1-5-2019

Meta-analysis of the molecular associations of mucinous colorectal cancer.

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Citation

Reynolds IS, Furney SJ,Kay EW,McNamara DA, Prehn JA, Burke JP. A systematic review and meta-analysis of the molecular associations of mucinous colorectal cancer. British Journal of Surgery. 2019;106(6):682-691

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Title: A systematic review and meta-analysis of the molecular associations of mucinous colorectal cancer.

Short Running Head: KRAS, BRAF, MSI & CIMP Status in Mucinous Adenocarcinoma

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Submission Category: Systematic Review & Meta-Analysis

Word count: Abstract: 200 Text: 2,632

Funding: Funding for this study was provided by the Beaumont Hospital Colorectal Research Trust

Previous Communication: This paper is not based on previous communication to a society or meeting

Abstract

Background: Mucinous differentiation is present in 5-15% of colorectal adenocarcinomas. This subtype of colorectal cancer responds poorly to chemoradiotherapy and is associated with a worse prognosis. There is a lack of consensus regarding the genetic aetiology underpinning this cancer subtype. The aim of this study is to use meta-analytical techniques to clarify the molecular associations of mucinous colorectal cancer.

Methods: This study adhered to MOOSE guidelines. Databases were searched for studies comparing KRAS, BRAF, MSI, CIMP, p53 and p27 status between patients with mucinous and non-mucinous colorectal adenocarcinoma. A random-effects model was used for analysis.

Results: 46 studies describing 17,746 patients were included. Mucinous colorectal adenocarcinoma was positively associated with KRAS (OR: 1.46, 95% CI: 1.08-2.00, p=0.014) and BRAF (OR: 3.49, 95% CI: 2.50-4.87, p<0.001) mutation, microsatellite instability (OR: 3.976, 95% CI: 3.30-4.79, p<0.001) and CpG island methylator phenotype (OR: 3.56, 95% CI: 2.85-4.43, p<0.001) and negatively associated with altered p53 expression (OR: 0.457, 95% CI: 0.311-0.672, p<0.001).

Conclusion: These data imply the genetic origins of mucinous colorectal adenocarcinoma are predominantly associated with in BRAF, MSI and CIMP pathways. This pattern of molecular alterations may in part explain the resistance to standard chemotherapy regimens that is seen with mucinous adenocarcinoma.

Introduction

Colorectal cancer (CRC) is a frequently encountered malignancy with an estimated 135,430 cases diagnosed in the United States in 2017, furthermore there was 50,260 deaths from the disease in the USA alone during 2017 (1, 2). The corresponding figures for the UK are 41,700 and 16,000 respectively (3). Mucinous tumours comprise 5-15% of all tumours found in the colon and rectum (4). A mucinous tumour is defined as a tumour in which >50% of the lesion is composed of pools of extracellular mucin (5). When compared with non-mucinous adenocarcinoma, patients with mucinous tumours of the rectum have a reduced rate of pathological complete response (pCR) and tumour downstaging after neo-adjuvant chemoradiotherapy, in addition they are more likely to have a positive resection margin and in keeping with this they are known to have a poorer overall survival (6). Colon cancers with mucinous differentiation have also been shown to be associated with an increased risk of metastasis and death as well as resistance to oxaliplatin and irinotecan based chemotherapy (7). Currently the mechanism of resistance to chemoradiotherapy in mucinous tumours of the rectum remains unknown. We hypothesise that the underlying cause of resistance arises from alternative genetic mutations when compared to those with non-mucinous adenocarcinoma.

To date several studies have tried to determine the genetic aetiology of mucinous adenocarcinoma of the colon and rectum and this has resulted in multiple published papers with heterogeneous outcomes (8-10). Mutations in Kirsten rat sarcoma viral oncogene (KRAS) and v-Raf murine sarcoma viral oncogene homolog B (BRAF), inherited defects or epigenetic silencing of mismatch repair proteins (MMR) resulting in microsatellite instability (MSI) and the presence of the CpG island methylator phenotype (CIMP) are the most common genetic aberrations that have been studied to date (11-14). The presence or absence of each of these

genetic markers may have therapeutic and/or prognostic implications for patients with CRC. Currently those with metastatic disease who are KRAS wildtype can be offered treatment with an EGFR inhibitor such as cetuximab. Those with the BRAF v600e mutation may respond to treatment with vemurafenib. Nivolumab, an anti-programmed cell death-1 (PD-1) monoclonal antibody can be used for the treatment of MSI high or MMR deficient unresectable or metastatic colorectal cancers that have progressed following treatment with a fluoropyrimidine, oxaliplatin and irinotecan (15).

At present there is no published meta-analysis that has pooled together all the available data on the molecular characteristics of mucinous adenocarcinoma of the colon and rectum. To address this, a systematic review and meta-analysis of all studies comparing KRAS, BRAF, MSI, CIMP, p53 and p27 status between mucinous and non-mucinous colorectal adenocarcinoma was undertaken.

Methods

Literature Search and Study Selection

This systematic review and meta-analysis adhered to the recommendations of the MOOSE (Meta-analyses of Observational Studies in Epidemiology) statement(16). A systematic search of PubMed, Embase and the Cochrane Central Register of Controlled Trials was performed for all studies published that compared KRAS, BRAF, MSI, CIMP, p53 and p27 status between patients with non-mucinous adenocarcinoma and those with mucinous adenocarcinoma of the colon and rectum. The following search terms were used in the search algorithm: (Mucinous OR Mucin) AND (Colon OR Rectal OR Colorectal). The latest search was performed on the 17/07/2017. Two authors (I.S.R. and J.P.B.) independently examined the title and abstract of citations, the full texts of potentially eligible studies were obtained; disagreements were resolved by discussion or if needed by a third author (D.A.M). The reference lists of all articles that were retrieved were further screened for additional eligible publications.

Eligibility Criteria

Comparative studies of mucinous and non-mucinous adenocarcinoma of the colon and rectum containing data on KRAS, BRAF, MSI, CIMP, p53 and p27 status were eligible for inclusion. Any studies looking at the status of the above markers in mucinous adenocarcinoma only or with no comparative data were excluded. Any studies that did not correctly define mucinous adenocarcinoma according to the World Health Organisation (WHO) definition were excluded. Studies in which patients with mucinous components of <50% or studies where signet ring cell tumours were analysed with the mucinous adenocarcinoma group were also excluded. There were no language restrictions.

Data Extraction and Outcomes

The following information regarding each eligible study was recorded: authors' names, journal, year of publication, country/countries in which the study was undertaken, the method of how mucinous adenocarcinoma was defined, the number of patients with mucinous and non-mucinous adenocarcinoma of the colon or rectum, KRAS, BRAF, MSI, CIMP, p53 and p27 status.

Statistical Analysis

All pooled outcome measures were determined using a random-effects model as described by DerSimonian and Laird and the odds ratio (OR) was estimated with its variance and 95% confidence interval (CI). The random-effects analysis weighted the natural logarithm of each study's OR by the inverse of its variance plus an estimate of the between-study variance in the presence of between-study heterogeneity. As previously described, heterogeneity between ORs for the same outcome between different studies was assessed (17). This was through the use of the I^2 inconsistency test and the χ^2-based Cochran Q statistic test in which $p < 0{\cdot}05$ is taken to indicate the presence of significant heterogeneity (18). 95% prediction intervals (PI) were calculated and presented in parenthesis(19). Analyses were conducted using Comprehensive Meta-analysis version 2 (Biostat Inc, Englewood, NJ). The quality of included studies was assessed by using the Newcastle-Ottawa Scale. The quality of studies was evaluated by examining 3 items: patient selection, comparability of the 2 study groups and assessment of exposure (maximum score=9).

Results

Literature Review

The initial search yielded 13,575 papers, this was reduced to 8,516 after duplicates were removed. 8,349 articles were excluded by title and abstract alone leaving 167 papers for full-text review. 121 articles were deemed ineligible after full-text review, the remaining 46 articles with information on 17,746 patients were deemed suitable for inclusion in the systematic review and meta-analysis. All included studies had Newcastle Ottawa scores of between 6 and 9. The reasons as to why articles were excluded are listed in the literature search flow diagram [See Figure 1]. The details of the papers that were included in the review are available in Supplementary Table 1 (8-14, 20-58). The median frequency and range of molecular alterations for each marker are shown in Table 2.

KRAS Status

16 studies with data on a total of 4,975 patients (807 mucinous vs. 4,168 non-mucinous) were eligible for inclusion in the analysis of KRAS status in mucinous vs. non mucinous colorectal cancers (8-11, 14, 20, 21, 25, 27, 30, 33, 37, 39, 46, 52, 55). 3 of the 16 studies carried out extended RAS testing versus just exon 2 testing. Mucinous tumours were weakly associated with KRAS mutations (OR 1.46, 95% CI 1.08 – 2.00, p = 0.014) (95% PI: 0.51-4.14) [See Figure 2]. There was significant heterogeneity (Cochran Q: $p < 0.001$, I^2: 64.2).

BRAF Status

10 studies including data on 6,608 patients (863 mucinous vs. 5,745 non-mucinous) comparing BRAF mutation status between mucinous and non-mucinous colorectal tumours were deemed eligible for inclusion in the meta-analysis (8, 11-14, 21, 25, 27, 30, 39). Again

mucinous tumours were positively associated with BRAF mutations (OR 3.49, 95% CI 2.50 – 4.87, p < 0.001) (95% PI: 1.47-8.27) [See Figure 3]. There was significant heterogeneity (Cochran Q: p = 0.04, I^2: 48.8).

MSI Status

27 studies comparing MSI status between mucinous and non-mucinous colorectal tumours were included in the study (4, 8, 11-14, 20, 22, 25-29, 31, 32, 34, 36, 41, 47-51, 53, 54, 57, 58). This pooled analysis was the largest including data on 11,043 (1,431 mucinous vs. 9,612 non-mucinous) patients. Mucinous tumours of the colon and rectum were significantly more likely to be associated with microsatellite instability when compared with non-mucinous tumours (OR 3.98, 96% CI 3.30 – 4.79, p < 0.001) (95% PI: 2.41-6.56) [See Figure 4]. There was no significant heterogeneity (Cochran Q: p = 0.121, I^2: 24.8).

CIMP Status

6 studies were deemed suitable for inclusion in the analysis on CIMP status (8, 14, 23-25, 45). This analysis included a total of 3,433 patients (474 mucinous vs. 2,959 non-mucinous). Mucinous tumours were more likely to be associated with the CPG island methylator phenotype high status (OR 3.56, 95% CI 2.85 – 4.43, p < 0.001) (95% PI: 2.60-4.86) [See Figure 5]. There was no significant heterogeneity (Cochran Q: p = 0.717, I^2: 0.0).

p53/p27 Status

12 studies which detailed 2,234 patients were included in the analysis of p53 status (8, 20, 27, 35, 37, 40-42, 44, 48, 52, 56), (449 mucinous vs. 1,785 non-mucinous). Mucinous tumours were less likely to be associated with altered p53 expression (OR 0.46, 95% CI: 0.31-0.67,

p<0.001) (95% PI: 0.14-1.47) [See Figure 6A]. There was significant heterogeneity (Cochran Q: p = 0.011, I^2: 55.1).

3 studies which detailed 442 patients were included in the analysis of status p27 status(20, 41, 43), (124 mucinous vs. 318 non-mucinous). Mucinous tumours were not associated with altered p27 expression (OR 0.66, 95% CI: 0.05-8.02, p=0.74)[See Figure 6B]. There was significant heterogeneity (Cochran Q: p < 0.001, I^2: 95.8).

Discussion

The current study represents a distillation of all currently available evidence on the molecular associations of mucinous colorectal cancer. Mucinous colorectal adenocarcinoma was positively associated with BRAF mutation, microsatellite instability and CPG island methylator phenotype and negatively associated with altered p53 expression. It was also weakly associated with KRAS mutation although there was significant statistical heterogeneity associated with this result.

Sporadic colorectal cancer tends to develop through one of two distinct mechanisms. The first mechanism is caused by chromosomal instability. This pathway results from loss of heterozygosity at multiple tumour suppressor loci. This accounts for 80-85% of CRC. P53 mutations are frequently found in tumours with chromosomal instability (59). The second mechanism is known as microsatellite instability. These tumours have mismatch repair (MMR) deficiency resulting in an inability to repair single nucleotide DNA mismatches. MLH1 silencing is characteristic of sporadic MSI tumours (60). This group of tumours frequently has associated BRAF mutations. Mucinous adenocarcinoma tends to demonstrate the characteristics described above for MSI tumours. Recently a third mechanism has been described known as epigenetic instability. This pathway results in the aberrant methylation and silencing of tumour suppressor genes (61). The findings from our meta-analysis confirm that mucinous tumours are more likely to display MMR deficiency, BRAF mutations and epigenetic instability as demonstrated by the association with the CpG island methylator phenotype and hence tend to develop and progress through different molecular pathways when compared to the majority of sporadic colorectal cancers. Mucinous tumours appear to

occur more frequently in right-sided lesions and are associated with higher tumor stage and histological grade (62-64), a shared feature with BRAF associated colorectal cancer (65).

In the clinical setting subtypes of colorectal cancer are predominantly distinguished by histopathological features. This is in stark contrast to genetic and histopathological studies where subtypes of colorectal cancer are distinguished at a molecular level (2, 66). However, as DNA sequencing technologies evolve, it is becoming increasingly likely that molecular subtyping of colorectal cancer will be used to determine outcomes such as risk of recurrence, response to treatments and long-term prognosis(67). It is clear that a more in depth analysis of the molecular genetics of mucinous tumours of the colon and rectum is needed. The Cancer Genome Atlas (TCGA) colorectal cancer project has provided us with in-depth insight into the molecular mechanisms that are responsible for the initiation and progression of CRC, however it has not yet addressed the differences between histological subtypes (68). This will improve our understanding of the sequence of genetic events that occur when normal colonic epithelium transitions to mucinous adenocarcinoma and may facilitate the discovery of novel therapeutic targets for this cohort of patients who have limited treatment options. In the clinical setting we have already begun to divide patients into treatment groups based on their molecular status. The importance of molecular subtyping will continue to grow as subtype-based targeted interventions become more readily available. Guinney et al have described the consensus molecular subtypes (CMS) of colorectal cancer. They have described four different molecular subtypes of colorectal cancer, each with distinguishing features (2). Based on our findings it appears that mucinous colorectal tumours would be classified as CMS1. This subtype is hypermutated, microsatellite unstable and has strong immune activation, there is frequent occurrence of BRAF mutations found in the CMS1 group.

Classifying tumours in this manner provides us with insight into prognosis and it is known that CMS1 tumours tend to present with a higher histopathological grade and have a poor survival after relapse (2).

The mechanism of the poor response of mucinous colorectal cancers to traditional chemotherapeutic regimens is at present poorly understood, but the demonstrated association of this tumour subtype with MSI may in part provide an explanation. Retrospective studies show that the clinical behaviour of MSI high tumours is different than those without this characteristic. Studies examining adjuvant chemotherapy for patients with stage III MSI high tumours indicate that, unlike patients whose tumours demonstrate chromosomal instability, they experience no benefit with regimens containing fluorouracil (69). In the setting of mucinous rectal cancer, the lack of response to chemoradiotherapy could potentially be attributed to decreased sensitizing of tumour cells to radiotherapy due to reduced efficacy of 5-fluorouracil in mucinous tumours that are MSI high. The use of chemotherapeutic regimens containing irinotecan has shown promise in MSI high tumours (60) and novel immunotherapeutic agents such as ipilimumab have already been shown to be beneficial (70). In keeping with this the FDA has approved the use of pembrolizumab, an anti-programmed cell death-1 (PD-1) monoclonal antibody, for the treatment of unresectable or metastatic solid tumours that have been identified as being MSI-high or MMR deficient (dMMR). Shortly after this, nivolumab another anti-PD-1 monoclonal antibody, gained an accelerated approval in August 2017 for adult and paediatric patients with MSI-high or dMMR metastatic colorectal cancer that has progressed after standard chemotherapy (15). Finally the increased rate of KRAS mutations in patients with mucinous

adenocarcinoma means that this group of patients is less likely to benefit from epidermal growth factor receptor (EGFR) inhibitors if they do develop metastatic disease.

It is important to recognise the limitations of this meta-analysis including the significant statistical heterogeneity found in the KRAS, BRAF and p53 analysis. This heterogeneity may reflect the difference in study types included in the analysis, methodological differences between studies, unknown study characteristics and publication bias. Also of note were the different methods used across different studies to detect altered expression of p53 and p27. These differences, however, reflect real life clinical practice in that different laboratories often use different assays. With regard to the heterogeneity in the BRAF and p53 analyses it is noteworthy that the rate of BRAF mutations was higher in the mucinous groups in all included studies and the rate of p53 alterations was lower in the mucinous groups in all studies apart from one. Heterogeneity may have been underestimated in the CIMP analysis given that there are reported difficulties determining and assigning CIMP status due to the variety of markers currently used(71, 72). The majority of studies included in the analysis were retrospective in nature, this may potentially increase the risk of sampling bias and data collection bias. We have reported our results as odds ratios, it is important to recognise that odds ratios can often overestimate the effect when compared to relative risks (73).

In conclusion, mucinous adenocarcinoma of the colon and rectum are more likely to have BRAF mutations. Furthermore they are more likely to demonstrate MSI and be of the CPG island methylator phenotype and less likely to be associated with altered p53 expression. The progression of these subtypes of colorectal cancer along alternative genetic pathways may account in some part for the resistance to treatment and worse prognosis seen in mucinous

adenocarcinomas. In-depth research into the genetics of mucinous adenocarcinoma may help to identify potential therapeutic targets.

Tables & Figures Legends

1st Author	Year	Country	Study Type	Enrolment Interval	Total number	KRAS	BRAF	MSI (H)	CIMP (H)	P53 Data	P27 Data	NO
Jang, M.H.	2017	Korea	Retrospective	2011-2014	346	N/A	20	35	N/A	No	No	8
Andrici, J.	2016	Australia	Retrospective	1998-2011	2608	N/A	532	424	N/A	No	No	8
Wang, M.J.	2015	China/Sweden	Retrospective	1972-2009	1001	41	N/A	83	N/A	Yes	Yes	7
Rosty, C.	2013	Australia	Prospective	1990-1994	776	119	125	N/A	N/A	No	No	8
Jung, S.B.	2012	Korea	Retrospective	2004-2006	120	N/A	N/A	11	N/A	No	No	8
Nosho, K.	2008	USA	Retrospective	Not Stated	904	N/A	N/A	N/A	133	No	No	8
Kawasaki, T.	2008	USA/Japan	Retrospective	Not Stated	782	N/A	N/A	N/A	125	No	No	8
Tanaka, H.	2006	USA/Japan	Retrospective	Not Stated	83	30	21	14	20	No	No	8
Chang, SC.	2006	China	Prospective	1999-2000	213	N/A	N/A	19	N/A	No	No	8
Ogino, S.	2006	USA	Retrospective	Not Stated	450	N/A	N/A	N/A	78	No	No	7
Ashktorab, H.	2005	USA	Retrospective	1998-2001	51	N/A	N/A	22	N/A	No	No	7
Kazama, Y.	2005	Japan	Retrospective	1990-2003	78	N/A	N/A	14	N/A	No	No	7
Greenson, J.K.	2003	USA/Israel	Retrospective	1998-2002	526	N/A	N/A	52	N/A	No	No	7
Li, W.	2015	China	Retrospective	2011-2012	761	265	23	N/A	N/A	No	No	8
Ward, R.	2001	Australia/UK	Prospective	1993-1998	307	N/A	N/A	32	N/A	No	No	8
Alexander, J.	2001	USA	Retrospective	1985-1992	299	N/A	N/A	80	N/A	No	No	7

Zhang, H.	1998	Sweden	Retrospective	1982-1997	149	41	N/A	N/A	N/A	No	No	8
Messerini, L.	1997	Italy	Retrospective	Not Stated	74	N/A	N/A	34	N/A	No	No	7
1st Author	**Year**	**Country**	**Study Type**	**Enrolment Interval**	**Total number**	**KRAS**	**BRAF**	**MSI (H)**	**CIMP (H)**	**P53 Data**	**P27 Data**	**NO**
Hanski, C.	1992	Berlin/Japan/UK	Retrospective	Not Stated	76	N/A	N/A	N/A	N/A	Yes	No	7
Liddell, C.	2017	France	Retrospective	2004-2012	196	69	21	25	N/A	No	No	8
Yoon, Y.S.	2015	South Korea	Retrospective	2003-2007	2028	N/A	N/A	202	N/A	No	No	8
Ines, C.	2014	Tunisia	Retrospective	1995-2010	167	52	N/A	N/A	N/A	Yes	No	8
Langner, C.	2012	Austria	Retrospective	1992-2000	374	N/A	N/A	21	N/A	No	No	7
Pai, R.K.	2012	USA	Retrospective	2005-2010	181	78	20	N/A	N/A	No	No	8
Lam, A.K.	2008	Australia	Prospective	Not Stated	188	N/A	N/A	N/A	N/A	Yes	No	8
Inamura, K.	2015	USA	Retrospective	1976-2008	1336	440	179	190	200	No	No	8
Leopoldo, S.	2008	Italy	Prospective	1996-2000	156	N/A	N/A	35	N/A	Yes	Yes	8
Tozawa, E.	2007	Japan	Retrospective	Not Stated	152	N/A	N/A	N/A	N/A	Yes	No	8
Sarli, L.	2006	Italy	Prospective	1997-1999	108	N/A	N/A	N/A	N/A	No	Yes	8
Lan, Y.T.	2007	Taiwan	Retrospective	1999-2004	252	N/A	N/A	N/A	N/A	Yes	No	8
Ogino, S.	2006	USA	Retrospective	Not Stated	624	166	63	84	N/A	Yes	No	6
Lin, J.K.	2006	Taiwan	Prospective	1999-2000	255	112	N/A	N/A	N/A	No	No	8
Yearsley, M.	2006	USA	Prospective	Not Stated	482	N/A	N/A	69	N/A	No	No	7
Park, S.Y.	2006	South Korea	Retrospective	1993-2004	194	N/A	N/A	27	N/A	Yes	No	8

Song, G.A.	2005	USA	Retrospective	Not Stated	73	30	7	20	15	Yes	No	6
Wright, C.L.	2003	New Zealand	Retrospective	2000-2002	447	N/A	N/A	80	N/A	No	No	8
1st Author	**Year**	**Country**	**Study Type**	**Enrolment Interval**	**Total number**	**KRAS**	**BRAF**	**MSI (H)**	**CIMP (H)**	**P53 Data**	**P27 Data**	**NO**
Ismael, N.E.	2017	Egypt	Retrospective	2012-2015	48	N/A	N/A	14	N/A	No	No	8
Bazan, V.	2002	Italy	Prospective	1988-1992	160	74	N/A	N/A	N/A	No	No	7
Feeley, K.M.	1999	Ireland	Retrospective	1991-1992	50	N/A	N/A	5	N/A	No	No	8
Zhang, H.	1999	Sweden	Retrospective	1972-1986	293	41	N/A	N/A	N/A	Yes	No	7
Furlan, D.	1998	Italy	Prospective	1995-1996	100	N/A	N/A	9	N/A	No	No	9
Forster, S.	1998	Germany	Retrospective	1991-1993	20	N/A	N/A	7	N/A	No	No	8
Albanese, I.	1997	Italy	Prospective	Not Stated	60	28	N/A	N/A	N/A	No	No	7
Laurent-Puig, P.	1991	France	Prospective	Not Stated	99	39	N/A	N/A	N/A	No	No	7
Georgescu, C.V.	2007	Romania	Retrospective	2005-2006	41	N/A	N/A	N/A	N/A	Yes	No	8
Suh, J.H.	2002	South Korea	Retrospective	Not Stated	61	N/A	N/A	19	N/A	No	No	7

Supplementary Table 1 – Details of Included Studies

Molecular Marker	Mucinous	Non-Mucinous
KRAS Mutation	41.57% (26.92-72.73%)	33.00% (23.62-57.58%)
BRAF Mutation	29.08% (7.73-46.15%)	9.77% (0.00-19.96%)
MSI High	33.33% (0.00-63.64%)	10.64% (0.00-37.5%)
CIMP High	36.36% (33.33-41.38%)	13.56% (11.11-17.64%)
P27 Alteration	43.18% (30.00-58.33%)	51.27% (18.75-87.5%)
P53 Alteration	28.38% (0.00-75.68%)	51.07% (32.20-80.13%)

Table 2 - Frequency of Molecular Alterations (Displayed as Median with Range in brackets)

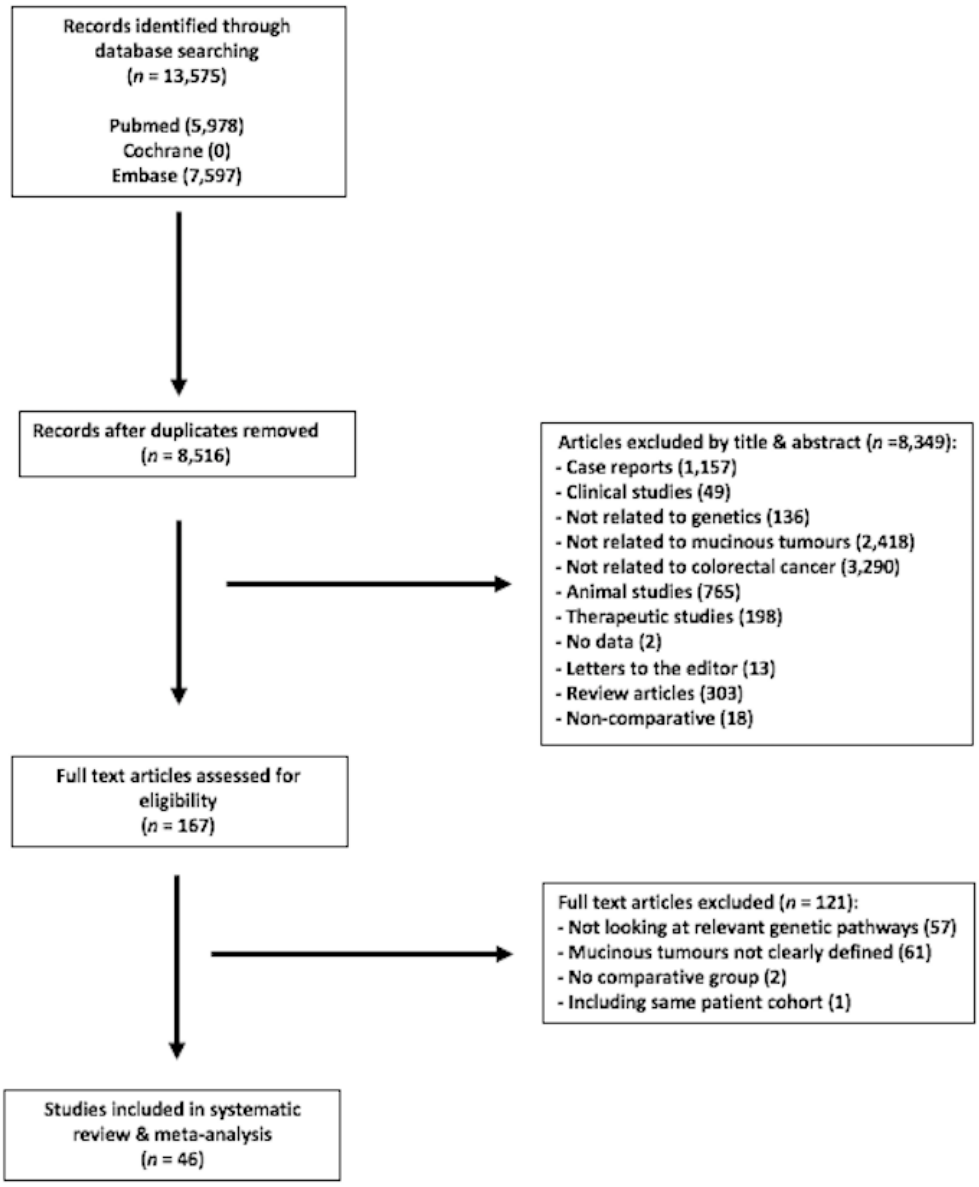

Figure 1 – Literature Search Flow Diagram

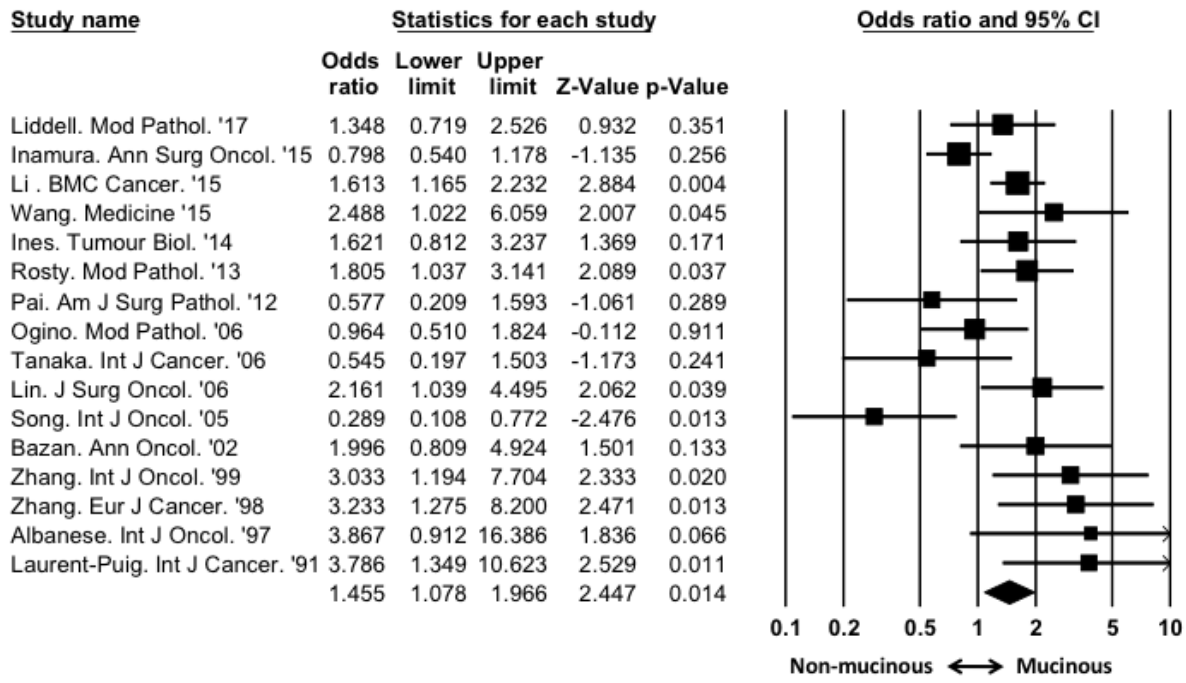

Figure 2 – Forest Plot For KRAS (n = 4,975, p = 0.014, Cochran Q: 41.9, df: 15, P<0.001, I^2: 64.2)

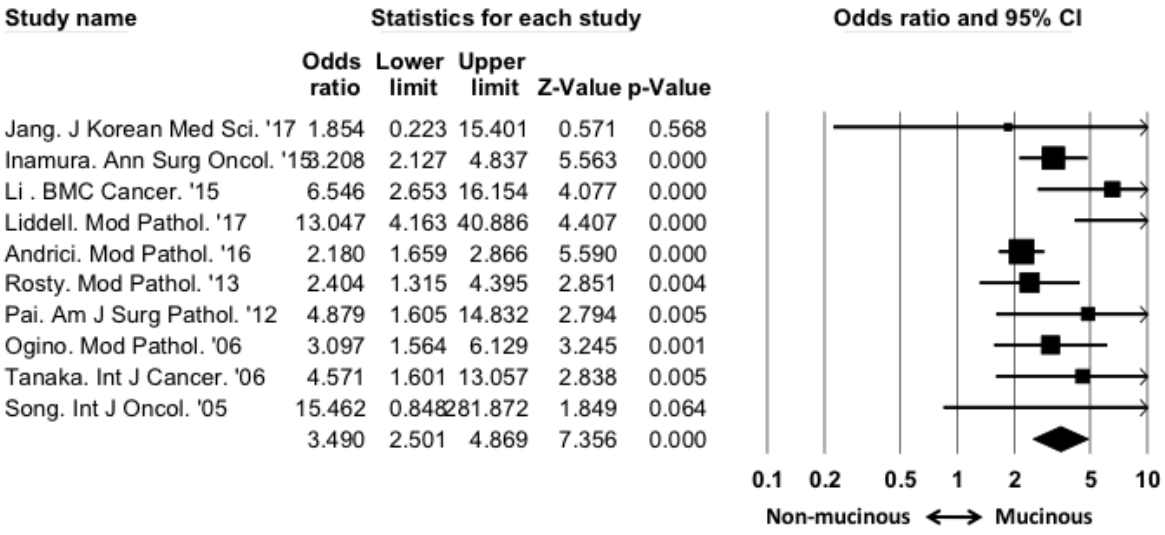

Figure 3 – Forest Plot For BRAF (n = 6,608, p<0.001, Cochran Q: 17.6, df: 9, P=0.04, I^2: 48.8)

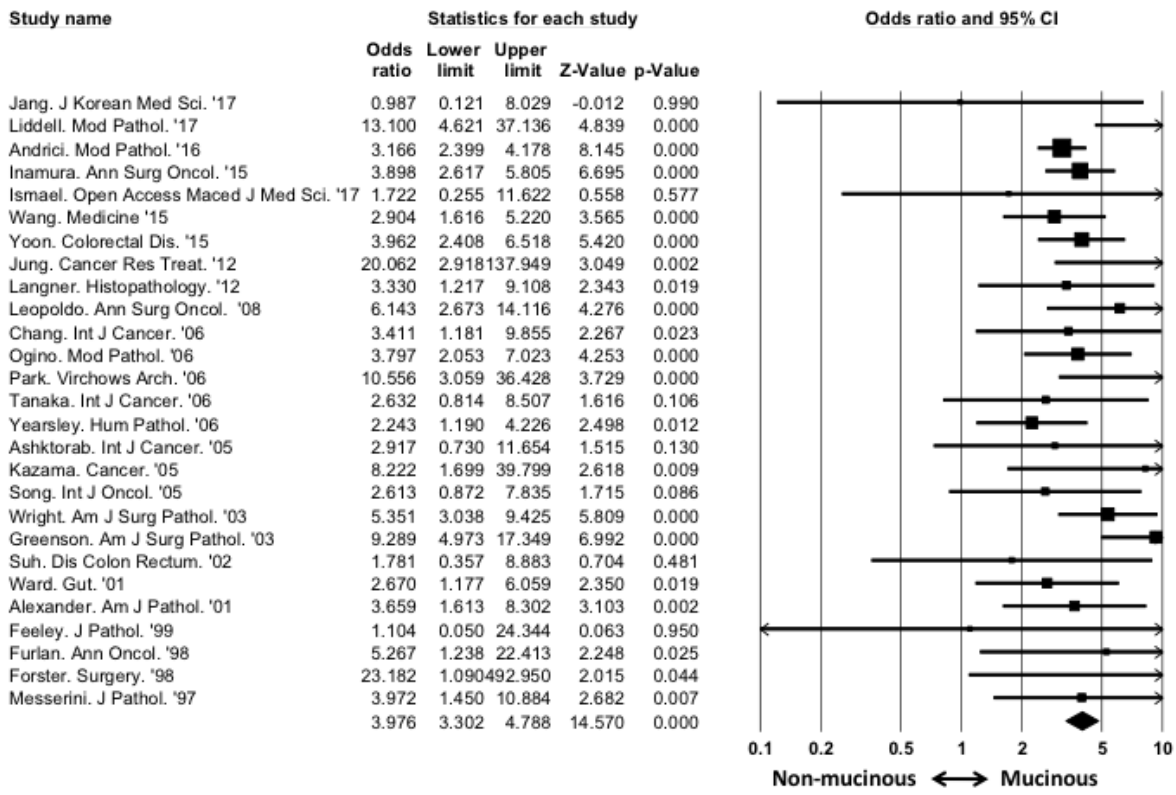

Figure 4 – Forest Plot For MSI (n = 11,043, p<0.001, Cochran Q: 34.6, df: 26, P=0.121, I^2: 24.8)

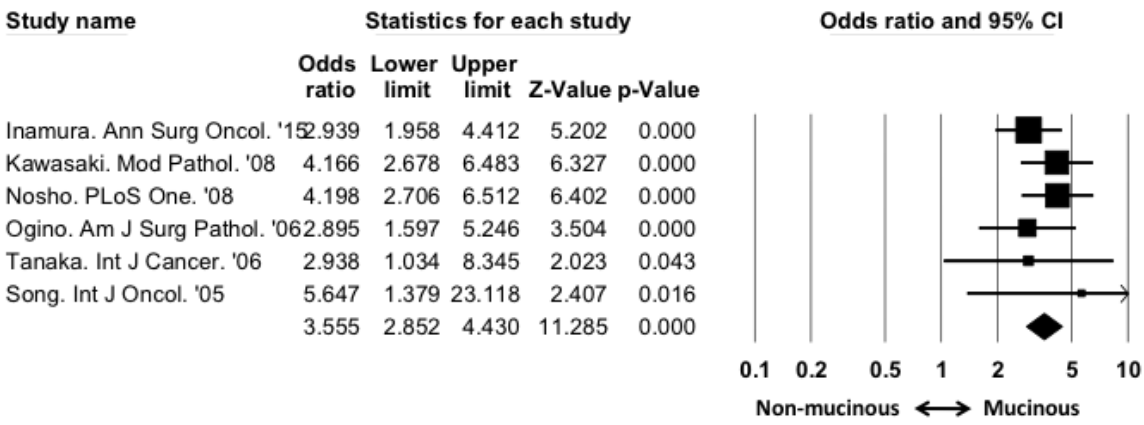

Figure 5 – Forest Pot For CIMP (n = 3,433, p<0.001, Cochran Q: 2.9, df: 5, P=0.717, I^2: 0.0)

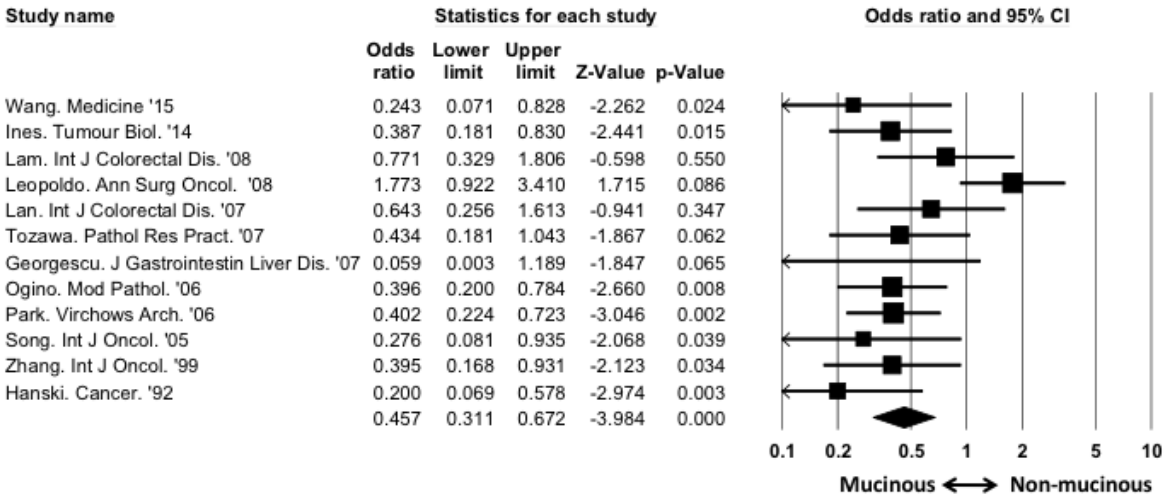

Figure 6A – Forest Pot For P53 (n = 2,234, p<0.001, Cochran Q: 24.5, df: 11, P=0.011, I^2: 55.1)

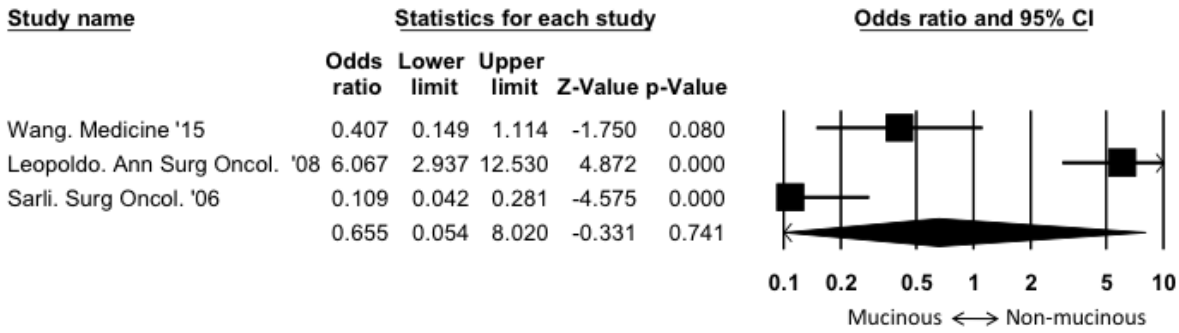

Figure 6B – Forest Pot For P27 (n= 442, p=0.74, Cochran Q: 54.2, df: 3, P<0.001, I^2: 95.8)

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Additional Information

Ethics approval and consent to participate

- Ethical approval was not deemed necessary for this study.

Consent for publication

- Consent for publication was not needed as no individual person's data was included in the study.

Availability of data

- Data can be provided to anyone who requests it by emailing the corresponding author

Disclaimer:

- There are no conflicts of interest.

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- Study concept and design – J.P. Burke, J.H.M. Prehn & S.J. Furney
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