

# Role of DNA mismatch repair genes in lung and head and neck cancer (Review)

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**Abstract.** The role of the DNA repair mechanism is to protect genetic material from destabilization. A defect in the DNA mismatch repair (MMR) mechanism has been associated with both hereditary and sporadic tumors. The dysregulation of MMR gene expression has been reported in lung, and in head and neck sporadic tumors. However, the mechanisms through which defects in the DNA MMR mechanism promote lung, and head and neck cancer remain unclear. Environmental factors and epigenetic alterations can significantly alter the ability of cells to repair genetic damage. The loss or a low expression of MMR genes allows for the survival of cells carrying a significant amount of genetic alterations, some in proto-oncogenes or genes regulating the cell cycle. The dysregulation or malfunction of the MMR mechanism has also been linked to alterations in response to chemotherapy. The investigation of MMR dysregulations in lung, and head and neck carcinomas may contribute to a better understanding of their biological role in the development and progression of

these types of cancer, and may thus also improve their diagnostic, prognostic and therapeutic value.

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## 1. Mismatch repair genes and their role in carcinogenesis: The functions of the mismatch repair system

DNA repair gene products function together to protect the destabilization of genetic material by errors that occur during DNA replication. In this manner, they participate in preventing the multistep process of the neoplastic transformation of normal cells to the tumorigenic phenotype (1-7). Genetic damage to the DNA mismatch repair (MMR) mechanism may lead to microsatellite instability (MSI), a common finding in hereditary forms of cancer, such as hereditary nonpolyposis colorectal cancer (HNPCC) and hereditary endometrial cancer (8-16). Defects in the DNA repair mechanism have been observed in hereditary forms of cancer, and have been linked to specific syndromes. Previous studies have demonstrated that MSI and/or the loss of expression of MMR proteins or low levels of mRNA, are common findings in a number of sporadic cancers, such as lung, endometrial, ovarian and gastric cancers, where a loss of expression of MMR proteins or low levels of mRNA are common results of MMR gene promoter methylation (16-24). Similarly, there have been reported cases of the increased expression of

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*Abbreviations:* MMR, mismatch repair; MSI, microsatellite instability

*Key words:* MMR, lung cancer, head and neck cancer

MMR genes in sporadic colon, prostate or urinary bladder cancers, supporting the theory of their complex role in carcinogenesis (25-30).

**DNA MMR mechanism.** The main role of the MMR mechanism is to recognize and repair single-base mismatch errors, such as insertion, deletion and mis-incorporation that can occur during DNA replication. The recognition of the error and subsequent activation of the mechanism depends on the enzymatic complex of the proteins, MutS, MuH and MutL (31). The MutS complex has the ability to recognize mismatched nucleotides and bind to the damaged DNA. The MuH complex attaches to the hemimethylated sites along the impaired fragment, while the MutL complex activates the MutH peptide, which acts as a mediator between MutS and MutH (4-6).

One of the most crucial part of this system is the mutS-Homolog 2 (*MSH2*) gene. *MSH2* codes a protein which participates in the formation of two functional heterodimers: MSH2-MSH6 (MutS $\alpha$ ) and MSH2-MSH3 (MutS $\beta$ ) that recognize DNA mismatches (base-base mismatches and short insertion-deletion loops) and large DNA loops (insertion-deletion) respectively (Fig. 1).

Mismatch errors promote ATP hydrolysis, resulting in a change in the configuration of the MSH2-MSH6 complex to slide from its DNA binding site and to perform the repair. The complex acts as ATPase by hydrolyzing ATP. The release of the complex from the DNA does not depend on its activity as ATPase (1,30).

In addition to the DNA damage recognition complexes, the mismatch DNA repair mechanism in humans includes MutL complexes that relate to MLH1-MLH3 and MLH1-PMS2 heterodimers (possibly also the MLH1-PMS1 complex) (32,33). MLH1-MLH3 binds to MutS $\alpha$  (MSH2-MSH6) by converting it to a large complex. The MutL complexes interact with the damage recognition complexes and with other proteins that function in the action of the MMR mechanism, such as exonucleases (for example EXO1), DNA polymerases ( $\delta$  and  $\epsilon$ ), replicating agents, helicases and PCNA to repair the damage (32).

**DNA MMR deficiency and carcinogenesis.** Defects in DNA recognition complexes of the MMR system (MutS $\alpha$  and MutS $\beta$ ) have been observed in humans. Specifically, a deficiency in the expression of DNA MMR genes is almost always followed by the alteration of the number of short tandem repeats, known as MSI, and this leads to the development of a number of known types of carcinomas (34-36) (Fig. 2).

**Defective DNA MMR mechanism leads to carcinogenesis.** In hereditary forms of cancer, the defective repair mechanism can be caused by inherited mutations in coding regions of a replication gene allele and loss of heterozygosity (LOH) or acquired mutations in the other allele, and is depicted as a loss of MMR expression or increased MSI (30). The most common recognized and studied syndrome is Lynch syndrome characterized by the alteration of *hMSH2* and *hMLH1* genes altered, respectively, in 60 and 30% of patients with Lynch syndrome (37). These mutations are responsible for MSI. Notably, both Lynch syndrome and MSI are predisposing conditions for the development of several tumors. In particular, patients with type I Lynch syndrome are more susceptible to the development of colorectal cancer, while patients with

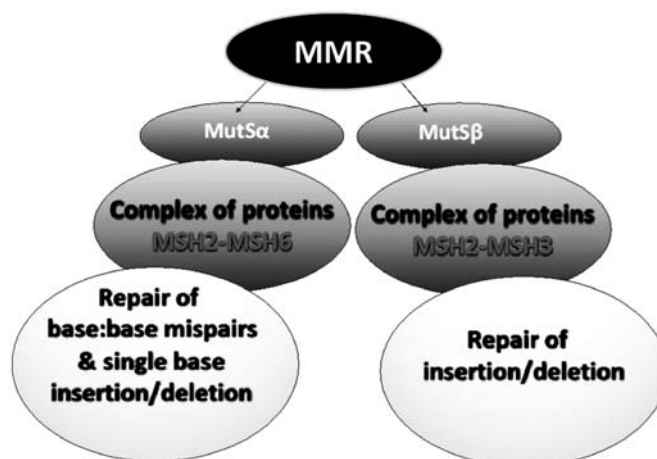


Figure 1. MMR complexes. The most crucial protein complexes of the DNA MMR mechanism that recognize the DNA mismatches are MutS $\alpha$  and MutS $\beta$ . The MutS $\alpha$  recognizes base substitution, mutations and short DNA loops, while MutS $\beta$  recognizes large DNA loops. MMR, mismatch repair.

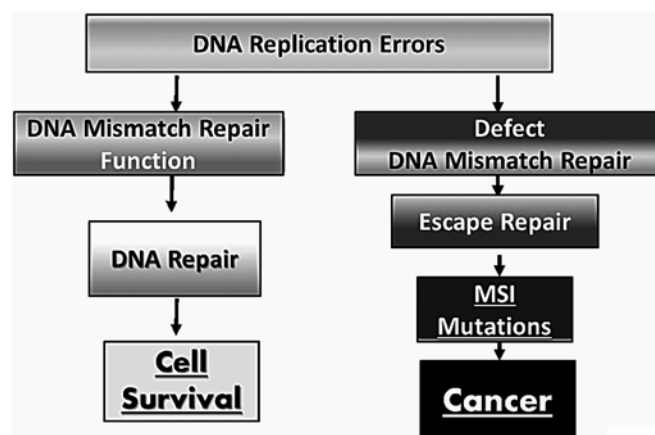


Figure 2. Dysregulated DNA MMR mechanism and carcinogenesis. Replication errors can be repaired by a functional DNA MMR mechanism that is capable of recognizing and successfully repairing these errors. The dysregulated DNA MMR mechanism cannot recognize the DNA replication errors leading to genetic alterations, such as MSI and/or mutations in functional sites of DNA that subsequently may lead to carcinogenesis. MMR, mismatch repair; MSI, microsatellite instability.

type II Lynch syndrome have an increased risk of developing gastric cancer, urological malignancies, cholangiocarcinoma and colon cancer (38-42)

Besides this syndrome, other genetic and epigenetic alterations in the *hMSH2*, *hMLH1*, *hMSH6*, *hMSH3*, *hPMS2* and *hPMS1* genes can cause damage to the DNA repair mechanism resulting in increased levels of genetic instability recognized as elevated MSI rates (32).

Recent studies have demonstrated that numerous sporadic tumors exhibit MSI without harboring any mutations in the repair genes (43), probably due to epigenetic alterations in the transcription and translation of MMR genes (30,44). Specifically, in sporadic cancers, the loss or low levels of MMR protein expression or mRNA depicted as increased MSI, due to the hypermethylation of *hMLH1*, or in some cases the *hMSH2* promoter, causes the suppression of their expression and compromises the function of the MMR mechanism (22,30).

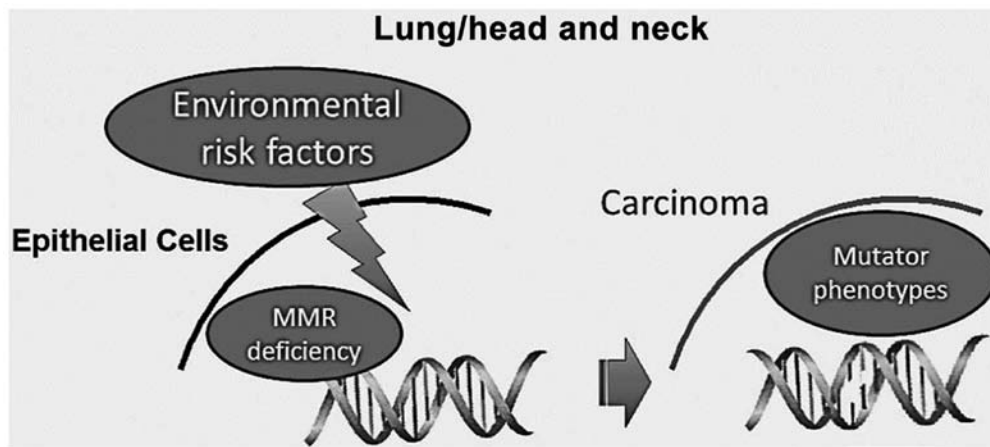


Figure 3. Environmental risk factors and DNA MMR deficiency may lead to neoplastic transformation in lung and head and neck. Lung and head and neck normal epithelial cells are often exposed to different environmental risk factors. Repetitive exposure of these cells to various risk factors may result to the accumulation of replication errors due to a defective DNA MMR mechanism. DNA mismatch repair deficiency may lead to a mutator phenotype escaping cell control and resulting to a neoplastic transformation of normal cells giving genesis to cancer. MMR, mismatch repair.

In general, the loss or low expression of MMR is evidence of a faulty repair mechanism constituting either a genetic background of cancer transformation in hereditary cancers, allowing for an increased MSI in cells, or promoting the tumorigenic pathway in sporadic cancers, allowing for the survival of cells carrying a significant amount of genetic alterations, some in proto-oncogenes or genes regulating the cell cycle.

The *hMSH2* and *hMLH1* genes are the most frequently involved in such changes. In fact, the MSH2 protein is normally expressed 3-5-fold above the protein levels of MLH1 expression, which in turn can be expressed 1.5-2.5-fold over PMS2 (45). In general the expression levels of MSH2 determine the expression levels of MSH3 and MSH6. Loss or low levels of MSH2 result in unstable MSH3 and MSH6 structures that are reflected by low levels of their proteins in the cells. Mutations in *hMLH1* can also result in low levels of MLH1 and PMS2 proteins, showing their interrelated relationship (PMS2 interacts with MLH1).

Defects that lead to the suppression of MSH6 expression, may result in an increase in MSH3 mRNA and protein expression. It is clear that balanced expression levels of components of the MMR system are essential for effective repair functions (2,32,45). Vageli *et al* previously demonstrated an association between an imbalanced mRNA phenotype of MMR genes and cancer progression in human lung, colorectal and urinary bladder cancer (18,46-49).

*The importance of MMR function as an indicator of chemotherapeutic resistance.* MSH2 protein, which is a major factor of base pair recognition error in DNA, under physiological conditions, is equated between the nucleus and cytoplasm. MSH2 has a high affinity for binding to the damaged site, which can be repaired, while having low affinity binding for non-severe damage that does not allow for cell viability, leading to apoptosis. The process of the initiation of apoptosis by MMR protein components appears to be related to their concentration in the nucleus. The concentration of MSH2 in the nucleus is a criterion for triggering apoptosis. Therefore, the loss or decreased expression levels of MSH2, will lead to

an insufficient nuclear concentration of MMR and therefore, in the inability of cells carrying extensive DNA damage to undergo apoptosis (31,50).

A number of studies have demonstrated that there is an association between the transcriptional activity of MMR mechanisms and the development of resistance to chemotherapy. Specifically, there is evidence that the inactivation of *hMLH1* by promoter hypermethylation promotes chemotherapeutic resistance (36,51,52). On the other hand, an imbalance in MMR mRNA phenotypes has been suggested to be of possible prognostic value in adjuvant chemotherapy treatment in non-small cell lung carcinomas (NSCLCs). Specifically, specific increased MMR mRNA phenotypes exhibit a trend for improved survival following chemotherapy, compared to other decreased mRNA phenotypes, which appear to be more effective in combination with post-operative chemotherapy (46).

*Epigenetic regulation of MMR mechanism.* The efficacy of the MMR mechanism can be strongly influenced by various environmental factors and epigenetic alterations that can significantly affect the ability of cells to repair genetic damage. These risk factors are able to determine profound epigenetic alterations, including the modulation of DNA methylation levels and microRNA (miRNA or miR) expression levels (53). The epigenetic regulation of MMR may be also associated with the composition of gut microbiota. This may strongly influence the development of several pathologies, including cancer (54,55).

*MMR and methylation.* Westwood *et al* demonstrated that the promoter hyper-methylation of *MLH1* was responsible for the additional loss of *MSH2* and *MSH6* expression in sporadic colorectal cancer (56). Other researchers have demonstrated that the methylation of MMR genes is responsible for the development of other tumors, such as extra mammary Paget's disease, where the authors demonstrated a significant correlation between reduced MSH2 expression and its promoter hyper-methylation (57). In general, several studies have demonstrated a strong association between methylation phenomena and Lynch syndrome, highlighting how patients with this syndrome have an increased risk of

DNA mutation accumulation due to genetic alterations typical of Lynch syndrome and the modulation of MMR induced by hyper-methylation (58).

**miRNA and MMR regulation.** As regards miRNAs, they are short (approximately 22 nucleotides in length) single-stranded non-translational RNAs that contribute to the regulation of gene expression (59). Specifically, they have the ability to target specific mRNAs, destabilizing or inhibiting their translation. The precursors of miRNAs, also known as pri-miRNAs, are produced in the nucleus by RNA polymerase II from the non-coding regions of the genome, and undergo a process of maturation in the nucleus, by the Drosha-DGCR8 complex, leading to hairpin RNAs known as precursor miRNAs (pre-miRNAs). The pre-miRNAs are then transported from the nucleus to the cytoplasm by exportin-5 and cut by Dicer to form single-strand miRNAs, the final functional form of miRNA. The majority of miRNAs inhibit the translation of their target genes by binding with an imperfect matching to their 3'UTR. In some cases, miRNAs can inhibit the protein expression of their target genes by binding the 3'UTR with a perfect match, leading to mRNA cleavage (60). The function of miRNAs in either case is to inhibit the protein expression of their genes. Therefore, these small regulatory molecules have been proposed as useful targets for the treatment of several pathological conditions, as well as markers for early neoplastic changes (61-66).

In particular, miRNAs have been shown to be involved in the regulation of numerous genes associated with various physiological processes, including MMR genes (67-72). Mao *et al* demonstrated that MutLa could function as a stimulatory factor for miRNA processing (73), while Valeri *et al* demonstrated the capability of miR-155 to alter both the expression and stability of the MMR pathway, supporting a regulatory role of miR-155 in the MMR mechanism (74). Zhong *et al* suggested that miRNAs play an important role in modulating the cell cycle by targeting *hMSH2* in lung cancer (75).

Overall, the unbalance of the MMR mechanism and the acquisition of new oncogenic mutations are the result of different genetic and epigenetic alterations. These alterations in expression can be identified using innovative and high-sensitive techniques (76), providing information with which to predict the risk of cancer onset and to identify novel biomarkers and therapeutic targets.

## 2. DNA mismatch repair deficiency in lung, and head and neck cancer

Lung and head and neck carcinogenesis is strongly associated with known risk factors, such as alcohol, tobacco smoking and oncoviruses, causing DNA damage. It is considered that the extensive accumulation of genetic alterations in DNA by these environmental risk factors may lead to an abnormal DNA damage response (DDR), which can result in cell death, chromosomal instability and unregulated proliferation (77-80). Therefore, the proper function of the various DNA repair mechanisms is essential for the elimination of these harmful effects, maintaining the DNA integrity (Fig. 3).

**Head and neck cancer and MMR.** Thus far, a number of studies have suggested that polymorphisms of MMR are associated with an increased risk of developing head and neck

cancer (81-83). A number of studies have shown that head and neck squamous cell carcinomas (HNSCCs) very often exhibit MSI. Notably, it has been observed that HNSCCs exhibit MSI at higher rates than other solid tumors, such as esophageal, breast and gastric carcinomas (21-24,40,84,85). Demokan *et al*, also showed that high levels of MSI in HNSCC are strongly associated with hypermethylation of *hMLH1* and *hMSH2* (81).

As has been discussed above, the dysfunction of the MMR mechanism can lead to MSI and to the accumulation of mutations in proto-oncogenes or tumor-suppressor genes, increasing the risk of malignant development and progression. It has already been demonstrated that the decreased expression of the *MSH2* gene causally increases the frequency of MSI (86,87). Furthermore, the investigation of the *MSH2* protein level in surgical specimens of head and neck carcinoma have revealed an association between low *MSH2* levels and locoregional metastasis, as well as a worse survival (88).

In addition, a specific polymorphism of the MMR genes, *MLH1*, *MSH2* and *MSH3*, as well as *EXO1*, has been suggested to have prognostic value for HNSCC, particularly among smokers (89). Moreover, a recent study also provided evidence that a single nucleotide polymorphism in the *hMLH1* promoter was associated with tobacco-related oral squamous cell carcinoma (90).

The hypermethylation of the *hMLH1* promoter, leading to the inactivation of the gene, has been shown to be an important epigenetic mechanism and has been linked to numerous human malignancies. The promoter hypermethylation of the *hMLH1* gene has been also demonstrated in HNSCC (91-93). Specifically, Tawfik *et al* demonstrated that the loss of *MLH1* protein was not an uncommon finding in HNSCC, and the common mechanism involved the methylation of the CpG island of its promoter (94).

### Lung cancer and MMR

**Role of MMR in NSCLC.** Previous studies have demonstrated a reduced expression of *MSH2* or *MLH1* genes at the protein or mRNA level in >50% of lung adenocarcinomas, associated with a poor survival and an increase in MSI (95-96). Kanellis *et al* evaluated the protein expression levels of MMR genes in fine-needle aspiration (FNA) specimens derived from various types of lung cancer. Their study demonstrated that that NSCLCs, and particularly squamous cell carcinomas, exhibited reduced *MSH2* protein levels at relatively high rates compared to small cell carcinomas (97).

Although in the majority of cases, the decreased expression of MMR genes is attributed to epigenetic silencing, other studies have indicated that MMR deficiency may act as a 'second hit', accelerating the development of lung tumors in mice that carry the *K-ras*<sup>LA1/+</sup> mutation (98). Specifically, Downey and Jirik, using a murine animal model, recently demonstrated that a deficiency in MMR genes can act in concert with the extremely common *K-ras* mutation, enhancing tumor development (98).

Wang *et al* suggested that *hMLH1* was the major altered MMR gene involved in NSCLC tumorigenesis, and that the methylation of its promoter was the most common mechanism for its dysregulation (19). Others have also suggested that the hypermethylation of *hMSH2* has prognostic value, particularly for non-smoking females (96). Although a reduction in the expression of MMR genes has been linked to a poor prognosis,

Scartozzi *et al* demonstrated that a decreased MLH1 protein expression was associated with a statistically significant improvement in survival compared to normal MLH1 protein levels (99). A well-accepted explanation for similar findings does not exist thus far; however, it is possible that the loss of MLH1 leads to the accumulation of a tremendous number of replication errors, leading to a decrease in the division rates of cells. Finally, Takahashi *et al* demonstrated that the expression of the MLH1 and MSH2 proteins was significantly reduced in cigarette-related (cigarette-exposed) lung cancer, demonstrating high replication error rates (100).

*MMR deficiency affects the chemotherapy treatment of NSCLC.* Taking into consideration that platinum-based chemotherapy is commonly used for advanced NSCLC (101), the determination of molecular markers indicating chemotherapeutic resistance has tremendous clinical value, potentially leading to the evidence-based selection of chemotherapy and the improvement of survival rates. A significant number of pre-clinical and clinical data have suggested that the inactivation of *hMLH1* and *hMSH2* promotes resistance to cisplatin and carboplatin-based chemotherapy, but not oxaliplatin-based chemotherapy (99,102,103). According to Vageli *et al*, reduced mRNA levels of MMR are not beneficial for cisplatin chemotherapy, resulting in low survival rates in patients with NSCLC (46). Although the underlying mechanism of this phenomenon is not yet clear, it is considered that an efficient MMR system recognizes the DNA adducts produced by cisplatin and by its attempt to process the extensive number of adducts, activates a sequence of signals that lead to apoptosis (102). As a result, the dysfunction of the MMR system compromises the ability of the cell to detect chemotherapy-induced DNA damage and as a consequence, the ability to undergo apoptosis. On the other hand, pre-clinical analyses have suggested the ability of cells to recognize and react to the DNA damage induced by the drug oxaliplatin does not rely on the MMR system (103). Scartozzi *et al* provided evidence based on clinical data that a decrease in the expression levels of MMR genes does not influence the sensitivity to oxaliplatin (99), confirming previously published pre-clinical data (103).

More recently, some studies have focused on the prognostic significance of a defective MMR mechanism and MSI in several types of cancer, including NSCLC (104,105), and the responsiveness of patients towards immune check-point inhibitors (106-108). Overall, it is well-recognized that patients with MSI and/or defective MMR have generally a durable complete response (106). Furthermore, MSI has been shown to be a good predictive biomarker for immunotherapy efficacy in several types of cancer treated with pembrolizumab or nivolumab, including NSCLC, advanced melanoma, renal cell carcinoma, bladder cancer, etc (107,108). As regards NSCLC, studies have demonstrated a high response rate following treatment with the immune check-point inhibitor, pembrolizumab, in the KEYNOTE-001 and KEYNOTE-024 trials (109,110). Further clinical trials consisting of a sufficient number of NSCLCs patients and adequate follow-up are necessary to verify the efficacy of pembrolizumab in patients with microsatellite instability high/deficiency MMR (MSI-H/dMMR).

*Role of MMR in small-cell lung carcinomas (SCLCs).* The role of the MMR mechanism in SCLC is not yet clear. Data exploring the expression levels of MMR genes and MSI in

SCLC specimens have yielded controversial results. Although Pylkkänen *et al* reported rates of MSI close to zero (111), a number of other studies have shown that the prevalence of MSI in SCLC can reach up to 76% (112-114). These differences have been attributed to the different microsatellite loci analyzed (112,115). Kanellis *et al* reported that small cell carcinomas did not exhibit a significant reduction in MSH2 levels (97). In addition, Hansen *et al* examined the activity of MMR genes at the mRNA and protein level and their association with MSI in 17 SCLC cell lines, supporting that there was a heterogeneous expression pattern of MMR genes and that MMR consequent MSI was not that common in SCLC (115).

### 3. Conclusion

DNA repair deficiency is a hallmark in cancer development and may affect the therapeutic outcomes. Sporadic head and neck and lung tumors often exhibit genetic alterations due to an inadequate mismatch DNA repair mechanism. The mechanisms through which defects in the DNA MMR mechanism promote lung, and head and neck cancer are not yet clear. To the best of our knowledge, the present review article is the first attempt to summarize what is known in the literature about the dysregulation of this mechanism and its role in these types of cancer. This review supports the further investigation of alterations in the expression of mismatch DNA repair genes, at both the transcriptional and translational level, in head and neck, and lung sporadic tumors, clarifying their prognostic and diagnostic value, as well as their therapeutic potential as novel targets.

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writing of the original draft. DPV, SGD, TKN, LF, AT and GL reviewed and edited the article. All authors have read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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