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Review article

Genomic instability and the link to infertility: A focus on microsatellites and genomic instability syndromes

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ABSTRACT

Infertility is associated to multiple types of different genomic instabilities and is a genetic feature of genomic instability syndromes. While the mismatch repair machinery contributes to the maintenance of genome integrity, surprisingly its potential role in infertility is overlooked. Defects in mismatch repair mechanisms contribute to microsatellite instability and genomic instability syndromes, due to the inability to repair newly replicated DNA. This article reviews the literature to date to elucidate the contribution of microsatellite instability to genomic instability syndromes and infertility. The key findings presented reveal microsatellite instability is poorly researched in genomic instability syndromes and infertility.

Introduction

Infertility is defined as either primary or secondary infertility. Primary infertility refers to the failure of pregnancy after one year without the use of birth control methods [1], whilst secondary infertility refers to the failure of conceiving another child after the first [2]. Furthermore, recurrent miscarriage (RM), is defined as three or more consecutive miscarriages [3–4]. Infertility is reported to affect approximately 15% of couples globally [5]. Genetic analyses has provided strong evidence for the role of gene variants and chromosomal aberrations [6–14]. These data suggest that individuals harbouring factors that predispose them to genomic instability have decreased fertility rates.

Genomic instability can manifest as multiple types of aberrations within the human genome. These aberrations are evident at the chromosomal or gene level as aneuploidy [15], translocations [16], inversions [17], deletions [18]. Genomic instability is most pronounced in patients with genomic instability syndromes due to variant(s) of gene(s) responsible for DNA repair. Genomic instability and genomic instability syndromes may be accompanied with microsatellite instability (MSI) [19–20,10,21].

Microsatellites

Microsatellites, the "genetic fingerprints" of the human genome, contains variable numbers of repeated 1–6 base pair units (bp), encompass approximately 3% of the human genome [22]. Generally, microsatellites are located within the intron regions (Fig. 1) of a gene [23], the 5' and 3' untranslated regions (5'UTR and 3'UTR) [24], intergenic spaces [25], and present in exon regions [26]. Additionally, for humans specifically, microsatellites can be observed adjacent to transcriptional start sites [27], translation initiation sites [28], and around CpG DNA methylation sites [27]. High levels of variability between individuals at microsatellite sites lends microsatellites to different applications including DNA profiling [29–31], and studies of disease [32–34]. Also, these may not be actively conserved in the human genome, as microsatellites are in regions that are not vital to gene function and hence, they are prone to variation (Fig. 1). DNA replication slippage may also be one of the factors involved in MSI occurrence [35–37].

The hypermutable state of microsatellites is partially due to the mismatch repair (MMR) system, correcting DNA sequences by acting as a "proof-reader" (Fig. 2) if there is a sequence error. The other

Abbreviations: microsatellite instability, (MSI); recurrent miscarriage, (RM); primordial germ cells, (PGCs); double stranded breaks, (DSBs); Bloom syndrome, (BS); mosaic variegated aneuploidy syndrome, (MVA); Lynch syndrome, (LS); colorectal cancer, (CRC); mouse embryo fibroblasts, (MEFs); mismatch repair, (MMR); sister chromatid exchange, (SCE); idiopathic recurrent miscarriage, (IRM); preimplantation genetic testing, (PGT).

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contributor is the simple nature of a microsatellite sequence as discussed above. DNA errors recognised by the MMR pathway include insertions [46], deletions [43], and mis-incorporations [47] of a nucleotide base. Defects within the MMR pathway is to MSI. MSI in turn is associated with several diseases and syndromes including cancers [48–51], inflammatory diseases including Crohn's disease [32,52] and Behçet's syndrome [33]. It should also be acknowledged that "hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome (LS) is the most common disorder with MSI" [53–60], yet the role of MSI in infertility is poorly understood.

Research to date evidenced links between genomic instability syndromes and gene variants and infertility [61–74]. In contrast, the specific link between MSI and infertility/RM, independent of genomic instability syndromes, is poorly understood. Therefore, we aim to highlight upon the importance of MSI in infertility by reviewing the literature on 1) the role of MSI during gametogenesis, early embryogenesis, and implantation, 2) MSI in genomic instability syndromes on infertility and 3), the known impact of specific MSI sites on infertility independent of genomic instability syndromes. We show that MSI contributes to infertility in genomic instability syndromes and independent of genomic instability syndromes. However, data is limited and more research in this area is warranted.

Microsatellite instability in germ cell development

MSI may disrupt normal foetal development through different mechanisms including prior to conception during gametogenesis, through influencing germ cell mosaicism and during early embryogenesis and implantation. We describe the role of MSI in each of these three stages in turn below.

Gametogenesis and microsatellite instability

Gametogenesis is the process of diploid or haploid cells entering cellular division and undergoing differentiation to mature haploid gametes. In male's, primordial germ cells (PGCs) enter mitosis to produce diploid cells (2n) and meiotic events for spermatogenesis [75]. In females, PGCs are required to produce primary oocytes through a series of meiotic events [76]. During the DNA replication stages, replicative errors may occur, leading to variation in nucleotide sequences and MSI sites.

Previous work has shown MMR genes can contribute to abnormal chromosomal synapse formations during chromosomal recombination during meiosis, leading to MSI at D6Mit59, D7Mit91, D19Mit36 and D1Mit62 in mice [77]. It was also reported that male mice deficient in PMS2, a key protein for DNA mismatch repair, had reduced spermatozoa and reduced fertility, whilst *PMS2*$^{-/-}$ females were fertile. Furthermore, spermatozoa were physiologically abnormal with misshaped, truncated, and irregular flagella with abnormally shaped heads. It was concluded that spermatogenesis is disrupted at the primary spermatocyte stage during meiosis I [77]. These data suggests that MSI may also disrupt human spermatogenesis and contribute to infertility.

Data relating to MMR gene variants and their impact on gametogenesis in humans are limited. However, a study by Ferrás et al. (ref 78), investigated variants of *hMLH3*, which encodes a protein key for DNA mismatch repair. Of 300 male patients studied, 13 showed spermatogenic arrest at the primary spermatocyte stage and one of the 13 had family history of HNPCC. Between them, these 13 patients had four missense variants including; 2896T/C and 2531C/T and eight intronic (IVS9 + 66G/A) within *hMLH3* [78]. Despite of these variants, MSI was not observed in the BAT-34 microsatellite.

MMR genes have also been reported to have a regulatory role within oogenesis. *Exo1*$^{-/-}$ female mice have been reported to be sterile due to meiotic defects, with an increased susceptibility to lymphomas and MSI [79]. The method of detecting MSI were from DNA of EXO1$^{-/-}$ mice by PCR using end-labelled primers and electrophoresis [79]. Mutation rate analysis involved studying mutation rates of the *HRPT* locus. Embryonic stem cells exhibited either EXO1$^{-/-}$, MSH2$^{-/-}$ and wild-type genotype, the cell line studies identified a 30-fold increase (0.6×10^{-6}) of MSI occurrences in EXO1$^{-/-}$ cells. MSH2$^{-/-}$ cells exhibited a 150-fold increase (3.0×10^{-6}) for mutation rates compared to wild-type cells [79]. Cells in meiosis I entered prophase I, but chiasmata was absent in metaphase I, leading to meiotic failure. The size of oocytes was smaller in *Exo1*$^{-/-}$ mice, but the oocytes had normal progression through diplonema, despite of the lack of a functioning EXO1 protein. At day 2 postpartum,

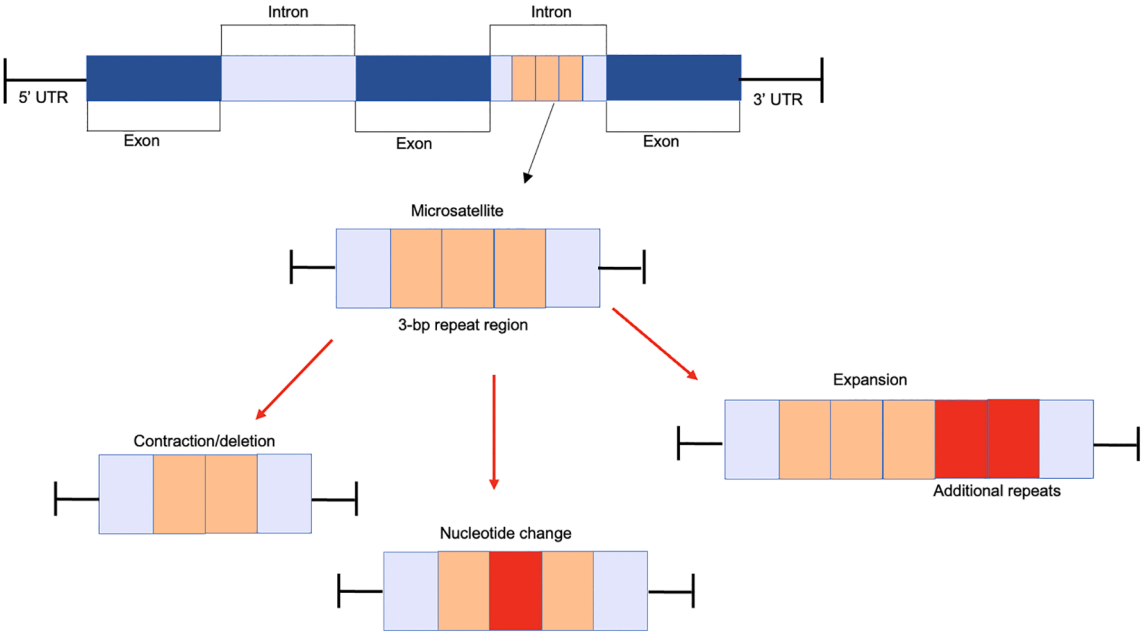

Fig. 1. Microsatellite structure and microsatellite instability. Within the intron, 5′UTR and 3′UTR of a gene, microsatellites can be identified which contain short (1–6 bp) regions repeated a variable number of times (orange segments). In individuals with defects in the mismatch repair pathway (MMR) (red arrows) this can lead to three outcomes; contraction because of a gene deletion [38], a nucleotide change [39], or additional repeats are added [40] (red segments). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oocytes were reported to be in dictyate arrest. Additionally, MSI was detected within the microsatellites; D7Mit91, D17Mit123, and at the *uPAR* gene [79]. MSI and the effects on oogenesis in humans as not been explored but from these data suggests oogenesis in humans may also be affected by MSI.

Microsatellite instability and germ cell mosaicism

Mosaicism can be described as the presence of two or more genetically different sets of somatic and germline cells that contain 46 chromosomes [80], or a variation in the number of chromosomes [81]. Germline mosaicism is known to be a contributing factor to infertility in males. Jaruzelska et al. (ref 81) reported a 45 X mosaicism, within 65 men with azoospermia and 23 men with oligozoospermia. However, MSI was not reported upon within this study.

Despite the study of Jaruzelska et al. [81] described above, reports investigating the impact of germline mosaicism/MSI and infertility in humans are lacking. However, *de novo* germline mosaicism within *MSH2* was identified in one patient with a Lynch-like syndrome [82]. Due to the *de novo* variant, the patient developed endometrial cancer (aged 45) and colorectal cancer (CRC) (aged 60). Further analysis of the CRC of this patient, showed MSI and loss of expression in the genes *MSH2* and *MSH6* [82]; similar analysis was not performed for the endometrial cancer. It should be acknowledged that previous research identified that MSI can be detected with endometrial cancer [83]. One of the clinical complications with endometrial cancer is there is an increased risk of infertility. This is due to patients with endometrial cancers having a thinner endometrial wall compared to those without cancer [84] causing reduced embryo implantation in endometrial cancer patients; however, MSI was not studied in these cancer patients [84]. In other research in MSI and cancer, Black et al. (ref 85), reported 20% of 473 endometrial cancer patients contained MSI associated with defective DNA repair [85]. Whilst Kanopiene et al. (ref 86) reported on MSI status of 100 of 109 endometrial cancers showing endometrioid type histology. From the 100 endometrial cancers, 17 demonstrated MSI-high status and 83 showed MSI-stable status. Furthermore, MSI-high cancers were linked to the pathology of deep myometrial invasion and higher histological gradings of endometrioid type cancers. These findings were identified using the Promega MSI analysis system which utilises five

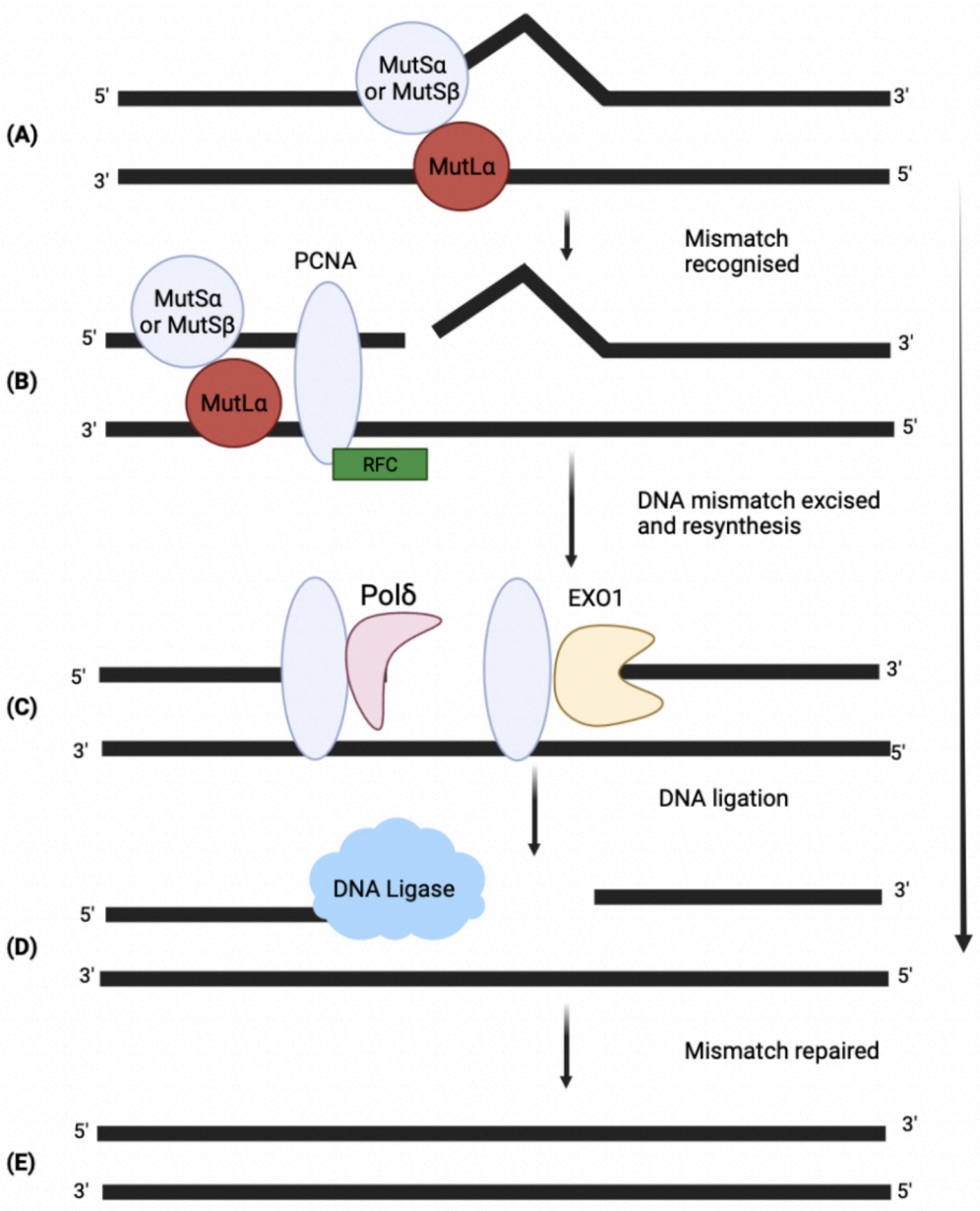

Fig. 2. The DNA mismatch repair pathway. After DNA replication has occurred the newly replicated DNA is proofread by the MMR pathway for mismatch errors. **A)** DNA mismatches are detected by the MutSα (a heterodimer of MSH2 and MSH6) or the MutSβ complex (a heterodimer of MSH2 and MSH3). These recruit MutLα (a MLH1-PMS2 heterodimer) [41,42]. **B)** PCNA interacts with MutLα for DNA MMR repair, by which PCNA binds to DNA acting as a sliding clamp to allow DNA polymerase δ (Pol δ) and EXO1 to bind to the mismatch region [41], whilst the recruitment of replication factor C (RFC) acts as the clamp loader for Pol δ and EXO1 [44]. **C)** After the binding of EXO1 to DNA, the mismatch error is excised from the DNA whilst, Pol δ replicates the DNA to compliment the 3′ →5′ strand. **D)** After the binding of DNA ligase to the DNA strand, the enzyme ligates the newly synthesised strand, replacing the region where the mismatch occurred. **E)** The DNA containing the mismatch error is repaired. Figure has been adapted from [45], with permission.

mononucleotide microsatellites (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) [86].

In testicular cancers, germline mosaicism remains to be investigated. The presence of MSI in testicular cancer is reported in two studies. Huddart et al. (ref 87), reported multiple MSI within two tetranucleotide microsatellites (vWFa and vWFb), two trinucleotide microsatellites (TFIID and AR) and one dinucleotide marker (D16S303), in human testicular tumours [87]. Another study reported MSI within testicular cancer complimented with reduced *MLH1* and *MSH2* expression [51]. It remains unclear whether germ cell mosaicism is accompanied with the presence of MSI. Surprisingly, neither study reported upon the fertility status of the male patients, as fertility is known to be affected by testicular cancer [88].

Embryo implantation/postzygotic microsatellite instability through mitotic and meiotic instability

After fertilisation, the preimplantation embryo then enters the womb for implantation. Throughout the development to a fully developed foetus, a series of cellular division events occur. These events are regulated by two spindle assembly checkpoint genes, *BUB1* and *CEP57* (discussed below), ensuring chromosomal segregation is achieved [70,89–90]. Mice with defective maternally-derived *Bub1* show embryonic aneuploidy and subsequent embryo loss after implantation [91–92]. Paternal inheritance of $Bub1^{m/m}$ (Bub1 mutant) is not associated with embryo mitotic defects in mice [91].

In relation to MMR, recent research has suggested that MLH1 and MLH3 are also required for normal disjunction during meiosis. Singh et al. (ref 93), used computational analysis in different human genomic databases to investigate variants associated with MLH1 (E268G, K618E, V326A and V716M) and MLH3 (A3914T, R93Q and R1230H). These variants were then created in mice for phenotypic analysis [93]. To summarise the results, $MLH3^{41230H}$ males were completely infertile due to spermatocyte arrest and reduced testicular size, whilst in female mice, $MLH1^{E268G}$, $MLH1^{V326A}$, $MLH1^{K618E}$, $MLH1^{K618T}$, and $MLH1^{V716M}$, produced offspring where the size of subsequent litters reduced over time. $MLH1^{K618T/K618T}$ females had fewer follicles, thus, suggesting chromosomal crossover events during meiosis were reduced. Further effects reported included that decreased chromosomal crossover events lead to increased aneuploidy and embryo loss, however the exact mechanism remains unanswered [93]. In $MLH3^{R1230H/R1230H}$ male mice, testicular size was reduced with complete sperm absence. Screening for MSI within mutant mice did not reveal MSI occurrences but data were not shown [93]. Previous work suggests mice that Do not have a *MLH1* functional gene, have MSI and show increases in morbidity, lymphoma development, and different gastrointestinal related tumours [94].

Genomic instability syndromes

Genomic instability syndromes arise from heritable variants within genes involved in DNA repair mechanisms which can affect the fidelity of the genome during cell replication and cell division [95], leading to different genomic aberrations as discussed above. Variants within MMR genes leads to MSI as discussed above and a feature of many genomic instability syndromes. We here, review evidence to discern whether MSI contributes to infertility/ RM or is coincident with it but not causative in the context of genomic instability syndromes. Here we have discussed three different genomic instability syndromes (Table 1); Mosaic Variegated Aneuploidy syndrome (MVA) [70], Bloom syndrome (BS) [99], and Lynch syndrome (LS) [68].

Aneuploidy and MVA

Aneuploidy is associated with the genomic instability syndrome MVA1 (OMIM #257300) (associated with *BUB1B*) and MVA2 (OMIM #614114) (associated with *CEP57*). MVA1 is due to variants within the *BUB1B* gene [70,89–90]. The BUB1B protein regulates the spindle assembly checkpoint during cellular division and therefore, maintains genomic stability by delaying cell division until full chromosome segregation is achieved [100–101]. Variants within the *BUB1B* gene lead to monosomy or trisomy [102–103].

Table 1
Summary of genomic instability syndromes.

Genomic Instability Syndrome	Genes Mutated	Gene summary or gene variant outcome	Reference
Mosaic Variegated Aneuploidy	*BUB1* and *CEP57*	Defective chromosomal segregation leading to chromosomal aneuploidy.	[70,72]
Lynch Syndrome	*MSH2*, *MLH1*, *PMS2*, *MSH6*	Variants in these MMR genes gives arise to a range of different cancers associated to Lynch syndrome, including colorectal cancers.	[96,59]
Bloom Syndrome	*BLM*	Essential for homologous recombination for DNA double strand break repair. Variants of *BLM* can lead to leukaemia and Burkitt lymphoma.	[97–98]

Patients who have a mutation in the BUB1B gene are diagnosed with MVA1 [19,104]. The research on MVA1 shows fertility status can be unaffected and patients can successfully conceive (and this syndrome is heritable) [13,105]. There is no research linking MSI to infertility in MVA1 in human patients. Baker et al. (ref 106), generated $Bub1b^{-}$ (knockout) $Bub1b^{H}$ (hypomorphic) and $Bub1b^{+}$ (wildtype) mice, investigating the role for Bub1b, resembling MVA1. The $Bub1b^{-/-}$ offspring died during early embryo development, $Bub1b^{-/H}$ offspring survived birth but died 12 h after birth. Furthermore, mouse embryo fibroblasts (MEFs) were also examined and revealed Bub1bH/H MEFs had deficient cell cycle arrest and aneuploidy, whilst $Bub1b^{-/H}$ MEFs had higher aneuploidy compared to Bub1bH/H. Male Bub1bH/H mice were infertile due to a 4-fold reduction in sperm compared to $Bub1b^{+/+}$ mice. $Bub1b^{+/+}$ mice were reported to be fully fertile. The crossing of $Bub1b^{H/H}$ with $Bub1b^{+/+}$ mice led to a reduction in the number of fertilised eggs [106]. Therefore, these data show that Bub1b is essential for spindle assembly checkpoints and defects cause aneuploidy; MSI was not reported upon. In humans, infertility associations with MVA1 have not been reported to date.

Mosaic variegated aneuploidy syndrome (MVA2) (OMIM #614114) is caused by variants within the *CEP57* gene, and this is a diagnostic biomarker [72]. CEP57 protein functions to maintain microtubule stability by ensuring that spindle microtubules are attached to chromosomes for proper segregation [107–108]. A case study found secondary infertility could be a clinical feature of MVA2, but this is complicated by the consanguineous nature of the couple involved [72]. At the time of writing this review, MSI has not been detected within MVA1 or MVA2.

Bloom syndrome

Bloom syndrome (BS) (OMIM #210900) is the product of mutations to the causative gene, *BLM*. The BLM protein functions to repair DNA double stranded breaks (DSBs) via homologous recombination [97,109]. Therefore, the presence of a *BLM* variant is associated with an increased rate of MSI, due to abnormal expression patterns of the *BLM* gene [110,111]. Furthermore, other research not related to BS specifically, has associated MMR defects for DSB repair gene transcripts with MSI [112]. BS is an autosomal recessive syndrome which phenotypically presents as red rashes around the facial area, short stature and elongated limbs [113]. BS predisposes patients to other complications including type 2 like diabetes mellitus [114], sun-sensitive skin [115], moderate to severe immunodeficiency [115], and cancers including leukaemia and Burkitt lymphoma [116,110].

BLM gene variants in BS have also been linked to infertility. While

two case studies have revealed that female patients with BS are able to successfully achieve pregnancy and conceive [117–118], male patients with BS have been reported to be infertile with oligozoospermia or azoospermia [119]. El Ghamrasni et al. (ref 120) reported a higher occurrence of genomic instability including chromosome breaks and chromosome fragmentation of dicentric chromosomes in *Blm*$^{-/-}$ and *Mus81*$^{-/-}$ mice compared to wildtype mice. This study did not report on MSI [120]. However, this suggests MSI may be associated with BS as *BLM* is a DNA repair participating gene.

BS is also associated with higher rates of sister chromatid exchange (SCE), a process by which sister chromatids break and re-join [121]. For instance, Howell and Davis [122] reported SCE counts between 31 and 70 in BS patient, compared to normal chorionic cells that contained 6 to 8 SCE counts; these discrepancies can be used to diagnose BS [122]. Other work has suggested that the rates of SCE in BS patients are between 10 and 12 times more than those without BS [123]. In mice models of BS, telomere length was observed to be reduced due to the loss of telomerase activity and a loss in telomeric repeats in fibroblast cells [124]. Therefore, telomeric dysregulation could also be a molecular characteristic within BS patients. Previous research has found short telomere length associated with MSI [125–128]. The mechanism linking these two processes is not entirely clear.

DNMT1 codes for DNA methyltransferase 1 which participates in DNA methylation [129–130]. DNA methylation involves the attachment of methyl groups to CpG islands, suppressing gene expression [131–133]. In relation to MSI in BS, Guo et al. (ref 20), screened for MMR deficient genes in blm-deficient embryonic stem cells. This analysis revealed *Dnmt1* as a putative novel MMR gene, as dnmt1-defiicent cells exhibited MSI and *Dnmt1* expression was not detectable [20]. A similar study also reported *DNMT1* as a novel MMR gene, using dnmt1-deficient mouse embryonic stem cells [134]. These data also suggest that there is a potential postzygotic predisposition to MSI in BS patients; whether this contributes to infertility remains unclear.

Only one study attempted to analyse MSI in six unrelated BS patients, by PCR and cell line analysis [135]. This study used eight different cell lines, from six unrelated patients which included five BS fibroblast cell lines (GM02520A, GM03402A, GM03498C, GM03509A, GM03510), one BS SV40-transformed fibroblast cell line (GM08505B) and two BS EBV-transformed lymphoblastoid B cell lines (GM03403D, GM04408A) where the cell lines GM03498C and GM04408A came from the same patient [135]. This analysis reported that MSI could not be detected in all six patients.

Patients with BS are predisposed to an increased risk of leukaemia and Burkitt lymphoma. Both cancer types arise in childhood in BS patients [98]. Of note, MSI has been reported within a BS-associated lymphoma. In this study, two Japanese siblings aged 23 and 25 of consanguineous parents were studied [136]. Genomic analysis revealed MSI within the *p53* gene and a 3-bp deletion in the *BLM* gene [136]. MSI in non-cancerous cells was not evaluated in this report.

Lynch syndrome

Lynch syndrome (LS) (OMIM #120435) is associated with variants in *MLH1* [137],*MSH2* [138],*MSH6* [68], and/or *PMS2* [139]. These variants contribute to the onset of multiple cancers including endometrial [96], breast [140], colorectal [141], and prostate [142]. Recently, for the first time, testicular cancer was found to be associated with LS patients [143]. Testicular cancer may not be a common cancer to occur in male LS patients.

Infertility is associated with LS. A case study revealed that females diagnosed with LS and CRC, have decreased fertility across a broad age range (15 to 47) compared to those with CRC but without LS [144]. This was expressed as "children per woman and is calculated as the sum of age-specific fertility ages 15 to 49". This is based on a total fertility rate value defined as "an average number of children produced of a hypothetical cohort of women had by the end of their reproductive life, if they children at the population age-specific rates during their whole life" [144]. In LS male patients decreased fertility was also observed in the same age range [144]. Even though mutations in MMR genes have been linked to male infertility contributing to oligozoospermia and azoospermia [145–148], research to date has not addressed how LS is specifically linked to male infertility.

LS is associated with other cancers as identified above in this section. Limited research thus far has reported MSI in LS. For instance, Latham et al. (ref 59), studied 15,409 patients with more than 50 different types of cancers present in LS patients. 53 of 326 (16.3%) had high levels of MSI (MSI-H), 13 of 699 (1.9%) has low MSI (MSI-l) and 37 of 14,020 (0.3%) showed microsatellite stable phenotype (MSS). Furthermore, 33 of 66 (50%) had primary tumours excluding CRC. In LS-related tumours containing MSI (MSI-H/l), 12 of 32 (37.5%) were urothelial, 26 of 137 (19%) were CRC, 2 of 13 (15.4%) were gastric cancers. In those with high or low levels of MSI (MSI-H/I) pancreatic cancers 34 of 824 (4.1%); only 5 of these 34 (14.7%) were LS related [59], involving five germline MMR genes (*MLH1, MSH2, MSH6, PMS2* and *EPCAM*). MSI can be detected in non-LS related breast [149], colorectal [150], prostate [151] and testicular cancers [87]. Therefore, further research is important for the investigation of MSI. However, as Latham et al. (ref 59) revealed, LS is complicated by the presence of LS and non-LS related cancers which may prove difficult to address MSI related studies in LS patients.

Microsatellite instability in infertility and recurrent miscarriage

In this section we have discussed MSI occurrences in infertile patients independent of genomic instability syndromes. After searching the literature, we found four papers [152–155], that reported links between MSI and infertility/RM, independent of genomic instability syndromes. We have identified that from these four studies that MSI may be a common feature in idiopathic infertility. These studies reveal MSI is associated with infertility/RM and spontaneous abortions. The association of MSI within preimplantation failure in humans remains unclear. Some molecular mechanisms have recently been proposed for the potential reasons for preimplantation failure [156], discussed further below.

In the study of Kiaris et al. (ref 152) MSI was investigated at two different distal sites per spontaneously aborted embryo and compared against those of a normal birth. Thirty spontaneously aborted embryonic tissues were selected at the time of rejection and frozen until DNA analysis. Eight different microsatellite markers were selected: D6S344, D7S531, HRM, D12S94, D13S175, TCRD, THRA1 and D19S49. MSI was reported in 12 (40%) of the 30 specimens. In aborted embryonic tissue samples, in samples coming from those without a normal childbirth 61% (11 of 18 cases) had MSI. In samples that came from a normal birth, only 8% (1 of 12 cases) had MSI. THRA1 and D6S344 presented instability in 5 of the cases whilst D7S531 did not present MSI [152]. Both THRA1 and D6S344 have been found to be highly polymorphic microsatellite markers in other studies [157–160]. This provides evidence that MSI increases at the embryonic stages of pregnancy. Thus, MSI analyses should not be overlooked during genetic analysis of infertile patients and in embryos.

A second report focused on infertility associated with an MSI site in the *CTLA-4* gene [153]. The protein product of the *CTLA-4* gene (CTLA-4 protein) is an inhibitory receptor that maintains T-cell homeostasis through suppressive mechanisms [161–162]. Tsai et al. (ref 153) hypothesised that a microsatellite residing within the 3′UTR of the *CTLA-4* gene, compromised of an AT(n) repeat region (between 16 bp and 46 bp total length), leads to mRNA instability thereby leading to altered CTLA-4 protein expression. In turn this may alter immune tolerance of the foetus in utero and predispose to foetal rejection and infertility. To test this hypothesis, 60 couples were studied, each with a minimum of 3 unexplained spontaneous miscarriages and compromising a total cohort of 51 liveborn children and 10 abortuses. Cord blood and placental tissues from both liveborn and aborted foetuses

were genotyped for the CTLA-4 microsatellite locus. This revealed that the shorter alleles, inherited from the maternal side, were associated with increased foetal survival. Thus, mRNA transcribed from the longer alleles were shown to be intrinsically unstable, with reduced expression [153]. Other studies have also revealed a link between *CTLA-4* gene expression patterns and infertility but did not report upon MSI [163–164]; whether protein expression is significantly altered by the CTLA-4 MSI site remains to be seen.

A third study aimed to evaluate MSI incidence in 35 spontaneously aborted (between 6 and 20 weeks of gestation) embryonic tissues [154]. This study compared seven different microsatellite markers between parents and embryos; D3S1234, D4S194, D10S109, HRM, TCRD, THRA1 and D17S855. In total,19 of the patients who had a history of spontaneous abortion, had at least one instability in at least one of the microsatellite markers in aborted tissue samples [154]. This is supporting the idea that MSI in an embryo contributes to spontaneous abortions, alike to Tsai et al. [153].

Finally, a pilot study aimed to identify genetic loci that could be contributing factors for idiopathic recurrent miscarriage (IRM) [155]; 45 patients with IRM in the first trimester were included along with 44 healthy controls that were matched by sex and ethnicity. Of the 403 evaluable microsatellite markers studied, 17 were different between IRM patients and controls of which D6S446 (6q27), D9S1776 (9q31.1) and DXS1226 (Xp22.11), were found to have an association with IRM. Further analysis to identify genes around these sites, revealed 26 genes including *TNFSF8* and *TNFSF15* which are linked to immune function [155]. Of note, other members of the TNF superfamily are present at the maternal-foetal interface and likely play a key role in preventing embryonic rejection [165,166]. Despite these findings, confirmatory studies with patients with IRM of other ethnicities should be conducted to confirm if MSI is present in the same microsatellites or to reveal other unstable microsatellites.

We did not find any research assessing MSI in preimplantation embryos. A recent review suggests there are several potential molecular mechanisms including DNA damage and repair, aneuploidy and meiotic recombination that could contribute to genomic instability within a preimplantation embryo [156] and which may lead to spontaneous abortion. More research within this area is clearly needed.

Discussion and conclusion

Genomic instabilities are a result of chromosomal aberrations serving as a genomic predisposition for genomic instability syndromes. Microsatellites are 1–6 bp units, encompassing approximately 3% of the human genome [22]. MSI is a result of mutations to genes in the DNA MMR pathway [18,167–168]. The aim of this review was to discuss research into the role of MSI and genomic instability syndromes in infertility/RM. Our findings reveal that MSI is poorly investigated in genetic analyses of patients with genomic instability syndromes and infertility, including patients with infertility/RM independent of genomic instability syndromes. Potential reasons that may account for this are that; 1) MSI is not a core component of genetic analyses for infertility or genomic instability, 2) current methods used in genetic analyses may not identify MSI as a genetic feature, 3) MSI may happen during preimplantation which may lead to technical and ethical barriers for analyses.

Preimplantation genetic testing (PGT) is used in assisted reproduction methods to determine the genetic profile of embryos and to reveal whether an embryo is at risk of inheriting a disease or syndrome. PGT can be used to identify monogenic diseases (PGT-M) [169], chromosomal structural rearrangements (PGT-SR) [170] and aneuploidy (PGT-A) [170–171]. However, these current PGT methods do not address investigate MSI within infertile patients and/or in the embryo. From the studies that have been reviewed here we suggest that MSI may be a genomic instability feature a contributor to idiopathic infertility.

To conclude, we comprehensively reviewed limited research to date to reveal the role of microsatellite instability in genomic instability syndromes and infertility. We suggest the need for further research to define the role of microsatellite instability in infertility, including microsatellite instability in preimplantation embryos. We therefore propose the use of microsatellite instability as a biomarker of underlying DNA repair deficiencies resulting in unexplained infertility.

Key messages

The involvement of microsatellite instability in genomic instability syndromes and infertility is poorly understood. Whether infertility is a clinical phenotype of genomic instability syndromes remains unclear.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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