtype by functional complementation, as an intermediate step before a mutation screen is undertaken [36]. Although knowing the disease gene facilitates mutation screening, retroviral transduction has some drawbacks in comparison to direct mutation screening: (i) growing, MMC-sensitive cells either from a cell line or fresh blood sample are required, which are not always easy to obtain; (ii) overexpression of some FA proteins (e.g., FANCM and FANCP) may be toxic for cells; (iii) novel genetic subtypes that emerge after all known groups have been excluded and cannot be readily distinguished from false negatives, that is, transductions that for some unknown reason have failed to cause complementation; (iv) the method requires relatively advanced laboratory facilities and technology. However, functional assignment of complementation group can rapidly be provided by laboratories with capability for this type of analysis [37], which has greatly facilitated reliable genotyping for over 95% of FA patients for which viral constructs are available.

3.4. Genetic Counseling. All patients with a diagnosis of FA confirmed by mutation analysis should be referred for genetic counselling, together with their parents and siblings. Mutation testing should be performed in all sibs regardless of any clinical symptoms. A complete pedigree, including a cancer history anamnesis, should be prepared. Mutation carriers might be at increased cancer risk (see Section 3.7) whose aspect should be included in the counseling (see Section 3.7).

FA patients themselves usually have decreased fertility. Women usually have late menarche, irregular menses, and early menopause. However, pregnancies in women with FA have been described, and therefore women should be adequately informed about the risks for their offspring, which is mainly related to an increase in pregnancy-related complications [53].

Sibs of the parents of an FA patient often request carrier screening to assess their risk of getting a child with FA. If a sib appears to be carrier, this risk is still minimal because of the very low carrier frequency in the population. In the US the carrier frequency has been estimated to be about 1 in 181 [54]. The risk of a proven carrier to get a child with FA is therefore about 1 in 724. However, in small communities or in consanguineous couples this risk is much higher, and mutation screening in spouses of proven carriers may be indicated.

3.5. Prenatal Diagnosis. Prenatal diagnosis of FA is relatively straightforward after the pathogenic mutations in a given family have been identified. Fetal cells can be obtained by chorionic villus sampling (CVS) during weeks 10–12 of the pregnancy or by amniocentesis, which is performed between weeks 14 and 16. However, CVS may be preferred as the diagnosis will be known at an earlier stage. If the mutation is not known, a chromosomal breakage test on fetal material may be performed [55], but this test may be considered less reliable than screening for mutations in the fetal material. Alternatively, flow cytometric testing of MMC sensitivity in amniotic cell cultures might be an option; however this technique is only available in a limited number of specialized laboratories [56]. Occasionally, FA may be suspected by fetal ultrasound imaging and confirmed by parental carrier testing when the family is not yet known to carry a risk for FA [57].

3.6. Genotype-Phenotype Correlation. FA is considered as one disease, and the question may be raised whether all fifteen genetic subtypes equally conform to the clinical FA phenotype. Genotype-phenotype correlation studies comparing the 3 most common groups A, C, and G indicated modest phenotypic differences, which were rather correlated with the relative severity of the mutations [23]. However, bias due to the ethnic distribution of the studied population is very well possible. Other studies reported significant differences between FA-A/G versus FA-C [58]. Cases in group FA-D1 (mutated in BRCA2) and FA-N (mutated in PALB2) present with a distinct, relatively severe, phenotype that is characterized by the development of leukemia at very young age (median 2.2 years) and by pediatric cancers such as nephroblastoma (Wilms tumor) or medulloblastoma [40–44]. The observations that one of the pathogenic mutations in BRCA2 in FA-D1 patients is hypomorphic and that mice with biallelic null alleles in Brca2 are embryonic lethals suggest that the BRCA2 protein serves a function that is essential for survival.

Different mutations in the same gene may be associated with divergent phenotypes, as illustrated by the two FANCC mutations, c.711+4A>T and c.67delG. The former (splice-site) mutation is associated with a relatively severe phenotype in Ashkenazi Jewish people [19] although the associated phenotype was reportedly less severe in patients of Japanese
ancestry [20]. The carrier frequency for this mutation in the Ashkenazi population is relatively high (1 in 87), which has led to the recommendation of carrier detection to prevent disease [59]. In the Netherlands more than 50% of FA cases are homozygous for the FANCC frameshift mutation c.67delG. The phenotype associated with this mutation, like other exon 1 mutations, seems relatively mild, as these patients rarely have skeletal abnormalities and show a relatively late age of onset of their marrow failure [24]. Awareness of such genetically determined phenotypic differences may help in clinical decision making, including the counselling of patients and families.

### 3.7. Cancer Risk in Heterozygous Mutation Carriers

An important issue is whether FA mutation carriers are at increased risk to develop cancer or other types of disease. Overall, there is no increased risk for cancer among FA heterozygotes [60, 61]. However, the situation is different in some of the less prevalent FA subtypes. The FA-D1 subtype is caused by mutations in BRCA2 [62] which is a well-known breast and ovarian cancer predisposition gene [63]. In FA-D1 one of the mutations will be hypomorphic because biallelic “severe” mutations are supposed to be lethal [26]. Therefore, one of the parents of a FA-D1 patient will be a heterozygous carrier of a “severe” inactivating BRCA2 mutation and may thus have an increased risk for breast cancer and other BRCA2-associated cancers. Whether the parent with the hypomorphic mutation is also at increased risk is unknown: in breast cancer families these hypomorphic mutations are considered as variants with unknown clinical significance. Two other genes involved in FA and related to breast and ovarian cancer predisposition are PALB2/FANCN [64, 65] and RAD51C/FANCO [66]. Although cancer patients have been identified with germ-line mutations in these genes, an accurate estimate of the relative cancer risk for mutation carriers is still lacking.

Another special case is represented by female FANCB mutation carriers, who are supposed to consist of 50% FA-like cells due to silenced expression of the wild type FANCB allele by the random process of X inactivation that occurs during early embryonic development. Nevertheless, in the few female FANCB mutation carriers studied so far, inactivation appeared strongly skewed towards the mutated allele [67]. This suggests that FA cells have a poor chance to survive next to unaffected cells in the same tissue, and these FA cells may therefore not give an increased cancer risk. However, the data are scarce at present so that no firm conclusions can be drawn regarding the cancer risk of female FANCB mutation carriers [60].

### Conflict of Interests

The authors do not declare any conflict of interests related to this study.

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