Trends in Genetics

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REVIEWS

Review

The Fanconi Anemia Pathway and Fertility

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Fanconi anemia (FA) is a life-threatening syndrome characterized by bone marrow failure and cancer predispositions. The past two decades have seen an explosion of data in the FA field, both in humans and other organisms, following the cloning of 22 FA genes. A common but notably understudied clinical feature of the disease is the reduced fertility of individuals with FA. This review focuses on the known causes of reduced fertility in FA, and integrates them with the emerging role of the FA pathway in double-strand break (DSB) repair at meiosis in a variety of organisms, as well as providing future directions for research and diagnostics.

Highlights

Individuals with FA have reduced fertility.

Primordial germ cell proliferation is defective in FA males and females, and maintenance of spermatogonial stem cells is defective in males with FA.

Undiagnosed cases of FA may be identified through genetic testing of individuals with particular classes of reduced fertility.

FA proteins participate in the DSB repair model of genetic recombination at meiotic prophase.

Reduced Fertility in Individuals with FA

Fanconi anemia (FA, see Glossary) is a rare autosomal recessive disorder characterized by progressive **bone marrow failure** (BMF), an extremely high predisposition to cancers such as **head and neck squamous cell carcinoma** and leukemia, reduced fertility, and other congenital abnormalities [1]. It has been reported that approximately half of female FA patients are infertile and males are rarely fertile [2,3]. Because of the life-threatening nature of BMF and cancer, these topics are extensively studied relative to fertility. However, because life expectancy for individuals with FA is increasing into adulthood as a result of clinical advances, there is growing interest in the reasons for – and solutions to overcome – reduced fertility in FA.

Female FA patients can carry healthy children to term; however, given the reduced life expectancy in FA, less opportunity is available to have children. The observed rate of female FA patients 16–48 years of age who have conceived is 15% compared to 50% in the general population [3]. Pregnancies are more complex than in non-FA women, for example owing to pregnancy-induced cytopenias and the small pelvis of the FA mother, and maintenance of pregnancy may be decreased [3]. It is possible that the female FA patients who have children have a milder form of FA [3]. For example, some patients are diagnosed with FA during pregnancy owing to the development and detection of hematological abnormalities.

Female FA patients generally have normal menarche but have premature menopause reflective of a reproductive clock that is ticking faster than that of non-FA individuals. In female FA patients the reduced fertility manifests as **primary ovarian insufficiency** (POI). An additional reproductive barrier and concern for female FA patients is greatly increased rates of gynecological cancers [4].

Males with FA have a marked reduction in fertility. There are no well-documented cases of males with FA having children, however reference is made to at least three cases [3,5]. In FA males the clinical symptoms related to decreased reproductive capacity present as abnormal and severely decreased spermatogenesis, and are often accompanied with diagnoses of **non-obstructive azoospermia** and **Sertoli cell-only syndrome** [6], the male phenotype corresponding to POI, which reflects the lack of **spermatogonial** stem cells (Figure 1A, Key Figure, and Table 1). Illustrating the point of equivalent non-obstructive azoospermia and POI phenotypes, the same homozygous mutation in FANCM, p.Gln1701*, was suspected in independent studies as being causal for POI in females [7] and for non-obstructive azoospermia in males [8].

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Molecular Mechanisms of the FA Pathway

Mutations in 22 genes are currently known to cause FA in humans. The FA pathway is traditionally known for its essential role in DNA **interstrand crosslink** (ICL) repair [1,9,10], and this has been extended recently to include replication fork protection [11–14]. The FA pathway is active during S phase, as it is during DNA replication when DNA ICLs are detected, and their continued presence subsequently impedes progression through to mitosis. The repair of ICLs is achieved by the FA pathway [1] or the NEIL3 pathway [15] in vertebrates. For the FA pathway, ICL recognition leads to the mono-ubiquitination of each protomer of the heterodimer FANCI–FANCD2. The mono-ubiquitination is achieved by the FA **core complex**, a multicomponent ubiquitin ligase, comprising the catalytic component (FANC-B, –L, FAAP100) [16–18], the substrate adaptor (FANC-C, -E, -F), and FANC-A, -G, and FAAP20, that is proposed to localize the core complex to the nucleus [19] and promote strand-exchange activity at sites of **double-strand breaks** (DSBs) [20]. Although the FA pathway is well studied for its direct role in DNA repair, the precise link between its role in DNA repair and fertility has not been extensively described in humans. The large reduction in gametes suggests that the source of the problem is any combination of **primordial germ cell** (PGC) specification, PGC proliferation, germ cell maintenance, and **meiotic** progression. Interestingly, all mouse models of FA reported to date also involve a significant reduction in fertility, and have enabled studies that are not feasible in humans, particularly relating to embryonic germ cell development.

PGC Development

The development of PGCs is relatively indistinguishable in males and females until the development of the gonads [21,22]. In mice (*Mus musculus*) the PGC lineage is established in early embryogenesis, forming a cluster of ~30–40 cells at embryonic day (E) 7.0. These cells proliferate to approximately 1000 cells and then migrate to the gonadal ridge between E8.5 and E10.5 (Figure 2) [22,23]. By E13.5, this group of cells has undergone a rapid expansion to approximately 25 000 cells, which will later be a source of cells for spermatozoa or oocyte production [22]. In male mice, PGCs enter mitotic arrest after this mass proliferation, until postnatal day (P) 10 when they commence spermatogenesis to generate the first population of spermatozoa at approximately 3 weeks of age, whereas at around E13.5–14.5 in females, PGCs enter meiosis and arrest at diplotene until ovulation [22]. Mechanisms underlying human germ cell development are difficult to study because of ethical considerations. For humans, the earliest stages of PGC development are observed at around weeks 3–5 of embryonic development [24], approximately equivalent to mouse PGC E10.5 [22]. Estimates suggest that between weeks 4 and 9 PGCs expand from 1000 to about 450 000 in females and 150 000 in males [24]. At approximately week 5 the gonadal ridge has been colonized by the migrated PGCs, and by 8 weeks differentiation of the testis and ovaries is visible [22]. One study estimated that, by the end of the fifth month of gestation, the number of PGCs has reached 10 000 000 for **oogonia.** At the equivalent stage in males, 3 000 000–4 000 000 prespermatogonia have been produced [24]. In females at around 11–12 weeks of gestation, oogonia enter meiosis [22] and arrest at diplotene. Beginning at menarche and ending at menopause, oogonia will complete meiosis and differentiate into mature oocytes approximately once per month. By contrast, spermatozoa are produced from puberty on from a continually self-renewing pool of stem cells.

Loss of Germ Cells in FA Patients and Mice

Reduced fertility, hypogonadism, and **seminiferous tubule** hypoplasia have been reported as features of FA patients for over 60 years [25–27]. Similar phenotypes have been recapitulated in independent studies of >20 mouse mutants of the *Fanc* genes (Table 1). FA and FA-like phenotypes in humans and mouse models are homozygous recessive. Two exceptions are *Fancb* (X-linked [28,29]) and *Uaf1* {(homozygous embryonic lethal). *UAF1* is not a *bona fide* FA

Glossary

Bone marrow failure: occurs in individuals when there is insufficient production of red, white, or platelet blood cells.
Core complex: a multisubunit ubiquitin ligase which mono-ubiquitinates FANCD2 and FANCI at DNA lesions.
Crossover (CO): a large reciprocal exchange of DNA, which occurs between chromatids of homologous chromosomes in meiotic prophase I.
Crossover interference: a phenomenon where the probability of a meiotic CO in one genetic interval decreases the probability of another crossover in a physically adjacent genetic interval, in a distance-dependent manner.
Double-strand break (DSB): a DNA lesion where both strands of the double helix are severed. DSBs are highly toxic because they can lead to genome rearrangements.
Fanconi anemia (FA): a rare autosomal recessive disease characterized by bone marrow failure, congenital defects, cancer predisposition, and reduced fertility. FA cells are hypersensitive to ICLs.
Head and neck squamous cell carcinoma: a cancer in the squamous cells located in the mucous membranes and outer layers of the skin. The cancers are often in located in the mouth, pharynx, or genitalia.
Homologous recombination (HR): an accurate type of DNA repair in which a homologous DNA sequence is used as a repair template.
Interstrand crosslink (ICL): a covalent bond between nucleotides of opposite DNA strands that is induced by endogenous or exogenous DNA-damaging agents. ICLs block DNA strand separation and hence inhibit replication and transcription.
Meiosis: a specialized cell division in which two rounds of DNA segregation follow a single round of DNA replication. Maternal and paternal chromosomes are recombined and segregated to produce genetically unique haploid gametes. In mammals meiosis takes place in the gonads.
Non-homologous end joining (NHEJ): a type of DNA repair which

gene; however, loss of one copy of mouse *Uaf1* phenocopies aspects of FA [30]}. FA mouse litter sizes – if obtainable – are often reduced, and the window of fertility is shortened for both sexes [31–36]. The reduction in fertility correlates with hypogonadism in both sexes as per humans. The direct reason for the reduction in gonad size is not clear, even if it is generally understood that in humans and mice a decrease in spermatozoa production can correlate with a reduction in testis volume. When directly quantified, spermatozoa numbers – if above zero – are extremely low compared to controls, and this is what results in Sertoli cell-only diagnoses in humans [6,8,25,37,38]. Similarly, female FA patients and mouse models show decreased follicle numbers [34,39–44], a primary diagnostic criterion of POI.

The massive reduction in gametes can be traced back to two points in gametogenesis. The first is during PGC proliferation at the gonadal ridge in early embryonic development (Figure 2), and the second is during maintenance of the germ cell source, which is more relevant in post-pubescent males owing to the continual nature of spermatogenesis. Regarding PGC proliferation, FA mouse models develop a PGC lineage and undergo normal PGC migration to the gonadal ridge. The only exception to date is *MmFancv/Rev7* [43] where some PGC apoptosis can been seen during migration to the gonadal ridge. In all FA mouse models tested, at the stage of PGC expansion at the gonadal ridge, a drastically decreased rate of PGC proliferation was observed [29,31,42,45]. The rapid proliferation and DNA replication of the PGCs – from 150 cells to 25 000 between E8.5 and E13.5 [46] – would be highly dependent on the FA pathway. It is unlikely that individuals with a defective FA pathway would be able to tolerate the programmed rapid proliferation – especially considering the excessive G2/M arrest when FA cells encounter replication stress [47,48] – and therefore it is reasonable to hypothesize that a perturbed FA pathway would lead to a reduction in the development and maintenance of PGCs via apoptosis.

Beyond the extensive FA mouse mutant data, indirect support for the FA pathway being essential during PGC expansion comes from two studies that analyzed the effect of mitomycin C (MMC)-induced DNA ICLs in wild-type mice during gametogenesis. In one study, MMC injection into pregnant mice at E6.75–7.0 mimicked FA in the pups, with a reduction in gonadal volume at E13.5, atrophic seminiferous tubules, reduced follicle numbers, and a large reduction in PGC numbers between E8.5 and E13.5 [46]. Further, fully grown mouse oocytes, which do not actively replicate, were not sensitive to the ICL-inducing agent MMC [49]. In addition, the MMC treatment did not activate the FA pathway in mature oocytes – as measured by FANCD2 foci formation – until DNA replication resumed after fertilization. These observations underline the role of the FA pathway during S-phase as a crucial component to allow efficient gametogenesis.

After birth, the fertility of male FA mice decreases rapidly compared to non-FA littermates [40–42,45,50] (Figure 1A). In these mice, the progressive reduction in spermatogonial stem cells (SSCs) in the seminiferous tubules was reported to take place primarily via apoptotic pathways [29,31,33,35,39,51–57] and sometimes via reduced proliferation [42,50]. Close examination of mouse *Fancm* male and female germ cells revealed that, in this model, a partial rescue phenotype of male germ cell numbers is observed when crossing *Fancm* with *p53* and other mutants of DNA damage checkpoint genes. The results of these experiments suggest a multistep model in which some of the difference in germ cell numbers can be accounted for by reduced proliferation, some in an *ATM–p53–p21*-dependent pathway, and that there is sexual dimorphism for the phenotypes [42]. Female FA mice can also lose follicles faster than control mice as they age [31,37,38], and there is an acceleration of follicular decline in women carrying *FANCS/BRCA1*, *FANCD1/BRCA2* [58], and possibly *FANCM* [7] mutations (Figure 1B). However, because most oocytes remain diplotene-arrested, the loss in follicles cannot be correlated with defects due to active replication. One possible reason for the loss in

ligates two DNA DSB ends without a need for homology.
Non-obstructive azoospermia (NOA): complete absence of spermatozoa in the semen.
Oogonium: a diploid undifferentiated female germ cell which gives rise to oocytes.
Primary ovarian insufficiency (POI): reduced fertility as a result of premature loss of ovarian function. POI can be linked to few to no remaining follicles, autoimmune disorders, and hormone imbalance.
Primordial germ cell (PGC): a diploid stem cell precursor to spermatozoa or oocytes.
Seminiferous tubules: tubules within the testes where spermatozoa are produced.
Sertoli cell-only syndrome (SCOS): a male condition where Sertoli cells, which support developing sperm, are the only cells present in the seminiferous tubules of the testes. SCOS is diagnosed by azoospermia and testicular biopsy.
Spermatogonium: a diploid undifferentiated male germ cell which gives rise to spermatozoa.
Synapsis: an event in early meiotic prophase I where two homologous chromosomes are physically aligned and connected by a proteinaceous structure called the synaptonemal complex.
Synaptonemal complex (SC): a meiosis-specific structure that connects juxtaposed homologous chromosomes along their axes.
XY body: at meiosis in male mammals, the X and Y chromosomes condense to form the sex body/XY body where transcription is repressed. The XY chromosomes lack homology and are unsynapsed except for at the pseudoautosomal region.
***ZMM* genes:** a group of largely epistatic genes which encode the proteins that are required for the majority of COs in most species. *ZMM*-dependent COs display interference.

Key Figure

Representation of Gametogenesis in Fanconi Anemia (FA) and Non-FA

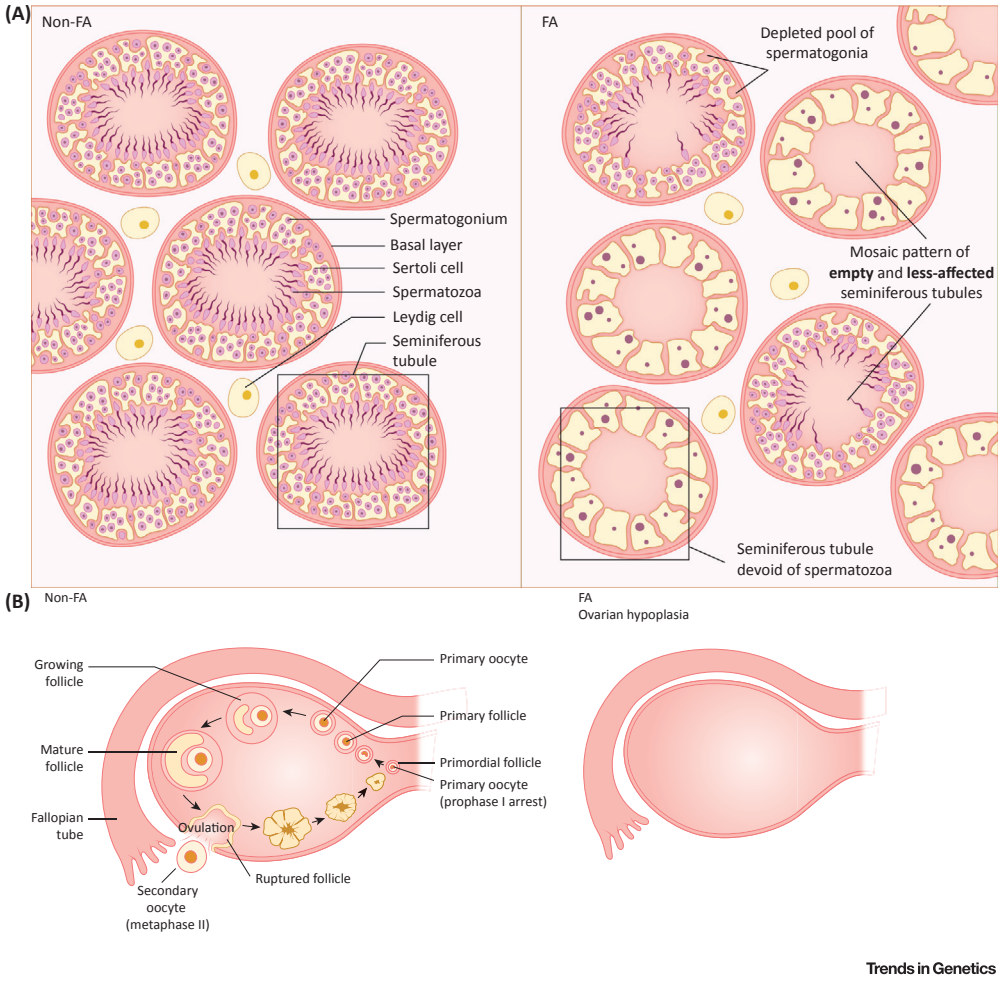

Figure 1. Representation of how gametogenesis occurs in FA and non-FA individuals in males and females. (A) Sertoli cells in the seminiferous tubule support the development of spermatogonia. Spermatogonia undergo meiosis, producing haploid cells, which then differentiate into spermatozoa. (B) In females, the primordial follicles arrest in diplotene in meiotic prophase I until ovulation. During ovulation, the primordial follicle resumes meiotic progression until metaphase II, when the oocyte is released into the fallopian tube. The remains of the follicle develop into the corpus luteum.

follicles may be that mechanisms involved in a prefollicle quality control, which maintain a 'memory' of meiotic defects that occurred before the diplotene arrest, guide oocytes with unrepaired DNA damage down apoptotic pathways [59].

Most seminiferous tubules of FA male mice had mosaic phenotypes where some tubules contained developing spermatogonia and others were completely devoid of SSCs and had a Sertoli cell-only phenotype [31,34,35,39–41,60]. The less-affected tubule sections appeared to be able to undergo differentiation into spermatozoa, as a basis for residual low-level fertility. This

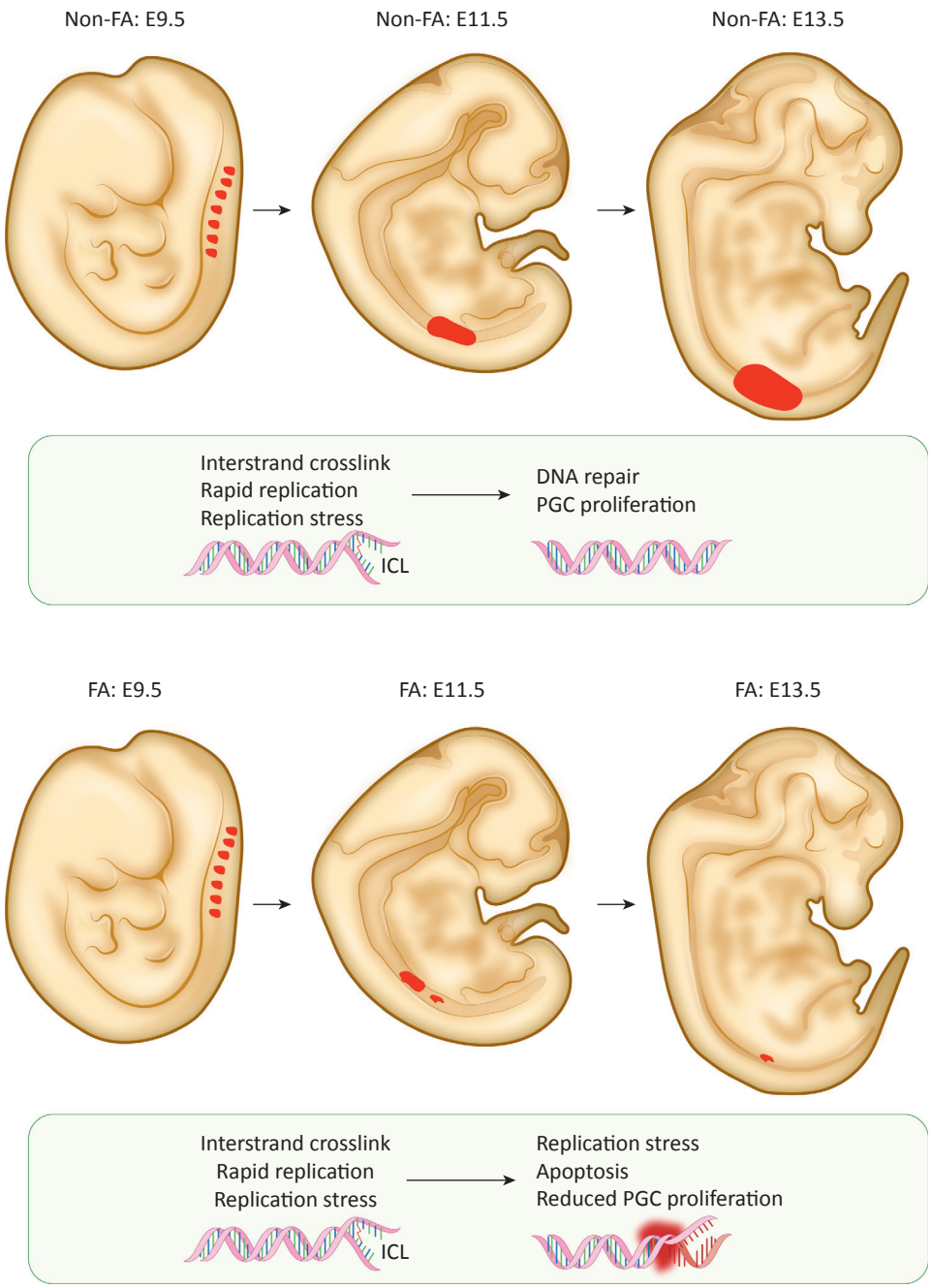

Figure 2. Primordial Germ Cell (PGC) Proliferation and Development in Fanconi Anemia (FA) and Non-FA Mouse Models. At approximately embryonic (E) day E9.5, PGCs migrate along the hindgut to the gonadal ridge. PGCs undergo mass proliferation until ~E13.5 that has a strong requirement for a functional FA pathway. FA mouse models are unable to sustain this proliferation and PGCs undergo apoptosis, leading to a reduction in germ cell pool, gametes, and fertility. PGCs are represented in red. Representative illustrations: not drawn to scale. Abbreviation: ICL, interstrand crosslink.

Table 1. Reproductive Phenotypes in Female and Male FA Mouse Models

	Alias	Refs	Testes	Spermatozoa	Male germ numbers	Females germ numbers	Ovaries	PGC apoptosis	Litter size	Meiosis
Fanca		[31,66,106]	Reduced weight. Mosaic tubules		Reduced	Reduced	Reduced ovary size. Reduced follicles	Yes	Reduced	No FANCD2 foci are formed during meiosis
Fancb		[29,66]	Reduced weight. Mosaic tubules		Reduced			Yes	Male sterility	No FANCD2 foci are formed during meiosis, unchanged MLH1 foci, subtly altered RAD51 dynamics
Fancc		[45,66,107,108]	Reduced weight. Tubules are atrophic and mosaic		Reduced	Reduced	Reduced follicles		Reduced	No FANCD2 foci are formed during meiosis
Fancd1	*Brca2*	[53,77,109]	Reduced weight	Reduced	Reduced	Reduced	Reduced ovary size. Reduced number of follicles	Yes	Male and female sterility	Persistent γ-H2AX foci and reduced RAD51 and DMC1 foci
Fancd2		[39,66]	Reduced weight. Mosaic tubules		Reduced		Reduced number of follicles	Yes		
Fance		[32,60]	Reduced weight. Mosaic tubules		Reduced		Reduced ovary weight. Reduced number of follicles		Reduced	
Fancf		[37]			Reduced		Smaller ovaries. Reduced numbers of follicles			
Fancg	*Xrcc9*	[34,40]	Reduced weight. Mosaic tubules	Reduced number of spermatozoa	Reduced		Reduced number of follicles		Reduced	
Fancj	*Brip1/Bach1*	[50]	Reduced weight	Reduced number of spermatozoa	Reduced			Mild		Increased number of MLH1 foci, increased number of chiasmata, upregulated BLM

Table 1. (continued)

	Alias	Refs	Testes	Spermatozoa	Male germ numbers	Females germ numbers	Ovaries	PGC apoptosis	Litter size	Meiosis
Fancl	*Pog/Phf9*	[110–112]	Reduced weight. Mosaic tubules	Reduced number of spermatozoa	Reduced	Reduced	Reduced number of follicles		Female sterility Males: allele- and age-dependent	
Fancm		[8,41,42,52]	Reduced weight. Tubules atrophic and mosaic	Reduced number of spermatozoa	Reduced	Reduced	Reduced follicles	Yes: C-terminal truncation [52] No: C142R [42]	Reduced	
Fancn	*Palb2*	[33,53]	Reduced weight. Mosaic tubules					Yes	Reduced	Persistent DSBs and apoptosis in zygotene. Decreased XY **synapsis**
Fanco	*Rad51C*	[54] Embryonic lethal. Knockout/ neo-hypomorph analyzed	Reduced weight	Reduced	Reduced		Ovulation defect. Fewer corpora lutea	Yes	Fertile and infertile mice. Reduced embryo number in infertile females.	Spermatocytes: reduced MLH1, persistent γ-H2AX, reduced RAD51. Arrest in meiosis I. Chromosome fragmentation. Oocytes: precocious separation of sister chromatids at metaphase II
Fancp	*Slx4/Btbd12*	[35,55]	Reduced weight	Reduced	Reduced		No follicles	Yes	Females are sterile. Males near-sterile	Increased MLH1/3. Unchanged number of chiasmata
Fancr	*Rad51*	[51]		Reduced	Embryonic lethal	Embryonic lethal		Yes		RAD51 labels meiotic DSBs. siRNA injection against RAD51 leads to p53-dependent apoptosis and reduced MLH1 foci

Table 1. (continued)

	Alias	Refs	Testes	Spermatozoa	Male germ numbers	Females germ numbers	Ovaries	PGC apoptosis	Litter size	Meiosis
Fancs	*Brca1*	[56] (*Brca1*$^{-/-}$ *p53*$^{+/-}$)	Reduced weight	None	None			Yes	Fertile females. Sterile males.	Spermatocytes: reduced RAD51 foci. Normal DMC1 foci. Normal synapsis. Lack of chiasmata and MLH1 in males. Pachytene arrest in males. Oocytes: MLH1 normal
Fancu	*Xrcc2*	[38]	Reduced weight	No spermatozoa			Reduced follicles	Yes	Reduced. Male sterility	
Fancv	*Rev7*	[43]	Reduced weight. Tubules atrophic	None	PGCs lost during migration	PGCs lost during migration	Atrophic, no follicles	Yes, during migration	Sterile	
Fancw	*Rfwd3*	[44]	Few spermatozoa	Reduced			Atrophic, no follicles		Male and female sterility	
Faap20		[36]	Reduced weight				Smaller ovaries. Reduced number of follicles		Reduced	
Usp1		[57]	Reduced weight. Tubules atrophic				Reduced number of oocytes	Yes	Male sterility	
Uaf1		[30] (*Uaf1*$^{+/-}$)	Reduced weight				Reduced number of oocytes			

raises the question of how long comparable SSCs could be maintained in FA males (see Outstanding Questions). We view this as an important biological question because it is estimated that a sperm has undergone 7–25-fold more chromosome replications than the egg it will fertilize, depending on the age of the individuals [61]. Therefore, with increasing age, defects in spermatogenesis – due to increasing mutational load and apoptosis of SSCs – would become more severe over time. This highlights why fertility defects would be exacerbated in males with FA, but also points to an area of research that could be tractable for prolonging any window of fertility.

The FA Pathway Genes in the DSB Repair Model of Meiotic Prophase

Programmed DSBs form at the onset of meiosis. Some FA pathway genes have been demonstrated to play a role in this DSB repair; however, no clear unifying and essential role for the FA core complex genes has been shown at this point. This is perhaps not surprising because the FA core complex as described in humans arose late in evolution in vertebrates [62–64] and meiotic DSB repair is a well conserved process [65]. Hence the FA core complex components have not been identified in classic meiotic forward-genetic screens in any species because FA core complex mutant phenotypes during meiosis in mouse spermatocytes [29,31,66] are subtle compared to the catastrophic effects of, for example, chromosome mis-segregation or unrepaired DSBs in *SPO11* or *MRE11* mutants, respectively, which can be observed for example in plant species which do not have a strict pachytene checkpoint [67]. Nevertheless, core complex mutants have meiotic phenotypes that have been detected across species. These defects include possible perturbation of the obligatory **crossover** (CO; Box 1) [62,68], altered genetic map distances [68–73], and finer regulatory roles and adaptations to the meiotic program that are needed in species with non-homologous sex chromosomes [29,66].

Initiation of Meiotic DSB Repair

The FANCD1/BRCA2 complex has been demonstrated to function early in the repair of meiotic DSBs with cofactors RAD51 and DMC1 (Figure 3). It was shown in the model species *Arabidopsis thaliana* (arabidopsis) and *Caenorhabditis elegans* that BRCA2 is essential for DSB repair, with massive meiotic chromosome fragmentation and aggregation in the mutants respectively, which was SPO11-dependent [74–76]. Similar phenotypes of persistent DSBs were observed in *Brca2* mutant male mice [77] and *Drosophila melanogaster* females [78]. Studies in arabidopsis and human have shown that *BRCA2* physically interacts with DMC1 and RAD51 [74,79,80], which is consistent with data from male mice, *C. elegans*, and arabidopsis showing that *BRCA2* is required for localization of RAD51 and DMC1 to meiotic DSBs [74–77].

Box 1. The DSB Repair Model of Meiotic Crossover Formation

Meiosis is a modification of cell division in which ploidy is halved, such that the normal DNA complement is restored upon fertilization. Ploidy reduction occurs by one round of DNA replication being followed by two successive rounds of chromosome segregation. The first meiotic division requires the balanced segregation of homologs. Each pair of homologs requires sister-chromatid cohesion and at least one 'obligatory CO' – a large reciprocal exchange of genetic material – to allow correct segregation. Without COs or cohesion, unbalanced chromosome segregation leads to decreased fertility and aneuploid gametes [65,113,114]. CO formation is initiated by programmed DSB formation by the topoisomerase-related SPO11 homologs [115–119]. DSBs are resected to leave a 3′ single-strand overhang, onto which RecA homologs RAD51 and DMC1 load to initiate HR, which will lead to either a CO or non-CO repair product. DSBs during meiosis vastly outnumber COs in most species studied, and only a minority of DSBs will form COs [65,120–122]. Most COs in mammals and other eukaryotes require a group of genes collectively referred to as the ***ZMMs***. *ZMM*-dependent COs display positive interference, a phenomenon where the occurrence of a CO in one genetic interval reduces the probability of a CO occurring in an adjacent interval. These COs are referred to as class I COs. Another minor CO pathway with distinct genetic requirements, for example *MUS81*, produces COs without detectable interference (class II COs) [65].

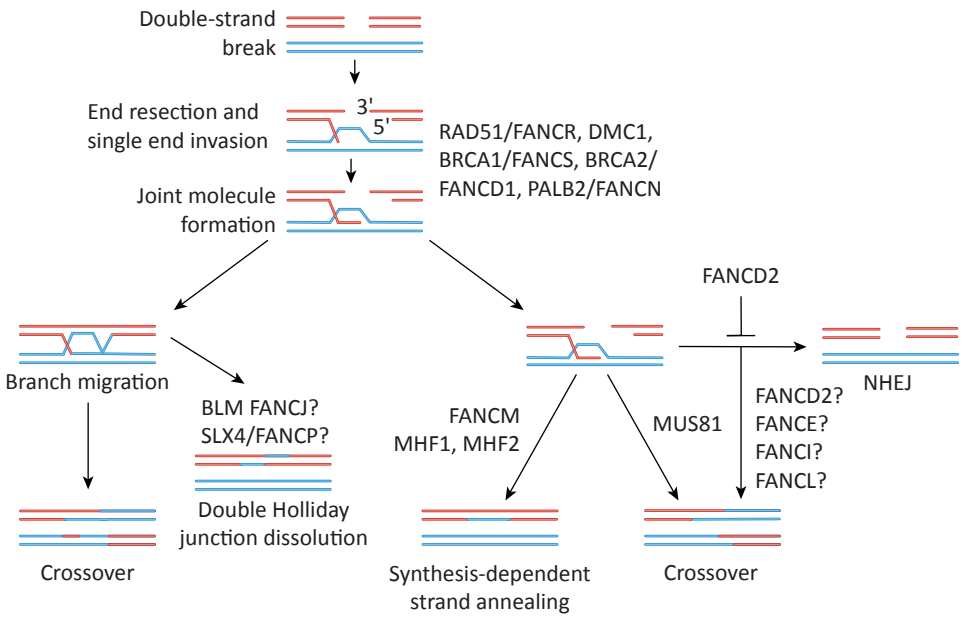

Figure 3. Model of Meiotic Double-Strand Break (DSB) Repair with a Focus on the Involvement of the Fanconi Anemia (FA) Pathway. A model of meiotic DSB repair inspired by multiple species, in particular mouse, arabidopsis, budding yeast, and *C. elegans*. Meiotic DSBs are resected, which leads to strand invasion and extension. A subset of intermediates form double Holliday junctions, most of which are resolved as interference-sensitive crossovers (class I COs). In an alternative pathway D-loop intermediates are destabilized, or these intermediates can form single Holliday junctions which can be resolved as interference-insensitive COs (class II) or non-COs. Non-homologous end-joining (NHEJ) is actively suppressed during meiotic DSB repair. Question marks over particular proteins indicate areas that remain to be understood but where there is evidence supporting roles for FA proteins in the balance between different pathway choices.

It is known in human somatic cells that mono-ubiquitinated FANCD2 and BRCA2 physically interact [81]. Further, one of the first molecular mechanistic links between the FA pathway and meiosis was the observation of colocalization of FANCD2 and BRCA1 in mouse spermatocytes [82]. We speculate that understanding the interaction between BRCA2–FANCD2 may provide clues that will allow future research to bridge the gap between data relating to the roles of BRCA2–RAD51–DMC1 and the FANCD2–FA core complex in meiotic DSB repair.

The FA Core Complex and FANCI–FANCD2 in Meiosis

In somatic cells, the FA core complex plays a key role in DNA ICL repair [1,9]. It does not seem likely that this role is essential in DSB repair at meiosis. Most meiotic phenotypic findings of core complex mutants come from non-mammalian systems. Mutant mouse strains exist for homologs of all components of the human FA core complex, and these have been used in a limited number of studies for analyses of male meiosis. The major findings from these studies are that mutations in the core complex genes do not provoke meiotic arrest in male mice, that the core complex is necessary for localization of FANCD2 to the **XY body**, and that the temporal localization of FANCD2 is different between XY and autosomes [29,66], the significance of which will be interesting to determine. One possibility for the X and Y chromosomes is that the FA gene products push DSB repair to the sister chromatid because interhomolog COs are not a possibility in XY regions which are outside the pseudo-autosomal region, that represent the majority of the physical length of the X and Y chromosomes. Evidence suggests that, in male mice, FANCD2 may localize to the sites of meiotic DSBs on XY axes as FANCD2 colocalizes

with RAD51 in a mostly *SPO11*-dependent manner [66]. This meiotic FANCD2–RAD51 colocalization somewhat parallels the role of human and chicken FANCD2 in stabilizing RAD51 in somatic cells [83], suggesting a conserved role for this interaction.

It is possible that functional redundancy between DNA repair pathways can mask the meiotic phenotypes of FA core complex mutants. The use of model organisms may permit more exhaustive multimutant studies which exacerbate phenotypes of FA mutants, and hence allow the detection of new functions. For example, seminal work in *C. elegans* demonstrated that the *FANCD2* homolog *fcd-2* suppresses **non-homologous end-joining** (NHEJ) during meiosis [84], as well as in somatic human, chicken, and *C. elegans* cells [84,85]. However, this meiotic role of *fcd-2* is cryptic when *msh-4* (a *ZMM* gene) or *syp-2* (a **synaptonemal complex** central region protein) are present, because illegitimate NHEJ was only observed in *fcd-2 msh-4* and *fcd-2 syp-2* double mutants, and not in the respective single mutants. CO frequencies were unchanged in the *fcd-2* mutant; however, there was an increase in SPO11-dependent RAD51 foci, which suggests altered repair dynamics. Similarly, DMC1 foci – a meiotic specific paralog of RAD51 that is not found in *C. elegans* – are increased in *fancd2* mutant arabidopsis, whereas RAD51 foci are unchanged in arabidopsis *fancd2* [68]. Slightly reduced CO frequencies were observed at two of four tested loci in arabidopsis *fancd2*. This was reflected in a reduction in chiasmata (cytological representation of a CO) on the three largest arabidopsis chromosomes, and a slight reduction in fertility, which correlated with low levels of homolog mis-segregation at the first meiotic division. However, **crossover interference** appeared unchanged in arabidopsis *fancd2*, as measured both genetically and cytologically. These findings suggest that FANCD2 promotes a type of CO that is independent of the two classic pathways to meiotic CO formation in many eukaryotes, or is partially redundant with the class II/MUS81-dependent pathway. It would be interesting to know if the meiotic *fcd-2* and arabidopsis *fancd2* phenotypes are dependent on FANCD2 ubiquitination. Using reverse- and forward-genetic approaches, a low frequency of mis-segregating homologs were detected in mutants for arabidopsis homologs of the FA genes *FANCE*, *FANCL*, *FANCD2*, and *FANCI* [62,68,86]. In particular, the arabidopsis *fancl* and *fance* phenotypes are consistent with the hypothesis that the FA core complex and *FANCD2* ubiquitination may be required for normal CO control in some species. A limited number of studies investigated the role of the FA core complex in mammalian meiosis [29,31,66], and the studies discussed above highlight how this area will be an interesting avenue for basic meiosis research questions and clinical projects focused on mis-segregation of homologs and resulting aneuploidies.

CO-Limiting Properties of FA Proteins

Model organisms have revealed meiotic roles of FA proteins which are nearly impossible to uncover in mammals owing to the strong fertility defects that occur earlier in gametogenesis in mammals. FANCM has been shown to limit CO frequencies in arabidopsis, *Brassica* crops, rice, pea, fission yeast, and *Drosophila* females [69–72,87]. The increase in COs in arabidopsis *fancm* mutants come from a genetic pathway that is dependent on *MUS81*, but not on the *ZMMs*, and these additional COs do not show interference. This is consistent with the idea that FANCM limits a normally very minor pathway of COs. The cofactors of arabidopsis FANCM, MHF1 and MHF2, also limit COs. CO frequencies are increased in *mhf2* and *mhf1* mutants but to a lesser extent than *fancm* [62]. Similar observations were made in fission yeast where an increase in COs associated with a gene conversion event were increased in *fml1/FANCM, mhf1*, and *mhf2* mutants via a pathway parallel to MUS81 [72]. Further, in *Drosophila*, the spontaneous mitotic COs which occur in *Fancm* mutants are dependent on MUS81 [87]. Intriguingly, in both *Drosophila* females [87] and plants [71,88,89], the increase in meiotic COs is

not homogenous along the length of any chromosome, highlighting that there are many levels of regulation on the path to CO formation that have different genetic requirements.

Phenotypic parallels can be observed between mutants of FANCM, MHF1, and MHF2 homologs with respect to assays that measure either COs at meiosis or sister-chromatid exchanges in chicken, mouse, and human somatic cells [41,90,91]. This CO-limiting role of FANCM–MHF1–MHF2, be it meiotic or somatic, differs from that of the FA core complex and FANCI–D2. The increase of COs versus non-COs in *FANCM* mutants seems to reflect 'directing' of repair intermediates to a particular pathway, as opposed to an essential requirement for FANCM in DSB repair. We speculate that the meiotic CO-limiting role of FANCM–MHF1–MHF2 was adapted early in sexually reproducing species from its somatic role as a mechanism to limit the generation of diversity from meiotic COs from one generation to the next such that favorable haplotypes are not disrupted too frequently [92,93].

However, FANCM may not be the only CO-limiting mechanism at meiosis from the FA pathway. Analysis of spermatocytes from mouse mutants for helicase-encoding gene *Fancj* showed a slight increase in MLH1 foci formation and chiasmata [50]. *Slx4*/*Fancp* mutant male mice also show a slight increase of MLH1 and MLH3 foci, and *Slx4*/*Fancp* mutant female mice had increased MLH1 foci; however, chiasmata numbers were not different from control littermates [35]. These data support that the idea that *Fancj* and *Slx4*/*Fancp* may limit class I CO formation at meiosis, and it would be interesting in future studies to perform genetic analysis of the meiotic products in mice mutant for *Fancj* and *Slx4* to test this hypothesis. It also would be interesting to investigate if there is any link between the CO-limiting roles of arabidopsis *FANCM* in the formation of *MUS81*-dependent COs, and the roles of *Fancj* and *Slx4*/*Fancp* in the formation of what may be *ZMM*-dependent COs in mice.

These studies highlight how FA proteins, and possibly their respective subcomplexes, are important to control several steps of meiotic recombination both at the initiation of DSB repair and in the later stages of CO maturation in diverse species [35,50,62,69]. Although the restricted comparability of meiotic DSB repair models across species limits cross-species inferences, the evidence suggests that the FA proteins are important to regulate the balance between class I and class II COs [35,50,62,68,69,72,87] in a way that differs significantly from the traditionally described role of these proteins in mammalian DNA ICL repair [1,9]. However, broadly, mutants of FA core complex genes and *FANCI–FANCD2* mutants tend to exhibit phenotypic differences from mutants of the *FANCM* anchor complex and of the *FANC* genes that have direct functions in **homologous recombination** (HR).

We speculate that the meiotic functions of FA gene products are largely masked by strong PGC phenotypes in FA mouse models. Techniques which inject siRNA or complementing proteins into the seminiferous tubules of mice [51] could allow uncoupling of the meiotic and PGC maintenance phenotypes, with the possible advantage of being more cost- and time-effective than generating conditional mutant mouse strains. This technique has already been used to decipher the genetic mechanisms of spermatocyte depletion with *Rad51/Fancr* mutants [51].

Opportunities for Diagnosing FA via Reproductive Phenotypes and Other Future Directions

Approximately 12.5–20% of couples globally are reported to be infertile, and 2.5–12% of men are reported to be infertile [94]. The genetics of human male infertility has become more tractable in recent years with the increased ease of DNA sequencing [95,96], and DNA repair genes are emerging as key players in fertility [97]. It is appreciated that there is a strong genetic

basis for male infertility, accounting for at least 15% of cases, of which many are diagnosed as azoospermia [96]. It has been established that the majority individuals with FA have severely reduced fertility (e.g., azoospermia in males) [2,3,25,98]; however, reduced fertility is historically not the manifestation of the disease which leads to an FA diagnosis. Diagnostic tests for FA are requested when congenital abnormalities, cytopenias, or childhood cancers are detected [1,2,98]. However, several recent reports describe patients with mutations in *FANCA* [6], *FANCM* [52], or *XRCC2*/*FANCU* [38] who were only clinically diagnosed with FA after they presented with non-obstructive azoospermia and Sertoli cell-only syndrome. These case studies demonstrate that infertility phenotypes represent a new approach to identifying individuals with FA before they develop hematological symptoms or malignancies. Early diagnosis and appropriate follow-up will allow lifestyle modifications such as a reduction in the intake of products which lead to increased aldehyde levels, thereby providing opportunities to improve long-term health in FA.

Concluding Remarks: New Technologies and Possibilities for Improved Reproductive Success in Patients with FA

This review highlights many reports showing a large reduction in PGCs and an acceleration of the reproductive clock in FA. However, there are new opportunities on the horizon, and we speculate below about how new technologies could increase reproductive possibilities in FA individuals with reduced or even abolished PGCs. Despite the great potential of the scientific possibilities that are emerging, notably gene editing of the germline for therapeutic purposes, the ethical implications must be considered. Further, the cost of gene therapies is likely to pose highly restrictive barriers to the uptake of new technologies. It is also important to consider that conditioning regimes for hematopoietic stem cell transplants lead to long-term effects on fertility [99], and clinical research into fertility preservation post-chemotherapy will benefit both FA patients and the general population.

FA mouse models collectively show very strong subfertile phenotypes where there is rapid attrition of PGCs compared to non-FA controls. The observation that some sections of seminiferous tubules develop more normally than others [31,34,35,39–41,60] could be explored further to inform future studies focused on developing *in vitro* fertilization (IVF) approaches for males with FA. However, it is currently unclear if cryopreservation of gametes from young adult male or female FA patients can be reliably used for IVF. Strategies to prevent the loss of germ cells may be facilitated by clinical trials currently aimed at improving other pathological manifestations of FA. Two Phase II clinical trials are assessing the efficiency of quercetin and metformin for chemoprevention for squamous cell carcinoma in patients with FA and improved hematologic response, respectively, and TGF-β inhibition also presents a promising target [100]. If these compounds prove efficient in treating one manifestation of the disease, it will be interesting to observe if the treatments could modify other manifestations of FA such as infertility, particularly if taken as a prophylactic. Two gene therapy clinical trials underway in Spain (Phase I/II) and the USA (Phase I) are establishing the safety of gene therapy in *FANCA* patients, also with a view to improve hematological response. Future efforts could employ strategies that reimplant corrected germ cells into FA patients. Further into the future, several groups are actively working on human *in vitro* gametogenesis [101], which would see induced pluripotent potent stem cells (iPSCs) from an individual passed through meiosis to the maturation of spermatozoa and oocytes that could be used in IVF. In the case of FA, the genetic defect causing FA could be corrected before generating iPSCs and subsequent healthy gametes. Such strategies may be compatible with current IVF methodology; however, this discussion is highly speculative and, importantly, other major ethical barriers regarding germ cell modification would need intense investigation and discussion. It has been a founding belief

Outstanding Questions

Science has led to significant advances which have resulted in increased life expectancies for individuals with FA. However, the extended life expectancy is not due to treatments specific for FA but instead to improved bone marrow transplant outcomes as well as early detection and treatment of cancer. The FA research community is working rapidly towards targeted treatments and gene therapy, which will result in an intensified desire for fertility. We believe that there are several areas where there is value to be gained both clinically and for fundamental research.

How can reproductive defects help to prioritize patients who should be considered for FA diagnostic tests? Non-obstructive azoospermia/primary ovarian insufficiency are often idiopathic with no family history. A significant number of these cases are suspected to have heterogeneous genetic causes. Exome-sequencing approaches are revealing mutations in DNA repair genes as one of the likely causes, but there are limitations for this to be useful clinically, including cost, variants of unknown significance that require functional validation from specialist researchers or diagnostic laboratories, and individuals with non-obstructive azoospermia/primary ovarian insufficiency may present to fertility clinics where diagnosis may be missed if the treating physicians are not familiar with FA.

Have gametes from adults with FA acquired any suppressor mutations that have allowed the cell lineage to prosper longer than those that underwent apoptosis? The genomes of descendants of FA fathers, and possibly FA mothers, could hold the answer to this question, as may the offspring of FA mouse models.

How has the meiotic DSB repair machinery integrated the FA proteins over the course of evolution? In species with a full or partial FA core complex, does mono-ubiquitination of FANCD2 and FANCI play a role in meiotic DSB repair? Genetic and cytological data indicated that the FA proteins have been co-opted at a variety of stages in the DSB repair model of

of the human gene therapy field that the human germline should not be genetically modified [102], although this notion may become increasingly tested [103]. A non-exhaustive list of issues which must be discussed related to germline editing include societal views of genetic modification for therapeutic purposes versus enhancement and how the two scenarios are distinguished, the consequences of unintended off-target effects of gene editing, modification of the gene pool, lack of consent from the unborn child, philosophical views on the difference between current IVF selection protocols and genome modification, equity of access, and other philosophical concerns [98,102,103]. In particular, it would be important to consider the consequences of creating embryos from cells that have acquired extensive genomic instability such as chromosomal translocations, duplications and deletions. Is the cause of FA-related infertility linked to the acquisition of excessively deleterious genomic instability? Could studies of the health and genomic organization of children already born to female FA patients help to answer this question? Individuals with FA have already been trailblazers in receiving new medical treatments – the first cord blood transplant recipient from an HLA-matched sibling [104] and the first combined preimplantation genetic diagnosis (PGD) for a genetic disorder with HLA matching [105]. The FA field may again be a pioneer for future fertility solutions that will benefit many people.

CO formation across diverse species (Figure 3) beyond their somatic role in ICL repair. The role of the FA core complex in mammalian meiosis has not been extensively studied; however, this is likely to be an interesting avenue for basic meiosis research.

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References

1. Ceccaldi, R. *et al.* (2016) The Fanconi anaemia pathway: new players and new functions. *Nat. Rev. Mol. Cell Biol.* 17, 337–349
2. Auerbach, A.D. (2009) Fanconi anemia and its diagnosis. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* 668, 4–10
3. Alter, B.P. *et al.* (1991) Fanconi's anaemia and pregnancy. *Br. J. Haematol.* 77, 410–418
4. Alter, B.P. (2003) Cancer in Fanconi anemia, 1927–2001. *Cancer* 97, 425–440
5. Liu, J.M. *et al.* (1991) Fanconi anemia presenting unexpectedly in an adult kindred with no dysmorphic features. *Am. J. Med.* 91, 555–557
6. Krausz, C. *et al.* (2018) From exome analysis in idiopathic azoospermia to the identification of a high-risk subgroup for occult Fanconi anemia. *Genet. Med.* Published online June 14, 2018. http://dx.doi.org/10.1038/s41436-018-0037-1
7. Fouquet, B. *et al.* (2017) A homozygous FANCM mutation underlies a familial case of non-syndromic primary ovarian insufficiency. *eLife* 6, 1–17
8. Kasak, L. *et al.* (2018) Biallelic recessive loss-of-function variants in FANCM cause non-obstructive azoospermia. *Am. J. Hum. Genet.* 103, 200–212
9. Deans, A.J. and West, S.C. (2011) DNA interstrand crosslink repair and cancer. *Nat. Rev. Cancer* 11, 467–480
10. Knipscheer, P. *et al.* (2009) The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair. *Science* 2, 1698–1701
11. Schlacher, K. *et al.* (2011) Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. *Cell* 145, 529–542
12. Schlacher, K. *et al.* (2012) A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51–BRCA1/2. *Cancer Cell* 22, 106–116
13. Kais, Z. *et al.* (2016) FANCD2 maintains fork stability in BRCA1/2-deficient tumors and promotes alternative end-joining DNA repair. *Cell Rep.* 15, 2488–2499
14. Michl, J. *et al.* (2016) FANCD2 limits replication stress and genome instability in cells lacking BRCA2. *Nat. Struct. Mol. Biol.* 23, 755–757
15. Semlow, D.R. *et al.* (2016) Replication-dependent unhooking of DNA interstrand cross-links by the NEIL3 glycosylase. *Cell* 167, 1–14
16. Van Twest, S. *et al.* (2017) Mechanism of ubiquitination and deubiquitination in the Fanconi anemia pathway. *Mol. Cell* 65, 247–259
17. Rajendra, E. *et al.* (2014) The genetic and biochemical basis of FANCD2 monoubiquitination. *Mol. Cell* 54, 858–869
18. Alpi, A.F. *et al.* (2008) Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCL, and FANCI. *Mol. Cell* 32, 767–777
19. Garcia-Higuera, I. *et al.* (2000) The Fanconi anemia proteins FANCA and FANCG stabilize each other and promote the nuclear accumulation of the Fanconi anemia complex. *Blood* 96, 3224–3230
20. Benitez, A. *et al.* (2018) FANCA promotes DNA double-strand break repair by catalyzing single-strand annealing and strand exchange. *Mol. Cell* 71, 621–628
21. Bashamboo, A. *et al.* (2017) Anomalies in human sex determination provide unique insights into the complex genetic interactions of early gonad development. *Clin. Genet.* 91, 143–156
22. Saitou, M. and Miyauchi, H. (2016) Gametogenesis from pluripotent stem cells. *Stem Cell* 18, 721–735
23. Cantú, A.V. and Laird, D.J. (2017) A pilgrim's progress: seeking meaning in primordial germ cell migration. *Stem Cell Res.* 24, 181–187
24. De Felici, M. *et al.* (2013) Origin, migration, and proliferation of human primordial germ cells. In *Oogenesis* (Coticchio, G., ed.), pp. 19–37, Springer
25. Bargman, G.J. *et al.* (1977) Studies of malformation syndromes of Man XLVII: disappearance of spermatogonia in the Fanconi anemia syndrome. *Eur. J. Pediatr.* 168, 163–168

26. Cowdell, R.H. *et al.* (1955) Constitutional anemia (Fanconi's Syndrome) and leukemia in two brothers. *Blood* 10, 788–801
27. Nilsson, L.R. (1960) Chronic pancytopenia with multiple congenital abnormalities (Fanconi's anaemia). *Acta Paediatr.* 49, 518–529
28. Meetei, A.R. *et al.* (2004) X-linked inheritance of Fanconi anemia complementation group B. *Nat. Genet.* 36, 1219–1224
29. Kato, Y. *et al.* (2015) FANCB is essential in the male germline and regulates H3K9 methylation on the sex chromosomes during meiosis. *Hum. Mol. Genet.* 24, 5234–5249
30. Park, E. *et al.* (2013) Inactivation of Uaf1 causes defective homologous recombination and early embryonic lethality in mice. *Mol. Cell. Biol.* 33, 4360–4370
31. Wong, J.C.Y. *et al.* (2003) Targeted disruption of exons 1 to 6 of the Fanconi anemia group A gene leads to growth retardation, strain-specific microphthalmia, meiotic defects and primordial germ cell hypoplasia. *Hum. Mol. Genet.* 12, 2063–2076
32. Fu, C. *et al.* (2016) Primary ovarian insufficiency induced by Fanconi anemia E mutation in a mouse model. *PLoS One* 11, 1–13
33. Simhadri, S. *et al.* (2014) Male fertility defect associated with disrupted BRCA1–PALB2 interaction in mice. *J. Biol. Chem.* 289, 24617–24629
34. Yang, Y. *et al.* (2001) Targeted disruption of the murine Fanconi anemia gene, Fancg/Xrcc9. *Blood* 98, 3435–3440
35. Holloway, J.K. *et al.* (2011) Mammalian BTBD12 (SLX4) protects against genomic instability during mammalian spermatogenesis. *PLoS Genet.* 7, e1002094
36. Zhang, T. *et al.* (2015) Loss of Faap20 causes hematopoietic stem and progenitor cell depletion in mice under genotoxic stress. *Stem Cells* 33, 2320–2330
37. Bakker, S.T. *et al.* (2012) Fancf-deficient mice are prone to develop ovarian tumours. *J. Pathol.* 226, 28–39
38. Yang, Y. *et al.* (2018) XRCC2 mutation causes meiotic arrest, azoospermia and infertility. *J. Med. Genet.* 55, 628–636
39. Houghtaling, S. *et al.* (2003) Epithelial cancer in Fanconi anemia complementatioin group D2 (FANCD2) knockout mice. *Genes Dev.* 17, 2021–2035
40. Koomen, M. *et al.* (2002) Reduced fertility and hypersensitivity to mitomycin C characterize Fancg/Xrcc9 null mice. *Hum. Mol. Genet.* 11, 273–281
41. Bakker, S.T. *et al.* (2009) Fancm-deficient mice reveal unique features of Fanconi anemia complementation group M. *Hum. Mol. Genet.* 18, 3484–3495
42. Luo, Y. *et al.* (2014) Hypersensitivity of primordial germ cells to compromised replication-associated DNA repair involves ATM–p53–p21 signaling. *PLoS Genet.* 10, e1004471
43. Watanabe, N. *et al.* (2013) The REV7 subunit of DNA polymerase ζ is essential for primordial germ cell maintenance in the mouse. *J. Biol. Chem.* 288, 10459–10471
44. Knies, K. *et al.* (2017) Biallelic mutations in the ubiquitin ligase RFWD3 cause Fanconi anemia. *J. Clin. Invest.* 127, 3013–3027
45. Nadler, J.J. and Braun, R.E. (2000) Fanconi anemia complementation group C is required for proliferation of murine primordial germ cells. *Genesis* 27, 117–123
46. Tam, P.P.L. and Snow, M.H.L. (1981) Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. *J. Embryol. Exp. Morphol.* 64, 133–147
47. Ceccaldi, R. *et al.* (2011) Spontaneous abrogation of the G2DNA damage checkpoint has clinical benefits but promotes leukemogenesis in Fanconi anemia patients. *J. Clin. Invest.* 121, 184–194
48. Heinrich, M.C. *et al.* (1998) DNA cross-linker-induced G2/M arrest in group C Fanconi anemia lymphoblasts reflects normal checkpoint function. *Blood* 91, 275–287
49. Yuen, W.S. *et al.* (2012) DNA double strand breaks but not interstrand crosslinks prevent progress through meiosis in fully grown mouse oocytes. *PLoS One* 7, 1–9
50. Sun, X. *et al.* (2016) FancJ (Brip1) loss-of-function allele results in spermatogonial cell depletion during embryogenesis and altered processing of crossover sites during meiotic prophase I in mice. *Chromosoma* 125, 237–252
51. Dai, J. *et al.* (2017) Meiotic knockdown and complementation reveals essential role of RAD51 in mouse spermatogenesis. *Cell Rep.* 18, 1383–1394
52. Yin, H. *et al.* (2018) A homozygous FANCM frameshift pathogenic variant causes male infertility. *Genet. Med.* Published online August 29, 2018. http://dx.doi.org/10.1038/s41436-018-0127-0
53. Hartford, S.A. *et al.* (2016) Interaction with PALB2 is essential for maintenance of genomic integrity by BRCA2. *PLoS Genet.* 12, 1–27
54. Kuznetsov, S. *et al.* (2007) RAD51C deficiency in mice results in early prophase I arrest in males and sister chromatid separation at metaphase II in females. *J. Cell Biol.* 176, 581–592
55. Crossan, G.P. *et al.* (2011) Disruption of mouse Slx4, a regulator of structure-specific nucleases, phenocopies Fanconi anemia. *Nat. Genet.* 43, 147–152
56. Xu, X. (2003) Impaired meiotic DNA-damage repair and lack of crossing-over during spermatogenesis in BRCA1 full-length isoform deficient mice. *Development* 130, 2001–2012
57. Kim, J.M. *et al.* (2009) Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. *Dev. Cell* 16, 314–320
58. Lin, W. *et al.* (2017) Ovarian aging in women with BRCA germline mutations. *J. Clin. Endocrinol. Metab.* 102, 3839–3847
59. Qiao, H. *et al.* (2018) Impeding DNA break repair enables oocyte quality control. *Mol. Cell* 72, 211–221
60. Fu, C. *et al.* (2016) Dearth and delayed maturation of testicular germ cells in Fanconi anemia E mutant male mice. *PLoS One* 11, 1–14
61. Crow, J.F. (2000) The origins, patterns and implications of human spontaneous mutation. *Nat. Rev. Genet.* 1, 40–47
62. Girard, C. *et al.* (2014) FANCM-associated proteins MHF1 and MHF2, but not the other Fanconi anemia factors, limit meiotic crossovers. *Nucleic Acids Res.* 42, 9087–9095
63. Zhang, X.Y. *et al.* (2009) Xpf and not the Fanconi anaemia proteins or Rev3 accounts for the extreme resistance to cisplatin in *Dictyostelium discoideum*. *PLoS Genet.* 5
64. Dong, H. *et al.* (2015) Update of the human and mouse Fanconi anemia genes. *Hum. Genomics* 9, 32
65. Hunter, N. (2015) Meiotic recombination: the essence of heredity. *Cold Spring Harb. Perspect. Biol.* 7, a016618
66. Alavattam, K.G. *et al.* (2016) Elucidation of the Fanconi anemia protein network in meiosis and its function in the regulation of histone modifications. *Cell Rep.* 17, 1141–1157
67. Puizina, J. *et al.* (2004) Mre11 deficiency in *Arabidopsis* is associated with chromosomal instability in somatic cells and Spo11-dependent genome fragmentation during meiosis. *Plant Cell* 16, 1968–1978
68. Kurzbauer, M.-T. *et al.* (2018) *Arabidopsis thaliana* FANCD2 promotes meiotic crossover formation. *Plant Cell* 30, tpc.00745.2017
69. Crismani, W. *et al.* (2012) FANCM limits meiotic crossovers. *Science* 336, 1588–1590
70. Blary, A. *et al.* (2018) FANCM limits meiotic crossovers in *Brassica* crops. *Front. Plant Sci.* 9, 368
71. Mieulet, D. *et al.* (2018) Unleashing meiotic crossovers in crops. *Nat. Plants* 4, 1010–1016
72. Lorenz, A. *et al.* (2012) The fission yeast FANCM ortholog directs non-crossover recombination during meiosis. *Science* 336, 1585–1588
73. Da Ines, O. *et al.* (2013) Effects of XRCC2 and RAD51B mutations on somatic and meiotic recombination in *Arabidopsis thaliana*. *Plant J.* 74, 959–970
74. Siaud, N. *et al.* (2004) Brca2 is involved in meiosis in *Arabidopsis thaliana* as suggested by its interaction with Dmc1. *EMBO J.* 23, 1392–1401

75. Martin, J.S. *et al.* (2005) RAD-51-dependent and -independent roles of a *Caenorhabditis elegans* BRCA2-related protein during DNA double-strand break repair. *Mol. Cell. Biol.* 25, 3127–3139
76. Seeliger, K. *et al.* (2012) BRCA2 is a mediator of RAD51- and DMC1-facilitated homologous recombination in *Arabidopsis thaliana*. *New Phytol.* 193, 364–375
77. Sharan, S.K. (2004) BRCA2 deficiency in mice leads to meiotic impairment and infertility. *Development* 131, 131–142
78. Klovstad, M. *et al.* (2008) *Drosophila* Brca2 is required for mitotic and meiotic DNA repair and efficient activation of the meiotic recombination checkpoint. *PLoS Genet.* 4, e31
79. Jensen, R.B. *et al.* (2010) Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature* 467, 678–683
80. Martinez, J.S. *et al.* (2016) BRCA2 regulates DMC1-mediated recombination through the BRC repeats. *Proc. Natl. Acad. Sci. U. S. A.* 113, 3515–3520
81. Wang, X. *et al.* (2004) Functional interaction of monoubiquitinated FANCD2 and BRCA2/FANCD1 in chromatin. *Mol. Cell. Biol.* 24, 5850–5862
82. Garcia-Higuera, I. *et al.* (2001) Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell* 7, 249–262
83. Sato, K. *et al.* (2016) FANCI–FANCD2 stabilizes the RAD51–DNA complex by binding RAD51 and protects the 5′-DNA end. *Nucleic Acids Res.* 44, 10758–10771
84. Adamo, A. *et al.* (2010) Preventing nonhomologous end joining suppresses DNA repair defects of Fanconi anemia. *Mol. Cell* 39, 25–35
85. Pace, P. *et al.* (2010) Ku70 corrupts DNA repair in the absence of the Fanconi anemia pathway. *Science* 329, 219–223
86. Capilla-Perez, L. *et al.* (2018) The HEM lines: a new library of homozygous *Arabidopsis thaliana* EMS mutants and its potential to detect meiotic phenotypes. *Front. Plant Sci.* 9, 1–9
87. Kenny Kuo, H. *et al.* (2014) *Drosophila* FANCM helicase prevents spontaneous mitotic crossovers generated by the MUS81 and SLX1 nucleases. *Genetics* 198, 935–945
88. Ziolkowski, P.A. *et al.* (2015) Juxtaposition of heterozygosity and homozygosity during meiosis causes reciprocal crossover remodeling via interference. *eLife* 4, e03708
89. Girard, C. *et al.* (2015) AAA-ATPase FIDGETIN-LIKE 1 and helicase FANCM antagonize meiotic crossovers by distinct mechanisms. *PLoS Genet.* 11, e1005369
90. Yan, Z. *et al.* (2010) A histone-fold complex and FANCM form a conserved DNA-remodeling complex to maintain genome stability. *Mol. Cell* 37, 865–878
91. Deans, A.J. and West, S.C. (2009) FANCM connects the genome instability disorders Bloom's syndrome and Fanconi anemia. *Mol. Cell* 36, 943–953
92. Hadany, L. and Comeron, J.M. (2008) Why are sex and recombination so common? *Ann. N. Y. Acad. Sci.* 1133, 26–43
93. Ritz, K.R. *et al.* (2017) Variation in recombination rate: adaptive or not? *Trends Genet.* 33, 364–374
94. Agarwal, A. *et al.* (2015) A unique view on male infertility around the globe. *Reprod. Biol. Endocrinol.* 13, 1–9
95. Krausz, C. *et al.* (2015) Genetics of male infertility: from research to clinic. *Reproduction* 150, R159–R174
96. Krausz, C. and Riera-Escamilla, A. (2018) Genetics of male infertility. *Nat. Rev. Urol.* 15, 369–384
97. Nagirnaja, L. *et al.* (2018) Genetic intersection of male infertility and cancer. *Fertil. Steril.* 109, 20–26
98. Frohnmayer, D. *et al.* (2014) *Fanconi Anemia: Guidelines for Diagnosis and Management.* (4th edn), Fanconi Anemia Research Fund
99. Borgmann-Staudt, A. *et al.* (2012) Fertility after allogeneic haematopoietic stem cell transplantation in childhood and adolescence. *Bone Marrow Transplant.* 47, 271–276
100. Zhang, H. *et al.* (2016) TGF-β inhibition rescues hematopoietic stem cell defects and bone marrow failure in Fanconi anemia. *Cell Stem Cell* 18, 668–681
101. Yamashiro, C. *et al.* (2018) Generation of human oogonia from induced pluripotent stem cells *in vitro*. *Science* 632, 356–360
102. Kohn, D.B. *et al.* (2016) Ethical and regulatory aspects of genome editing. *Blood* 127, 2553–2560
103. Adashi, E.Y. and Cohen, I.G. (2018) The ethics of heritable genome editing: new considerations in a controversial area. *JAMA* 12, 421–428
104. Gluckman, E. *et al.* (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N. Engl. J. Med.* 321, 1174–1178
105. Verlinsky, Y. (2001) Preimplantation diagnosis for Fanconi anemia combined with HLA matching. *JAMA* 285, 3130
106. Cheng, N.C. *et al.* (2000) Mice with a targeted disruption of the Fanconi anemia homolog Fanca. *Hum. Mol. Genet.* 9, 1805–1811
107. Whitney, M.A. *et al.* (1996) Germ cell defects and hematopoietic hypersensitivity to gamma-interferon in mice with a targeted disruption of the Fanconi anemia C gene. *Blood* 88, 49–58
108. Chen, M. *et al.* (1996) Inactivation of Fac in mice produces inducible chromosomal instability and reduced fertility reminiscent of Fanconi anaemia. *Nat. Genet.* 12, 448
109. Weinberg-Shukron, A. *et al.* (2018) Essential role of *BRCA2* in ovarian development and function. *N. Engl. J. Med.* 379, 1042–1049
110. Agoulnik, A.I. *et al.* (2002) A novel gene, Pog, is necessary for primordial germ cell proliferation in the mouse and underlies the germ cell deficient mutation, gcd. *Hum. Mol. Genet.* 11, 3047–3053
111. Lu, B. and Bishop, C.E. (2003) Mouse GGN1 and GGN3, two germ cell-specific proteins from the single gene Ggn, interact with mouse POG and play a role in spermatogenesis. *J. Biol. Chem.* 278, 16289–16296
112. Lu, B. and Bishop, C.E. (2003) Late onset of spermatogenesis and gain of fertility in POG-deficient mice indicate that POG is not necessary for the proliferation of spermatogonia. *Biol. Reprod.* 69, 161–168
113. Ottolini, C.S. *et al.* (2015) Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates. *Nat. Genet.* 47, 727–735
114. Petronczki, M. *et al.* (2003) Un ménage à quatre. *Cell* 112, 423–440
115. Keeney, S. *et al.* (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* 88, 375–384
116. Grelon, M. *et al.* (2001) AtSPO11-1 is necessary for efficient meiotic recombination in plants. *Eur. Mol. Biol. Organ. J.* 20, 589–600
117. de Massy, B. (2013) Initiation of meiotic recombination: how and where? Conservation and specificities among eukaryotes. *Annu. Rev. Genet.* 47, 563–599
118. Vrielynck, N. *et al.* (2016) A DNA topoisomerase VI-like complex initiates meiotic recombination. *Science* 351, 939–944
119. Robert, T. *et al.* (2016) The TopoVIB-Like protein family is required for meiotic DNA double strand break formation. *Science* 351, 943–949
120. Martini, E. *et al.* (2006) Crossover homeostasis in yeast meiosis. *Cell* 126, 285–295
121. Cole, F. *et al.* (2012) Homeostatic control of recombination is implemented progressively in mouse meiosis. *Nat. Cell Biol.* 14, 1–8
122. Mercier, R. *et al.* (2015) The molecular biology of meiosis in plants. *Annu. Rev. Plant Biol.* 66, 297–327