
The material cannot be used for any other purpose without further permission of the publisher and is for private use only.

There may be differences between this version and the published version. You are advised to consult the publisher’s version if you wish to cite from it.

http://eprints.gla.ac.uk/245653/

Deposited on 15 July 2021
Novel Methods of Risk Stratifying Patients for Metachronous, Pre-Malignant Colorectal Polyps: A Systematic Review.

Mark S. Johnstone\textsuperscript{a}, Gerard Lynch\textsuperscript{b}, James Park\textsuperscript{a}, Stephen McSorley\textsuperscript{a}, Joanne Edwards\textsuperscript{b}

\textsuperscript{a} Academic Unit of Surgery, School of Medicine, University of Glasgow
\textsuperscript{b} Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow

Corresponding author: Mark Johnstone
Email: mark.johnstone2@ggc.scot.nhs.uk
Address: New Lister Building, Glasgow Royal Infirmary, 8-16 Alexandra Parade, Glasgow, G31 2ER

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors declare they have no conflicts of interest.

Abstract word count: 150

Manuscript word count: 4989
Abstract

Introduction

Despite conventional measures of future polyp risk (histology, dysplasia, size, number), surveillance places a burden on patients and colonoscopy services. We aimed to review novel risk stratification techniques.

Methods

A systematic literature review was performed for studies using genomics, transcriptomics, IHC or microbiome as markers of metachronous polyp risk.

Results

4165 papers underwent title, 303 abstract and 215 full paper review. 25 papers were included. 49 mutations/ SNPs/ haplotypes in 23 genes/ chromosomal regions (KRAS, APC, EGFR, COX1/2, IL23R, DRD2, CYP2C9/24A1/7A1, UGT1A6, ODC, ALOX12/15, PGDH, SRC, IGSF5, KCNS3, EPHB1/ KY, FAM188b, 3p24.1, 9q33.2, 13q33.2) correlated with metachronous adenoma / advanced adenoma risk. Expression levels of 6 proteins correlated with metachronous adenoma (p53, β-catenin, COX2, Adnab-9, ALDH1A1) or sessile serrated polyp (ANXA10) risk.
Conclusion

Although genomic and IHC markers correlated with metachronous polyp risk, it seems likely that a panel of novel markers will be required to refine this risk.

Keywords: metachronous, recurrence, future, polyp, adenoma, colorectal
**Introduction**

Colorectal cancer is the 4\textsuperscript{th} most common cancer in the UK, with approximately 41,000 new cases and 16,000 deaths each year [1]. Colorectal malignancies are known to originate from precursor lesions in the form of polyps [2]. There are two main types of colorectal polyps with recognised malignant potential: adenomas and serrated polyps [3-5]. Over time a small proportion of these benign polyps become increasingly dysplastic and eventually malignant via two principal pathways: adenomas via the classic adenoma-carcinoma (~70%) sequence and sessile serrated polyps via the serrated polyp pathway (~30%) [3, 6]. On a molecular level there are 2 main classification systems: the Consensus Molecular Subtypes (CMS1 MSI Immune, CMS2 Canonical, CMS3 Metabolic and CMS4 Mesenchymal) and the Colorectal Cancer Intrinsic Subtypes (CRIS-A, CRIS-B, CRIS-C, CRIS-D, CRIS-E). Each subtype is characterised by a series of genetic and epigenetic changes [7-9] with malignant transformation estimated to take 7 to 15 years, making polyps an excellent target for cancer prevention [6]. By removing benign dysplastic polyps endoscopically prior to malignant transformation, it should be possible to reduce colorectal cancer incidence, a theory supported by bowel cancer screening data [10].

Premalignant polyps are common, occurring in 25-50% of all patients at screening age (50-74 years) [5]. Additionally it is estimated that 20-50% of patients who undergo polypectomy will develop future polyps[11], hence surveillance colonoscopy is widely recommended[6]. However, as a large proportion of patients will never develop metachronous polyps and as few will progress to malignancy, it is inefficient and unnecessary to subject all patients to surveillance [5]. It is preferable instead to risk stratify patients and only perform surveillance on those with a higher likelihood of developing metachronous pre-malignant polyps or
cancer[2]. The British Society of Gastroenterology and Association of Coloproctology of
Great Britain and Ireland post-polypectomy surveillance guidelines risk-stratify patients
based on polyp histology, grade of dysplasia, size and number (Figure 1) [5]. The presence of
high-grade dysplasia[12-17], larger polyp size[12-15] and an increasing number of
adenomata found at index colonoscopy[12-15, 17-20], have been associated with a greater
risk of future advanced adenoma and colorectal cancer. Other factors which clinicians may
consider in their overall risk assessment are presence of villous histology[12, 13, 15, 17, 21],
increasing age[22], male sex[22], high BMI[22], smoking[23], hypertension[23, 24] and a
raised systemic inflammatory response[25], which have all been associated metachronous
polyp risk. Additionally, aspirin[26-32], NSAIDs[32-35], calcium supplementation[36-45]
and good compliance with a high-fibre, -fruit and -vegetable and low-fat diet[46] have been
shown to be protective. Current risk stratification measures are insufficient and surveillance
still places a large burden on patients and endoscopy services, with surveillance accounting
for 100,000 of the 700,000 colonoscopies performed in England each year[47]. By further
refining risk stratification it may be possible to relieve pressure on endoscopy services and
avoid unnecessary invasive investigations.

The INCISE (Integrated Technologies for Improved Polyp Surveillance) project is a
retrospective, multi-partner collaborative project which aims to combine patient
characteristics with tissue analysis of polyps, including digital pathology,
immunohistochemistry (IHC), genomic and transcriptomics, integrated using machine
learning, to better predict future polyp risk and improve current surveillance protocols.
Therefore, the aim of this systematic review was to evaluate existing studies that have used
genomics, transcriptomics, IHC and polyp microbiome as markers of metachronous polyp
risk.
Methods

A systematic review was performed following PRISMA guidelines. The primary outcomes of interest were the presence of any metachronous polyps or adenomata, and the presence of high risk metachronous polyps or adenomata, at follow up. A secondary outcome of interest was the presence of metachronous colorectal cancers.

A search for other systematic reviews was performed first. One narrative review that addressed polyp characteristics, patient factors, chemopreventive medications, diet and gene polymorphism was identified [11]. No study was identified which addressed the wide range of novel methods of risk stratification as was intended to be explored in the current study.

Next a systematic literature review was performed of PubMed from inception until August 2020 inclusive, using the following MeSH terms: “colorectal”, “polyp”, “adenoma” “metachronous”, “recurrence”, “future risk”, “mutation”, “genetics”, “genome”, “mRNA”, “transcriptome”, “expression”, “immunohistochemistry”, “IHC” and “microbiome.” Observational studies, randomised control trials and systematic review and meta-analyses were included that used genomics, transcriptomics, immunohistochemistry or polyp microbiome as novel markers of metachronous colorectal polyp risk. Narrative reviews, animal studies, conference abstracts, non-English studies and those not addressing our primary outcomes of interest were excluded. Study titles were screened for relevance followed by a review of selected abstracts and full texts. Reference lists from identified studies were also searched for other eligible studies.
Results

A total of 4165 papers were identified using the systematic search protocol. Each title was reviewed followed by abstract review of 303 papers and full paper review of 215 (Figure 2). 25 papers met the inclusion criteria, with 19 pertaining to genomic markers (Table 1) and 6 pertaining to immunohistochemical markers (Table 2) of metachronous polyp risk. No papers were identified that used transcriptome or microbiome as novel markers of future polyp risk.

Genomic Markers of Metachronous Polyp Risk

19 papers were identified that addressed risk stratification for metachronous polyps using genetic markers including individual gene mutations, single nucleotide polymorphisms and haplotypes (Table 1).

Key Proto-Oncogenes and Tumour Suppressor Genes - KRAS, BRAF, APC, EGFR.

Juárez et al[48] investigated whether the KRAS or BRAF mutation status of index polypectomy specimens could determine the risk of developing metachronous advanced neoplasia at surveillance colonoscopy. In this retrospective study, 995 polyps from 308 patients were sequenced for KRAS mutations at exon 2 and for BRAF mutations at codon 600 (V600E) and divided into three groups: at least one KRAS mutated polyp (22.8%), at least one BRAF mutated polyp (14.9%) and wild type (62.3%). Patients with both KRAS and BRAF mutant status were excluded. On multivariate analysis KRAS mutation was associated with the development of metachronous advanced polyps (OR 2.27, 95% CI: 1.15-4.46, p=0.018) and more specifically with the development of metachronous advanced adenomas.
BRAF mutation status had no impact on the development of metachronous advanced polyps. In Nusko et al[49]’s study of 54 patients, KRAS mutant index adenoma status did not impact overall metachronous adenomas rate. However, having a KRAS mutated index adenoma (OR 4.00, 95% CI: 1.18-13.6, p=0.0265) or an index adenoma ≥20mm (p=0.0259) were predictors of developing metachronous adenomas >5mm. On multivariate logistic regression KRAS mutation did not retain significance as an independent predictor of metachronous adenoma >5mm (OR 3.92, 95% CI: 0.82-18.72, p=0.0871), while adenoma ≥20mm did (p=0.0084). Benamouzig et al[50] found no significant difference in metachronous adenoma rate between KRAS mutated and wild type polyp in their study of 104 adenomas (37.78% vs 33.33%, p>0.05).

Egan et al[51] investigated the impact of 5 SNPs in the APC tumour suppressor gene (rs2229992, rs42427, rs459552, rs465899 and rs2229995) in their study of 1399 patients. No individual SNP had a significant impact, however a haplotype consisting of all five SNPs (TGACC for rs222992, rs42427, rs459552, rs465899 and rs2229995 respectively) significantly reduced the metachronous adenoma rate as compared to the common haplotype (CAATC) (OR 0.73, 95% CI: 0.57-0.94). A truncated TA haplotype (TA rs2229992 and rs459552) was also associated with a reduced metachronous adenoma rate compared to the common CA haplotype (OR 0.73, 95% CI: 0.59–0.91). For the risk of metachronous advanced adenoma, the TGACC but not the truncated TA haplotype retained significance (OR 0.63, 95% CI: 0.42–0.94 and OR 0.76, 95% CI: 0.54-1.07).

Kraus et al[52] genotyped a number of target genes including EGFR in their study of 117 patients taken from a NSAID chemoprevention trial. Among patients on placebo they found
that rs7801956 SNP in the EGFR gene significantly increased the risk of metachronous adenoma (HR 2.65, 95% CI: 1.03-6.86, p=0.04).

**COX**

Cyclooxygenase 1 (COX1) and cyclooxygenase 2 (COX2) catalyse prostaglandin synthesis and play a key role in inflammation [53, 54]. COX2 activation stimulates cell proliferation and angiogenesis and inhibits apoptosis. COX2 expression is elevated in ~50% of colorectal adenomas and >85% of colorectal cancers and increased expression is associated with poorer survival in colorectal cancer [53]. Both COX1 and COX2 are inhibited by aspirin and NSAIDs, which have been associated with a reduced metachronous adenoma rate and reduced incidence of colorectal cancer[54]. Hubner et al[54] investigated the COX 1 rs3842787 SNP and the COX2 rs20417 SNP in 546 patients from the UK Colorectal Adenoma Prevention trial. In the parent study patients were randomised to aspirin, folate, both or placebo and scoped at 3 years. Neither SNP of interest had an impact on metachronous adenoma rate or influenced the observed benefit of taking aspirin. While in a similar study by Barry et al[53] the COX2 rs20417 SNP also had no impact on metachronous adenoma rate, CC genotype for rs5277 (RR 1.49, 95% CI: 1.00-2.23) and AG genotype for the rs4648310 (RR 1.35, 95% CI: 1.03-1.77) SNPs in the COX 2 gene significantly increased metachronous adenoma risk as compared to GG and AA wildtypes respectively. Kraus et al[52], mentioned previously, assessed SNPs in both COX1 and COX2. Examining only the patients on placebo in this NSAID chemoprevention trial, they found that the rs10306110, rs10306122, rs10306164, rs1236913, rs1330344 and rs3119773 SNPs in COX1 and rs4648268 and rs689469 SNPs in the COX2 gene significantly increased metachronous adenoma rate.
**Interleukins (IL-1β, IL-6, IL-8, IL-10, IL23R)**

Chronic inflammation, under the influence of both pro- and anti-inflammatory cytokines, is known to drive colorectal carcinogenesis [55]. Bobe et al [55] explored the impact of SNPs in the promoter regions of IL-1β, IL-6, IL-8 and IL-10 on the rate of metachronous adenoma. 808 patients were recruited from the intervention arm of the Polyp Prevention Trial, an RCT investigating a low-fat, high-fibre, -fruit and -vegetable diet. SNPs investigated were IL-1β (rs16944), IL-6 (rs1800795), IL-8 (rs4073), and IL-10 (rs1800872, rs1800871 and rs1800896). No SNP in isolation could predict metachronous adenoma risk. In Hubner et al.’s [54] study mentioned previously they examined the IL-10 rs1800872 SNP and found no impact on metachronous adenoma rate. Sansbury et al [56] conducted a study exploring a similar set of SNPs to Bobe et al (IL-1β rs16944, IL-6 rs1800795, IL-8 rs4073 and IL-10 rs1800896 and rs1800871) in 1723 patients. In agreement with Bobe et al, no individual SNP impacted metachronous adenoma rate. Kraus et al [52] mentioned previously, found two SNPs in the IL23R gene (rs10889675 and rs6683455) significantly increased the risk of developing metachronous adenoma (HR 3.08, 95% CI: 1.05-9.04, p=0.04 and HR 2.51, 95% CI: 1.06-5.96, p=0.04 respectively).

**Dopamine**

Murphy et al [57] noted that three SNPs in the dopamine D2 receptor (DRD2) have previously been associated with colorectal cancer risk: rs1799732, rs6277 and rs1800497. Additionally, they linked the rs1799732 CT genotype to increased metachronous adenoma rate (OR 1.30, 95% CI: 1.01-1.69) and the rs1800497 TT genotype to increased advanced adenoma rate (OR 2.4, 95% CI: 1.11-5.20).
Cytochrome P450

The Cytochrome P450 enzyme family catalyse the metabolism of a variety of endogenous and exogenous compounds. CYP2C9 is involved in the metabolism of 10-30% of commonly used medications and gene polymorphisms have been associated with colorectal adenoma and cancer risk and may modify the protective effects of aspirin and NSAIDs[58]. Barry et al[58] screened 928 patients participating in an aspirin and folate chemoprevention trial for the rs1799853 and rs1057910 SNPs in the CYP2C9 gene. While the rs1799853 SNP had no impact (RR 1.09, 95% CI: 0.91-1.31), the rs1057910 variant allele increased the risk of metachronous adenoma (RR 1.47, 95% CI 1.19-1.83). Likewise, the risk for metachronous advanced lesions or multiple (≥3) adenomas was not impacted by the rs1799853 SNP (RR 1.29, 95% CI: 0.89-1.85) but was increased by the rs1057910 SNP (RR=1.79, 95% CI=1.16–2.75). Presence of any variant CYP2C9, increased the risk of metachronous adenoma (RR 1.29, 95% CI: 1.09-1.51, p=0.002) and the risk of metachronous advanced lesions or multiple adenoma (RR 1.64, 95% CI: 1.18-2.28, p=0.003). Hubner et al[59] genotyped the same CYP2C9 SNPs in 546 patients, but found no significant interaction with metachronous adenoma rate.

Other cytochrome P450 genes investigated include CYP24A1 and CYP27B1, involved in the synthesis and metabolism of vitamin D. Vitamin D deficiency has been associated with colorectal adenoma and cancer[60]. Hibler et al[60] investigated 11 SNPs in CYP24A1 and 1 SNP in the CYP27B1 gene (Table 1) in 1,188 patients. A TT genotype in the rs927650 SNP of the CYP24A1 gene was associated with increased metachronous adenoma risk as compared to CC wildtype (OR 1.38, 95% CI: 1.01-1.89). No other SNP had a significant impact.
Finally, cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the conversion of cholesterol to bile acids. While bile acids such as chenodeoxycholic acid and deoxycholic acid have been implicated in colorectal carcinogenesis, ursodeoxycholic acid (UDCA) has been shown to be protective[61]. Wertheim et al[61] examined six SNPs in the CYP7A1 gene (Table 1) among 703 patients from a UDCA randomised chemoprevention trial. Of the six SNPs, the GG genotype for rs8192871 was the only to significantly impact metachronous adenoma risk (OR 0.41, 95% CI: 0.19-0.89 as compared to AA wildtype). Additionally they found the CCGTAG haplotype (rs10957057, rs8192879, rs8192877, rs11786580, rs8192871 and rs13251096) increased metachronous adenoma risk (OR 1.89, 95% CI: 1.00-3.57) as compared to the common CTACAG haplotype.

UGT1A6

UDP-glucuronyosyltransferase isoenzyme 1A6 (UGT1A6) is an enzyme involved in the metabolism of aspirin. Two SNPs in the UGT1A6 gene (rs2070959 and rs1105879) are known to be associated with lower enzyme activity and variant alleles may enhance the chemopreventive effects of aspirin [62]. Hubner et al[59] found the presence of either SNP significantly reduced the risk of metachronous colorectal neoplasia (RR 0.68, 95% CI: 0.52-0.89).

ODC

Polyamines are a group of cations implicated in carcinogenesis and ornithine decarboxylase (ODC) is the rate limiting enzyme in polyamine synthesis. The ODC gene is a target of the
MYC transcription factor which is overexpressed following loss of APC early in colorectal carcinogenesis. Aspirin is known to induce polyamine catabolism and may be a chemopreventive mechanism [63]. Hubner et al[63] genotyped 546 patients from the UKCAP aspirin and folic acid chemoprevention trial for the rs2302615 SNP in the ODC gene. Neither GA genotype (RR 0.92, 95% CI: 0.70-1.21) nor AA genotype (RR 0.43, 95% CI: 0.16-1.15) had an impact on metachronous adenoma risk as compared to GG wildtype. In a similar study by Martinez et al[64] involving 688 patients, GA genotype had no impact however AA genotype significantly reduced the risk of metachronous adenoma (OR 0.48, 95% CI: 0.24-0.99, p=0.05). Finally in the study by Barry et al[65] involving 973 patients, neither variant homozygotes (AA) nor heterozygotes (GA) had an altered risk of metachronous adenoma as compared to wild type (GG). Carrying at least one A allele was shown to significantly reduce the risk of metachronous adenoma in patients randomised to aspirin in this study.

MTHFR

Folate deficiency has been linked with colorectal neoplasia. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism and polymorphisms in the MTHFR gene have previously been associated with reduced colorectal cancer risk in folate deficient individuals[66]. Levine et al[66], investigated two SNPs in the MTHFR gene (rs1801133 and rs11801131) known to be associated with reduced enzyme activity. Neither SNP was associated with metachronous adenoma risk. Folate supplementation did not affect this outcome.
Kraus et al[52] screened a large numbers of genes for SNPs impacting metachronous adenoma risk (Table 1). The rs2073438 SNP in the ALOX12 gene, rs4796535 in ALOX15, rs45567139 in PGDH and rs6063022 in SRC all significantly increased the risk of metachronous adenoma. The rs2292350 SNP in the ALOX12 gene significantly decreased metachronous adenoma risk. These genes are involved in arachidonate and leukotriene synthesis, regulation of prostaglandin synthesis and EGFR signalling[52].

**Genome Wide Association Study (GWAS)**

One GWAS was identified which aimed to identify SNPs associated with metachronous advanced adenoma. Wang et al[67] created a discovery set of 1406 patients from the Adenoma Prevention with Celecoxib trial and a validation set of 4175 familial colorectal adenoma or colorectal cancer cases and 5036 controls from the CORGI, Scotland, VQ58 and Australia GWAS consortia. 19 SNPs with moderate to strong association with metachronous advanced adenoma risk were identified (OR≥2) (Table 1). In the validation phase, the rs1535989 SNP was additionally associated with CRC development (OR 1.12, 95% CI: 1.019-1.23, p=0.019).
Immunohistochemistry/ Protein Expression Markers of Metachronous Polyp Risk

6 studies were identified that used immunohistochemistry (IHC) to assess target protein expression as potential markers of metachronous polyp risk (Table 2).

**P53**

Mutations in the p53 tumour suppressor gene are a late event in colorectal carcinogenesis and generally result in overexpression of the gene product[68-70]. Brand et al[70] studied the expression of p53 of index polypectomy specimens from 109 patients from the German 5-ASA Polyp Prevention Study, a randomised, placebo-controlled mesalazine adenoma chemoprevention trial. They reported that nuclear expression of p53 was significantly associated with metachronous adenoma risk at 3 years (72.3% vs 20.5% of patients with and without metachronous adenoma had positive p53 nuclear staining, OR 10.15, p=0.001).

Sheikh et al[71] conducted a similar study only including patients with index adenomas with high-grade dysplasia. 83.3% of patients who developed metachronous adenoma showed p53 positivity in their index polyp as compared to 50.0% of patients with no metachronous adenoma (p=0.025). In contrast, studies by Vernillo et al[72] and Benamouzig et al[50] found no association between p53 expression and metachronous polyp risk.

**β-Catenin**

β-catenin is a key protein in the pro-proliferative Wnt signalling pathway[68]. Increased expression of β-catenin is present in the majority of colorectal adenomas and nearly all
colorectal cancers [70]. Brand et al[70] described above, found nuclear β-catenin expression to be associated with metachronous adenoma risk at 3 years (OR 3.49, p=0.002).

**COX2**

Benamouzig et al[50] assessed COX2 expression in 219 index adenomas from 136 patients participating in a double-blind aspirin chemoprevention RCT. While strong overall COX2 expression had no significant association with the risk of metachronous adenoma (42.0% vs 45.0% of patients with and without metachronous adenomas had strong overall COX2 expression, p>0.05), strong deep stromal COX2 expression was able to predict metachronous adenoma (42.0% vs 25.0% of patients with and without metachronous adenoma had strong deep stromal COX2 expression, p=0.04). On multivariate analysis deep stromal COX2 expression was an independent predictor of metachronous adenoma (OR 2.78, 95% CI: 1.18 to 6.25, p=0.02). Brand et al[70] described previously, also found COX2 positivity to be associated with metachronous adenoma risk at 3 years (OR 3.53, p=0.001).

**Ki67**

Ki67 is a nuclear protein only present in cycling cells and whose expression is used as a marker of cellular proliferation. Increased Ki67 expression has been associated with colorectal adenomas with high grade dysplasia[72]. Vernillo et al[72] found no association between Ki67 expression and risk of metachronous polyps in their study of 78 adenoma from 51 patients.
Adnab-9

Adnab-9 is a monoclonal antibody developed to react to an adenoma-associated antigen expressed early in the adenoma-carcinoma sequence. It stains at-risk, dysplastic, non-invasive colorectal epithelium but not invasive tumour tissue and has been shown to correlate with future cancer risk[71]. In Sheikh et al’s [71] study, as described previously, including patients with index adenomas with high grade dysplasia only, 76.4% of patients who developed metachronous adenoma stained positive for Adnab-9 at IHC as compared to 38.8% of patients without metachronous adenoma (p=0.024).

Cyclin D1

Cyclin D1 is a proto-oncogene with important roles in regulating cell cycle progression[73]. It is activated by β-catenin, a key component of the Wnt-signalling pathway known to be upregulated in colorectal carcinogenesis[50] and increased cyclin D1 expression has been associated with more advanced colorectal malignancies and reduced overall survival[74]. In the study by Benamouzig et al[50] they found 66.7% of patients with high cyclin D1 expression developed metachronous adenoma as compared to 50.8% of patients with low cyclin D1 expression but this did not reach statistical significance.

Annexin A10

Annexin A10 (ANXA10) is a calcium and phospholipid binding protein with roles in growth regulation, cell division, apoptosis and differentiation. High ANXA10 expression has been shown to occur more frequently in sessile serrated polyps as compared to hyperplastic polyps
and may differentiate these effectively. It is more highly expressed by serrated colonic carcinomas as opposed to conventional colon cancers[75]. Macaron et al[75] used IHC to assess the expression of ANXA10 in 179 patients with either a sessile serrated or hyperplastic polyp. Patients with high levels of ANXA10 expression within their index polyp had an increased risk of metachronous sessile serrated polyp at follow-up colonoscopy (HR 2.7, p=0.048), particularly in the proximal colon (HR 4.0, p=0.02). The rate of metachronous adenomatous polyp was similar between the groups (18.8% vs 19.4%, p=0.52).

**ALDH1A1**

A number of solid tumours have been shown to possess cells with stem cell-like properties including ability to self-renew and multipotency. These stem-like cells are believed to possess tumour initiation and maintenance capabilities and have been reported to be present in premalignant adenomas. Aldehyde dehydrogenase isoform 1A1 (ALDH1A1) is a well-recognised biomarker for the presence of stem-like cells[76]. Bartley et al[76] performed IHC using ALDH1A1 on index polyps taken from placebo-arm patients from two polyp prevention trials. 20 polyps from 20 patients were used to form an exploratory set and 89 polyps from 76 patients known to be high risk for metachronous adenomas acted as a validation set. In both sets, patients who developed metachronous adenoma had a significantly higher expression of ALDH1A1 compared to those without metachronous adenomas (mean ALDH1A1 labelling index 22.5% vs 15.0%, p=0.03 for the validation set).
Combination of IHC Markers.

As discussed previously, Brand et al[70] found the expression of β-catenin, COX2 and p53 to all individually be associated with the risk of metachronous adenomas. They therefore combined these three markers to explore whether collectively they represent a more powerful predictor. Of the 109 study participants, 26 (23.9%) patient’s adenomas were triple-negative, while 83 (76.1%) patient’s adenomas were positive for at least one marker. Only 3 of 26 (11.5%) triple-negative patients developed metachronous adenomas while 53 of 83 (63.8%) patients with at least one positive marker did. This translated into a negative predictive value of 88.5% and a sensitivity of 94.6% for freedom from metachronous adenoma and an OR 13.54 for metachronous adenoma.
Discussion

This study provides a comprehensive literature review for novel markers of metachronous polyp risk. 19 papers exploring 94 individual mutations, SNPs or haplotypes as predictors of future polyp risk in 33 different genes or non-coding chromosomal regions were identified. Six papers were found that attempted to predict metachronous polyp risk using IHC to measure the expression levels of eight different target proteins and one combination of target proteins. While the results are promising, no clear definitive marker of future polyp risk has been identified.

Genomic markers are the most studied. It is important to note that only three studies directly assessed the genomics of index polyp tissue [48-50], while most studies used the presence of germline SNPs, as determined from blood samples, to assess the influence of target genes on metachronous polyp risk. While several positive genetic markers were identified, most individual mutations or SNPs were not able to significantly predict future polyp risk. Additionally, it was not uncommon for one study to find a mutation or SNP to be an accurate predictor, while a second study has disputed this positive finding.

Perhaps one of the most intriguing papers is that by Juárez et al[48] which examined the KRAS and BRAF mutation status of index polyps. KRAS and BRAF are key proto-oncogenes, frequently activated in colorectal carcinogenesis. While BRAF had no impact, KRAS mutant status was significantly associated with the development of metachronous advanced polyps and advanced adenomas. Of note patients with index polyps with both KRAS and BRAF mutations were excluded and it is not clear how this may influence risk. KRAS mutant status is already routinely tested in patients with metastatic colorectal cancer
being considered for treatment with the anti-EGFR monoclonal antibody, cetuximab. This testing may feasibly be applied to polypectomy specimens to refine risk stratification for colonoscopic surveillance. It should be noted that two smaller studies did not find KRAS mutant status to correlate with metachronous adenoma risk [49, 50]. Other positive genomic markers of note included the TGACC (rs222992, rs42427, rs459552, rs465899 and rs2229995) and TA (rs2229992 and rs459552) haplotypes in the APC gene which significantly reduced metachronous adenoma rate [51], as well as numerous SNPs in the COX1 and COX2 genes which increased this risk (Table 1) [52, 53].

A number of protein expression level markers of metachronous polyp risk have also been identified including the key tumour suppressor gene p53 [70, 71], β-catenin [70], Adnab-9 [71] and ALDH1A1 [76]. The important role of COX2 has been reconfirmed at the protein expression level [50, 70] and ANXA10 has been identified as a marker specific to metachronous sessile serrated polyp risk [75]. The study by Brand et al[70] is perhaps the most interesting IHC-based paper in that it combined the expression of β-catenin, COX2 and p53 into a single powerful predictor. Only 11.5% of patients who were triple-negative for these markers developed metachronous adenomas compared to 63.8% of patients positive for at least one. It seems likely that a wide panel of markers may have to be combined in this manner in order to accurately predict metachronous polyp risk.

No studies were identified which examined the microbiome in the context of metachronous polyp risk. There is a rapidly expanding literature relating the colonic microbiome to polyps and colorectal cancer in the contexts of either detection and diagnosis[77], or in carcinogenesis pathways[78]. Indeed, there are studies which link specific species of bacteria such as Fusobacterium nucleatum to serrated and traditional adenomas, increasing dysplasia.
and early cancer[79]. Furthermore, it may be that different types of polyp are associated with
different microbiomic landscapes[80]. Although there remains debate as to whether such
dysbiosis is causal, or is an epiphenomenon of colonic tumorigenesis, there is evidence that
altering the microbiome can have effects on established colorectal cancer in animal
models[81]. Therefore, further of investigation of the role of the microbiome in metachronous
polyp risk would seem important.

Likewise, no papers were identified that used transcriptomics to predict future polyp risk.
Studies have however compared polyp subtypes and advancement in the context of their
transcriptome. Druliner et al[82] used RNA sequencing to compare polyps with or without an
adjacent synchronous cancer (cancer adjacent polyp (CAP) and cancer free polyp (CFP)
respectively). CAPs showed significantly higher levels of CXCL5, GREM1, IGF2, CTGF
and PLAU expression, as compared to CFPs. Several of these genes are known to play a role
in colorectal carcinogenesis. Chang et al[83] performed RNA sequencing on 301 adenomas
and 88 serrated polyps to establish whether the colorectal cancer CMS classification could be
applied to premalignant polyps. They found that adenomas predominantly displayed a CMS2-
like phenotype with WNT and MYC activation, while hyperplastic and serrated polyps most
commonly displayed CMS1-like phenotype with strong immune activation. Both of these
studies give us important insights into the transcriptomic landscapes of premalignant
colorectal polyps, however the application of transcriptomics to assess future polyp risk
represents a significant gap in the literature.

While no other systematic review was identified which addressed the wide range of novel
methods of risk stratification as was explored in the current study, a narrative review by Hao
et al did examine gene polymorphism studies as well as conventional influencers of metachronous polyp risk such as polyp characteristics, patient factors, chemopreventive medications and diet [11]. In agreement with the present study they found the rs5277 CC and rs4648310 AG genotypes in the COX2 gene, the rs927650 TT or TC genotype in the CYP24A1 gene and the presence of any variant allele (rs1057910 or rs1799853) in the CYP2C9 gene, to increase the risk of metachronous adenoma compared to wildtype. Additionally, they found the rs1799732 CT or rs1800497 TT genotypes in the DRD2 gene increased the risk of metachronous advanced adenoma. They concluded that most individual gene polymorphisms did not alter metachronous polyp risk independently but may alter this risk when combined with an intervention; dietary changes being the focus of that review.

The main limitation of the current systematic review is the generally small sample size of each identified study, with 20 of 25 included studies involving less than 1000 patients and all six IHC-based studies involving less than 200 patients. Additionally, 19 of the 25 studies were published prior to 2010. It is possible to argue that the results may not be entirely applicable to current practice given advancements in colonoscopic technology, the introduction of bowel cancer screening programmes, altered surveillance guidelines and the more recent recognition of sessile serrated polyps as a distinct malignant precursor. Indeed, the vast majority of included papers focussed on metachronous adenoma rate only. Additionally, there was heterogeneity in length of follow up and most of the patients in the genomic studies were recruited from RCTs examining a chemopreventive medication or dietary change which may limit generalisability.

The strengths of the current study include the systematic nature of the literature review with, with two authors participating in the title, abstract and full paper appraisal. Additionally, this
is the first paper to systematically review for studies that have used genomics, transcriptomics, immunohistochemistry and microbiome as novel techniques of metachronous polyp risk stratification. It has identified a gap in the literature in terms of a definitive multi-modal novel metachronous polyp risk prediction tool.

**Conclusion**

A variety of genomic and immunohistochemical markers which significantly correlated with metachronous polyp risk have been identified within the literature. It seems likely that future research will have to amalgamate a panel of novel markers in order to develop a score able to accurately determine future polyp risk. The INCISE project is a large, retrospective, multi-partner collaborative project which aims to use patient characteristics, digital pathology, immunohistochemistry, genomic and transcriptomic features, merged using machine learning, to predict future polyp risk[84]. It is hoped that the technology used in this study could be applied in clinical practice to markedly refine surveillance protocols. This may reduce the number of unnecessary, invasive colonoscopies performed, reduce the burden on stretched endoscopy services and simultaneously increase the detection yield for high risk metachronous polyps.
References

22. Di Rollo, D.G., An investigation into the role of adenoma and host-specific factors on the incidence and recurrence of colorectal neoplasia, in Academic Unit of Colorectal Surgery, Glasgow Royal Infirmary, College of Medical, Veterinary and Life Sciences. 2020, University of Glasgow.


84. *Integrated Technologies for Improved Polyp Surveillance - About INCISE.* [cited 15/02/2021]; Available from: [https://www.gla.ac.uk/research/az/incise/aboutincise/](https://www.gla.ac.uk/research/az/incise/aboutincise/).
Patients with a higher risk of metachronous polyps or future colorectal cancer following polypectomy are those with either:

- Two or more premalignant polyps including at least one advanced polyp, defined as:
  - Serrated polyp ≥10mm or containing any grade of dysplasia.
  - Adenoma ≥10mm or containing high-grade dysplasia.

- Five or more premalignant polyps.

Patients who meet these criteria at index colonoscopy and are aged <75 years should have a single surveillance colonoscopy at 3 years.

Patients with a life expectancy <10 years or in general those aged >75 years should not have surveillance colonoscopy due to:

- Low chance of developing a metachronous polyp which progresses to a symptomatic cancer in their lifetime.
- Increased risk of colonoscopy-related complications.

Patients without these high-risk features should be discharged to the bowel cancer screening programme.
Figure 2. PRISMA Flow Chart

Studies identified by search strategy (n=4154)

Additional records identified through other sources (reference lists) (n=11)

Total (n=4165)

Duplicates (n=39)

Excluded (n=190)
  - Review article of conventional risk factors and SNPs (n=1)
  - Assessment of a genomic marker’s influence on a chemopreventative medication only (n=3)
  - Comparison of a genomic and an IHC marker’s influence on polyp morphology, size and grade of dysplasia without assessment of metachronous polyp risk (n=1)
  - Assessment of a IHC marker’s influence on future cancer risk only (n=1)
  - Comparison of IHC marker between normal tissue, polyps and cancer without assessment of metachronous polyp risk (n=3)
  - Assessment of a microbiome marker’s influence on a chemopreventative medication only (n=1)
  - Comparison of a microbiome marker between normal tissue, polyps and cancer without assessment of metachronous polyp risk (n=8)
  - Assessment of a microbiome marker’s influence on future cancer risk only (n=1)
  - Only included metachronous polyps located in same region of colorectum as index polyp (n=1)
  - Non-English (n=2)
  - Irrelevant (n=167)

Studies for title review (n=4126)

Studies for abstract review (n=303)

Studies for full paper review (n=215)

Studies included in qualitative synthesis (n=25)

Studies pertaining to genomic markers of metachronous polyp risk (n=19)

Studies pertaining to IHC markers of metachronous polyp risk (n=6)
Table 1. Genomic markers of metachronous polyp risk.

<table>
<thead>
<tr>
<th>Gene/Chromosome Region</th>
<th>Mutation or SNP</th>
<th>Paper</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KRAS</strong> Mutations in Exon 2 (codon 12 and 13)</td>
<td>rs2229992 (C486T)</td>
<td>Egan</td>
<td>Increased risk of metachronous advanced polyps (OR 2.27, 95% CI: 1.15-4.46, p=0.018). Increased risk of metachronous advanced adenoma (OR 2.23, 95% CI: 1.02-4.85, p=0.044).</td>
</tr>
<tr>
<td></td>
<td>rs42427 (A1678G)</td>
<td>Egan</td>
<td>Neither CT genotype (OR 1.12, 95% CI: 0.87-1.45) nor TT genotype (OR 0.72, 95% CI: 0.51-1.02) had an impact on metachronous adenoma risk as compared to CC wildtype.</td>
</tr>
<tr>
<td></td>
<td>rs459552 (A1822T)</td>
<td>Egan</td>
<td>Neither AT genotype (OR 1.16, 95% CI: 0.91-1.48) nor TT genotype (OR 1.21, 95% CI: 0.74-1.99) had an impact on metachronous adenoma risk as compared to AA wildtype.</td>
</tr>
<tr>
<td></td>
<td>rs465899 (T1960C)</td>
<td>Egan</td>
<td>Neither TC genotype (OR 1.07, 95% CI: 0.83-1.38) nor CC genotype (OR 0.78, 95% CI: 0.55-1.12) had an impact on metachronous adenoma risk as compared to TT wildtype.</td>
</tr>
<tr>
<td></td>
<td>rs2229995 (C2502T)</td>
<td>Egan</td>
<td>CT genotype (OR 1.13, 95% CI: 0.62-2.06) had no impact on metachronous adenoma risk as compared to CC wildtype. 0 patients in the study had the TT genotype to allow for comparison.</td>
</tr>
<tr>
<td><strong>TGACC Haplotype (a)</strong></td>
<td></td>
<td>Egan</td>
<td>TGACC haplotype significantly reduced risk of metachronous adenoma (OR 0.73, 95% CI: 0.57-0.94). TGACC haplotype significantly reduced risk of metachronous advanced adenoma (OR 0.63, 95% CI: 0.42-0.94).</td>
</tr>
<tr>
<td><strong>TA Haplotype (b)</strong></td>
<td></td>
<td>Egan</td>
<td>TA haplotype significantly reduced risk of metachronous adenoma (OR 0.73, 95% CI: 0.59-0.91). No impact on risk of metachronous advanced adenoma (OR 0.76, 95% CI: 0.54-1.07).</td>
</tr>
<tr>
<td><strong>APC</strong> rs7801986 (G&gt;A, intron 4)</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 2.65, 95% CI: 1.03-6.86, p=0.04).</td>
<td></td>
</tr>
<tr>
<td><strong>EGFR</strong> rs3842787 (C507T)</td>
<td>Hubner</td>
<td>Neither CT genotype (OR 1.01, 95% CI: 0.66-1.54) nor TT genotype (OR 0.91, 95% CI: 0.14-6.07) had an impact on metachronous neoplasia risk as compared to CC wildtype.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs10306110 (A&gt;G, near gene-5')</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 15.46, 95% CI: 1.58-151.67, p=0.02).</td>
</tr>
<tr>
<td></td>
<td>rs10306122 (T&gt;C, intron)</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 15.58, 95% CI: 1.59-152.67, p=0.02).</td>
</tr>
<tr>
<td></td>
<td>rs10306164 (G&gt;T, intron)</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 2.53, 95% CI: 1.02-6.29, p=0.05).</td>
</tr>
<tr>
<td></td>
<td>rs1236913 (C&gt;T, W8R)</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 3.78, 95% CI: 1.32-10.80, p=0.01).</td>
</tr>
<tr>
<td></td>
<td>rs1330344 (G&gt;A, near gene-5')</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 2.75, 95% CI: 1.12-6.75, p=0.03).</td>
</tr>
<tr>
<td></td>
<td>rs3119773 (T&gt;G, T&gt;C)</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 4.58, 95% CI: 1.69-12.44, p=0.01).</td>
</tr>
<tr>
<td><strong>COX1</strong> rs20417 (G765C)</td>
<td>Hubner</td>
<td>Neither GC genotype (OR 0.96, 95% CI: 0.70-1.30) nor CC genotype (OR 1.32, 95% CI: 0.66-2.62) had an impact on metachronous neoplasia risk as compared to GG wildtype.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs2745557 (C&gt;T, intron 1)</td>
<td>Barry</td>
<td>Neither CT genotype (RR 1.11, 95% CI: 0.94-1.31) nor CC genotype (RR 0.97, 95% CI: 0.64-1.46) had an impact on metachronous neoplasia risk as compared to GG wildtype.</td>
</tr>
<tr>
<td></td>
<td>rs5277 (G&gt;C, exon 3)</td>
<td>Barry</td>
<td>While GC genotype had no impact on metachronous adenoma risk (OR 1.05, 95% CI: 0.89-1.24), CC genotype significantly increased metachronous adenoma risk (RR 1.49, 95% CI: 1.00-2.23) as compared to GG wildtype.</td>
</tr>
</tbody>
</table>
rs20432 (T>G, intron 5) | Barry | Neither TG genotype (RR 1.09, 95% CI: 0.93-1.29) nor GG genotype (RR 1.21, 95% CI: 0.85-1.72) had an impact on metachronous adenoma risk as compared to TT wildtype.

rs468310 (A>G, near gene-3') | Barry | AG genotype increased risk of metachronous adenoma (RR 1.35, 95% CI: 1.03-1.77) as compared to AA wildtype.

rs468268 (G>A, intron) | Kraus | Increased risk of metachronous adenoma (HR 3.73, 95% CI: 1.26-11.11, p=0.02).

rs5275 (T>C, exon 10-3' UTR) | Barry | Neither TC genotype (RR 0.96, 95% CI: 0.82-1.12) nor CC genotype (RR 1.04, 95% CI: 0.84-1.29) had an impact on metachronous neoplasia risk as compared to TT wildtype.

Kraus | No significant impact on metachronous adenoma risk (HR 3.09, 95% CI: 0.88-10.82, p=0.08).

rs689469 (G>A, 3' UTR) | Kraus | Increased risk of metachronous adenoma (HR 2.65, 95% CI: 1.03-6.86, p=0.04).

IL-1β | rs16944 (C511T) | Bobe | Neither CT genotype (OR 0.90, 95% CI: 0.66-1.24) nor TT genotype (OR 0.83, 95% CI: 0.49-1.41) had an impact on metachronous adenoma risk as compared to CC wildtype.

Sanisbury | Neither CT genotype (OR 0.92, 95% CI: 0.74-1.15) nor TT genotype (OR 0.91, 95% CI: 0.64-1.29) had an impact on metachronous adenoma risk as compared to CC wildtype.

IL-6 | rs1800795 (G174C) | Bobe | Neither GC genotype (OR 1.25, 95% CI: 0.89-1.75) nor CC genotype (OR 1.19, 95% CI: 0.74-1.91) had an impact on metachronous adenoma risk as compared to GG wildtype.

Sanisbury | Neither GC genotype (OR 1.25, 95% CI: 0.99-1.57) nor CC genotype (OR 0.85, 95% CI: 0.61-1.19) had an impact on metachronous adenoma risk as compared to GG wildtype.

IL-8 | rs4073 (T231A) | Bobe | Neither AT genotype (OR 1.16, 95% CI: 0.80-1.68) nor AA genotype (OR 1.03, 95% CI: 0.66-1.60) had an impact on metachronous adenoma risk as compared to TT wildtype.

Sanisbury | Neither AT genotype (OR 1.18, 95% CI: 0.92-1.52) nor AA genotype (OR 1.05, 95% CI: 0.77-1.42) had an impact on metachronous adenoma risk as compared to TT wildtype.

IL-10 | rs1800872 (C592A) | Hubner | Neither CA genotype (OR 1.11, 95% CI: 0.83-1.47) nor AA genotype (OR 1.24, 95% CI: 0.74-2.07) had an impact on metachronous neoplasia risk as compared to CC wildtype.

Bobe | Neither CA genotype (OR 0.92, 95% CI: 0.67-1.26) nor AA genotype (OR 1.45, 95% CI: 0.83-2.53) had an impact on metachronous adenoma risk as compared to CC wildtype.

rs1800896 (G1082A) | Bobe | Neither AG genotype (OR 0.93, 95% CI: 0.66-1.31) nor GG genotype (OR 1.06, 95% CI: 0.68-1.63) had an impact on metachronous adenoma risk as compared to AA wildtype.

Sanisbury | Neither AG genotype (OR 0.98, 95% CI: 0.75-1.27) nor GG genotype (OR 1.01, 95% CI: 0.75-1.36) had an impact on metachronous adenoma risk as compared to AA wildtype.

rs1800871 (C819T) | Bobe | Neither CT genotype (OR 0.90, 95% CI: 0.66-1.23) nor TT genotype (OR 1.35, 95% CI: 0.79-2.33) had an impact on metachronous adenoma risk as compared to CC wildtype.

Sanisbury | Neither CT genotype (OR 1.05, 95% CI: 0.85-1.31) nor TT genotype (OR 1.13, 95% CI: 0.72-1.76) had an impact on metachronous adenoma risk as compared to CC wildtype.

IL23R | rs10898675 (C>A, intron) | Kraus | Increased risk of metachronous adenoma (HR 3.08, 95% CI: 1.05-9.04, p=0.04).

rs6683455 (T>C, near gene-5') | Kraus | Increased risk of metachronous adenoma (HR 2.51, 95% CI: 1.06-5.96, p=0.04).

rs7518660 (G>A, intron) | Kraus | No significant impact on metachronous adenoma risk (HR 1.34, 95% CI: 0.54-3.32, p=0.53).

DRD2 | rs1799732 (141 C>del) | Murphy | While TT genotype had no impact on metachronous adenoma risk (OR 1.25, 95% CI: 0.57-2.75), CT genotype significantly increased metachronous adenoma risk (OR 1.30, 95% CI: 1.01-1.69) as compared to CC wildtype.

rs6277 (C957T) | Murphy | Neither CT genotype (OR 1.00, 95% CI: 0.77-1.29) nor TT genotype (OR 0.94, 95% CI: 0.69-1.26) had an impact on metachronous adenoma risk as compared to CC wildtype.

rs1800497 (A>G, TaqIA) | Murphy | Neither CT genotype (OR 1.00, 95% CI: 0.80-1.26) nor TT genotype (OR 0.98, 95% CI: 0.59-1.65) had an impact on metachronous adenoma risk as compared to CC wildtype.

While CT genotype had no impact on metachronous advanced adenoma risk (OR 1.09, 95% CI: 0.69-1.72), TT genotype significantly increased metachronous advanced adenoma risk (OR 2.40, 95% CI: 1.11-5.20) as compared to CC wildtype.

CYP2C9 | rs1799853 (C430T, CYP2C9*2) | Barry2 | No significant impact on metachronous adenoma risk (RR 1.09, 95% CI: 0.91-1.31, p=0.33).
<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP/Allele Details</th>
<th>Study</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1057910 (A1075C, CYP2C9*3)</td>
<td>Barry2</td>
<td>Increased risk of metachronous adenoma (RR 1.47, 95% CI: 1.19-1.83, p=0.001). Increased risk of metachronous advanced lesions or multiple adenoma (RR 1.79, 95% CI: 1.16-2.75, p=0.008).</td>
<td></td>
</tr>
<tr>
<td>rs1799853 (C430T, CYP2C9<em>2) or rs1057910 (A1075C, CYP2C9</em>3)</td>
<td>Hubner2</td>
<td>Presence of any variant CYP2C9, had no impact on risk of metachronous colorectal neoplasia (RR 1.09, 95% CI: 0.82-1.44) as compared to patients with homozygous wild-type genotype.</td>
<td></td>
</tr>
<tr>
<td>rs6013905 (T&gt;C)</td>
<td>Hibler</td>
<td>Neither TC genotype (OR 0.97, 95% CI: 0.76-1.25) nor CC genotype (OR 1.01, 95% CI: 0.49-2.07) had an impact on metachronous adenoma risk as compared to TT wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs2585428 (G&gt;A)</td>
<td>Hibler</td>
<td>Neither AG genotype (OR 0.85, 95% CI: 0.66-1.10) nor AA genotype (OR 0.86, 95% CI: 0.63-1.18) had an impact on metachronous adenoma risk as compared to GG wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs2296241 (A&gt;G)</td>
<td>Hibler</td>
<td>Neither AG genotype (OR 1.01, 95% CI: 0.77-1.32) nor GG genotype (OR 1.22, 95% CI: 0.90-1.66) had an impact on metachronous adenoma risk as compared to AA wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs2762939 (G&gt;C)</td>
<td>Hibler</td>
<td>Neither CC genotype (OR 1.11, 95% CI: 0.88-1.40) nor CC genotype (OR 0.59, 95% CI: 0.36-0.97) had an impact on metachronous adenoma risk as compared to GG wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs3051736 (G&gt;A)</td>
<td>Hibler</td>
<td>GA genotype had no impact on risk of metachronous adenoma (OR 1.98, 95% CI: 0.70-5.62) as compared to GG wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs6022999 (A&gt;G)</td>
<td>Hibler</td>
<td>Neither AG genotype (OR 0.98, 95% CI: 0.77-1.25) nor GG genotype (OR 0.70, 95% CI: 0.43-1.13) had an impact on metachronous adenoma risk as compared to AA wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs4809958 (T&gt;G)</td>
<td>Hibler</td>
<td>Neither GT genotype (OR 0.95, 95% CI: 0.74-1.21) nor GG genotype (OR 0.98, 95% CI: 0.49-1.96) had an impact on metachronous adenoma risk as compared to TT wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs276942 (A&gt;G)</td>
<td>Hibler</td>
<td>Neither AG genotype (OR 1.19, 95% CI: 0.83-1.71) nor GG genotype (OR 0.20, 95% CI: 0.02-1.66) had an impact on metachronous adenoma risk as compared to AA wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs927650 (C&gt;T)</td>
<td>Hibler</td>
<td>While TC genotype had no impact on metachronous adenoma risk (OR 1.30, 95% CI: 0.99-1.70), TT genotype significantly increased metachronous adenoma risk (RR 1.38, 95% CI: 1.01-1.89) as compared to CC wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs6013897 (T&gt;A)</td>
<td>Hibler</td>
<td>Neither AT genotype (OR 0.90, 95% CI: 0.66-1.22) nor AA genotype (OR 0.85, 95% CI: 0.51-1.39) had an impact on metachronous adenoma risk as compared to TT wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs4809960 (T&gt;C)</td>
<td>Hibler</td>
<td>Neither TC genotype (OR 1.04, 95% CI: 0.82-1.32) nor CC genotype (OR 1.05, 95% CI: 0.67-1.66) had an impact on metachronous adenoma risk as compared to TT wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs4646536 (T&gt;C)</td>
<td>Hibler</td>
<td>Neither CT genotype (OR 0.89, 95% CI: 0.70-1.13) nor CC genotype (OR 1.04, 95% CI: 0.71-1.53) had an impact on metachronous adenoma risk as compared to TT wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs10957057 (C&gt;T)</td>
<td>Wertheim</td>
<td>Neither CT genotype (OR 0.89, 95% CI: 0.52-1.50) nor TT genotype (OR 0.52, 95% CI: 0.10-2.81) had an impact on metachronous adenoma risk as compared to CC wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs8192879 (C&gt;T)</td>
<td>Wertheim</td>
<td>Neither CT genotype (OR 1.47, 95% CI: 0.90-2.40) nor TT genotype (OR 1.47, 95% CI: 0.76-2.84) had an impact on metachronous adenoma risk as compared to CC wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs8192877 (A&gt;G)</td>
<td>Wertheim</td>
<td>Neither AG genotype (OR 1.47, 95% CI: 0.88-2.43) nor GG genotype (OR 1.03, 95% CI: 0.22-4.86) had an impact on metachronous adenoma risk as compared to AA wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs11786580 (C&gt;T)</td>
<td>Wertheim</td>
<td>Neither CT genotype (OR 1.13, 95% CI: 0.71-1.82) nor TT genotype (OR 1.19, 95% CI: 0.44-3.20) had an impact on metachronous adenoma risk as compared to CC wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs8192871 (A&gt;G)</td>
<td>Wertheim</td>
<td>While AG genotype had no impact on metachronous adenoma risk (OR 0.78, 95% CI: 0.49-1.26), GG genotype significantly reduced metachronous adenoma risk (RR 0.41, 95% CI: 0.19-0.89) as compared to AA wildtype.</td>
<td></td>
</tr>
<tr>
<td>rs13251096 (G&gt;A)</td>
<td>Wertheim</td>
<td>Neither GA genotype (OR 0.70, 95% CI: 0.43-1.13) nor AA genotype (OR 0.54, 95% CI: 0.28-1.05) had an impact on metachronous adenoma risk as compared to GG wildtype.</td>
<td></td>
</tr>
<tr>
<td>CCGTAG Haplotype (c)</td>
<td>Wertheim</td>
<td>CCGTAG haplotype significantly increased risk of metachronous adenoma (OR 1.89, 95% CI: 1.00-3.57) as compared to common CTACAG haplotype.</td>
<td></td>
</tr>
<tr>
<td>UGT1A6</td>
<td>Hubner2</td>
<td>Presence of any variant UGT1A6 allele, had a significantly reduced risk of metachronous colorectal neoplasia (RR 0.68, 95% CI: 0.52-0.89) as compared to patients with homozygous wild-type genotype.</td>
<td></td>
</tr>
<tr>
<td>ODC</td>
<td>Hubner3</td>
<td>Neither GA genotype (RR 0.92, 95% CI: 0.70-1.21) nor AA genotype (RR 0.43, 95% CI: 0.16-1.15) had an impact on metachronous adenoma risk as compared to GG wildtype.</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>rsID</td>
<td>authors</td>
<td>effect</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>---------</td>
<td>--------</td>
</tr>
<tr>
<td>GPX1</td>
<td>rs1050450</td>
<td>Kraus</td>
<td>No significant impact on metachronous adenoma risk (HR 0.46, 95% CI: 0.19-0.72, p=0.04).</td>
</tr>
<tr>
<td>IGSF5</td>
<td>rs2837156</td>
<td>Wang</td>
<td>Increased risk of advanced metachronous adenoma (OR 2.38, 95% CI: 1.00-5.68, p=0.05).</td>
</tr>
<tr>
<td>MTHFR</td>
<td>rs1801133 (C677T)</td>
<td>Levine</td>
<td>Neither GA genotype (RR 0.39, 95% CI: 0.19-0.81) nor AA genotype (RR 0.37, 95% CI: 0.15-0.89) had an impact on metachronous adenoma risk as compared to GG wildtype.</td>
</tr>
<tr>
<td>ALOX12</td>
<td>rs11078659 (G&gt;A, intron)</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 2.33, 95% CI: 0.70-7.81, p=0.17).</td>
</tr>
<tr>
<td>PGDH</td>
<td>rs7349744 (G&gt;A, intron)</td>
<td>Kraus</td>
<td>No significant impact on metachronous adenoma risk (HR 0.19, 95% CI: 0.08-0.41, p=0.05).</td>
</tr>
<tr>
<td>ALOX15</td>
<td>rs4796535 (A&gt;G, intron)</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 3.56, 95% CI: 1.39-9.56, p&lt;0.01).</td>
</tr>
<tr>
<td>SRC</td>
<td>rs46063022 (C&gt;T, near gene-5')</td>
<td>Kraus</td>
<td>Increased risk of metachronous adenoma (HR 3.38, 95% CI: 1.35-8.50, p&lt;0.01).</td>
</tr>
<tr>
<td>3p24.1</td>
<td>rs1381392</td>
<td>Wang</td>
<td>Increased risk of advanced metachronous adenoma (OR 2.01, 95% CI: 1.52-2.65, p=7.4x10^-3).</td>
</tr>
<tr>
<td>KCNS3</td>
<td>rs11886781</td>
<td>Wang</td>
<td>Increased risk of advanced metachronous adenoma (OR 2.16, 95% CI: 1.61-2.91, p=2.1x10^-3).</td>
</tr>
<tr>
<td>EPB1, KY</td>
<td>rs13085889</td>
<td>Wang</td>
<td>Increased risk of advanced metachronous adenoma (OR 1.77, 95% CI: 1.37-2.29, p=8.8x10^-3).</td>
</tr>
<tr>
<td>PLXNA4</td>
<td>rs1424593</td>
<td>Wang</td>
<td>Decreased risk of advanced metachronous adenoma (OR 0.56, 95% CI: 0.44-0.73, p=9.1x10^-2).</td>
</tr>
<tr>
<td>9q33.2</td>
<td>rs16909065</td>
<td>Wang</td>
<td>Increased risk of advanced metachronous adenoma (OR 2.59, 95% CI: 1.71-3.96, p=3.6x10^-2).</td>
</tr>
<tr>
<td>13q33.2</td>
<td>rs1535989</td>
<td>Wang</td>
<td>Increased risk of advanced metachronous adenoma (OR 2.59, 95% CI: 1.71-3.96, p=3.7x10^-2).</td>
</tr>
<tr>
<td>FAM188b</td>
<td>rs17781398</td>
<td>Wang</td>
<td>Decreased risk of advanced metachronous adenoma (OR 0.19, 95% CI: 0.08-0.43, p=9.6x10^-1).</td>
</tr>
</tbody>
</table>

**Note:**
a - TGLAC (rs2229992, rs4242427, rs459552, rs4655099, rs2229995); b - TA (rs2229992, rs495552); c - CCCTAG (rs10957057, rs1892879, rs1892877, rs11786590, rs1892871, rs13251096).
<table>
<thead>
<tr>
<th>Protein</th>
<th>Paper</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p53</strong></td>
<td>Brand</td>
<td>Nuclear p53 expression was associated with a significantly increased risk of metachronous adenoma (72.3% of patients who developed metachronous adenoma had positive p53 nuclear staining vs 20.5% of patients without metachronous adenoma, OR 10.15, p=0.001).</td>
</tr>
<tr>
<td></td>
<td>Sheikh</td>
<td>p53 expression was associated with a significantly increased risk of metachronous adenoma (83.3% of patients who developed metachronous adenoma stained positive for p53 vs 50.0% of those without metachronous adenoma, p=0.025).</td>
</tr>
<tr>
<td></td>
<td>Vernillo</td>
<td>No association between p53 expression and metachronous adenoma risk (p&gt;0.05).</td>
</tr>
<tr>
<td></td>
<td>Benamouzig</td>
<td>No association between p53 expression and metachronous adenoma risk (62.0% of patients who developed metachronous adenoma stained positive for p53 vs 51.0% of those without metachronous adenoma, p&gt;0.05).</td>
</tr>
<tr>
<td><strong>β-catenin</strong></td>
<td>Brand</td>
<td>Nuclear β-catenin expression was associated with significantly increased risk of metachronous adenoma (OR 3.49, p=0.002).</td>
</tr>
<tr>
<td><strong>COX2</strong></td>
<td>Brand</td>
<td>COX2 expression was associated with significantly increased risk of metachronous adenoma (OR 3.53, p=0.001).</td>
</tr>
<tr>
<td></td>
<td>Benamouzig</td>
<td>Deep stromal COX2 expression was associated with a significantly increased risk of metachronous adenoma (OR 2.78, 95% CI: 1.18 to 6.25, p=0.02).</td>
</tr>
<tr>
<td><strong>Ki-67</strong></td>
<td>Vernillo</td>
<td>No association between Ki-67 expression and metachronous adenoma risk (p&gt;0.05).</td>
</tr>
<tr>
<td><strong>Adnab-9</strong></td>
<td>Sheikh</td>
<td>Adnab-9 expression was associated with a significantly increased risk of metachronous adenoma (76.4% of patients who developed metachronous adenoma stained positive for Adnab-9 vs 38.8% of those without metachronous adenoma, p=0.024).</td>
</tr>
<tr>
<td><strong>Cyclin D1</strong></td>
<td>Benamouzig</td>
<td>No association between cyclin D1 expression and metachronous adenoma risk (67.0% of patients who developed metachronous adenoma stained positive for cyclin D1 vs 50.0% of those without metachronous adenoma p&gt;0.05).</td>
</tr>
<tr>
<td><strong>Annexin A10</strong></td>
<td>Macaron</td>
<td>ANXA10 expression was associated with a significantly increased risk of metachronous sessile serrated polyps (HR 2.7, p=0.048). There was no association between ANXA10 expression and metachronous adenomas (p=0.52).</td>
</tr>
<tr>
<td><strong>Aldehyde Dehydrogenase Isoform 1A1 (ALDH1A1)</strong></td>
<td>Bartley</td>
<td>ALDH1A1 expression was associated with a significantly increased risk of metachronous adenomas (mean ALDH1A1 labelling index 22.5% for patients who developed metachronous adenoma vs 15.0% for those without metachronous adenoma, p=0.03).</td>
</tr>
<tr>
<td><strong>Combination of β-catenin, COX2 and p53.</strong></td>
<td>Brand</td>
<td>Positivity for ≥1 of these markers was associated with a significantly increased risk of metachronous adenoma as compared to triple negativity (OR 13.54, p&lt;0.001)</td>
</tr>
</tbody>
</table>
Authors

Mr Mark Johnstone is general surgical registrar currently working as a clinical research fellow at the Glasgow Royal Infirmary Academic Unit of Surgery. He undertook his undergraduate training at the University of Glasgow, completing an intercalated BSc in cancer science before graduating with honours from Glasgow Medical School in 2014. He completed 2 years of academic foundation training and 2 years of core surgical training and was appointed as the surgical Glasgow Academic Training Environment (GATE) Trainee in 2016. Mark gained Membership of Royal College of Surgeons (Glasgow) in 2017 before being appointed as a general surgical speciality registrar in the West of Scotland in 2018.

Dr Gerard Lynch is the INCISE Project Manager. As the Project Manager for the INCISE project, he supports the wider INCISE Management team, coordinates the various work packages and is responsible for delivering operational management. Gerard has over 14 years’ experience in Biomedical research and project management. He completed his PhD in Biochemistry at the University of Leeds. After working as a research scientist at Memorial Sloan Kettering in NYC for six years, he returned to the UK and has been working at the University of Glasgow since 2018.

Mr James Park is a clinical lecturer and consultant in colorectal and general surgery. He completed his undergraduate degree in medicine in 2007 at University of Glasgow, and subsequently a undertook a clinical research fellow post in Glasgow Royal Infirmary, completing a PhD focusing on the systemic environment and tumour microenvironment of colorectal cancer. He is a member of the Cancer Research UK Early Detection Early Career Researcher Scotland steering committee.
Mr Stephen McSorley is a Clinical Lecturer and Honorary Specialty Training Registrar in General Surgery based at the University of Glasgow. He qualified from the University of Glasgow medical school in 2009 and undertook his foundation and core surgical training in the West of Scotland. He was made Member of the Royal College of Surgeons (Glasgow) in 2012, before completing his PhD in colorectal cancer surgery at the University of Glasgow and Glasgow Royal Infirmary in 2019.

Professor Joanne Edwards is Professor of Translational Cancer Pathology, in Institute of Cancer Sciences, University of Glasgow, working closely with Academic Unit of Surgery, Glasgow Royal Infirmary and Glasgow Research Tissue Facility, Queen Elizabeth University Hospital. Joanne has a BSc in Pharmacology from University of Glasgow and a PhD in Molecular Biology from University of St Andrews. Notably, despite being a non-clinical researcher, her contribution to the field of cancer biomarkers resulted in her being elected as a Fellow of the Royal College of Pathologists. She has a successfully funded portfolio of multi-disciplinary peer-reviewed research, over 170 publications, over 10,000 citations and an H index of 53. She is director of the INCISE project.