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REVIEW

Fanconi anaemia genes and susceptibility to cancer

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Fanconi anaemia (FA) is a rare recessive disorder associated with chromosomal fragility, aplastic anaemia, congenital abnormalities and a high risk of cancer, including acute myeloid leukaemia and squamous cell carcinomas. The identification of 11 different FA genes has revealed a complex web of interacting proteins that are involved in the recognition or repair of DNA interstrand crosslinks and perhaps other forms of DNA damage. Bi-allelic mutations in *BRCA2* are associated with a rare and highly cancer-prone form of FA, and the DNA helicase BRIP1 (formerly BACH1) is mutated in FA group J. There is little convincing evidence that FA heterozygotes are at increased risk of cancer, but larger studies are needed to address the possibility of modest risk effects. Somatic inactivation of the FA pathway by mutation or epigenetic silencing has been observed in several different types of sporadic cancer, and this may have important implications for targeted chemotherapy. Inhibition of this pathway represents a possible route to sensitization of tumours to DNA crosslinking drugs such as cisplatin.
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The Fanconi anaemia syndrome

Fanconi anaemia (FA) is an inherited disorder associated with progressive aplastic anaemia, multiple congenital abnormalities and predisposition to malignancies including leukaemia and solid tumours (Fanconi, 1967). The developmental abnormalities include radial aplasia, hyperpigmentation of the skin, growth retardation, microphthalmia and malformation of the kidneys. The disorder generally presents as aplastic anaemia between the ages of 5–10 years, but the diagnosis may be made much earlier if characteristic developmental abnormalities are present or if there is a family history, or much later if the haematological symptoms are very mild. FA is inherited mainly as an autosomal recessive trait, but is genetically heterogeneous, with multiple complementation groups that include an X-linked form (Meetei *et al*., 2004). It is a rare disease with an incidence of 1 in 200 000–400 000 live births (Joenje and Patel, 2001), and a heterozygote frequency of around 1 in 250. However, it is more common in some populations, with carrier frequencies of about 1 in 90 reported in Ashkenazi Jews and the Afrikaans-speaking population of South Africa (Rosendorff *et al*., 1987; Verlander *et al*., 1995). At the cellular level, FA is considered to be a chromosomal fragility syndrome. Cells from FA patients are hypersensitive to DNA interstrand crosslinking agents such as mitomycin C and diepoxybutane (Schroeder *et al*., 1964), and the increased chromosomal breakage elicited by these agents forms the basis for a laboratory diagnostic test. Clinical, genetic and functional aspects of FA have been the subject of detailed reviews (Joenje and Patel, 2001; Tischkowitz and Dokal, 2004; Thompson *et al*., 2005; Taniguchi and D'Andrea, 2006). The main focus of this review is the association of mutations in FA genes with susceptibility to cancer.

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Genetics of FA

Complementation analysis of cell lines from different FA patients has led to the description of at least 12 groups, named FA-A, B, C, D1, D2, E, F, G, I, J, L and M, with the corresponding genes named as *FANCA*-*FANCM* (Table 1). The FA genes were cloned using a variety of strategies including functional complementation of FA cell lines, positional cloning, candidate gene screening and the identification of novel proteins from immunoprecipitates of other FA proteins. The FA proteins form nuclear multi-protein complexes referred to collectively as the FA pathway (Taniguchi and D'Andrea, 2006). Interest in this pathway was stimulated by the discovery that the gene for *FANCD1* is *BRCA2* (Howlett *et al*., 2002); mono-allelic mutations in *BRCA2* cause susceptibility to breast and other cancers (this issue, 2006), whereas bi-allelic mutations cause Fanconi anaemia. Most FA genes have a wide spectrum of mutations in FA patients that include deletions, frameshifts, stop codons, splice-site mutations and missense mutations (Joenje and Patel, 2001). FA-A is the most common group world-wide, but there are population-specific differences as a result of founder mutations. Thus, for example, most Ashkenazi Jewish patients are from group C and are homozygous for a

Table 1 Fanconi anaemia genes and proteins

Gene	*Incidence in FA patients (%)*[a]	*Chromosomal location*	*Protein size kDa*	*Protein motif/function*
FANCA	66.0	16q24.3	163	NLS
FANCB	0.8	Xp22.31	95	NLS
FANCC	9.5	9q22.3	63	
FANCD1/BRCA2	3.3	13q12.3	384	NLS, BRC repeats, BLAT
FANCD2	3.3	3p25.3	166	NLS
FANCE	2.5	6p21.3	59	NLS
FANCF	2.0	11p15	42	
FANCG	8.7	9p13	68	
FANCJ/BRIP1	1.6	17q22–q24	130	DEAH-box helicase
FANCL	0.4	2p16.1	43	WD40, PHD-finger, ubiquitin ligase
FANCM/Hef	0.4	14q21.3	250	helicase, endonuclease?, translocase

FA genes and proteins. [a]From Levitus *et al*. (2004). *FANCH* has been withdrawn, *FANCI* has not yet been identified, and the *FANCK* symbol has not been utilized. NLS: nuclear localization signal.

splice-site mutation (IVS4 + 4A > T) in *FANCC* (Whitney *et al*., 1993), and other examples have been identified in Afrikaners, Spanish gypsies and sub-Saharan Africans (Tipping *et al*., 2001; Callen *et al*., 2005; Morgan *et al*., 2006).

There is some evidence for a correlation between either complementation group or mutation type and the severity of the clinical phenotype. The IVS4 + 4A > T mutation is associated with a severe FA phenotype in Ashkenazi Jews (Yamashita *et al*., 1996; Gillio *et al*., 1997), but the same mutation produces a much milder phenotype in Japanese patients, suggesting that modifier genes or environmental factors contribute to disease severity (Futaki *et al*., 2000). A comparison of the more common FA groups A, C and G found a higher incidence of acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) in FA-G (Faivre *et al*., 2000), and *FANCD1/BRCA2* mutations produce a particularly cancer-prone phenotype (see below). Variations in clinical severity are consistent with the finding that different patient-derived mutations in the *FANCA* gene have variable effects on the function of the FA pathway (Adachi *et al*., 2002). The clinical phenotype may also be modified by somatic mosaicism in which a functional *FANC* allele is generated by intragenic recombination or gene conversion in compound heterozygotes or, remarkably, in mutation homozygotes by a compensatory secondary mutation in *cis* which restored the function of the mutant allele (Waisfisz *et al*., 1999).

Function of the FA pathway

The association of the cellular phenotype of FA with increased chromosome breakage and hypersensitivity to DNA interstrand crosslinkers and of the clinical phenotype with a strong predisposition to cancer led to the assumption that the FA proteins had some role in the recognition or repair of DNA damage. However, the identification of the first FA genes provided few functional insights since their encoded proteins had no obvious connections with known protein families. Several did, however, contain nuclear localization signals, consistent with a role in DNA repair (Table 1). The identification of the *FANCD1* gene as *BRCA2* provided the first direct link between FA proteins and DNA repair, given this protein's involvement in homologous recombination and the regulation of the RAD51 recombinase. Some of the newer FA genes have been more informative, with *FANCL* having a PHD finger motif that is associated with ubiquitin ligase activity (Meetei *et al*., 2003), *FANCJ* (*BRIP1*) being a DNA-dependent ATPase and 5′–3′ helicase (Levitus *et al*., 2005; Levran *et al*., 2005), and *FANCM* being related to the archeal protein Hef, which has functional endonuclease and helicase domains (Meetei *et al*., 2005).

A group of at least eight FA proteins (A, B, C, E, F, G, L and M) form a nuclear complex (the FA core complex) that is required for the monoubiquitination of the FANCD2 protein. The ubiquitinated form of FANCD2 is translocated to sites of DNA damage in chromatin, where it is found in a complex with BRCA2/FANCD1 and FANCE (Wang *et al*., 2004), but its function is currently unknown. The FANCG protein also appears to function both inside and outside of the FA core complex, since it is found in a complex with BRCA2 and the homologous recombination repair protein XRCC3 that is independent of core complex formation or the ubiquitination of FANCD2 (Hussain *et al*., 2006). There appears to be extensive cross-talk between the FA pathway and other DNA repair pathways, since FA proteins have been found in complexes with BLM, RPA, TopoIIIα, NBS1, BRCA1 and ATR (reviewed by Surralles *et al*. (2004) and Taniguchi and D'Andrea (2006)). A broader function has been proposed for the FA proteins in monitoring oxidative stress and in the stabilization of stalled replication forks that arise during recombination and repair (Thompson *et al*., 2005). A current model of the FA pathway is shown in Figure 1. Although we now have more information about the nature and interactions of these proteins, it is clear that their precise roles in DNA repair have not yet been defined.

Other functions of individual FA proteins that are not directly related to DNA repair have been proposed, such

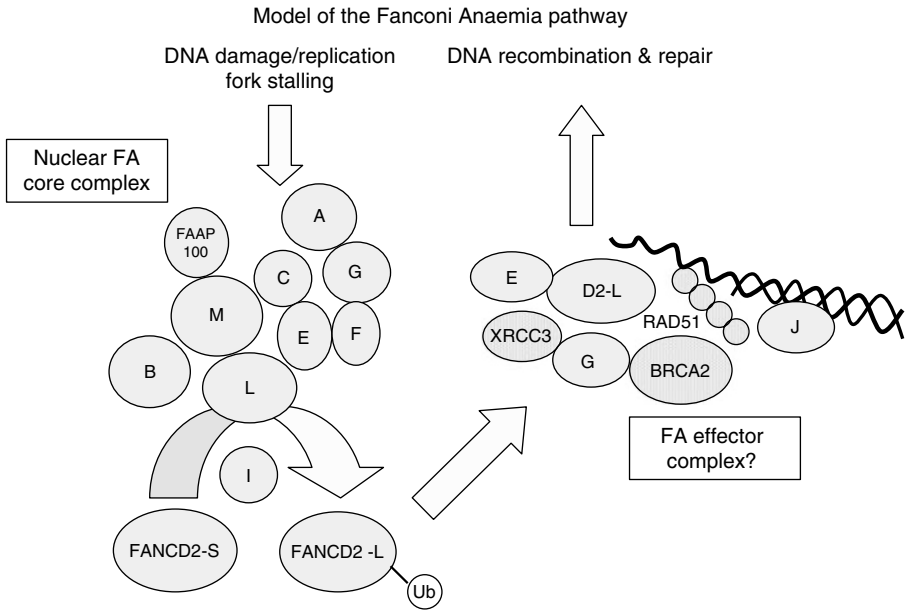

Figure 1 A model of the Fanconi Anaemia pathway. A large nuclear complex of at least eight FA proteins are required for the monoubiquitination of FANCD2. The FANCI protein, which has not yet been identified, does not appear to be needed for complex formation but is required for the modification of FANCD2. The Fanconi anaemia-associated protein FAAP100 has also not been identified, and may be encoded by a novel FA gene. The active ubiquitinated form of FANCD2 associates with DNA repair proteins such as BRCA2 in chromatin at sites of DNA damage, but its precise role is not known.

as prevention of oxidative damage by reactive oxygen species (Cumming *et al*., 2001), and in the regulation of apoptosis and signalling in response to cytokines and growth factors (Fagerlie *et al*., 2001). A large number of interactions between FA and other proteins have been described (Reuter *et al*., 2003), but the functional significance of many of these remains to be established.

Cancer in FA homozygotes

Cancer incidence

FA patients have a high risk of leukaemia and solid tumours. A literature review of over 1300 reported cases from 1927 to 2001 found that 9% had leukaemia, mainly AML, 7% had MDS, 5% had solid tumours and 3% had liver tumours (Alter, 2003). The distribution of the solid tumours was very unusual compared to the general population, since more than half were of the head and neck, oesophagus or vulva. The liver tumours were generally not malignant, and were thought likely to be related to prolonged androgen therapy. A retrospective cohort study of 145 FA patients showed that nine had developed AML and 14 had solid tumours, most of which were of the head and neck, oesophagus, vulva or cervix (Rosenberg *et al*., 2003). The ratio of observed to expected cancers was 50 for all cancers, 48 for solid tumours and 785 for leukaemia. The cumulative incidence to age 48 was 10% for leukaemia and 29% for a solid tumour. A larger study of 754 patients in the International Fanconi Anemia Registry (IFAR) found neoplasms in 23%, with haematological neoplasms (mainly AML or MDS) in 15.9% and non-haematological tumours in 10.5%, with about 3% of patients having more than one neoplasm during their lifetime (Kutler *et al*., 2003b). The most common non-haematological tumours were squamous cell carcinomas (SCC) of the head and neck, vulva and cervix. Importantly, only three of the 19 patients with a head and neck SCC had a history of alcohol or tobacco abuse, which are the major risk factors for these tumours. The cumulative incidence of haematological and non-haematological neoplasms was 33 and 28%, respectively by age 40. The standardized incidence ratio for head and neck SCC (HNSCC) in the IFAR registry was 500, with a median patient age of 31 years (Kutler *et al*., 2003a).These patients had poor tolerance for radiotherapy and chemotherapy, and the tumours were aggressive. Examples have been reported of young cases of apparently sporadic HNSCC with no known risk factors, who had a severe reaction to radiotherapy and who were later diagnosed as FA (Alter *et al*., 2005). There is also evidence that conditioning for stem cell transplants in FA patients is associated with an increased risk of SCC of the head, neck and oesophagus (Rosenberg *et al*., 2005).

Cancers in FA-D1

It is clear that FA is generally associated with strong predisposition to leukaemia and solid tumours, especially of the head and neck. However, clinical and molecular characterization of FA-D1 families with bi-allelic mutations in *BRCA2* has revealed an even stronger cancer-prone phenotype in the 16 confirmed D1 kindreds reported to date (Howlett *et al*., 2002; Offit *et al*., 2003; Hirsch *et al*., 2004; Wagner *et al*., 2004;

Reid *et al*., 2005). Early onset leukaemia was common, with a median onset at 2.2 years of age compared to 13.4 years for all other FA patients in the IFAR registry (Wagner *et al*., 2004); this was mainly AML, but there were also cases of T- and B-cell acute lymphoblastic leukaemia. There were nine cases with brain tumours (mainly medulloblastomas), and five with Wilms tumours (summarized in Reid *et al*. (2005)). It was proposed initially that FA-D1 would occur in individuals who had at least one partially functional *BRCA2* allele (Howlett *et al*., 2002). This would be consistent with the fact that complete knockout of *brca2* is an early embryonic lethal in the mouse (Ludwig *et al*., 1997; Sharan *et al*., 1997), whereas some mice homozygous for a C-terminal truncating *brca2* mutation are viable but succumb to lymphomas (Connor *et al*., 1997; Friedman *et al*., 1998). The frequency of *BRCA2* mutation carriers in the general population is estimated to be around 1 in 1000 (Thompson and Easton, 2004), which corresponds to a mutant allele frequency of 0.0005 and a predicted homozygote frequency in the absence of selection of 1 in 4 million. As the incidence of FA in most populations is around 1 in 250,000 and FA-D1 accounts for about 3.3% of all FA cases (Levitus *et al*., 2004), this corresponds to an incidence of FA-D1 of 0.53 in 4 million, which is about half of the predicted incidence. Thus, it is possible that only a subset of mutant *BRCA2* alleles is compatible with survival to term in humans. Perinatal lethality has been observed in *fancd2* and *fancc* knockout mice (Houghtaling *et al*., 2003; Carreau, 2004), so this may be a general feature of the FA pathway. However, an assessment of the location of *BRCA2* mutations in FA-D1 patients in comparison with *BRCA2* carriers does not support a bias towards carboxy-terminal mutations in FA-D1 (Reid *et al*., 2005). Also, the nature of the mutations in many of the reported FA-D1 cases would predict early truncation of the BRCA2 protein expressed from both alleles, and its absence has been confirmed in some cases by Western blot analysis (Hirsch *et al*., 2004).

Molecular basis and tissue specificity

The association of haematopoietic stem cell failure and a high risk of cancer with a chromosomal instability syndrome is not surprising since excessive chromosome breakage might be expected to lead to unrepaired DNA damage and apoptosis, or to mutations which could confer a selective growth advantage to a progenitor cell. Why cells of myeloid origin are particularly affected, leading to MDS or AML, is not known; perhaps myeloid precursors are particularly susceptible to forms of DNA damage that are recognized or repaired by the FA pathway. The unusual spectrum of solid tumours, with a preponderance of squamous cell carcinomas of the oral cavity and vulva or cervix is also unexplained. Evidence both for and against an involvement of human papilloma virus (HPV) in SCCs from FA patients has been presented (Kutler *et al*., 2003c; van Zeeburg *et al*., 2004). If even a significant minority of these tumours are HPV positive, there will be a strong case for prophylactic HPV vaccination in FA patients. Another possible route to carcinogenesis is an interaction of the environmental toxins to which the mucous membranes of the oral cavity and genital areas are exposed with a constitutional deficiency of the FA patient to detoxify such agents or to repair DNA damage consequent to the exposure. In a clinical context, it will be important to establish what proportion of young patients who have HNSCC in the absence of major risk factors are in fact undiagnosed FA, since this will have a major impact on their clinical management. The highly cancer-prone phenotype of FA-D1 patients is not unexpected given the central role occupied by BRCA2 in homologous recombination repair. However, the high incidence of brain tumours in these patients is not characteristic of FA patients in general, or of *BRCA2* mutation carriers, and remains to be explained, as does the association with familial Wilms tumour (Reid *et al*., 2005).

Cancer in FA heterozygotes

Epidemiology

The possibility that FA heterozygotes might be at increased risk of cancer was first suggested by Swift (1971), and it is a reasonable hypothesis that a modest reduction in DNA repair efficiency or loss of a second FA allele in some tissues might lead to the development of a tumour. Support for this hypothesis comes from the now well established increase in risk of breast cancer in heterozygotes for mutations in the *ATM* gene (reviewed in this issue, 2006). There is also some experimental support for differences in FA heterozygotes. The mean index of DEB-induced chromosome breakage was higher in obligate FA heterozygotes relative to controls (0.13 ± 0.06 versus 0.07 ± 0.09), although there was overlap between these groups (Pearson *et al*., 2001). Also, comet assays of FA heterozygotes showed an increased release of fragmented DNA after exposure to X-irradiation compared to controls (Djuzenova *et al*., 2001). However, Swift's original findings of an increased cancer risk in FA relatives were not confirmed in a later and larger study of relatives in 25 FA families (Swift *et al*., 1980). There were 48 deaths from cancer among blood relatives of the index cases, with 56.2 expected, and no excess of cancers were observed in obligate heterozygotes. A smaller study of the relatives of nine FA patients did not detect an increase in cancer risk (Potter *et al*., 1983). A recent study of 36 British families (Hodgson *et al*., 2004) found no increase in cancer in relatives of FA patients, with 55 cancers observed and 56.95 expected (O/E 0.97, 95% CI 0.71–1.23). Thus there is no current evidence from epidemiological studies that FA heterozygotes are at increased risk of cancer. However, the existing studies are small, and have insufficient power to detect a modest increase in cancer risk. The increase in the risk of breast and other cancers as a result of heterozygosity for *BRCA2* mutations in the FA-D1 group would be difficult to detect in such studies, as this represents only about 3% of all FA

families. A multi-centre, international collaboration is needed, and this could be strengthened by a study design that involved FA families with known mutations that would allow molecular testing for carrier status in relatives with a diagnosis of cancer. This would have the added advantage of assessing whether cancer risk was related to a subset of complementation groups and specific mutations.

Leukaemia

Another approach to this problem has been to look for germline FA gene mutations in cancer patients who do not have FA in the expectation that a subset of cancers in the general population may be associated with mutations in the FA pathway. A screen for *FANCC* mutations in remission blood samples from cases of sporadic childhood leukaemias did not detect any pathogenic mutations in 97 cases of AML or 91 of ALL, but there was a marginally significant increase in the frequency of a single nucleotide polymorphism (S26F) in the AML group (Barber *et al*., 2003). This group also analysed the *FANCG* gene in 107 children with sporadic AML and found a pathogenic splice site mutation in one case together with an unclassified variant (S588F), although whether the second mutation was in *cis* or in *trans* could not be determined (Meyer *et al*., 2006). Mutation screens of *FANCA* in adult sporadic cases of AML detected a low frequency of both missense mutations (Condie *et al*., 2002) and heterozygous exonic deletions (Tischkowitz *et al*., 2004), but remission samples were not available to determine whether these were germline or somatic mutations. Taken together, these studies provide little support for a contribution of inherited FA gene mutations to susceptibility to AML, but a comprehensive screen for mutations in all 11 of the known FA genes would be required to resolve this question.

Breast cancer

The major role of *BRCA2*/*FANCD1* in susceptibility to breast and other cancers such as cancer of the prostate and pancreas is well established (this issue, 2006). There have been several studies of the other known FA genes in relation to breast cancer susceptibility. Two different approaches have been used; one has been to look for highly penetrant FA gene mutations in families with multiple cases of breast cancer, and the other to analyse single nucleotide polymorphisms (SNPs) in FA genes for association with sporadic breast cancer. A comprehensive mutation screen of the *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF* and *FANCG* genes was carried out in 88 familial breast cancer families who were negative for BRCA1/2 mutations (Seal *et al*., 2003). No clear pathogenic mutations were detected, but two missense variants were found (one in FANCA and one in FANCE) that were absent in controls and segregated with breast cancer. The authors concluded that FA gene mutations, other than in BRCA2, are unlikely to make a significant contribution to the risk of familial breast cancer. The *FANCD2* gene was screened for mutations in an Australian cohort of 30 non-BRCA1/2 familial breast cancer families, but no pathogenic mutations were detected (Lewis *et al*., 2005). An association study of seven SNPs in the *FANCA*, *FANCL* and *FANCD2* genes was carried out to determine whether any of these SNPs conferred susceptibility to breast cancer in the Spanish population (Barroso *et al*., 2006). A marginally significant association of a synonymous SNP in *FANCD2* with breast cancer was observed, but independent replication of such findings is essential to validate their significance.

A gene that is variously known as *BACH1*/*BRIP1*/*FANCJ* and is located on chromosome 17q22-24 has been screened extensively for involvement in breast cancer susceptibility. This was originally identified as a protein that binds to the BRCT repeats of BRCA1, and was called BACH1 (Cantor *et al*., 2001). Its name was later changed to *BRIP1* (BRCA1 interacting protein 1) since, unfortunately, another gene that encodes a leucine zipper transcription factor and is located on chromosome 21q22.11 was also called *BACH1*. *BRIP1* was later identified as the gene that is mutated in FA-J patients, so is also called *FANCJ*. The original report on *BRIP1*/*BACH1* found two missense mutations (P47A and M299I) in early onset familial breast cancer cases (Cantor *et al*., 2001), and the P47A mutation was later shown to abrogate the ATPase and helicase activity of the protein (Cantor *et al*., 2004).

Several subsequent studies screened the *BRIP1* coding sequence in large numbers of *BRCA1*/*2* negative familial breast cancer families from populations in Northern Europe (Luo *et al*., 2002; Karppinen *et al*., 2003; Vahteristo *et al*., 2006), North America (Rutter *et al*., 2003) and Australia (Lewis *et al*., 2005). This resulted in the detection of one frameshift mutation and several missense mutations, none of which segregated with breast cancer in the families. The conclusion from these studies was that germline pathogenic mutations in *BRIP1* are extremely rare or absent in familial breast cancer.

SNPs in *BRIP1* were also tested for association with sporadic breast cancer. A study of 19 SNPs in eight DNA repair genes found a marginally significant association of homozygosity for a non-synonymous SNP, S919P, in *BRIP1* in breast cancer up to age 50 in a North American population, but the association was not significant when all cases up to age 70 were included (Sigurdson *et al*., 2004). The S919P SNP was not associated with breast cancer risk in two subsequent studies (Garcia-Closas *et al*., 2006; Vahteristo *et al*., 2006).

Pancreatic cancer

The involvement of *BRCA2* in the FA pathway and its known association with an increased risk of pancreatic cancer prompted mutation screening of other FA genes in sporadic and familial pancreatic cancer. Mutation analysis of the *FANCC* and *FANCG* genes in 44 pancreatic tumours from young onset cases selected for loss of heterozygosity (LOH) at these genes detected

the missense mutation D195V in *FANCC* (van der Heijden *et al*., 2003); the mutation was also present in the patient's germline DNA, but its effect on FANCC protein function is not known (Verlander *et al*., 1994). Some of the subsequent screens of germline DNA for mutations *FANCC*, *FANCG* and *FANCA* in familial pancreatic cancer did not detect any pathogenic mutations (Rogers *et al*., 2004a, b). However, mutation screening of *FANCC* and *FANCG* in germline DNA from 421 unselected pancreatic cases identified heterozygous truncating mutations in *FANCC* in two young onset cases, and none in *FANCG* (Couch *et al*., 2005). The tumours from these two cases had LOH of the wild-type allele, and no truncating *FANCC* mutations were detected in 658 controls. As *FANCC*, *FANCG* and *BRCA2*/*FANCD1* mutations together account for only about 21% of all cases of FA (Levitus *et al*., 2004), it is possible that constitutional heterozygosity for FA gene mutations may contribute to susceptibility to pancreatic cancer in a small but significant minority of young onset cases.

Somatic inactivation of the FA pathway

Acute myeloid leukaemia

Several studies have examined the question of whether somatic mutations in the FA pathway might contribute to the pathogenesis of sporadic cancers (Lyakhovich and Surralles, 2005). Reduced levels of FA proteins or absence of one or more FA proteins and their complexes was observed in five of 10 AML cell lines and 11 of 15 primary AML samples (Xie *et al*., 2000). One of the cell lines (CHRF-288) was hypersensitive to MMC and lacked the FANCF protein. A follow-up study of this cell line showed that the MMC sensitivity could be corrected by transfection with the *FANCF* gene, and that *FANCF* expression in these cells was silenced not by mutation, but by extensive methylation of its promoter region (Tischkowitz *et al*., 2003). Hypersensitivity to MMC and inactivation of the FA pathway with loss of FANCD2 ubiquitination and reduced amounts of nuclear FA proteins was reported in another AML cell line (Lensch *et al*., 2003). In this case, MMC sensitivity was partially corrected by transduction with the *FANCA* gene, but no mutation or methylation of *FANCA* was detected, so the basis of this acquired FANCA dysfunction was unresolved. The deletion of all or part of the *FANCA* gene in four of 101 adult sporadic AMLs was associated with a reduction in *FANCA* expression, but no pathogenic mutations were observed in the intact *FANCA* allele (Tischkowitz *et al*., 2004).

Other cancers

Interest in the possible involvement of the FA pathway in cancers other than AML was stimulated by the finding of cisplatin sensitivity and a lack of ubiquitinated FANCD2 in two of 25 ovarian cancer cell lines, which was corrected by transfection with *FANCF* (Taniguchi *et al*., 2003). The lack of *FANCF* expression was correlated with hypermethylation of the *FANCF* CpG island, and *FANCF* methylation was also observed in four of 19 primary ovarian tumours. A screen for *FANCF* hypermethylation using a methylation-specific PCR of bisulphite-modified DNA from primary tumours detected methylation in 13 of 89 head and neck SCCs and 22 of 158 non-small-cell lung carcinomas (Marsit *et al*., 2004), but the effect of this on the level of *FANCF* expression and the function of the FA pathway was not examined. *FANCF* hypermethylation was also observed in 27 of 91 (30%) primary cervical cancers, in three of nine cervical cancer cell lines and in 0 of 20 samples of normal cervical epithelia (Narayan *et al*., 2004); six of the nine cell lines had reduced expression of *FANCF* mRNA, and this correlated with hypersensitivity to MMC. Two pancreatic cancer cell lines have been described that are MMC sensitive and defective in *FANCD2* monoubiquitination (van der Heijden *et al*., 2004). One line had acquired a homozygous deletion of *FANCC* and the other a truncating mutation in *FANCG*, and in both cases the cellular FA phenotype could be corrected by transduction with the relevant wild-type FA gene.

The association of pathogenic mutations or epigenetic silencing of FA genes with MMC sensitivity and lack of monoubiquitinated FANCD2 in cell lines from some AMLs and ovarian and pancreatic cancers, coupled with restoration of a functional FA pathway upon transduction with the relevant FA gene, is quite compelling evidence that inactivation of this pathway has a role in the development of a subset of these types of tumour. However, since these well-characterized examples of loss of the FA pathway are in transformed cell lines, they could be late events in tumour progression. Detailed examination of the function of this pathway in primary tumours will be required to resolve the timing of these events.

A target for chemotherapy?

As DNA interstrand crosslinking (ICL) agents are an important class of chemotherapeutics, it is possible that selective inhibition of the FA pathway in tumours may sensitize them to drugs such as cisplatin and melphalan. Transfection of a dominant-negative form of *FANCA* into cancer cell lines disrupted FANCD2 monoubiquitination and produced a two- to threefold sensitization of the cells to cisplatin (Ferrer *et al*., 2004). Melphalan-resistant myeloma cells had reduced ICL formation and enhanced ICL repair, and small interfering RNA (siRNA) knock down of *FANCF* in melphalan-resistant cells partially reversed their drug resistance (Chen *et al*., 2005). A cell-based screening strategy has been developed which identified several small-molecule inhibitors of the FA pathway that may have utility in sensitizing cancer cells to ICL-based therapy (Chirnomas *et al*., 2006). Finally, since BRCA2-deficient cells and tumours are acutely sensitive to inhibitors of poly(ADP-ribose) polymerase (Bryant *et al*., 2005; Farmer *et al*., 2005), it would be interesting to see whether this property is shared by tumours that are deficient in other components of the FA pathway.

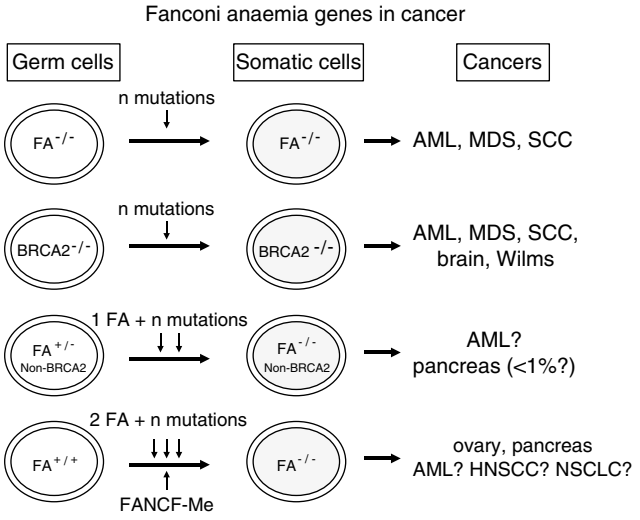

Figure 2 Fanconi anaemia genes in cancer. FA patients ($FA^{-/-}$) have bi-allelic FA gene mutations in their germline, which results in genomic instability and additional mutations in their somatic cells and the development of leukaemia and solid tumours. The $BRCA2^{-/-}$ genotype strongly predisposes to cancer. In FA heterozygotes ($FA^{+/-}$) a somatic mutation in the other allele of the corresponding FA gene would inactivate the FA pathway and could lead to apoptosis or to the acquisition of further mutations and a proliferative advantage. Cells with no FA germline mutations ($FA^{+/+}$) would require multiple mutations or epigenetic events to inactivate the FA pathway.

Conclusions

There has been major progress in unravelling the complexities of the FA pathway in the past few years, with the identification of new FA genes, the discovery that *FANCD1* is *BRCA2*, and that the FANCJ and FANCM proteins have DNA helicase or DNA translocase activity. However, much remains to be done to define the functions of these proteins and to understand how they contribute to DNA recombination and repair. The use of proteomics to dissect the components of FA protein complexes (Meetei *et al*., 2005) and model organisms such as chicken DT40 cells to knockout combinations of FA genes (Yamamoto *et al*., 2003; Bridge *et al*., 2005; Mosedale *et al*., 2005) are beginning to yield valuable insights into how these proteins combine to recognize or repair DNA damage.

A scheme for the involvement of germline and somatic mutations of the FA genes in the development of cancer is shown in Figure 2. In homozygotes the lack of a functional FA pathway will lead to mutations or chromosomal breakage and rearrangements in some cells. The cellular response in most cases is likely to be apoptosis, but some cells will acquire a proliferative advantage and develop into a tumour. The unusual spectrum of solid tumours in these patients is unexplained. Heterozygotes for a germline FA mutation would require a somatic mutation or loss of heterozygosity involving the other allele of the same FA gene to disrupt the pathway and generate genomic instability. At present, it seems unlikely from the limited data available that heterozygosity for mutations in any of the FA genes except *BRCA2* confers strong susceptibility to cancer, but a large collaborative epidemiological and molecular study is needed to examine the possibility that mutation carriers have a modest increase in cancer risk. Extensive mutation screening of the full complement of FA genes in germline DNA from young onset cases of sporadic cancers such as AML and squamous cell carcinomas of the head, neck and cervix, although laborious, will also help to address this question. The majority of the population will have two functional copies of their FA genes, and would therefore require somatic mutation of both alleles of the same FA gene to inactivate the pathway. An important exception to this is the *FANCB* gene which is located on the X chromosome (Meetei *et al*., 2004). All males are effectively FA carriers, and only one somatic mutation will therefore be required for loss of function of the FA pathway, and in females one copy would be subject to X-inactivation. The biological significance of somatic mutations of FA genes and the silencing of *FANCF* by hypermethylation in some sporadic cancers needs further investigation. If a broader role for inactivation of the FA pathway in cancer can be established, it may have important implications for future therapeutic strategies.

The advances of the past few years have moved the Fanconi anaemia syndrome from the study of a relatively obscure chromosome breakage disorder into the mainstream of DNA repair and cancer research, and have the potential to yield novel insights into the cellular response to DNA damage and the pathogenesis of some forms of human cancer.

References

Adachi D, Oda T, Yagasaki H, Nakasato K, Taniguchi T, D'Andrea AD *et al*. (2002). Heterogeneous activation of the Fanconi anemia pathway by patient-derived FANCA mutants. *Hum Mol Genet* **11**: 3125–3134.

Alter BP, Joenje H, Oostra AB, Pals G. (2005). Fanconi anemia: adult head and neck cancer and hematopoietic mosaicism. *Arch Otolaryngol Head Neck Surg* **131**: 635–639.

Alter BP. (2003). Cancer in Fanconi anemia. *Cancer* **97**: 425–440.

Barber LM, McGrath HEN, Meyer S, Will AM, Birch JM, Eden OB *et al*. (2003). Constitutional sequence variation in the Fanconi anaemia group C (*FANCC*) gene in childhood acute myeloid leukaemia. *Br J Haematol* **121**: 57–62.

Barroso E, Milne RL, Fernandez LP, Zamora P, Arias JI, Benitez J *et al*. (2006). FANCD2 associated with sporadic breast cancer risk. *Carcinogenesis* May 5; (E-pub ahead of print).

Bridge WL, Vandenberg CJ, Franklin RJ, Hiom K. (2005). The BRIP1 helicase functions independently of BRCA1 in the Fanconi anemia pathway for DNA crosslink repair. *Nat Genet* **37**: 953–957.

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E *et al*. (2005). Specific killing of BRCA2-deficient

tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434**: 913–917.

Callen E, Casado JA, Tischkowitz MD, Bueren JA, Creus A, Marcos R *et al*. (2005). A common founder mutation in FANCA underlies the world's highest prevalence of Fanconi anemia in Gypsy families from Spain. *Blood* **105**: 1946–1949.

Cantor S, Drapkin R, Zhang F, Lin Y, Han J, Pamidi S *et al*. (2004). The BRCA1-associated protein BACH1 is a DNA helicase targeted by clinically relevant inactivating mutations. *Proc Natl Acad Sci USA* **101**: 2357–2362.

Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S *et al*. (2001). BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* **105**: 149–160.

Carreau M. (2004). Not-so-novel phenotypes in the Fanconi anemia group D2 mouse model. *Blood* **103**: 2430.

Chen Q, van der Sluis PC, Boulware D, Hazlehurst LA, Dalton WS. (2005). The FA/BRCA pathway is involved in melphalan-induced DNA interstrand cross-link repair and accounts for melphalan resistance in multiple myeloma cells. *Blood* **106**: 698–705.

Chirnomas D, Taniguchi T, de la Vega M, Vaidya AP, Vasserman M, Hartman AR *et al*. (2006). Chemosensitization to cisplatin by inhibitors of the Fanconi anemia/BRCA pathway. *Mol Cancer Ther* **5**: 952–961.

Condie A, Powles RL, Hudson CD, Shepherd V, Bevan S, Yuille MR *et al*. (2002). Analysis of the Fanconi anaemia complementation group A gene in acute myeloid leukaemia. *Leuk Lymphoma* **43**: 1849–1853.

Connor F, Bertwistle D, Mee PJ, Ross GM, Swift S, Grigorieva E *et al*. (1997). Tumorigenesis and a DNA repair defect in mice with a truncating Brca2 mutation. *Nat Genet* **17**: 423–430.

Couch FJ, Johnson MR, Rabe K, Boardman L, McWilliams R, de Andrade M *et al*. (2005). Germ line Fanconi anemia complementation group C mutations and pancreatic cancer. *Cancer Res* **65**: 383–386.

Cumming RC, Lightfoot J, Beard K, Youssoufian H, O'Brien PJ, Buchwald M. (2001). Fanconi anemia group C protein prevents apoptosis in hematopoietic cells through redox regulation of GSTP1. *Nat Med* **7**: 814–820.

Djuzenova CS, Rothfuss A, Oppitz U, Spelt G, Schindler D, Hoehn H *et al*. (2001). Response to X-irradiation of Fanconi anemia homozygous and heterozygous cells assessed by the single-cell gel electrophoresis (comet) assay. *Lab Invest* **81**: 185–192.

Fagerlie S, Lensch MW, Pang Q, Bagby Jr GC. (2001). The Fanconi anemia group C gene product: signaling functions in hematopoietic cells. *Exp Hematol* **29**: 1371–1381.

Faivre L, Guardiola P, Lewis C, Dokal I, Ebell W, Zatterale A *et al*. (2000). Association of complementation group and mutation type with clinical outcome in Fanconi anemia. *Blood* **96**: 4064–4070.

Fanconi G. (1967). Familial constitutional panmyelocytopathy, Fanconi's anemia (F.A.). I. Clinical aspects. *Semin Hematol* **4**: 233–240.

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB *et al*. (2005). Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434**: 917–921.

Ferrer M, de Winter JP, Masterbroek DC, Curiel DT, Gerritsen WR, Giaccone G *et al*. (2004). Chemosensitizing tumor cells by targeting the Fanconi anemia pathway with an adenovirus overexpressing dominant-negative FANCA. *Cancer Gene Ther* **11**: 539–546.

Friedman LS, Thistlethwaite FC, Patel KJ, Yu VP, Lee H, Venkitaraman AR *et al*. (1998). Thymic lymphomas in mice with a truncating mutation in Brca2. *Cancer Res* **58**: 1338–1343.

Futaki M, Yamashita T, Yagasaki H, Toda T, Yabe M, Kato S *et al*. (2000). The IVS4+4 A to T mutation of the Fanconi anemia gene FANCC is not associated with a severe phenotype in Japanese patients. *Blood* **95**: 1493–1498.

Garcia-Closas M, Egan KM, Newcomb PA, Brinton LA, Titus-Ernstoff L, Chanock S *et al*. (2006). Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses. *Hum Genet* **119**: 376–388.

Gillio AP, Verlander PC, Batish SD, Giampietro PF, Auerbach AD. (1997). Phenotypic consequences of mutations in the Fanconi anemia FAC gene: an International Fanconi Anemia Registry study. *Blood* **90**: 105–110.

Hirsch B, Shimamura A, Moreau L, Baldinger S, Hag-alshiekh M, Bostrom B *et al*. (2004). Association of biallelic *BRCA2/FANCD1* mutations with spontaneous chromosomal instability and solid tumors of childhood. *Blood* **103**: 2554–2559.

Hodgson SV, Easton DF, Ball J, Mathew CG, Tischkowitz MD. (2004). *A study of cancer incidence in British Fanconi anemia families* (Abstract 406, American Society of Human Genetics Meeting, Toronto, Canada, 26–30.10.2004).

Houghtaling S, Timmers C, Noll M, Finegold MJ, Jones SN, Meyn MS *et al*. (2003). Epithelial cancer in Fanconi anemia complementation group D2 (*Fancd2*) knockout mice. *Genes Dev* **17**: 1933–1936.

Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, de Die-Smulders C *et al*. (2002). Biallelic inactivation of *BRCA2* in Fanconi anemia. *Science* **297**: 606–609.

Hussain S, Wilson JB, Blom E, Thompson LH, Sung P, Gordon SM *et al*. (2006). Tetratricopeptide-motif-mediated interaction of FANCG with recombination proteins XRCC3 and BRCA2. *DNA Repair* **5**: 629–640.

Joenje H, Patel KJ. (2001). The emerging genetic and molecular basis of Fanconi anaemia. *Nat Rev Genet* **2**: 446–457.

Karppinen SM, Vuosku J, Heikkinen K, Allinen M, Winqvist R. (2003). No evidence of involvement of germline *BACH1* mutations in Finnish breast and ovarian cancer families. *Eur J Cancer* **39**: 366–371.

Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG *et al*. (2003a). High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. *Arch Otolaryngol Head Neck Surg* **129**: 106–112.

Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF *et al*. (2003b). A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood* **101**: 1249–1256.

Kutler DI, Wreesman VB, Goderdhan A, Ben-Porat L, Satagopan J, Ngai I *et al*. (2003c). Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst* **95**: 1718–1721.

Lensch MW, Tischkowitz M, Christianson TA, Reifsteck CA, Speckhart SA, Jakobs PM *et al*. (2003). Acquired FANCA dysfunction and cytogenetic instability in adult acute myelogenous leukemia. *Blood* **102**: 7–16.

Levitus M, Rooimans MA, Steltenpool J, Cool NFC, Oostra AB, Mathew CG *et al*. (2004). Heterogeneity in Fanconi anemia: evidence for 2 new genetic subtypes. *Blood* **103**: 2498–2503.

Levitus M, Waisfisz Q, Godthelp BC, de Vries Y, Hussain S, Wiegant WW *et al*. (2005). The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. *Nat Genet* **37**: 934–935.

Levran O, Attwooll C, Henry RT, Milton KL, Neveling K, Rio P *et al*. (2005). The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. *Nat Genet* **37**: 931–933.

Lewis AG, Flanagan J, Marsh A, Pupo GM, Mann G, Spurdle AB *et al*. (2005). Mutation analysis of FANCD2, BRIP1/BACH1, LMO4 and SFN in familial breast cancer. *Breast Cancer Res* **7**: R1005–R1016.

Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A. (1997). Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev* **11**: 1226–1241.

Luo L, Lei H, Du Q, von Wachenfeldt A, Kockum I, Luthman H *et al*. (2002). No mutations in the *BACH1* gene in *BRCA1* and *BRCA2* negative breast-cancer families linked to 17q22. *Int J Cancer* **98**: 638–639.

Lyakhovich A, Surralles J. (2005). Disruption of the Fanconi anemia/BRCA pathway in sporadic cancer. *Cancer Lett* **232**: 99–106.

Marsit CJ, Liu M, Nelson HH, Posner M, Suzuki M, Kelsey KT. (2004). Inactivation of the Fanconi anemia/BRCA pathway in lung and oral cancers: implications for treatment and survival. *Oncogene* **23**: 1000–1004.

Meetei AR, de Winter JP, Medhurst AL, Wallisch M, Waisfisz Q, van de Vrugt HJ *et al*. (2003). A novel ubiquitin ligase is deficient in Fanconi anemia. *Nat Genet* **35**: 165–170.

Meetei AR, Levitus M, Xue Y, Medhurst AL, Zwaan M, Ling C *et al*. (2004). X-linked inheritance of Fanconi anemia complementation group B. *Nat Genet* **36**: 1142–1143.

Meetei AR, Medhurst AL, Ling C, Xue Y, Singh TR, Bier P *et al*. (2005). A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nat Genet* **37**: 958–963.

Meyer S, Barber LM, White DJ, Will AM, Birch JM, Kohler JA *et al*. (2006). Spectrum and significance of variants and mutations in the Fanconi anaemia group G gene in children with sporadic acute myeloid leukaemia. *Br J Haematol* **133**: 284–292.

Morgan NV, Essop F, Demuth I, de Ravel T, Jansen S, Tischkowitz M *et al*. (2006). A common Fanconi anemia mutation in black populations of sub-Saharan Africa. *Blood* **105**: 3542–3544.

Mosedale G, Niedzwiedz W, Alpi A, Perrina F, Pereira-Leal JB, Johnson M *et al*. (2005). The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway. *Nat Struct Mol Biol* **12**: 763–771.

Narayan G, Arias-Pulido H, Nandula SV, Basso K, Sugirtharaj DD, Vargas H *et al*. (2004). Promoter hypermethylation of FANCF: disruption of Fanconi anemia-BRCA pathway in cervical cancer. *Cancer Res* **64**: 2994–2997.

Offit K, Levran O, Mullaney B, Mah K, Nafa K, Batish SD *et al*. (2003). Shared genetic susceptibility to breast cancer, brain tumors, and Fanconi anaemia. *J Natl Cancer Inst* **95**: 1548–1551.

Pearson T, Jansen S, Havenga C, Stones DK, Joubert G. (2001). Fanconi anemia: a statistical evaluation of cytogenetic results obtained from South African families. *Cancer Genet Cytogenet* **126**: 52–55.

Potter NU, Sarmousakis C, Li FP. (1983). Cancer in relatives of patients with aplastic anemia. *Cancer Genet Cytogenet* **9**: 61–65.

Reid S, Renwick A, Seal S, Baskcomb L, Barfoot R, Jayatilake H *et al*. (2005). Biallelic *BRCA2* mutations are associated with multiple malignancies in childhood including familial Wilms tumour. *J Med Genet* **42**: 147–151.

Reuter TY, Medhurst AL, Waisfisz Q, Zhi Y, Herterich S, Hoehn H *et al*. (2003). Yeast two hybrid screens imply involvement of Fanconi anaemia proteins in transcription regulation, cell signalling, oxidative metabolism and cellular transport. *Exp Cell Res* **289**: 211–221.

Rogers CD, Couch FJ, Brune K, Martin ST, Philips J, Murphy KM *et al*. (2004A). Genetics of the *FANCA* gene in familial pancreatic cancer. *J Med Genet* **41**: er126.

Rogers CD, van der Heijden MS, Brune K, Yeo CJ, Hruban RH, Kern SE *et al*. (2004B). The genetics of FANCC and FANCG in familial pancreatic cancer. *Cancer Biol Ther* **3**: 167–169.

Rosenberg PS, Greene MH, Alter BP. (2003). Cancer incidence in persons with Fanconi anemia. *Blood* **101**: 822–826.

Rosenberg PS, Socié G, Alter BP, Gluckman E. (2005). Risk of head and neck squamous cell cancer and death in patients with Fanconi anemia who did and did not receive transplants. *Blood* **105**: 67–73.

Rosendorff J, Bernstein R, Macdougall L, Jenkins T. (1987). Fanconi anemia: another disease of unusually high prevalence in the Afrikaans population of South Africa. *Am J Med Genet* **27**: 793–797.

Rutter JL, Smith AM, Dávila MR, Sigurdson AJ, Giusti RM, Pineda MA *et al*. (2003). Mutational analysis of the *BRCA1*-interacting genes *ZNF350*/*ZBR1* and *BRIP1*/*BACH1* among *BRCA1 and BRCA2*-negative probands from breast-ovarian cancer families and among early-onset breast cancer cases and reference individuals. *Hum Mutat* **22**: 121–128.

Schroeder TM, Anschutz F, Knopp A. (1964). Spontaneous chromosome aberrations in familial panmyelopathy. *Humangenetik* **1**: 194–196.

Seal S, Barfoot R, Jayatilake H, Smith P, Renwick A, Bascombe L *et al*. (2003). Evaluation of Fanconi anemia genes in familial breast cancer predisposition. *Cancer Res* **63**: 8596–8599.

Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C *et al*. (1997). Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* **386**: 804–810.

Sigurdson AJ, Hauptmann M, Chatterjee N, Alexander BH, Doody MM, Rutter JL *et al*. (2004). Kin-cohort estimates for familial breast cancer risk in relation to variants in DNA base excision repair, BRCA1 interacting and growth factor genes. *BMC Cancer* **4**: 9.

Surralles J, Jackson SP, Jasin M, Kastan MB, West SC, Joenje H. (2004). Molecular cross-talk among chromosome fragility syndromes. *Genes Dev* **18**: 1359–1370.

Swift M. (1971). Fanconi's anaemia in the genetics of neoplasia. *Nature* **230**: 370–373.

Swift M, Caldwell RJ, Chase C. (1980). Reassessment of cancer predisposition of Fanconi anemia heterozygotes. *J Natl Cancer Inst* **65**: 863–867.

Taniguchi T, D'Andrea AD. (2006). The molecular pathogenesis of Fanconi Anemia: recent progress. *Blood* **107**: 4223–4233.

Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG, Joenje J *et al*. (2003). Disruption of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian tumors. *Nature Med* **9**: 568–574.

Thompson D, Easton DF. (2004). The BRCA1 and BRCA2 genes. In: Eeles RA, Easton DF, Ponder BAJ, Eng C (ed). *Genetic Predisposition to Cancer*, 2nd ed. Arnold: London, pp. 256–276.

Thompson LH, Hinz JM, Yamada NA, Jones NJ. (2005). How Fanconi anemia proteins promote the four Rs: replication, recombination, repair and recovery. *Environ Mol Mutagen* **45**: 128–142.

Tipping AJ, Pearson T, Morgan NV, Gibson RA, Kuyt LP, Havenga C *et al*. (2001). Molecular and genealogical evidence for a founder mutation in Fanconi anemia families of the Afrikaner population of South Africa. *Proc Natl Acad Sci USA* **98**: 5734–5739.

Tischkowitz M, Ameziane N, Qaisfisz Q, de Winter JP, Harris R, Taniguchi T *et al*. (2003). Bi-allelic silencing of the Fanconi anaemia gene *FANCF* in acute myeloid leukaemia. *Br J Haematol* **123**: 469–471.

Tischkowitz M, Dokal I. (2004). Fanconi anaemia and leukaemia – clinical and molecular aspects. *Br J Haematol* **126**: 176–191.

Tischkowitz MD, Morgan NV, Grimwade D, Eddy C, Ball S, Vorechovsky I *et al*. (2004). Deletion and reduced expression of the Fanconi anemia *FANCA* gene in sporadic acute myeloid leukaemia. *Leukemia* **18**: 420–425.

Vahteristo P, Yliannala K, Tamminen A, Eerola H, Blomqvist C, Nevanlinna H. (2006). *BACH1* Ser919Pro variant and breast cancer risk. *BMC Cancer* **6**: 19.

van der Heijden MS, Brody JR, Gallmeier E, Cunningham SC, Dezentje DA, Shen D *et al*. (2004). Functional defects in the Fanconi anemia pathway in pancreatic cancer cells. *Am J Pathol* **165**: 651–657.

van der Heijden MS, Yeo CJ, Hruban RH, Kern SE. (2003). Fanconi anemia gene mutations in young-onset pancreatic cancer. *Cancer Res* **63**: 2585–2588.

van Zeeburg HJT, Snijders PJF, Joenje H, Brakenhoff RH. (2004). Re: Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst* **96**: 968–969.

Verlander PC, Kaporis A, Liu Q, Zhang Q, Seligsohn U, Auerbach AD. (1995). Carrier frequency of the IVS4+4 A→T mutation of the Fanconi anemia gene FAC in the Ashkenazi Jewish population. *Blood* **86**: 4034–4038.

Verlander PC, Lin JD, Udono MU, Zhang Q, Gibson RA, Mathew CG *et al*. (1994). Mutation analysis of the Fanconi anemia gene FACC. *Am J Hum Genet* **54**: 595–601.

Wagner JE, Tolar J, Levran O, Scholl T, Deffenbaugh A, Satagopan J *et al*. (2004). Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia. *Blood* **103**: 3226–3229.

Waisfisz Q, Morgan NV, Savino M, de Winter JP, van Berkel CGM, Hoatlin ME *et al*. (1999). Spontaneous functional correction of homozygous Fanconi anaemia alleles reveals novel mechanistic basis for reverse mosaicism. *Nat Genet* **22**: 379–383.

Wang X, Andreassen PR, D'Andrea AD. (2004). Functional interaction of monoubiquitinated FANCD2 and BRCA2/FANCD1 in chromatin. *Mol Cell Biol* **24**: 5850–5862.

Whitney MA, Saito H, Jakobs PM, Gibson RA, Moses RE, Grompe M. (1993). A common mutation in the FACC gene causes Fanconi anaemia in Ashkenazi Jews. *Nat Genet* **4**: 202–205.

Xie Y, de Winter JP, Waisfisz Q, Nieuwint AWM, Scheper RJ, Arwert F *et al*. (2000). Aberrant Fanconi anaemia protein profiles in acute myeloid leukaemia cells. *Br J Haematol* **111**: 1057–1064.

Yamamoto K, Ishiai M, Matsushita N, Arakawa H, Lamerdin JE, Buerstedde JM *et al*. (2003). Fanconi anemia FANCG protein in mitigating radiation- and enzyme-induced DNA double-strand breaks by homologous recombination in vertebrate cells. *Mol Cell Biol* **23**: 5421–5430.

Yamashita T, Wu N, Kupfer G, Korless C, Joenje H, Grompe M *et al*. (1996). Clinical variability of Fanconi anemia (type C) results from expression of an amino terminal truncated Fanconi anemia complementation group C polypeptide with partial activity. *Blood* **87**: 4424–4432.