Pathogenic mutations identified by a multimodality approach in 117 Japanese Fanconi anemia patients

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Supplemental Data

Supplemental Note: Case 66 Presentation

A 23-year-old man was admitted to the hospital because of a giant mediastinal tumor. He was born to unrelated healthy parents and had no significant past medical history. Physically, he presented with short stature (155cm, -2.7SD) and severe microcephaly (49.4cm, -5SD). Hematological and bone marrow examination were normal (neutrophils, 3.88×10⁹/L; hemoglobin, 14.1 g/dl; hematocrit, 42.2%; reticulocytes, 1.5%; platelets 244×10⁹/L). On the basis of a CT scan and mediastinal tumor biopsy, he was diagnosed with mediastinal T-cell lymphoblastic lymphoma (T-LBL). Induction chemotherapy consisting of cyclophosphamide, vincristine, daunorubicin, prednisone, and L-asparaginase was performed and he suffered from severe sepsis due to prolonged pancytopenia. After hematological recovery, a mitomycin C induced chromosomal breakage test showed an increased rate of chromosomal breakage. Targeted-exome sequencing identified the splice site mutation c.475+1G>A and the missense mutation c.7847C>T in FANCD1 (BRCA2). He was diagnosed as Fanconi anemia.

After induction chemotherapy, the T-LBL achieved a partial remission but he was found to have adenosquamous lung cancer and bilateral renal tubule-papillary adenoma. He underwent focal radiation therapy to the mediastinal lesion and reduced-intensity chemotherapy. However, he relapsed and died of T-LBL 15 months after the initial chemotherapy.
### Supplemental Table 1. Summary of 22 FA-related genes

| FA gene (Alternative name) | OMIM No. | FA/FBOC/FA-like* | Functions | this study (2018) | Rockefeller Fanconi Anemia Mutation Database (2008) | National Network of the Italian Association of Pediatric Hematology and Oncology (2014) |
|---------------------------|----------|-----------------|-----------|------------------|----------------------------------------------------|-----------------------------------------------------------------------------------|
| FANCA                     | 607139   | FA              | Component of the FA core complex | 58% | 57% | 85% |
| FANCB                     | 300515   | FA              | Component of the FA core complex | 3% | 0.9% | 1% |
| FANCC                     | 613899   | FA              | Component of the FA core complex | 1% | 15% | 3% |
| FANCD1 (BRCA2)            | 600185   | FBOC, FA       | HR repair, mediator function for RAD51, Protects stalled replication fork | 2% | 2.9% | 0% |
| FANCD2                    | 613984   | FA              | Monoubiquitylated by the FA core complex, Forms ID2 complex, Regulates the DNA damage response | 0% | 3.9% | 2% |
| FANCE                     | 613976   | FA              | Component of the FA core complex | 1% | 2.3% | 0% |
| FANCF                     | 613897   | FA              | Component of the FA core complex | 1% | 2% | 0% |
| FANCQ                     | 620956   | FA              | Component of the FA core complex | 25% | 11.0% | 9% |
| FANCI                     | 611360   | FA              | Monoubiquitylated by the FA core complex, Forms ID2 complex, Regulates the DNA damage response | 2% | 1.7% | 0% |
| FANCI (BRIP1)             | 605882   | FBOC, FA       | HR repair, DNA helicase | 0% | 2.4% | 0% |
| FANCL                     | 608111   | FA              | Component of the FA core complex, E3 ubiquitin ligase | 0% | 0.1% | 0% |
| FANCN (PALB2)             | 610355   | FBOC, FA       | Component of the FA core complex, DNA translocase | 0% | 0% | 0% |
| FANCO (RAD51C)            | 6092774  | FBOC, FA-like  | RAD51 paralog, HR repair, Stabilizes RAD51 nucleoprotein filament | 0% | ___*** | 0% |
| FANC (SLX1)               | 613278   | FA              | Resolves Holliday junctions, Nuclease regulation,  | 2% | ___*** | 0% |
| FANCP (ERCC4)             | 133520   | FA              | Incises DNA-ICL damage | 0% | ___*** | 0% |
| FANCR (RAD51)             | 179017   | FA-like         | HR repair, Protects stalled replication fork | 0% | ___*** | ___*** |
| FANCS (BRCA1)             | 113705   | FBOC, FA-like  | HR repair, Promotes RAD51 recruitment | 0% | ___*** | ___*** |
| FANCT (UBE27)             | 610638   | FA              | E2 ubiquitin-conjugating enzyme | 2% | ___*** | ___*** |
| FANCU (XRCC2)             | 600375   | FA-like         | RAD51 paralog, HR repair, Stabilizes RAD51 nucleoprotein filament | 0% | ___*** | ___*** |
| FANCV (MAD2L2/REV7)       | 604094   | FA              | Translesion DNA synthesis | 0% | ___*** | ___*** |
| FANCW (RFWD3)             | 614151   | FA              | HR repair, E3 ligase | 0% | ___*** | ___*** |

* FA-like genes cause a chromosome fragility syndrome with FA-related malformations but without bone marrow failure.3
** FANCM was originally thought to be FA gene but it turned out that biallelic FANCM mutations do not cause any overt FA phenotype but early onset cancer.5,6
*** These genes were not identified at the time of the publication.

FA, Fanconi anemia; FBOC, familial breast and ovarian cancer; HR, homologous recombination; ID2 complex, FANCD2-FANCI heterodimer; ICL, interstrand crosslink.
| Family No. | Case No. | Sex | Affected gene | Methods for identifying the mutations | Mutation 1 | Mutation 2 | ALDH2 genotype* | Hematological/Oncologic phenotype | Age at BMF/Malignancy diagnosis (months) | FA-features** | VACTERL-H | References /Comments |
|-----------|----------|-----|---------------|---------------------------------------|------------|------------|----------------|----------------------------------|----------------------------------------|-------------|----------|----------------------|
| 1         | 1        | F   | FANCA         | WES                                   | c.2870G>A  | g.2722_27207CT>G | GG | MDS              | 121/535                           | Yes | No         | 5, 10                |
| 2         | 2        | M   | FANCA         | WES                                   | c.1303C>T  | g.2435C     | GA | AML              | unknown/289                       | Yes | No         | 8, 10                |
| 3         | 3        | F   | FANCA         | WES                                   | c.2170A>C  | g.2722T>P   | GA | MDS              | unknown/143                       | Yes | No         | 8, 10                |
| 4         | 4        | M   | FANCA, MLPA   | WES, MLPA                             | c.2546delC | g.8849FlxX40 | GA | AA               | 37                                | Yes | No         | 5, 8, 10             |
| 5         | 5        | F   | FANCA         | WES                                   | c.1303C>T  | g.2435C     | AA | AA               | 26                                | Yes | No         | 5, 8, 10             |
| 6-1       | 6-1      | M   | FANCA         | WES                                   | c.3765+1G>T | g.2602toX12 | AA | AA               | 96                                | Yes | No         | 5, 10                |
| 6-2       | 6-2      | F   | FANCA         | MLPA                                 | c.3765+1G>T | g.2602toX12 | AA | AA               | 51                                | Yes | No         | 5, 10                |
| 7         | 7        | M   | FANCA, Sanger | WES, Sanger                           | c.4240_4241delAG | g.2602-1G>A | ab | AA               | 41/115                           | Yes | No         | 5, 7, 8, 10          |
| 8         | 8        | M   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | GA | AA               | 38                                | Yes | No         | 5, 8, 10             |
| 9-1       | 9-1      | M   | FANCA, Sanger | MLPA, Sanger                          | c.978,979delGA | g.2435C     | AA | MDS              | 60/192                           | Yes | No         | 5, 7, 10             |
| 9-2       | 9-2      | F   | FANCA, Sanger | MLPA, Sanger                          | c.978,979delGA | g.2435C     | AA | AA               | 92                                | Yes | No         | 5, 7, 10             |
| 9-3       | 9-3      | F   | FANCA         | MLPA                                 | c.978,979delGA | g.2435C     | AA | AA               | 45                                | Yes | No         | 5, 7, 10             |
| 10        | 10-1     | F   | FANCA         | WES                                   | c.2602-2A>T | g.2602toX12 | AA | AA               | 120                               | Yes | No         | 5, 8, 10             |
| 10-2      | 10-2     | F   | FANCA         | WES                                   | c.2602-2A>T | g.2602toX12 | AA | AA               | 48                                | Yes | No         | 5, 10                |
| 11        | 11       | M   | FANCA         | WES                                   | c.3569C>T  | g.2190X     | AA | AA               | 297                               | Yes | No         | 5, 10                |
| 12        | 12       | M   | FANCA         | WES                                   | c.3919_3920delT | g.2435C     | AA | MDS              | 144/145                           | Yes | No         | 5, 10                |
| 13        | 13       | F   | FANCA         | WES                                   | c.2546delC | g.8849FlxX40 | AA | AA               | 72/72                            | Yes | No         | 5, 7, 8, 10          |
| 14        | 14       | M   | FANCA         | WES                                   | c.2602-2A>T | g.2602toX12 | AA | AA               | 134                               | Yes | No         | 5, 8, 10             |
| 15        | 15       | F   | FANCA         | WES                                   | e.1007+2A>GT | g.2602toX12 | AA | AA               | 48/60                            | No  | No         | 10                  |
| 16        | 16       | F   | FANCA         | WES                                   | c.2546delC | g.8849FlxX40 | AA | AA               | 24                                | Yes | No         | 5, 7, 8, 10          |
| 17        | 17       | F   | FANCA         | WES                                   | e.190_191insT | g.2190X     | AA | AA               | 77                                | Yes | No         | 5, 10                |
| 18        | 18-1     | M   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | AA | MDS              | 0/12                             | Yes | No         | 5, 7, 10             |
| 18-2      | 18-2     | F   | FANCA         | Targeted-Seq                          | c.2546delC | g.8849FlxX40 | AA | MDS              | 69/69                            | Yes | No         | 7, 8, 10             |
| 19-1      | 19-1     | M   | FANCA         | Targeted-Seq                          | c.283+2T>C | g.2730_27310delCT | AA | AA               | 30                                | Yes | No         | 5, 8, 10             |
| 19-2      | 19-2     | M   | FANCA         | Targeted-Seq                          | c.283+2T>C | g.2730_27310delCT | AA | AA               | 16                                | Yes | No         | 7, 8, 10             |
| 20        | 20       | F   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | AA | MDS              | 77                                | Yes | No         | 5, 10                |
| 21        | 21       | F   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | AA | AML              | 21                                | Yes | No         | 5, 7, 10             |
| 22-1      | 22-1     | F   | FANCA         | Sanger                               | c.2840C>G  | g.2730_27310delCT | AA | AA               | 106                               | Yes | No         | 5, 10                |
| 22-2      | 22-2     | M   | FANCA         | Sanger                               | c.2840C>G  | g.2730_27310delCT | AA | AA               | 28/168                           | Yes | No         | 5, 10                |
| 23        | 23       | F   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | AA | AA/HNSCC          | 53/457                           | No  | No         | 5, 10                |
| 24        | 24       | M   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | AA | AA               | 22                                | Yes | No         | 5, 7, 10             |
| 25        | 25       | M   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | AA | AA               | 78                                | Yes | No         | 5, 7, 10             |
| 26        | 26       | M   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | AA | AML              | 114                               | Yes | No         | 5, 10                |
| 27        | 27       | F   | FANCA         | Sanger                               | c.2546delC | g.8849FlxX40 | AA | AML              | 62/311                           | Yes | No         | 5, 7, 10             |
| 28        | 28       | F   | FANCA         | Sanger                               | c.4124_4125delCA | g.2435C     | AA | AA               | 156/156                           | Yes | No         | 5, 10                |
| 29        | 29       | F   | FANCA         | Sanger                               | c.3765+1G>T | g.2435C     | AA | AA               | 72                                | Yes | No         | 5, 10                |
30 30 F FANCA Sanger c.2546delC p.S849FfsX40 c.2546delC p.S849FfsX40 GA AA 70 Yes Yes 5, 7, 10
31 31 F FANCA Sanger c.2546delC p.S849FfsX40 c.1567+1G>A aberrant splicing# GA MDS 82/82 Yes No 5, 10
32 32 F FANCA Sanger c.2546delC p.S849FfsX40 c.3720_3724del p.E1240fsX36 GA AA 88 Yes No 5, 10
33 33 M FANCA Sanger c.2546delC p.S849FfsX40 c.3720_3724del p.E1240fsX36 GA MDS 68/105 Yes No 10
34 34 F FANCA Sanger c.2546delC p.S849FfsX40 c.2602-1G>A aberrant splicing GA AML 60/282 Yes No 10
35 35-1 M FANCA Sanger c.2546delC p.S849FfsX40 c.2546delC p.S849FfsX40 AA MDS 0/4 Yes No 7, 10
35-2 M FANCA Sanger c.2546delC p.S849FfsX40 c.2546delC p.S849FfsX40 GA AA 21 Yes No 7, 10
36 36 M Sanger c.44_69del p.T13RxX13 c.2170A>C p.T724P GA MDS/4NSCC 108/348/348 Yes No 5, 10
37 37 F Sanger c.2546delC p.S849FfsX40 c.3296C>T p.Q1099X MDS 49/189 Yes Yes 5, 10
38 38 M WES, MLPA c.2840C>G p.S947X ex24-28del - GA AA 60 Yes No 5, 10
39 39 F FANCA Targeted-seq, MLPA Targeted-seq, MLPA c.462T>G p.Y154X ex6del - GA AA unknown No No 8
40 40 F Sanger c.2602-1G>A aberrant splicing# GA AML 108/384 Yes No 5, 10
41 41 M FANCA, MLPA c.2546delC p.S849FfsX40 ex37del - GA AML 136/736 Yes No 5, 10
42 42 F WES, MLPA c.4190G>C p.R1400P ex16_17del - GA AML 61/51 Yes No 5, 10
43 43 M Targeted-seq Targeted-seq c.2T>C p.M1T c.15G>A p.W6X GA AA 37 No No 7, 10
44 44 M Targeted-seq Targeted-seq c.2546delC p.S849FfsX40 c.2972delT p.P991fsX35 GA MDS 50/73 Yes Yes 7, 10
45 45-1 F Targeted-seq Targeted-seq c.4164C>A ex1_43del ex19_29del - GA AA 108 Yes No 10
45-2 F FANCA Targeted-seq, MLPA Targeted-seq, MLPA ex1_43del ex19_29del - GA AA 12 Yes No 10
46 46 F WES, MLPA WES, MLPA c.2546delC p.S849FfsX40 ex1_5del - GA AA unknown Yes No New case
47 47 M WES c.2546delC p.S849FfsX40 c.2546delC p.S849FfsX40 GA unknown unknown unknown unknown unknown 8
48 48 F WES c.4015_4017del CTC c.3638_3639delCTC p.L1213fsX64 p.P1133fsX64 GA unknown unknown unknown unknown unknown 8
49 49 F WES c.2546delC p.S849FfsX40 c.2546delC p.S849FfsX40 GA AA 71 Yes No 8
50 50 F WES c.2546delC p.S849FfsX40 c.2546delC p.S849FfsX40 GA AA 71 Yes No 8
51 51 F WES c.1464C>A c.1464C>A p.Y488X p.Y488X GA AA 157 Yes No 8
52 52 F WES, MLPA c.978_979delGA p.Q326fsX12 ex30del - GA unknown unknown unknown unknown unknown New case
53 53 M WES, MLPA c.978_979delGA p.Q326fsX12 ex30del - GA unknown unknown unknown unknown unknown New case
54 54 F WES c.2546delC p.S849FfsX40 not detected - GA AA 85 No No 8
55 55 F WES, MLPA c.2546delC p.S849FfsX40 not detected - GA AA 80 Yes No 8
56 56 F FANCA c.2316_2317+2T>A aberrant splicing# GA AA 59 Yes No 8
57 57 F Sanger c.2546delC p.S849FfsX40 not detected - GA MDS unknown/234 No No New case
58 58 F FANCA c.2546delC p.S849FfsX40 not detected - GA AA 82 Yes No New case
59 59 F FANCA c.2546delC p.S849FfsX40 not detected - GA AA 80 Yes No New case
60 60 M FANCR aCGH, Sanger complete loss - - GA AA 58 Yes Yes 5
61 61 M FANCR aCGH, Sanger complete loss - - GA MDS 24/51 Yes Yes 5, 10
62 62 M FANCR WES, RNA-seq c.1497G>T aberrant splicing (p.S500fsX14) - GA AA 96 Yes No 5
63 63 M FANCR WES c.516G>A p.W172X - not examined unknown unknown unknown unknown 8

**Current study identified causative FA gene mutation.**
| #  | Sex | Gene | WGS | cDNA change | Protein change | Splice change | AA | Gene change | Gene comment |
|----|-----|------|-----|-------------|---------------|--------------|----|-------------|--------------|
| 64 | F   | FANCC| WGS | c.1154+6G>A | aberrant splicing | p.(S386X) | 40 | AA | No | cancer gene mutations. |
| 65 | F   | FANCD2| WES | c.617+2A>G | aberrant splicing | p.R231X | No | 9 | No | 7 | Cancer gene mutations. |
| 66 | M   | FANCD2| Targeted-seq | c.478+1G>A | aberrant splicing | p.S261X | No | 9 | No | 5 | Current case identified cancer gene mutations. |
| 67 | F   | FANCC| WES | c.419T>C | aberrant splicing | p.L140P | Yes | 9 | Yes | 5 | Cancer gene mutations. |
| 68 | F   | FANCC| WES | c.484_485delCT | aberrant splicing | p.Y22X | Yes | 9 | Yes | New case |
| 69 | M   | FANCC| WES | c.1066C>T | aberrant splicing | p.G356X | Yes | 9 | Yes | New case |
| 70 | F   | FANCC| WES | c.1066C>T | aberrant splicing | p.G356X | Yes | 9 | Yes | New case |
| 71 | M   | FANCC| WES | c.1066C>T | aberrant splicing | p.G356X | Yes | 9 | Yes | New case |
| 72 | M   | FANCC| WES | c.1066C>T | aberrant splicing | p.G356X | Yes | 9 | Yes | New case |
| 73 | M   | FANCC| WES | c.307+1G>C | aberrant splicing | p.A101X | Yes | 9 | Yes | New case |
| 74 | F   | FANCC| WES | c.1066C>T | aberrant splicing | p.G356X | Yes | 9 | Yes | New case |
| 75 | M   | FANCC| WES | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 76 | F   | FANCC| WES | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 77 | F   | FANCC| WES | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 78 | F   | FANCC| WES | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 79 | F   | FANCC| WES | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 80 | M   | FANCC| WES | c.1066C>T | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 81 | F   | FANCC| WES | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 82 | M   | FANCC| WES | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 83 | F   | FANCC| WES | c.1066C>T | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 84 | M   | FANCC| Sanger | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 85 | M   | FANCC| Sanger | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 86 | F   | FANCC| Sanger | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 87 | M   | FANCC| Sanger | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 88 | M   | FANCC| WES | c.1886delC | aberrant splicing | p.W463SfsX5 | Yes | 9 | Yes | New case |
| 89 | F   | FANCC| Sanger | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 90 | M   | FANCC| WES | c.194delC | aberrant splicing | p.P65LeX7 | Yes | 9 | Yes | New case |
| 91 | F   | FANCC| WES | c.1066C>T | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 92 | M   | FANCC| Sanger | c.194delC | aberrant splicing | p.P65LeX7 | Yes | 9 | Yes | New case |
| 93 | M   | FANCC| Sanger | c.107+1G>C | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 94 | M   | FANCC| Sanger | c.1066C>T | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 95 | M   | FANCC| Sanger | c.1066C>T | aberrant splicing | p.Q356X | Yes | 9 | Yes | New case |
| 96 | M   | FANCC| WES | c.168-2A>G | aberrant splicing | p.S56fsX10 | Yes | 9 | Yes | New case |
| 97 | M   | FANCC| WES | c.3346_3347insT | aberrant splicing | p.S1116fsX16 | Yes | 9 | Yes | New case |
| No. | 98 | 99 | 100 | 101 | 102 | 103 | 104 |
|-----|----|----|-----|-----|-----|-----|-----|
| Genes | FANCN | FANCN | FANCN | FANCN | FANCN | FANCN | FANCN |
| Substitution Method | WES, RNA-seq | WES | Sanger | WES, aCGH | WES | WES | WES |
| cDNA Mutation | c.3350+5C>T | c.343delA | c.434delA | c.343delA | c.4C>G | c.4C>G | c.4C>G |
| AA | aberrant splicing (p.G1068VfsX5) | aberrant splicing (p.G1068VfsX5) | aberrant splicing (p.G1068VfsX5) | complete loss | aberrant splicing (p.E37RfsX49) | complete loss | not examined |
| GA | FANCN | FANCN | FANCN | FANCN | FANCN | FANCN | FANCN |
| FA features include physical abnormalities such as short stature, malformations or skin pigmentation. **FA features include physical abnormalities such as short stature, malformations or skin pigmentation.**

Novel mutations (not included in the Rockefeller FA mutation data base) are indicated in boldface type.

Effects of these splicing mutations are unverified.

5. **FA** features include physical abnormalities such as short stature, malformations or skin pigmentation.

AA, aplastic anemia; **ALDH2** wild type and the inactivating mutation (p.Glu504Lys) allele is referred to as G and A, respectively.

References in this table (the numbers are the same as the references in the main text)

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| DNA change | Location | Effect | Comments | No. of alleles | No. of patients | No.of unrelated families |
|------------|----------|--------|----------|---------------|----------------|-------------------------|
| **missense mutations** | | | | | | |
| c.2T>C     | exon 1   | p.M1T  | known mutation | 1 | 1 | 1 |
| c.1303C>T  | exon 14  | p.R435C | known mutation | 3 | 2 | 2 |
| c.2170A>C  | exon 24  | p.T724P | known mutation | 2 | 2 | 2 |
| c.2290C>T  | exon 25  | p.R764W | known mutation | 1 | 1 | 1 |
| c.252G>T   | exon 27  | p.Y843D | known mutation | 1 | 1 | 1 |
| c.2723_2725TCT>GCC | exon 28 | p.LS908_909RP | novel mutation | 1 | 1 | 1 |
| c.396T>G   | exon 40  | p.V1322G | novel mutation | 1 | 1 | 1 |
| c.4198C>T  | exon 42  | p.R1400C | known mutation | 2 | 2 | 1 |
| c.4199G>C  | exon 42  | p.R1400P | known mutation | 1 | 1 | 1 |
| **nonsense mutations** | | | | | | |
| c.15G>A    | exon 1   | p.W5X  | known mutation | 1 | 1 | 1 |
| c.462T>G   | exon 5   | p.Y154X | novel mutation | 1 | 1 | 1 |
| c.505G>T   | exon 5   | p.E169X | known mutation | 1 | 1 | 1 |
| c.1464C>A  | exon 15  | p.Y488X | novel mutation | 2 | 1 | 1 |
| c.2840C>G  | exon 29  | p.R947X | known mutation | 2 | 2 | 2 |
| c.2870G>A  | exon 30  | p.W957X | known mutation | 1 | 1 | 1 |
| c.2985C>T  | exon 33  | p.Q1099X | novel mutation | 1 | 1 | 1 |
| c.3565C>T  | exon 36  | p.Q1190X | known mutation | 1 | 1 | 1 |
| **small insertions/deletions** | | | | | | |
| c.44_69del | exon 1   | p.P15RfsX40 | known mutation | 1 | 1 | 1 |
| c.190_191insT | exon 3 | p.Y154X | novel mutation | 2 | 1 | 1 |
| c.978_978delGA | exon 11 | p.Q988X | known mutation | 5 | 3 | 30 |
| c.2544delC | exon 27  | p.S843X | known mutation | 41 | 33 | 30 |
| c.2594delA | exon 27  | p.R846X | novel mutation | 1 | 1 | 1 |
| c.2730_2731delCT | exon 28 | p.W911D | known mutation | 2 | 2 | 1 |
| c.2972delT | exon 30  | p.F997X | known mutation | 1 | 1 | 1 |
| c.3638_3639del | exon 37 | p.P1212X | known mutation | 1 | 1 | 1 |
| c.3720_3724del | exon 37 | p.R1240X | known mutation | 2 | 2 | 2 |
| c.3781_3785del | exon 38 | p.F1261X | novel mutation | 1 | 1 | 1 |
| c.3919_3920insT | exon 39 | p.Q1307X | known mutation | 1 | 1 | 1 |
| c.3931_3932delAG | exon 39 | p.S1311X | novel mutation | 1 | 1 | 1 |
| c.4015_4017delCTC | exon 41 | p.L1339del | known mutation | 1 | 1 | 1 |
| c.4042_4043insC | exon 41 | p.L1348X | novel mutation | 2 | 2 | 1 |
| c.4124_4125delCA | exon 41 | p.T1375del | known mutation | 1 | 1 | 1 |
| c.4240_4241delAG | exon 42 | p.Q1414X | known mutation | 1 | 1 | 1 |
| **splicing mutations** | | | | | | |
| c.283+2T>C | intron 3 | aberrant splicing | novel mutation | 2 | 2 | 1 |
| c.1005-2A>G | intron 11 | aberrant splicing | novel mutation | 1 | 1 | 1 |
| c.1567-1G>A | intron 16 | aberrant splicing | novel mutation | 1 | 1 | 1 |
| c.2316+2T>A | intron 25 | aberrant splicing | novel mutation | 1 | 1 | 1 |
| c.2802-2A>G | intron 27 | aberrant splicing | known mutation | 6 | 5 | 4 |
| c.3025+1G>A | intron 27 | aberrant splicing | known mutation | 4 | 4 | 4 |
| c.3765+1G>T | intron 37 | aberrant splicing | known mutation | 2 | 2 | 1 |
| c.4168-1G>C | intron 41 | aberrant splicing | known mutation | 1 | 1 | 1 |
| c.4168-2A>G | intron 41 | aberrant splicing | known mutation | 3 | 3 | 2 |
| **large deletions** | | | | | | |
| ex1-3 del  | —        | —      | —        | 1 | 1 | 1 |
| ex1-5 del  | —        | —      | —        | 1 | 1 | 1 |
| ex1-28 del | —        | —      | —        | 1 | 1 | 1 |
| ex1-43 del | —        | —      | —        | 2 | 2 | 1 |
| ex6 del  | —        | —      | —        | 1 | 1 | 1 |
| ex6-17 del | —        | —      | —        | 1 | 1 | 1 |
| ex19-29 del | —        | —      | —        | 2 | 2 | 1 |
| ex24-28 del | —        | —      | —        | 1 | 1 | 1 |
| ex30 del  | —        | —      | —        | 8 | 8 | 5 |
| ex30-31 del | —        | —      | —        | 1 | 1 | 1 |
| ex37 del  | —        | —      | —        | 1 | 1 | 1 |
| c.3765+827_3814del | intron 37-exon 38 | — | novel mutation | 1 | 1 | 1 |
| **large duplication** | | | | | | |
| ex11-15 dupi | —        | —      | —        | 1 | 1 | 1 |
| **Total** | | | | | | 130 |
| DNA change       | Location | Effect       | Comments          | No. of alleles | No. of patients |
|-----------------|----------|--------------|-------------------|----------------|----------------|
| **nonsense mutations** |          |              |                   |                |                |
| c.91C>T         | exon 2   | p.Q31X       | known mutation    | 2              | 2              |
| c.1066C>T       | exon 8   | p.Q356X      | known mutation    | 15             | 10             |
| **small deletions** |          |              |                   |                |                |
| c.194delC       | exon 3   | p.P65LfsX7   | known mutation    | 3              | 2              |
| c.907_908del    | exon 7   | p.L303GfsX5  | novel mutation    | 1              | 1              |
| c.1386delC      | exon 10  | p.W463GfsX55 | novel mutation    | 1              | 1              |
| **splicing mutations** |         |              |                   |                |                |
| c.307+1G>C      | intron 3 | aberrant splicing | known mutation | 34             | 21             |
| c.1637-15G>A    | intron 12| VUS          | novel mutation    | 1              | 1              |
| **Total**       |          |              |                   | 57             |                |

variant of unknown significance
Supplemental Figure 1. Proposed model for mechanism of microhomology-mediated end joining (MMEJ) to repair DNA double-strand break (DSB). This repair model consists of at least five steps: resection of the DSB ends by nuclease digestion, annealing of 3bp homologous regions, removal of heterologous flaps, and fill-in synthesis and ligation. The mutation is speculated to be created by two DSBs and subsequent religation of the two distant ends by MMEJ repair.
Supplemental Figure 2. Display of a cross section of RNA-sequencing (top) and Whole-exome sequencing (bottom). RNA sequence reads of exon 7 in FANCB and exon 12 in FANCN were absent for Case 62 (A) and Case 98 (B), respectively, which enabled us to identify exon skipping. WES analysis revealed a synonymous mutation (FANCB c.1497G>T) in Case 62, resulting in skipping of exon 7, and a homozygous mutation (PALB2 c.3350+5G>A) in intron 12 in Case 98, resulting in skipping of exon 12. These mutation variants were also verified by PCR and Sanger sequencing.
Supplemental Figure 3. Localization of mutation variants found in FANCA (A) or FANCG (B).
Case 63: c.516G>A (p.W172X)  
Case 62: c.1497G>T (p.S500AfsX14)  

Case 60: complete loss of FANCB gene (chrX g.14730104-14904216 del)  
Case 61: complete loss of FANCB gene (chrX g.14810970-14932973 del)  

Case 96: c.158-2A>G (p.S54fsX5)  
c.288G>A (p.C56fsX8)  

Case 97: c.3006+3A>G  
c.3346_3347insT (p.S1116FfsX16)  

Case 65: c.517-2A>G  
c.6952C>T (p.R2318X)  
c.7847C>T (p.S2616F)  

Case 66: c.475+1G>A  

Case 98: Homozygous c.3350+5C>T (p.G1068VfsX5)  

Supplemental Figure 4. Localization of mutation variants in FANCB (A), FANCI (B), FANCD1 (BRCA2) (C), and FANCN (PALB2) (D).
Supplemental References

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