Diet derived polycyclic aromatic hydrocarbons and its pathogenic roles in colorectal carcinogenesis

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Graphical abstract
Caption: Graphical abstract for the review article Diet derived polycyclic aromatic hydrocarbons and its pathogenic roles in colorectal carcinogenesis, highlighting associations that exist between genetic variants in genes associated with polycyclic aromatic hydrocarbon metabolism and DNA repair, and epidemiological studies linking these molecules to colorectal cancer.
Polycyclic aromatic hydrocarbons (PAH) are associated with colorectal cancer (CRC) in the population.

Polymorphisms in PAH metabolic enzymes are associated with CRC.

PAH metabolites create DNA adducts.

Variants of nucleotide excision repair are linked to CRC.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are molecules that contaminate meat products during the high-temperature cooking of meat. This study reviewed the pathogenic roles of meat derived polycyclic aromatic hydrocarbons in the carcinogenesis of colorectal cancer (CRC). Ingested PAHs undergo xenobiotic metabolism resulting in the activation of genotoxic metabolites that can induce DNA damage in the colorectum. Genetic polymorphisms in PAH xenobiotic enzymes...
are linked to the risk of CRC and suggest a role for PAH-meat ingestion in carcinogenesis of colorectal malignancies. Furthermore, PAH specific DNA adducts have been identified in colorectal cancer tissue and linked to high meat intake. DNA adduct resolution is mediated by the nucleotide excision repair, and polymorphisms within genes of this repair pathway and high meat intake are associated with increased CRC risk. In the literature, there is evidence from metabolic enzyme gene variants, DNA repair genes, PAH metabolites, and epidemiological studies suggesting PAH involvement in CRC.

**Keywords**
Polycyclic aromatic hydrocarbon, meat, colon, rectal, bowel, cancer, carcinoma, diet

### 1.0 Introduction

Colorectal cancer (CRC) occurs most frequently in regions with a high human development index (HDI) such as Europe, Australia/New Zealand, North America, and Eastern Asia, whilst countries with a low HDI record much lower incidences of CRC (International Agency for Research on Cancer, n.d.). The incidence and mortality of CRC are predicted to increase over the next 10 years, and this is largely attributed to an overall shift towards a Western lifestyle (Arnold et al., 2017). A Western diet is characterized by high consumption of preserved and processed foods, especially meats and fats as well as low levels of fish, legumes, and whole grains (Moss and Nalankilli, 2017; Singh et al., 2019). The consumption of red and processed meat is an established risk factor of CRC; however, it is not clear the mechanism by which red and processed meat intake contributes to colorectal carcinogenesis (Bouvard et al., 2015).

The International Agency for Research on Cancer (IARC) hypothesises that polycyclic aromatic hydrocarbons (PAHs), a class of chemical compound, might play a significant role in
meat-associated colorectal carcinogenesis (Bouvard et al., 2015). PAHs are compounds composed of hydrogens and carbons containing more than one ring structure, at least one of which is a benzene ring. PAH contamination in meat products is due to direct pyrolysis of food nutrients and smoke deposition from incomplete combustion reactions (Hamidi et al., 2016). PAHs commonly appear in meat exposed to thermal processes, in particular barbequing and grilling. Therefore, the consumption of meats that have undergone high-temperature cooking exposes the gastrointestinal tract (GIT) to higher levels of PAHs.

PAHs ingested via meat can reach the colorectum via three main routes: PAHs can be absorbed with triacylglycerides and enter the systemic circulation via chylomicron transport; PAHs can enter the hepatic portal venous system via fatty acid transport system to be metabolised at the liver to either enter the systemic circulation or the enterohepatic system; and/or movement through the alimentary canal (Kelly L Harris et al., 2013). As PAHs are lipophilic and hydrophobic, these compounds must be metabolised to form a more hydrophilic compound to allow the body to target it for excretion. Once metabolised, PAH-metabolites are directed to the large intestine to be excreted via the faeces. Figure 1 illustrates routes of bioavailability and locations of bioaccessibility of PAH/PAH-metabolites. PAHs via other routes such as inhalation is also linked to lung cancers (Moorthy et al., 2015), and PAHs have been associated with breast cancer (Korsh et al., 2015), however its ingestion is most associated with cancers of the GIT. Although there is a strong link between red and processed meat intake and CRC, PAH in meat and CRC is not well understood. Many studies use benzo[a]pyrene (B[a]P) as a model PAH as it is formed during the cooking of meat and is deemed a class I carcinogen (Humans, 2012). This review aims to collate available literature regarding the colorectal
carcinogenic mechanisms of dietary polycyclic aromatic hydrocarbons, genetic variations of PAH related genes in CRC, and current epidemiological evidence linking CRC to PAH.

2.0 Methods

Literature was systematically identified from Jan 1990 to March 2021 in four databases: PubMed, Scopus, Medline (via Web of Science), and EMBASE. The following terms were searched as full-text and Medical Subject Headings (MeSH): polycyclic aromatic hydrocarbon and colorectal (which includes colon and rectum). Only search results available as full-text and written in English were included. After screening for duplicates, 217 search results fit the aforementioned search terms. Articles that were excluded from this review include: i) review articles, ii) investigations of non-dietary PAH, iii) investigations not regarding cancers of the colon or rectum. Included in this review are original research articles investigating dietary PAH or directly-related substances in colon or rectal cancers. Thirty-one original research articles were found to be directly relevant to this review. Figure 2 details the literature search methodology.

3.0 Discussion

3.1 PAH and its metabolism

PAHs are compounds ubiquitous in the environment, present within the air, soil, water, and food products (Marinković et al., 2013). Despite the presence of PAHs in the air, the gastrointestinal tract is the primary site of PAH exposure (Maliszewska-Kordybach, 1999). Following its absorption, these compounds must undergo biological transformation into a more hydrophilic, and more readily excretable compound (McGinnity and Grime, 2017). PAHs are
xenobiotic substances, meaning they are not naturally produced or present in humans; thus PAHs undergo xenobiotic metabolism involving phase I and II metabolism. Phase I involves the creation of a functional group in the substrate (Testa, 2007), essentially forming a location on the PAH where a chemical bond can be formed readily. Phase II utilises the new functional group on the PAH-metabolite from Phase I, and conjugates it with a polar moiety to allow its excretion (Stanley, 2017).

Xenobiotic metabolism can be a double-edged sword; though the desired outcome is to rid the body of PAHs, biological reactions do not favour activation nor detoxification. Activating enzymes create substrates that are more reactive, whilst detoxifying enzymes create less reactive products (Dashwood, 2006; Stanley, 2017). Highly reactive metabolites can be dangerous as they are more likely to interact with their surroundings rather than being excreted safely. PAHs can undergo metabolic transformation via three distinct pathways (Figure 3), each potentially creating genotoxic metabolites that confer cancer risk. Details of the metabolism of PAHs are discussed in detail below.

3.2 PAH Phase I metabolism and CRC

Phase I metabolism describes the initial enzymatic transformation that a parent PAH molecule undergoes. Most xenobiotic Phase I metabolism is carried out by the cytochrome P450 superfamily of enzymes, particularly CYP1 family comprised of three functional genes (CYP1A1, CYP1A2, CYP1B2) (Stanley, 2017). The cytochrome P450 enzymes are intracellular membrane-bound proteins containing a heme pigment (cyto + chrome + P) (Lynch and Neff, 2007). CYP450 enzymes are generally monoxygenases which catalyse oxidation reactions involving the transfer of molecular oxygen to a substrate (equation 1) (Nam, 2003). Whilst most
CYP450 enzymes are expressed in the liver (CYP3A4, 2C9, 2C8, 2E1, 1A2), CYP1A1 and CYP1B1 are largely expressed extrahepatically (Zanger and Schwab, 2013).

**Equation 1:** RH + O₂ + 2e⁻ + 2H⁺ → ROH + H₂O (Nam, 2003)

Specific to PAHs, CYP450 enzymes act to create a functional site from a chemically inactive C-H bond on the benzene ring (Hrycay and Bandiera, 2015). Monooxygenation of a parent PAH molecule catalysed by CYP450 enzymes results in the formation of an arene oxide intermediate (Mooorthy et al., 2015). This is particularly true for PAHs with a bay region, which is the region that bears the most ring strain (Figure 4). CYP450s show a preference to create benzo-ring arene oxides in PAHs containing a bay region (Jerina et al., 1986). Due to the ring strain experienced at the epoxide site, this metabolic intermediate is highly reactive and is quickly acted upon via Phase II enzymes (Matsumoto and Katsuki, 2012). Thus, PAHs containing a bay region are much more likely to be converted to carcinogenic diol-epoxides.

Of the CYP450 enzymes, the CYP1A1 enzyme shows substrate preference for planar aromatic hydrocarbons (Stanley, 2017), and is, therefore, the main enzyme involved in the initial transformation of PAHs. CYP enzyme expression is inducible and is regulated by the aryl hydrocarbon receptor (AhR) (Go et al., 2015). AhR is a cytosolic receptor that is bound and activated by xenobiotics such as PAHs. Ligand activation of AhR by PAHs results in heterodimerization of AhR with aryl hydrocarbon receptor nuclear translocator (ARNT), which is able to move through the nucleus and induce transcription of xenobiotic response elements (XRE), including CYP1A1 (Go et al., 2015; Stanley, 2017). The induction of CYP1A1 by AHR promotes the conversion of a PAH to an epoxide metabolite. In addition to CYP1A1 induction, AHR has been associated with the gut immune system, microflora, and neural inflammation (Rothhammer and Quintana, 2019). Furthermore, its target genes of alcohol dehydrogenases and
phospholipase A2 suggest a broad role for AhR (Stanley, 2017). Thus, the activation of AhR via PAHs increase the formation of epoxide intermediates via CYP1 enzymes and may also play a role in carcinogenic or inflammatory pathways.

Supplementary to CYP1A1 induction by AHR, CYP1A1 expression can also be induced by PAHs via a p53-dependent mechanism (Willis et al., 2018), and is associated with the risk of CRC (Kiss et al., 2007; Lima et al., 2008; Sachse et al., 2002). Furthermore, CYP enzymes are highly polymorphic and genetic variants of these enzymes are associated with CRC; the CYP1A1 m4 variant (C2453A) was inversely associated with CRC risk (Little et al., 2006), and the m2 variant (A2455G) associated with a slightly decreased risk of CRC (Murtaugh et al., 2005). Moreover, there is significant evidence in the literature on CYP1A1 statistically interacting with meat associated colorectal carcinogenesis (Ferrucci et al., 2010; Goode et al., 2007; Little et al., 2006; Murtaugh et al., 2005; Wohak et al., 2016). The AHR gene, encoding for AhR, was not found to be significantly associated with CRC or meat (Gilsing et al., 2012; Wang et al., 2011). However, ARNT gene polymorphisms expressing an A-allele were inversely associated with the risk of colorectal cancer (33), thus suggesting the importance of CYP1A1 transcriptional regulation in response to PAH in CRC.

Beyerle et al. have demonstrated that CYP3A4, which is present both intra- and extrahepatically, is downregulated in colorectal cancer tissues compared to adjacent non-neoplastic colorectal mucosa tissues, and its increased expression was associated with high processed meat intake (Beyerle et al., 2020a). A weak interaction between B[a]P and CYP3A4 seemed to be associated with colorectal adenoma (Ferrucci et al., 2010). CYP3A4 is inducible via Pregnane X receptor (PXR), but PXR polymorphisms were not linked to CRC risk (V. Andersen et al., 2010). Therefore, it is possible that CYP3A4 can be induced by processed meat
intake and plays a role in the conversion of dihydrodiols to dihydrodiol epoxides of PAHs in the intestines. As PXR does not seem to be linked to colorectal carcinogenesis, it may be possible that meat-induced CYP3A4 transcription occurs through other transcription factors such as peroxisome proliferator-activated receptor α (PPARα).

Levels of PAHs undergoing phase I metabolism may vary depending on the regulation of CYP450 enzymes and the allelic alteration of enzyme activity, both of which have been associated with colorectal carcinogenesis (Table 1). Further research on these processes and the outcome of harbouring differing alleles must be investigated to understand the effects on gene and protein activity. Figure 5 shows the role of CYP450 enzymes in phase I metabolism and the downstream metabolite formation.

3.3 Phase II metabolism of PAHs lead to genotoxic metabolites

Upon completion of Phase I metabolism, the resultant metabolite will undergo Phase II metabolism. Phase II metabolism involves any subsequent reactions involving the alteration of a phase I metabolite (Stanley, 2017). The end product of xenobiotic metabolism of PAHs can lead to DNA damage and increase the risk of carcinogenesis via three main pathways; the CYP450/epoxide hydrolase, the aldo-keto-reductase, and the radical cation pathways (Figure 3).

CYP450/epoxide hydrolase pathway and CRC

The first pathway which an epoxide metabolite may potentially undergo is the CYP450/epoxide hydrolase pathway, otherwise known as the diol-epoxide pathway. As depicted in Figure 5, the substrates in blue represent those in the CYP450/epoxide pathway. The epoxide hydroxylase is an enzyme with substrate specificity towards the arene oxide site. After
forming an epoxide intermediate via CYP450, epoxide hydroxylase uses water to break the unstable epoxide group to convert the epoxide into a dihydro-diol (Rourke and Sinal, 2014). Dihydro-diols which undergo further monooxygenation reactions via CYP450 enzymes, form carcinogenic dihydrodiol epoxides.

Dihydrodiol epoxides are the ultimate carcinogens that can bind to exocyclic amino groups on purine bases (Ewa and Danuta, 2017). This binding to DNA forms bulky adducts that interfere with DNA replication. Unless these PAH-DNA adducts are repaired by the nucleotide excision repair (NER) pathway, PAH bulky adducts can be mutagenic and/or carcinogenic. In particular, bay region-PAH metabolites have been shown to exhibit much higher mutagenic behaviour than their K-region counterparts (Figure 4). Typical PAH compounds which follow the bay region activation pathway include B[a]P, benzo[a]anthracene (B[a]A), benzo[b]fluoranthene (B[b]F), and benzo[c]pyrene (B[c]P) (Shimada and Fujii-Kuriyama, 2004).

The key phase II enzyme involved in the CYP450/epoxide pathway is epoxide hydrolase (EH) as this pushes substrates towards the formation of carcinogenic dihydrodiol epoxides (Figure 5). Of the two main subfamilies of epoxide hydrolases, the microsomal form (mEH) is more relevant to PAH metabolism whilst the soluble form (sEH) participates in endogenous reactions with fatty acid derivatives (Huber, 2007). mEH shows broad substrate specificity and appears to be universally expressed in all tissues, thus allowing quick conversion of a highly reactive epoxide to a more stable and more water-soluble trans-dihydrodiol (Huber, 2007). The epoxide hydrolase 1 (EPHX1) gene (encoding mEH) and its regulatory elements are located on chromosome 1 (Václavíková et al., 2015). There is evidence linking colorectal carcinogenesis and mEH (44, 47, 54), whereby high predicted mEH activity was linked to cases of colorectal adenoma (CRA), which is a key step of colorectal carcinogenesis (Cortessis et al., 2001). These
findings support the hypothesis of PAH-induced carcinogenesis as higher mEH activity would promote the formation of dihydrodiols and dihydrodiol epoxides to result in more DNA adducts. Furthermore, high mEH coupled with high meat intake was associated with increased adenoma and CRC risk (Ferrucci et al., 2010; Gilsing et al., 2012; Goode et al., 2007; Ulrich et al., 2001). This is very suggestive of the CYP450/epoxide hydrolase pathway in transforming PAHs to carcinogenic metabolites and conferring CRC risk.

After the actions of epoxide hydrolase, the PAH-diol can take one of three routes for further transformation; it can be conjugated to glucuronic acid (targeting it for excretion), undergo another round of monooxygenation via CYP450 (creating PAH-diolepoxides), or it can enter the aldo-keto reductase pathway (Figure 5). Glucuronidation reactions are catalysed by uridine 5’-diphospho-glucuronosyltransferases (UGTs) composed of four superfamilies; UGT1, 2, 3, and 8 (Meech et al., 2019). UGT1 and UGT2 families appear to play the largest role in metabolism. Gilising et al. showed that UGT1A family gene expression was nominally associated with both CRA and CRC risk, and individuals with single nucleotide polymorphisms (SNPs) at the rs7569014 locus had significantly higher CRC risk (Gilsing et al., 2012). RNA expression of both UGT1A8 and -1A10 are highest in the gastrointestinal tract, especially in the colon (“Tissue expression of UGT1A10 - Summary - The Human Protein Atlas,” n.d., p. 10; “UGT1A8 protein expression summary - The Human Protein Atlas,” n.d., p. 8). A study by Beyerle et al. showed that UGT1A8 and UGT1A10 expression was downregulated in colorectal cancer tissue when compared to adjacent non-neoplastic mucosa tissue (Beyerle et al., 2020b). Therefore, decreased UGT1A activity could allow more PAH-diols to undergo monooxygenation instead of excretion, leading to higher amounts of PAH-diol epoxides being formed and increasing the likelihood of adduct and cancer formation.
If a PAH-diol-epoxide is formed, it can undergo two potential fates: these carcinogens can bind DNA and form bulky adducts, or they can be targeted for excretion by conjugating it to a glutathione group. This conjugation reaction is mediated by glutathione S-transferases (GSTs), encoded by GST genes. Five families of GSTs have been studied with respect to CRC and meat intake, with polymorphisms in GSTA1, GSTM1, and GSTT1 associated with CRC (Beyerle et al., 2020b; Ferrucci et al., 2010; Gilsing et al., 2012; Little et al., 2006). Specifically, downregulation of GSTA1 and GSTM1 was observed in cancer tissue compared to non-neoplastic mucosal tissue (Beyerle et al., 2020b), suggesting its potential importance in PAH-metabolite excretion.

There is substantial evidence linking all activating and detoxifying enzymatic components of the CYP450/epoxide hydrolase pathway to colorectal cancer. Variations of these genes involved and their association to colorectal adenoma and cancer are highly supportive of this pathway and its causative role in PAH-induced carcinogenesis.

**Aldo-keto-reductase pathway**

Aldo-keto-reductases (AKRs) are a large family of cytosolic redox catalysing enzymes (Barski et al., 2008), including dihydrodiol dehydrogenases (Jez et al., 1997). The aldo-keto-reductase pathway begins with the conversion of PAH-dihydriodols to unstable PAH-catechols via AKRs, which can undergo two rounds of autooxidation to produce redox-active \( \alpha \)-quinones (Sen and Field, 2013, p. 3). \( \alpha \)-quinones can create DNA adducts which could lead to DNA damage and carcinogenesis. Furthermore, byproducts of autooxidation reactions (hydrogen peroxide and oxygen free radicals) can induce oxidative DNA damage.
Catechols can be conjugated to glucuronic acid or sulfonate groups via phase II enzymes to promote export from the body. Sulfonation and glucuronidation are catalysed by sulfotransferase (SULT) and glucuronosyltransferases enzymes, respectively (Stanley, 2017). Thus polymorphisms in these genes may alter levels of conjugation and export. Higher levels of catechols being exported via the actions of SULT and UGT enzymes could result in less adduct formation and oxidative DNA damage.

Sulfotransferases are a superfamily of cytosolic enzymes, of which two forms (SULT1A1 and SULT1A2) have been noted to harbour functional genetic polymorphisms (Glatt, 2000). Studies investigating polymorphisms in SULT1A genes found no significant association with colorectal cancer risk, nor any interaction with meat intake (Ferrucci et al., 2010; Gilsing et al., 2012; Goode et al., 2007). This may be due to low levels of SULT1A protein expression in the intestines, as SULT1A is expressed mainly in the liver. The lack of association between CRC risk and SULT1A polymorphisms suggests that differing sulfonation levels of catechols in the AKR pathway do not contribute to colorectal carcinogenesis.

Sulfonation is not the only method of targeting catechols for excretion; glucuronidation via the UGT family of enzymes can also clear PAH-catechols from the body and deter oxidative stress and adduct formation, thereby decreasing cancer risk. As mentioned previously, polymorphisms in UGT1A confer higher CRC risk, and CRC tissue expresses lower UGT1A8 and UGT1A10 compared to adjacent non-neoplastic mucosal tissue (Beyerle et al., 2020b; Gilsing et al., 2012). Though UGT1A enzymes are linked to CRC, it is not possible via current evidence to determine whether this link is due to catechol clearance (AKR pathway), dihydrodiol clearance (CYP450/epoxide hydrolase and/or AKR pathway), or something else entirely.
A downstream enzyme of the AKR pathway is nicotinamide adenine dinucleotide phosphate (NADPH) dehydrogenase [quinone] 1 (NQO1) (Sen and Field, 2013). NQO1 catalyses the reduction of quinone by utilising NADPH (Ross and Siegel, 2017). Thus, NQO1 functions as a protective enzyme by preventing redox-active $o$-quinone adduct formation, promoting catechol formation and allowing for conjugation/excretion. Ferrucci et al. did not find an association between NQO1 SNPs and CRC, however did note a possible interaction with 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (meat-related mutagen) and risk of colorectal adenoma (CRA) (Ferrucci et al., 2010). A study by Gilsing et al. found that one SNP at the $NQO1$ gene was associated with CRC (Gilsing et al., 2012). Together, these studies show that meat intake can alter NQO1 expression and confer risk of CRC, and further studies are required to investigate the role of PAHs in this enzymatic conversion.

Radical cation pathway

The radical cation pathway is another potentially mutagenic pathway that ultimately leads to free radical formation and DNA damage (Cavaliere and Rogan, 1985). The previous two pathways involved CYP enzymes catalysing monooxygenation reactions by taking a single oxygen from $O_2$ and inserting it into the substrate to create a functional group (Hrycay and Bandiera, 2015). This reaction is dependent on the transfer of electrons from nicotinamide adenine dinucleotide phosphate (NADPH) via flavin cofactors (FAD and FMN) to activate the heme centre of P450 enzyme (Huang et al., 2015). However, in the absence of NADPH and $O_2$, CYP450 catalyses peroxidase-dependent hydroxylation of PAHs (Ewa and Danuta, 2017; Hollenberg, 1992). Furthermore, $H_2O_2$-dependent peroxidases and prostaglandin H synthase can also catalase one-electron oxidation of PAHs (Sen and Field, 2013). Through this process, a
radical cation is created. This electrophilic radical cation reacts with nucleophilic DNA at the N7 position of purines to form DNA adducts (Cavalieri and Rogan, 1995; Li et al., 1995). The formation of an adduct by a radical cation creates an unstable adduct, which is spontaneously cleaved at the glycosidic bond and released from DNA. This removal leaves an apurinic (AP) site, where a purine is not present where it should be within the sugar phosphodiester backbone (Sen and Field, 2013). The AP sites cause genetic instability, and are potentially lethal and mutagenic lesions that could lead to cancer formation (Boiteux and Guillet, 2004).

The formation of radical cations from PAHs generally only occur in instances where NADPH/O$_2$ are absent and is thus the least likely metabolic pathway for PAHs to undertake. Moreover, radical cations showed much lower mutagenicity compared to other PAH metabolites. Their limited ability to generate DNA damage and their contribution to oxidative stress may act in concert with other carcinogenic traits of PAH metabolism but is unlikely to be the sole cause of colorectal carcinogenesis. Due to the comparatively lower mutagenic and carcinogenic potential of PAH radical cations formed from this pathway, research with respect to meat intake and colorectal cancer is limited.

3.4 PAH metabolites cause DNA damage in the colon

Through three potential pathways, xenobiotic metabolism of PAHs results in metabolites capable of DNA damage. The CYP/EH, radical cation, and AKR pathways produce compounds able to form stable, unstable, and stable DNA adducts, respectively. In particular, adenine- and guanine-adducts are preferentially formed due to the electrophilic nature of such metabolites.
B[a]P, dibenz[a,h]anthracene (D[a,h]A), and dibenz[a,l]pyrene (D[a,l]P) treatments in HCT116 (colorectal cancer cells) resulted in detectable levels of diol-epoxides and PAH-DNA adducts (Wohak et al., 2012). PAH-DNA adducts in white blood cells were significantly higher in patients with CRA compared to healthy controls (Gunter et al., 2007), and is suggestive of a role of PAH in carcinogenic steps of CRC. Furthermore, (+)-anti-benzo[a]pyrene diol epoxide (anti-BPDE) adducts were present in mucosal tissue of the colon (Alexandrov et al., 1996), thus proving that PAH metabolites can bind DNA in the large intestine, and the diol-epoxide pathway does occur in the colon and is strongly linked to cancer. Opposingly, a small study by Al-Saleh et al did not detect BPDE-DNA adducts in cancerous, nor non-cancerous tissues of newly diagnosed CRC (Al-Saleh et al., 2008), though a small sample size may have limited this study.

Overall, there is substantial literature displaying evidence of PAH-induced DNA damage in colorectal cells and tissues. In particular, the metabolites and adducts detected in these studies indicate the importance of PAH ingestion via meat and its transformation via CYP450/EH pathway in CRC.

3.5 Aberrant DNA repair and PAH-CRC risk

PAHs undergo xenobiotic transformation to create toxic metabolites that form bulky DNA adducts. Such lesions are able to distort the DNA-helix and/or interrupt transcription (Marteijn et al., 2014). Nucleotide excision repair (NER) is employed by the cell to relieve DNA of bulky adducts (Cai et al., 2012). Genetic variation among individuals may place them at higher susceptibility to PAH-DNA adducts, and therefore increase their risk of PAH-induced mutagenesis and carcinogenesis.

DNA excision repair protein excision repair cross-complementation group 6, encoded by ERCC6, has ATPase activity and is involved in transcription-coupled excision repair via
interactions with transcription factors and other excision repair proteins (“ERCC6 ERCC excision repair 6, chromatin remodelling factor [Homo sapiens (human)] - Gene - NCBI,” n.d.). Individuals with a variant of this gene, ERCC6 1213G, were at a higher risk of CRC with increasing variant alleles (Berndt et al., 2006). ERCC6 1213G is hypothesised to dampen NER capacity (Berndt et al., 2006) and thus increase the likelihood of cancer risk if under attack by bulky adducts, such as those formed by dietary PAH. Furthermore, Joshi et al. found an association between CRC, meat intake, and a polymorphism in a closely related protein. Intake of heavily browned meat increased the risk of CRC, especially rectal cancer, in individuals carrying the lysine/lysine genotype in codon 751 in the excision repair cross complementation group 2 (XPD/ERCC2) gene and for aspartic acid/aspartic acid at codon 312 of ERCC2 (Joshi et al., 2009). This highlights the importance of ERCC2 and ERCC6 in DNA repair of bulky adducts whilst also showing that meat intake alters the risk of CRC dependent on the genetic variant of these genes.

In addition to the ERCC gene family linked to aberrant repair and CRC risk, individuals having at least one 492H allele on the XPC gene were also associated with a greater risk of CRC (Berndt et al., 2006). The protein product of XPC is the DNA repair protein complementing XP-C cells, which forms part of the Xeroderma pigmentosum complementation group C (XPC) DNA repair protein complex with UV excision repair protein RAD23 homolog B (RAD23B) and centrin-2 (CETN2) to stably repair DNA damage (Zhang et al., 2015). It is possible that individuals with at least one 492H allele on XPC do not form the XPC complex as appropriately and thus have a compromised ability to stably repair DNA damage potentially caused by PAHs, leading to persistent damage and higher CRC risk.
This section (3.4) focuses on the presence of PAH-bulky adducts in colorectal cells and tissues, and thus highlights the importance of reparations of bulky adducts. The nucleotide excision repair pathway and its associated proteins are critical in relieving the cell of DNA damage and mitigating genotoxic stress and cancer risk. Genetic variants of such NER proteins and its association with meat intake leading to greater risk of CRC further supports a PAH as a carcinogen in CRC.

3.6 Dietary PAHs are linked to CRC risk in population studies

Though many studies investigate meat intake and CRC, there is limited evidence available for the direct association between polycyclic aromatic hydrocarbons and colorectal carcinogenesis and cancer risk in the population (table 2). Of the available studies investigating dietary PAH and CRC, all studies utilised modified food frequency questionnaires (FFQ) to estimate dietary PAH intake. Thus, it is important to note that PAHs were not quantitatively measured and assessed against CRC. All available literature unanimously showed that dietary intake of red and processed meats that were either exposed to high-temperature cooking methods or cooked for longer was positively associated with CRC risk (table 2). Although not a direct correlation, PAHs are formed from high-temperature cooking methods, and thus, PAHs are indirectly associated with higher CRC risk.

Many studies linked their modified FFQ to mutational databases to derive levels of B[a]P taken through the diet (Cross et al., 2010; Ferrucci et al., 2009; Miller et al., 2013; Mosley et al., 2020; Sinha et al., 2005a, 2005b). Through the use of these modified FFQ coupled to mutagen databases, some studies have found no association with CRC risk (Cross et al., 2010; Ferrucci et al., 2009; Hofmann et al., 2013; Mosley et al., 2020; Shin et al., 2007), whilst others found B[a]P
intake from the diet to be positively associated with rectal cancer (Miller et al., 2013), colorectal adenoma (CRA) (Sinha et al., 2005a), and adenomas of the descending and sigmoid colons (Sinha et al., 2005b). Although using a FFQ-mutagen database is an established method and holds significant advantages (i.e. allowing estimates in a large population study), it also has some limitations. B[a]P mutagen measures are approximations of the truth, whose reliability may be compromised by the accuracy of the questionnaire and database. Furthermore, B[a]P is used as a representative PAH and does not account for many other PAHs present in meat. Despite such limitations, there is evidence linking B[a]P (as a representative PAH) to CRC.

Thus, the current literature investigating dietary PAH and CRC risk in the population is inconclusive. Whilst studies show that a Western diet high in meats and fats promote PAH-induced neoplasia in the colorectum of mice (Harris et al., 2009), rats (Harris et al., 2015; K.L. Harris et al., 2013), and cell models (Herbst et al., 2006; Tylíková et al., 2019), this relationship within the human population is not concrete. Given that all studies have positively linked consumption of high-temperature cooking and well-done meat intake to CRC risk (Cross et al., 2010; Ferrucci et al., 2009; Hofmann et al., 2013; Mosley et al., 2020; Shin et al., 2007; Sinha et al., 2005a, 2005b); PAHs are formed during such processes; and three of five case-control studies investigating B[a]P in the diet (Miller et al., 2013; Mosley et al., 2020; Shin et al., 2007; Sinha et al., 2005a, 2005b) showed an increased CRC risk (Miller et al., 2013; Sinha et al., 2005a, 2005b); a further inquiry into this relationship is warranted.

4.0 Conclusion

This review summarised the associations between dietary polycyclic aromatic hydrocarbons and CRCs. PAHs are present in meats cooked at a high temperature, which are strongly associated with CRC risk in population studies. Mechanisms of colorectal carcinogenicity lie in the
metabolites formed by xenobiotic transformation of parent PAHs. Such metabolites are able to induce DNA damage, primarily through the formation of bulky adducts. PAH metabolism is a complex process involving many enzymes across varying metabolic pathways; the genetic variation of xenobiotic metabolism enzymes may confer CRC risk in association with high meat intake. Additionally, nucleotide excision repair is essential in protecting the genome from bulky adducts and NER genes are found to be associated with CRC risk and are statistically altered by meat intake. Taken together, there is substantial evidence suggesting a role for PAHs in CRCs. To further elucidate this role, clinicopathological associations and specific molecular alterations could benefit future research. A better understanding of this could inform dietary practices to mitigate PAH ingestion and lead to lower incidences of CRC, particularly in countries with a Western diet.

Conflicts of interest
All authors declare they have no conflicts of interest.

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References


Figure 1: Bioaccessibility of PAHs. Ingestion of PAHs via consumption of meat cooked at high temperatures can be absorbed with dietary fats to enter the systemic circulation; the enterohepatic circulation; or can pass through the alimentary canal, all to ultimately reach the colorectum.
Figure 2: Literature search methodology. PRISMA flow diagram of literature search for included studies in this review of meat-related polycyclic aromatic hydrocarbons in colorectal cancer.
**Figure 3**: Polycyclic aromatic hydrocarbons can undergo metabolic activation via three overlapping pathways. Here B[a]P is depicted with B[a]P specific adducts.
Figure 4: Examples of polycyclic aromatic hydrocarbons and their regions of possible contortion. Bay regions experience the highest levels of ring strain, which is hypothesised to show the highest levels of carcinogenicity.
**Figure 5**: Xenobiotic transformation of PAHs via CYP/EH (blue), radical cation (light green), and AKR pathways (light red). This can lead to damaging effects (solid red), or conjugation can lead to excretion (solid green).
Table 1: Original studies investigating PAH xenobiotic genes in colorectal cancer, and whether this association statistically interacts with meat intake. Note: N-acetyltransferases have been included in this table due to their significant association with CRC and meat intake, however, these phase II enzymes are not involved with PAH metabolism but rather metabolism of heterocyclic aromatic amines, which are formed during browning of the meat.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>First author</th>
<th>CRC risk</th>
<th>Interaction with red or processed meat</th>
<th>Main Findings</th>
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<tbody>
<tr>
<td>AHR</td>
<td>Aryl hydrocarbon receptor</td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>Yes?</td>
<td>Suggestive modifying effect with meat in colorectal adenoma.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wang, H (Wang et al., 2011)</td>
<td>No</td>
<td>No</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Wohak, LE (Wohak et al., 2016)</td>
<td>-</td>
<td>No</td>
<td>PAH (B[a]P, D[a,h]A, D[a,l]P) treatment in HCT116 cells did not induce differing AHR expression across differing TP53 status.</td>
</tr>
<tr>
<td>ARNT</td>
<td>Aryl hydrocarbon receptor nuclear translocator</td>
<td>Wang, H (Wang et al., 2011)</td>
<td>Yes</td>
<td>No</td>
<td>A-allele at rs12410394 significantly inversely associated with CRC for GG, AG and AA genotypes.</td>
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<tr>
<td></td>
<td></td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>No</td>
<td>Yes</td>
<td>Possible interaction with nitrate/nitrite in relation to colorectal adenoma</td>
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<tr>
<td></td>
<td></td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Goode, EL (Goode et al., 2007)</td>
<td>No</td>
<td>Yes?</td>
<td>Evidence for interaction between meat intake and risk of colorectal polyp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Little, J (Little et al., 2006)</td>
<td>Yes</td>
<td>Yes</td>
<td>CYPIA1<em>4 (m4) variant inversely associated with CRC. Significant interaction associated with CYPIA1</em>2A, CYPA1A1<em>2C and CYPIA1</em>4 with meat intake.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Murtaugh, M (Murtaugh et al., 2005)</td>
<td>Yes</td>
<td>Yes</td>
<td>Slight decrease in CRC risk in individuals with CYPIA1 any<em>2 genotype coupled with white meat consumption. Women with CYPIA1 any</em>2 genotype and GSTM1 present genotype showed increased CRC risk with high red meat mutagen index.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wang, H (Wang et al., 2011)</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wohak, LE (Wohak et al., 2016)</td>
<td>-</td>
<td>Yes</td>
<td>CYPIA1 protein expression is induced by PAHs (B[a]P, D[a,h]A, D[a,l]P) in HCT116-TP53(+/+) cells</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>Cytochrome P450 1A1</td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>Wang, H (Wang et al., 2011)</td>
<td>Yes?</td>
<td>No</td>
<td>Carriers of the minor T-allele at rs11072508 showed slight decreased CRA risk.</td>
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<tr>
<td>CYP1B1</td>
<td>Cytochrome P450 1B1</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>No</td>
<td>Yes</td>
<td>Possible interaction with PhIP, and B[a]P in relation to colorectal adenoma</td>
</tr>
</tbody>
</table>

Note: CRC: colorectal cancer; PAH: polycyclic aromatic hydrocarbons; PhIP: Phenoxyisouquinoline; GSTM1: glutathione S-transferase M1; TP53: tumor protein p53; N-acetyltransferase; N-oxidase; NNAT: N-acetyltransferase 2; GSTM1: glutathione S-transferase M1; TP53: tumor protein p53; HCT116: human colorectal adenocarcinoma cell line.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Enzyme Function</th>
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<th>Reference 2</th>
<th>Reference 3</th>
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<tr>
<td><strong>CYP2A6</strong></td>
<td>Cytochrome P450 2A6</td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Wang, H (Wang et al., 2011)</td>
<td>No</td>
<td>No</td>
<td>-</td>
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<tr>
<td><strong>CYP2C9</strong></td>
<td>Cytochrome P450 2C9</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>Ward, M (Ward et al., 2007)</td>
<td>No</td>
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<td><strong>CYP2D6</strong></td>
<td>Cytochrome P450 2D6</td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
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<td><strong>CYP2E1</strong></td>
<td>Cytochrome P450 2E1</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>No</td>
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<td></td>
<td></td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>Yes?</td>
<td>Suggestive modifying effect with meat in colorectal adenoma.</td>
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<tr>
<td><strong>CYP2W1</strong></td>
<td>Cytochrome P450 2W1</td>
<td>Beyerle, J (Beyerle et al., 2020b)</td>
<td>No</td>
<td>Yes</td>
<td>Decreased expression in normal tissue with high red meat intake.</td>
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<tr>
<td><strong>CYP3A4</strong></td>
<td>Cytochrome P450 3A4</td>
<td>Beyerle, J (Beyerle et al., 2020b)</td>
<td>Yes</td>
<td>Yes</td>
<td>Downregulated in CRC tissue compared to non-neoplastic mucosa tissue. Increased expression in cancer tissue with high processed meat intake.</td>
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<tr>
<td></td>
<td></td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>No</td>
<td>Yes</td>
<td>Possible interaction with B[a]P in relation to colorectal adenoma</td>
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<tr>
<td><strong>EPHX1</strong></td>
<td>Microsomal epoxide hydrolase 1</td>
<td>Cortessis, V (Cortessis et al., 2001)</td>
<td>Yes</td>
<td>Yes</td>
<td>High predicted mEH activity was associated with adenoma. mEH effect is modified by well-done red meat intake.</td>
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<td></td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>Yes</td>
<td>Yes?</td>
<td>Associated with advanced colorectal adenoma. Suggestive modifying effect with meat in colorectal adenoma.</td>
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<tr>
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<td></td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>Yes</td>
<td>No</td>
<td>Nominally associated with colorectal adenoma CRA and CRC</td>
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33
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
<th>Study 4</th>
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<tr>
<td>GSTA1</td>
<td>Glutathione S-Transferase Alpha 1</td>
<td>Beyerle, J (Beyerle et al., 2020b)</td>
<td>No</td>
<td>Yes</td>
<td>Evidence for interaction between meat intake and colorectal polyp risk</td>
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<td></td>
<td>Ulrich, C (Ulrich et al., 2001)</td>
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<td>Yes</td>
<td>High meat intake was associated in a 2-fold risk of colorectal adenoma development, especially at exon 3 (Tyr113His).</td>
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<td>GSTM1</td>
<td>Glutathione S-Transferase Mu 1</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>Yes</td>
<td>No</td>
<td>Downregulated in CRC tissue compared to normal tissue.</td>
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<td></td>
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<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
<td>Nominally associated with colorectal adenoma</td>
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<td>GSTM3</td>
<td>Glutathione S-Transferase Mu 3</td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>Yes?</td>
<td>Suggestive modifying effect with meat in colorectal adenoma.</td>
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<tr>
<td>GSTP1</td>
<td>Glutathione S-Transferase Pi 1</td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
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<tr>
<td></td>
<td></td>
<td>Beyerle, J (Beyerle et al., 2020b)</td>
<td>No</td>
<td>No</td>
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<tr>
<td>GSTT1</td>
<td>Glutathione S-Transferase Theta 1</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>Yes</td>
<td>No</td>
<td>Associated with advanced colorectal adenoma (size of ( \geq 1 ) cm, high-grade dysplasia, or villous components, including tubulovillous)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Little, J (Little et al., 2006)</td>
<td>No</td>
<td>No</td>
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<td>NAT1</td>
<td>N-acetyltransferase 1</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>Yes</td>
<td>No</td>
<td>Associated with advanced colorectal adenoma (size of ( \geq 1 ) cm, high-grade dysplasia, or villous components, including tubulovillous)</td>
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<tr>
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<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>Yes</td>
<td>Yes</td>
<td>Nominally associated with colorectal adenoma and CRC. NAT1 polymorphism rs6586714 interacted with meat intake (MeIQx) in CRA. High MeIQx intake increased risk for GG genotype.</td>
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<tr>
<td></td>
<td></td>
<td>Goode, EL (Goode et al., 2007)</td>
<td>Yes?</td>
<td>No</td>
<td>Evidence for interaction with risk of hyperplastic polyps</td>
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<tr>
<td>NAT2</td>
<td>N-acetyltransferase 2</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>Yes</td>
<td>No</td>
<td>Associated with advanced colorectal adenoma (size of ( \geq 1 ) cm, high-grade dysplasia, or villous components, including tubulovillous)</td>
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<td>Gene</td>
<td>Reference/Study Details</td>
<td>Interaction with CRC/Adenoma</td>
<td>Interaction with Risk of Hyperplastic Polyps</td>
<td>Evidence Comments</td>
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<tr>
<td>NFkB1</td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
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<tr>
<td></td>
<td>Goode, EL (Goode et al., 2007)</td>
<td>Yes?</td>
<td>No</td>
<td>Evidence for interaction with risk of hyperplastic polyps</td>
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<tr>
<td></td>
<td>Andersen, V (Vibeke Andersen et al., 2010)</td>
<td>Yes</td>
<td>Yes</td>
<td>Carriers of NFkB -94 del (ATTG (rs28362491)) were at a higher risk of CRC than homozygous carries of insertion allele. These carriers had a 3% increase per 25g of meat per day, whilst homozygous insertion carriers showed no changes in CRC risk from meat intake interaction.</td>
<td></td>
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<tr>
<td>NQO1</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>No</td>
<td>Yes</td>
<td>Possible interaction with PhIP in relation to colorectal adenoma</td>
<td></td>
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<td></td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>Yes</td>
<td>No</td>
<td>Nominally associated with CRC</td>
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<td>NRIH3</td>
<td>Andersen, V (Vibeke Andersen et al., 2010)</td>
<td>No</td>
<td>No</td>
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<td>NR1I2</td>
<td>Andersen, V (Vibeke Andersen et al., 2010)</td>
<td>No</td>
<td>No</td>
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<tr>
<td>SULT1A1</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>No</td>
<td>No</td>
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<tr>
<td></td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
<td>-</td>
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<tr>
<td></td>
<td>Goode, EL (Goode et al., 2007)</td>
<td>Yes?</td>
<td>No</td>
<td>Evidence for interaction with risk of hyperplastic polyps</td>
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<td>SULT1A2</td>
<td>Ferrucci, L (Ferrucci et al., 2010)</td>
<td>No</td>
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<td></td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>No</td>
<td>No</td>
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<tr>
<td></td>
<td>Goode, EL (Goode et al., 2007)</td>
<td>No</td>
<td>No</td>
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<td>UGT1A</td>
<td>Gilsing, A (Gilsing et al., 2012)</td>
<td>Yes</td>
<td>Yes?</td>
<td>Nominally associated with colorectal adenoma and CRC. UGT1A rs7569014 significantly associated with CRC risk. Suggestive modifying effect with meat in colorectal adenoma.</td>
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<tr>
<td>UGT1A8</td>
<td>Beyerle, J (Beyerle et al., 2020b)</td>
<td>Yes</td>
<td>No</td>
<td>Downregulated in CRC tissue compared to non-neoplastic mucosa tissue.</td>
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<tr>
<td>UGT1A10</td>
<td>Beyerle, J (Beyerle et al., 2020b)</td>
<td>Yes</td>
<td>Yes</td>
<td>Downregulated in CRC tissue compared to non-neoplastic mucosa tissue. Increased expression in non-neoplastic mucosa tissue with high processed meat intake.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Journal articles investigating the association between colorectal cancer and estimated dietary polycyclic aromatic hydrocarbon intake.

<table>
<thead>
<tr>
<th>Year</th>
<th>First Author</th>
<th>Study design</th>
<th>Number of Cases</th>
<th>Organ/Tissue</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Sinha, R (Sinha et al., 2005a)</td>
<td>Case-control</td>
<td>146</td>
<td>Colorectal adenoma</td>
<td>Participants completed food frequency questionnaires including information regarding meat cooking techniques and meat doneness. High intake of B[a]P from meat and all food sources was associated with an increased risk of CRA.</td>
</tr>
<tr>
<td>2005</td>
<td>Sinha, R (Sinha et al., 2005b)</td>
<td>Case-control</td>
<td>3696</td>
<td>Colorectal adenoma</td>
<td>Participants completed food frequency questionnaires including information regarding meat cooking techniques and meat doneness. Well-done meat intake was linked to increased CRA risk. B[a]P was associated with increased risks for adenomas of the descending colon and sigmoid colon.</td>
</tr>
<tr>
<td>2007</td>
<td>Shin, A (Shin et al., 2007)</td>
<td>Case-control</td>
<td>1028</td>
<td>Colorectal polyps</td>
<td>Participants were recruited from the Tennessee Colorectal Polyp Study. Questionnaires were employed to determine meat intake and doneness. High total meat and red meat intake were positively associated with hyperplastic polyps. Well-done meat intake (total red) was positively associated with risk of large adenomas. No associations were found for B[a]P.</td>
</tr>
<tr>
<td>2009</td>
<td>Ferrucci, LM (Ferrucci et al., 2009)</td>
<td>Cross-sectional</td>
<td>158</td>
<td>Colorectal adenoma (CRA)</td>
<td>Women were recruited from a colonoscopy screening. Mutagenic meat components were estimated from food frequency and meat questionnaires which were linked to databases. Red meat and pan-fried meat intake was positively associated with CRA. B[a]P was not associated with colorectal adenoma.</td>
</tr>
<tr>
<td>2010</td>
<td>Cross, AJ (Cross et al., 2010)</td>
<td>Prospective</td>
<td>1995 724</td>
<td>Colon cancer Rectal cancer</td>
<td>PAH intake was represented by benzo[a]pyrene (B[a]P) was estimated using detailed dietary questionnaire and linked to mutagen databases. Red meat and processed meat intakes indicated elevated CRC risk. B[a]P was not associated with colorectal cancer.</td>
</tr>
<tr>
<td>2012</td>
<td>Miller, PE (Miller et al., 2013)</td>
<td>Case-control</td>
<td>989</td>
<td>Colorectal cancer</td>
<td>Meat-related components were determined via a food frequency questionnaire with a meat-specific module. Red and processed meat was positively associated with proximal colon cancer. Pan-fried red meat was positively associated with CRC. B[a]P was positively associated with rectal cancer.</td>
</tr>
<tr>
<td>2013</td>
<td>Hofmann, JN (Hofmann et al., 2013)</td>
<td>Case-control</td>
<td>343</td>
<td>Colorectal cancer</td>
<td>1-OHPG, a PAH metabolite, was analysed in CRC cases within the Shanghai Women’s Health Study. Eating foods there were cooked well-done was associated with elevated 1-OHPG. No significant differences were observed with CRC risk and urinary 1-OHPG levels.</td>
</tr>
<tr>
<td>2019</td>
<td>Mosley, DA (Mosley et al., 2020)</td>
<td>Case-control</td>
<td>2558</td>
<td>Colorectal polyps</td>
<td>Meat-intake and meat-doneness was assessed using an at-home booklet provided to participants. Well-done meat intake was 80% higher risk of developing serrated sessile lesions. No associations were observed for B[a]P intake and polyp risk.</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1: Bioaccessibility of PAHs. Ingestion of PAHs via consumption of meat cooked at high temperatures can be absorbed with dietary fats to enter the systemic circulation; the enterohepatic circulation; or can pass through the alimentary canal, all to ultimately reach the colorectum.

Figure 2: Literature search methodology. PRISMA flow diagram of literature search for included studies in this review of meat-related polycyclic aromatic hydrocarbons in colorectal cancer.

Figure 3: Polycyclic aromatic hydrocarbons can undergo metabolic activation via three overlapping pathways. Here B[a]P is depicted with B[a]P specific adducts.

Figure 4: Examples of polycyclic aromatic hydrocarbons and their regions of possible contortion. Bay regions experience the highest levels of ring strain, which is hypothesised to show the highest levels of carcinogenicity.

Figure 5: Xenobiotic transformation of PAHs via CYP/EH (blue), radical cation (light green), and AKR pathways (light red). This can lead to damaging effects (solid red), or conjugation can lead to excretion (solid green).