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ORIGINAL PAPER

# Chemoprevention in Lynch syndrome

**John Burn · John C. Mathers · D. Tim Bishop**

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**Abstract** CAPP1 tested aspirin 600 mg/day and/or resistant starch 30 g/day in 200 adolescent FAP carriers. Aspirin treatment resulted in a non-significant reduction in polyp number and a significant reduction in polyp size among patients treated with aspirin for more than 1 year. CAPP2 RCT used the same interventions in 937 Lynch syndrome patients, the first RCT to have cancer prevention as the primary endpoint. Aspirin did not reduce the risk of colorectal neoplasia in a mean treatment period of 29 months but double blind post intervention follow-up has revealed 48 participants developed 53 CRCs. Per protocol analysis showed 63 % fewer colon cancers with aspirin ($p = 0.008$) apparent from 4 years, with a similar effect on other LS cancers. Resistant starch was not beneficial at long term followup. CAPP3 will involve a double blind dose non-inferiority trial comparing 100, 300 or 600 mg daily in 3,000 gene carriers. We can now recommend aspirin in people at high risk of colorectal cancer.

**Keywords** Chemoprevention · Aspirin · Resistant starch · Mismatch repair gene defects · Genetic predisposition

J. Burn (✉)
Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK
e-mail: john.burn@ncl.ac.uk

J. C. Mathers
Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, UK

D. T. Bishop
Leeds Institute of Molecular Medicine, Leeds University, Leeds, UK

## Introduction

The CAPP studies had their origin in a small house near Newcastle when JB met a mother with familial adenomatous polyposis and her three children. Her son had an osteoma on his forehead, almost identical to the one on his mother's forehead, making it almost certain that he had inherited "the gene" even though his first endoscopy had been reported clear. It triggered the development of our genetically targeted trial programme, for here was someone with a genetic predisposition who could test with considerable statistical power the potential efficacy of simple chemoprevention strategies relevant both to these rare families and to the wider population. The realisation that loss of the APC gene function was an early trigger to colonic carcinogenesis strengthened the case and in 1993, CAPP1, as it became known was launched to test two interventions, aspirin and resistant starch as long term chemopreventive agents.

As the first trial was just getting under way, we were approached by a team of yeast geneticists led by Richard Kolodner to ask if we could provide DNA from a family with the still poorly understood hereditary non-polyposis colon cancer. They had recognised the genetic instability described in the literature as likely evidence of a failure of mismatch repair and needed affected individuals in which to test their theory. The result of this discovery process, described elsewhere in this book, was that we had our first family with Lynch syndrome (LS) in which we could offer a molecular diagnosis [1]. The relevance to our chemoprevention programme was clear; here were people at high risk of colorectal cancer who could be recruited to test the impact of our interventions and who unlike the FAP gene carriers had intact colons in adulthood and did not have thousands of polyps which were proving hard to evaluate in CAPP1.

CAPP2 (ISRCTN59521990) was the first large-scale genetically targeted chemoprevention trial. It was calculated to need 1,000 people with LS. This was achieved with the majority of recruits carried pathological DNA mismatch repair (MMR) gene variants. Fewer than 20 % were recruited on the basis that they belonged to a LS family based on the "Amsterdam criteria" [2] and had had a LS cancer previously treated.

CAPP1 and CAPP2 used the same factorial design which allows two interventions to be evaluated; resistant starch and/or aspirin. Aspirin was chosen based primarily on the early case control and observational studies suggesting its potential efficacy [3, 4]. The choice of resistant starch was based on supportive epidemiology. Cassidy et al. [5] reported a significant negative correlation between population starch intakes and colon-cancer incidence. A recent meta-analysis of 21 studies revealed a significant, dose dependent protective effect of dietary fibre against colorectal cancer (relative risk of 0.90 for each 10 g increase in intake; Confidence intervals 0.86–0.94) [6]. Resistant starch (RS) is the sum of starch and the products of starch digestion not absorbed in the small intestine of healthy individuals; it undergoes colonic fermentation to short-chain fatty acids including butyrate. RS supplementation can improve a number of potential biomarkers of CRC risk including faecal concentrations of total and secondary bile acids, which it lowers [7, 8]. Butyrate and non-steroidal anti-inflammatory drugs (NSAIDs) differ radically in the transcriptome and proteome modifications they produce in tumor cells [9, 10]. CAPP1 revealed a weakly significant impact of aspirin on size of largest observed polyp and a significant reduction in crypt length in those given RS [11].

The major value of CAPP1 was that it provided a training ground for the development of CAPP2.

## CAPP2 trial design

Between 1999 and 2005, 1,009 eligible LS gene carriers were recruited from 43 international centres, 937 participants started intervention in the CAPP2 study [12, 13] and 746 were included in the end of intervention analysis after a mean of 29 months treatment. The study had a preplanned design for 10 years follow-up. Of the 937 persons, 427 were randomized to aspirin, 434 to aspirin placebo (AP) and the remaining recruits were not randomized for the aspirin intervention having opted not to participate in this study limb (N = 76; almost all due to perceived aspirin sensitivity or history of peptic ulceration). All participants in this latter group were randomized to the RS or Resistant Starch Placebo (RSP) intervention only (Fig. 1; consort diagram).

Analysis of long term effect focused on 861 CAPP2 participants randomized to aspirin or AP from entry until the latest date for which the recruiters had information on cancer diagnosis, a time-point usually corresponding to the date of last surveillance attendance. Our analysis includes (1) the LS syndrome cancers included in the earlier report [12], (2) those that occurred subsequent to exit from the intervention phase and, (3) all cancers that occurred in persons without an exit colonoscopy which excluded them from the statistical analysis in our earlier report [12].

## CAPP2 statistical methods

Analysis was designed to test the primary hypothesis that aspirin would reduce the development of CRC (as primary outcome) and LS cancers (as secondary outcome) in participants randomized to aspirin (427) compared to those on placebo (434). The original protocol invited participants to continue with the original intervention for a further 2-year cycle following the initial 2 years.

Two approaches were taken: time to first CRC occurrence (our original focus) examined using life-table methods and Cox proportional hazards and second, Poisson regression modeling to investigate primary cancers at multiple anatomical sites. Poisson regression analysis takes into account the complete cancer history of the participant since randomization in contrast with the more restricted time to first event analysis.

For life-table analysis, end of follow-up was determined as (1) the time of first CRC diagnosis, if affected, or (2) the last recorded date at which the clinical status was known. Analyses included Cox proportional hazards models to estimate gender-adjusted hazard ratios (HR) and 95 % confidence intervals and Kaplan–Meier curves to assess non-parametrically the outcome differences between the aspirin and AP interventions. The assumption of proportional hazard was tested to assess compliance.

For the Poisson regression analysis, incidence rate ratios (IRR) for the effect of aspirin adjusted for gender were estimated from log-linear models for the number of primary cancers diagnosed after randomization; exposure time being that from randomization until date of last known clinical status.

All analyses used Stata v10. Designation of LS cancer spectrum was a clinical assessment, blinded to intervention, and based on a the recently published review of the LS phenotype [14]; endometrial, ovarian, pancreatic, small bowel, gall bladder, ureter, stomach and kidney cancers and cancer of the brain were included. A final analysis examined the total burden of LS related cancers in those who had been on intervention for at least 2 years (per protocol).

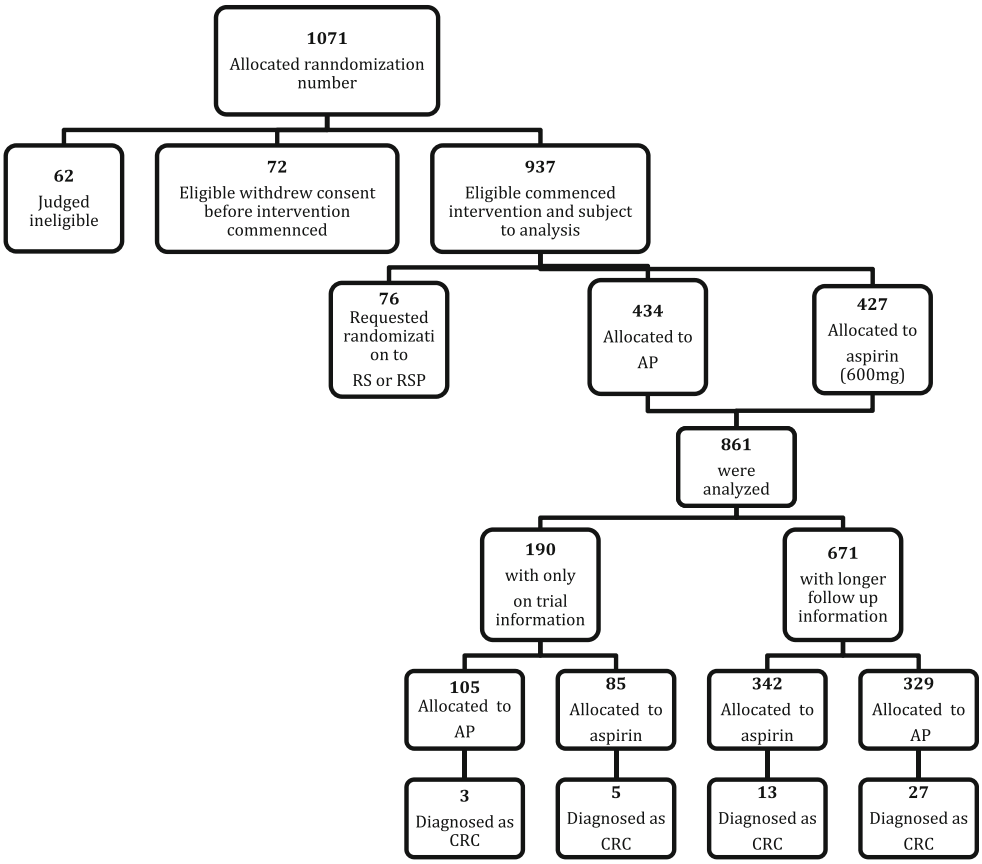


**Fig. 1** Consort diagram; the status of participants in the CAPP2 study

## Results

The mean observation period was 55.7 months (range 1–128 months) and 1 % of recruits were ≥10 years from randomization by the time of the analysis (Table 1).

Demographic data indicated no differences between those traced and not traced in the follow-up in respect of age, gender, randomization category, or geographical location though it is plausible that the development of a cancer made follow-up reporting more complete. There were no significant regional differences in CRC incidence (Chi squared [2] = 5.03, $p = 0.08$).

Overall, 40 people were diagnosed with CRC among those with post intervention information (13/342 allocated to aspirin and 27/329 allocated to AP). Another 8 CRC occurred among 190 (83 male and 107 female) individuals with intervention phase only information, (5/85 and 3/105 for aspirin and AP arm respectively).

Despite regular colonoscopy and polyp removal, 48 recruits developed CRC after randomization (Table 1). Of these, 18 received aspirin and 30 received AP. For the whole post-randomization period, the HR for CRC for aspirin was 0.63 (CI 0.35–1.13, $p = 0.12$) favouring protection in the aspirin group (Table 2; Fig. 2a). Five of the 48 people who developed CRC each had 2 primary colon cancers. Of these, one had received aspirin and 4 AP. Although the Intention to Treat time-to-event analysis showed a non-significant protective effect of aspirin, the Poisson regression taking into account the five multiple primary CRC participants (53 CRC) indicated a protective effect: IRR 0.56 (CI 0.32–0.99, $p = 0.05$). Because of this protective effect we re-estimated the protective effect with a per protocol analysis and obtained similar results.

Put simply, the per protocol analysis focuses on those participants who actually took the prescribed treatment for the time originally intended. This provides a better assessment of efficacy but is seen as inferior to the Intention to treat analysis because it does not identify whether people drop out due to side effects. With an over the counter preparation like aspirin this is less important because side effects are well understood.

We examined outcomes in those participants who took aspirin (or AP) for a minimum of 2 years defined as consumption of 1,400 (300 mg) tablets; rounded down from a 2 year total (1,461 tablets) to allow for early scheduling of the exit colonoscopy and/or occasional missed dosage. Based on this definition, 258 (30 %) and 250 (29.1 %) participants took aspirin and AP respectively for ≥2 years. The HR for those taking aspirin for ≥2 years was 0.41 (CI 0.19–0.86, $p = 0.02$, Table 2; Fig. 2b) and the IRR, 0.37

**Table 1** CAPP2 study population: numbers, time on study, time on follow-up and cancer burden according to aspirin use [2]

| | Aspirin | AP | Total |
|---|---|---|---|
| Number of participants | 427 | 434 | 861 |
| Months on CAPP2 intervention study (mean) (SD, range) | 25.0 (12.5) (0.8, 60.6) | 25.4 (14.2) (1.1, 74.4) | 25.2 (13.4) (0.8, 74.4) |
| Months since study entry (mean) (SD, range) | 56.6 (30.9) (0.8, 125.4) | 54.8 (31.8) (1.6, 128) | 55.7 (31.4) (0.8, 128) |
| *Number of participants with first CRC* | | | |
| Since randomization | 18 | 30 | 48 |
| Within 2 years of randomization | 10 | 10 | 20 |
| More than 2 years from randomization | 8 | 20 | 28 |
| *Number of participants with other LS cancers*[a] | | | |
| Since randomization | 16 | 24 | 40 |
| Within 2 years of randomization | 5 | 9 | 14 |
| More than 2 years from randomization | 11 | 15 | 26 |
| *Number of participants with one or more LS cancers (including CRC)* | | | |
| Since randomization | 34 | 52 | 86 |
| Within 2 years of randomization | 15 | 19 | 34 |
| More than 2 years from randomization | 19 | 33 | 52 |
| Number of participants with non-LS cancers | 19 | 19 | 38 |

[a] Two participants in placebo group had CRC and another LS cancer. These two participants are counted in the rows relating to both CRC and other LS cancers. In the row reporting to all Lynch syndrome cancers, these participants are counted only once

**Table 2** Cox proportional hazards analysis and Poisson regression for CRC cancer (adjusted for gender) based only on those randomized to aspirin or AP [2]

| Estimate of effect of | CRC | | CRC | |
|---|---|---|---|---|
| | HR (95 % CI)[a] | *p* value | IRR[c] (95 % CI) | *p* value |
| Intention to treat | | | | |
| Aspirin versus AP | 0.63 (0.35–1.13) | 0.12 | 0.56 (0.32–0.99) | 0.05 |
| Per protocol analysis | | | | |
| ≥2 years AP[b] | 1.0 | | 1.0 | |
| <2 years AP[b] | 0.62 (0.25–1.52) | 0.30 | 0.72 (0.32–1.59) | 0.41 |
| <2 years aspirin[b] | 1.07 (0.47–2.41) | 0.87 | 0.90 (0.42–1.91) | 0.77 |
| ≥2 years aspirin[b] | 0.41 (0.19–0.86) | 0.02 | 0.37 (0.18–0.78) | 0.008 |
| Cumulative aspirin dose | | | | |
| Units of 100 aspirin[d] | 0.97 (0.94–1.00) | 0.06 | 0.97 (0.94–1.00) | 0.03 |

[a] Cox proportional hazards analysis based on 48 participants with CRC involving a total of 53 cancer diagnoses: *HR* hazard ratio (95 % confidence interval)

[b] The threshold for 2 years intervention was consumption of more than 1,400 aspirin tablets; rounded down from a 2 year total of 1,461 tablets to allow for early scheduling of the exit colonoscopy and/or occasional missed dosage

[c] Incidence rate ratio (95 % confidence interval) from Poisson regression

[d] Units of 100 aspirin = the total number of aspirin taken divided by 100

(CI 0.18–0.78, $p = 0.008$) results similar to those for Poisson regression in the ITT analysis.

We explored the effect of compliance on outcome (important because noncompliance may be related to factors that also affect CRC risk) using per protocol analysis, and found those who took aspirin for ≥2 years had an incidence rate of 0.06 per 100 person-years compared with 0.13 per 100 person years among those who took aspirin <2 years. A similar analysis within the placebo group found no significant difference in CRC incidence between those who took AP for ≥2 years (0.14 per 100 person years) compared with those took AP for <2 years (0.10 per 100 person years).

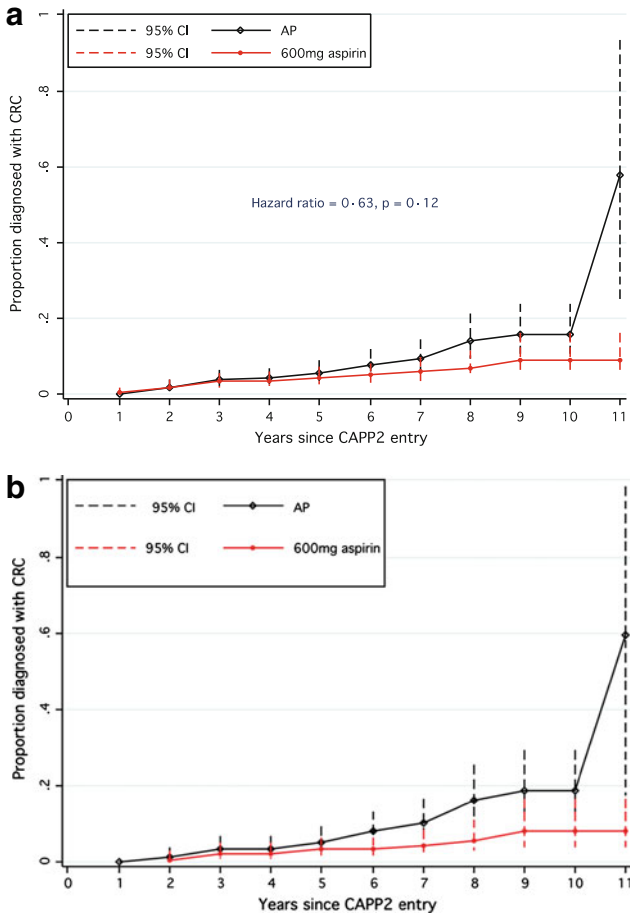


**Fig. 2** Time to first colorectal cancer in those randomized to aspirin compared with those randomized to AP. **a** Kaplan–Meier analysis was adjusted for gender. Each point on the plot shows the estimated cumulative incidence by years of follow-up together with the corresponding 95 % confidence interval. **b** Kaplan–Meier analysis was restricted to participants who had taken ≥2 years intervention and the analysis was adjusted for gender (HR 0.41 (CI 0.19–0.86), $p = 0.02$). Each point on the plot shows the estimated cumulative incidence by years of follow-up together with the corresponding 95 % confidence interval

The planned secondary analysis with other LS cancers as the secondary outcome also showed a trend to protection with aspirin; 18 participants developed endometrial cancer of whom 5 were randomized to aspirin and 13 to AP. In total, 38 participants developed cancer at a site other than the colorectum (additionally 2 participants had CRC and another LS cancer) of whom 16 were randomized to aspirin and 22 to AP. The HR for those randomized to aspirin was 0.63 (CI 0.34–1.19, $p = 0.16$, Table 3; and IRR was 0.63 (CI 0.34–1.16, $p = 0.14$) compared with AP group. Per protocol analysis showed that the HR for those who had taken aspirin for ≥2 years was 0.47 (CI 0.21–1.06, $p = 0.07$) with IRR = 0.49 (CI 0.23–1.05, $p = 0.07$) (Table 3).

Table 4 gives the combined analysis of all LS cancers including CRC. On intention to treat analysis, the HR was 0.65 (CI 0.42–1.00, $p = 0.05$ and IRR was 0.59 (CI 0.39–0.90, $p = 0.01$) while the per protocol analysis HR was 0.45 (CI 0.26–0.79, $p = 0.005$, Fig. 3) and IRR was 0.42 (CI 0.25–0.72, $p = 0.001$) supporting the protective effect of aspirin. Cox proportional hazards models analysis by cumulative aspirin consumption suggested a dose-response effect which was significant for non-CRC LS cancers ($p = 0.03$), LS cancers overall ($p = 0.007$) and a trend for CRC ($p = 0.06$, Tables 2, 3, 4). Corresponding outcomes from the Poisson regression analysis were also significant ($p = 0.03$ for non-CRC LS cancers, $p = 0.002$ for LS cancers overall and $p = 0.03$ for CRC).

To see if the apparent protective effect of aspirin might be due to unexpectedly high numbers of cancers in the AP group, we tested the risk of CRC for the non-randomized group (RS or RSP only) compared with the AP group. The CRC HR in this group was 1.4 times higher compared with AP [not statistically significant ($p = 0.4$)]. This gives further support to the protective effect of aspirin because it shows that the AP group did not have an unusually high cancer rate.

Where possible, details of adenoma development were collected in the post-intervention period. While incomplete, these data, gathered by blinded contributors' revealed no apparent effect of aspirin on numbers of participants who developed adenomas subsequent to the intervention phase i.e. 51 and 48 in the aspirin and AP groups respectively.

CRC was reported with equal frequency in those carrying *MLH1* and *MSH2* mutations (6.0 and 7.0 % respectively) while none of the *MSH6* mutation carriers developed CRC—in keeping with the anticipated milder phenotype. The remaining 163 recruits were diagnosed on the basis of Amsterdam Criteria [1] and had been treated for a LS-related neoplasia. Of these 7 (4.3 %) developed CRC. Overall, there was no evidence of difference in CRC incidence by presence of proven germ-line mutation (Chi squared [2] = 3.1, $p = 0.38$).

Eighteen (34 %) of 53 CRC diagnosed in aspirin or AP arms were Dukes stage A, 21 (39.6 %) Dukes B, 10 (18.9 %) had Dukes C and D, and 4 (7.5 %) were unknown. Twenty-seven (51 %) tumours were located in the ascending colon, transverse colon and splenic flexure, 6 (11.3) % in the descending colon, 12 (22.6 %) in the sigmoid and rectum, and 8 (15.1 %) were unknown. There was no significant difference in staging (Chi squared [3] = 2.92, $p = 0.40$) and tumour location (Chi squared [3] = 0.08, $p = 0.99$) between aspirin and AP groups.

## Toxicity

During the intervention phase adverse events in the aspirin and placebo groups were similar [12] with 11 significant gastrointestinal bleeds or ulcers in the aspirin group and 9 in the placebo group. No details of adverse events were available for

**Table 3** Cox proportional hazards analysis and Poisson regression for non-CRC Lynch syndrome cancers (adjusted for gender) based only on those randomized to aspirin or AP [2]

| Estimate of effect of | Non-CRC Lynch cancer | | Non-CRC LS cancer | |
|---|---|---|---|---|
| | HR (95 % CI)[a] | *p* value | IRR[c] (95 % CI) | *p* value |
| Intention to treat | | | | |
| Aspirin versus AP | 0.63 (0.34–1.19) | 0.16 | 0.63 (0.34–1.16) | 0.14 |
| Per protocol analysis | | | | |
| ≥2 years AP[b] | 1.0 | | 1.0 | |
| <2 years AP[b] | 0.96 (0.40–2.34) | 0.94 | 0.82 (0.35–1.96) | 0.66 |
| <2 years aspirin[b] | 1.11 (0.46–2.68) | 0.82 | 0.90 (0.38–2.14) | 0.81 |
| ≥2 years aspirin[b] | 0.47 (0.21–1.06) | 0.07 | 0.49 (0.23–1.05) | 0.07 |
| Cumulative aspirin dose | | | | |
| Units of 100 aspirin[d] | 0.96 (0.93–1.00) | 0.03 | 0.96 (0.93–1.00) | 0.03 |

[a] Cox proportional Hazards analysis based on 40 case: *HR* hazard ratio (95 % confidence interval)

[b] The threshold for 2 years intervention was consumption of more than 1,400 aspirin tablets; rounded down from a 2 year total of 1,461 tablets to allow for early scheduling of the exit colonoscopy and/or occasional missed dosage

[c] Incidence rate ratio (95 % confidence interval) from Poisson regression

[d] Units of 100 aspirin = the total number of aspirin taken divided by 100

**Table 4** Cox proportional hazards analysis and Poisson regression for all LS cancers (adjusted for gender) based only on those randomized to aspirin or AP [2]

| Estimate of effect of | All LS cancers | | All LS cancers | |
|---|---|---|---|---|
| | HR (95 % CI)[a] | *p* value | IRR[c] (95 % CI) | *p* value |
| Intention to treat | | | | |
| Aspirin versus AP | 0.65 (0.42–1.00) | 0.05 | 0.59 (0.39–0.90) | 0.01 |
| Per protocol analysis | | | | |
| ≥2 years AP[b] | 1.0 | | 1.0 | |
| <2 years AP[b] | 0.79(0.42–1.49) | 0.47 | 0.76 (0.43–1.37) | 0.36 |
| <2 years aspirin[b] | 1.13 (0.62–2.06) | 0.69 | 0.90 (0.51–1.59) | 0.71 |
| ≥2 years aspirin[b] | 0.45 (0.26–0.79) | 0.005 | 0.42 (0.25–0.72) | 0.001 |
| Cumulative aspirin dose | | | | |
| Units of 100 aspirin[d] | 0.97 (0.95–0.99) | 0.007 | 0.96 (0.94–0.99) | 0.002 |

[a] Cox proportional hazards analysis based on 86 participants with LS cancers involving a total of 93 cancer diagnoses: HR = hazard ratio (95 % confidence interval)

[b] The threshold for 2 years intervention was consumption of more than 1,400 aspirin tablets; rounded down from a 2 year total of 1,461 tablets to allow for early scheduling of the exit colonoscopy and/or occasional missed dosage

[c] Incidence rate ratio (95 % confidence interval) from Poisson regression

[d] Units of 100 aspirin = the total number of aspirin taken divided by 100

the post-intervention phase. There was also no significant difference in compliance (i.e. proportion of scheduled tablets not taken during the intervention phase) between the aspirin and AP groups for those with complete intervention phase data (Chi squared [1] = 1.27, $p = 0.20$) [12].

### Effects of Starch at long term follow up [15]

Information on longer term follow up was available for 714 persons whereas for 204 only "on trial" information was available. Demographic data indicate that there was no differences between those traced and not traced "post trial" in this follow-up in respect of sex, randomization category, or geographical location.

The median period of follow-up was 52.7 months (IQR 29.0–78.9 months), during which time, 53 persons developed CRC (27 in those given RS and 26 randomized to RSP). Forty-five of these cancers developed among those with long-term follow up data (23 and 22 cases among those allocated to RS and RSP respectively) and the remaining 8 cases occurred among individuals with intervention phase

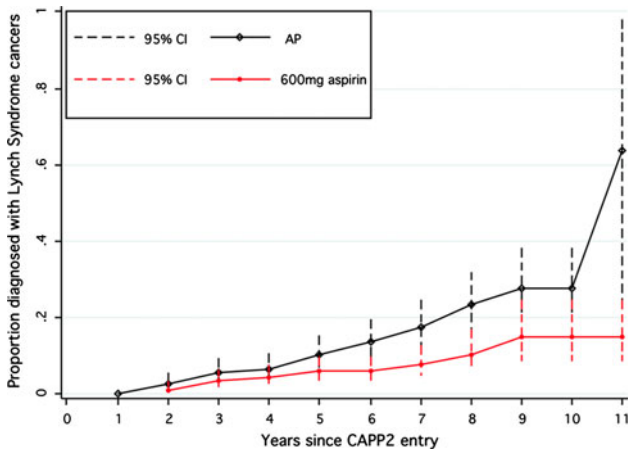


**Fig. 3** Time to first LS cancer in those randomized to aspirin compared with those randomized to the AP. In each case, Kaplan–Meier analysis was restricted to participants who had taken ≥2 years intervention and the analysis was adjusted for gender (HR 0.45 (CI 0.26–0.79), $p = 0.005$). Each point on the plot shows the estimated cumulative incidence by years of follow-up together with the corresponding 95 % confidence interval

only information (5 and 3 cases in those allocated to RS and to RSP respectively).

For the whole post randomization period, the hazard ratio for CRC (53 cases) among those randomized to RS was 1.40 (95 % CI 0.78–2.56, $p = 0.26$) showing no evidence for a protective effect of RS (Table 5; Fig. 4). The hazard ratio for CRC without adjusting for aspirin duration was not very different (1.33; 95 % CI 0.73–2.41). The ITT analysis by Poisson regression model that took into account the 8 multiple primary CRC participants also indicated no protective effect of RS (IRR = 1.15; 95 % CI 0.66–2.00, $p = 0.61$). There was no significant modification effect of aspirin taken for estimated hazard ratio for resistant starch ($p = 0.89$) and the mean of duration of aspirin use was similar for both RS and placebo (25.0 (±12.9) months and 25.0 (±12.5) months in RS and RSP arms respectively).

We re-estimated the potential protective effect of RS with a per protocol analysis and obtained similar results. For this analysis, we defined 2 years intervention as consumption of 1,400 (2 × 15 g/day) packs; this was rounded down from a 2 year total (1,461 packs) to allow for early scheduling of the exit colonoscopy and/or occasional missed dosage. The HR for those taking RS for <2 years was 2.38 (95 % CI 0.98–5.77, $p = 0.05$) which suggested a possible adverse effect of shorter-term RS treatment on CRC incidence in contrast, the HR for those taking RS treatment for ≥2 years was 1.09 (95 % CI 0.55–2.19, $p = 0.80$, Table 5; Fig. 4) and the IRR, 0.98 (95 % CI 0.51–1.88, $p = 0.95$).

A secondary analysis considered other LS cancers i.e. all LS cancers except CRC. In total, 41 participants developed cancer at a LS site other than the colorectum of whom 16 were randomized to RS and 25 to RSP. The HR for those randomized to RS was 0.72 (95 % CI 0.38–1.35, $p = 0.30$, compared with RSP group. Endometrial cancer was the commonest non-CRC cancer; 21 participants developed endometrial cancer of whom 10 were randomized to RS and 11 to RSP. Of note, no participants randomized to RS developed pancreatic or small bowel cancer compared with 5 and 3 respectively among those randomized to RSP. This apparent protection by RS is intriguing but, given the small numbers, may be a chance observation. The per protocol analysis showed that the HR for those allocated to RS who took treatment for <2 years was 0.87 (95 % CI 0.34–2.22, $p = 0.77$) whereas for those taking RS treatment for ≥2 years the HR was 0.63 (95 % CI 0.28–1.40, $p = 0.26$) with an IRR of 0.56 (95 % CI 0.26–1.23, $p = 0.15$).

**Table 5** Cox proportional hazards analysis and Poisson regression for CRC cancer (adjusted for sex and duration of aspirin taken) based only on those randomized to RS or RSP

| Estimate of effect of | CRC | | CRC | |
|---|---|---|---|---|
| | HR (95 % CI)[a] | *p* value | IRR[c] (95 % CI) | *p* value |
| Intention to treat | | | | |
| RS versus RSP | 1.40 (0.78–2.56) | 0.26 | 1.15 (0.66–2.00) | 0.61 |
| Per protocol analysis | | | | |
| RSP | 1.00 | | 1.00 | |
| <2 years RS[b] | 2.38 (0.98–5.77) | 0.05 | 1.59 (0.69–3.63) | 0.27 |
| ≥2 years RS[b] | 1.09 (0.55–2.19) | 0.80 | 0.98 (0.51–1.88) | 0.95 |
| Cumulative starch dose | | | | |
| Units of 100 RS | 1.01 (0.98–1.04) | 0.56 | 1.00 (0.98–1.03) | 0.72 |

[a] Cox proportional hazards analysis based on 53 participants with CRC involving a total of 61 cancer diagnoses

[b] The threshold for 2 years intervention was consumption of more than 1,400 starch packs; rounded down from a 2 year total of 1,461 packs to allow for early scheduling of the exit colonoscopy and/or occasional missed dosage

[c] Incidence rate ratio (IRR) from Poisson regression

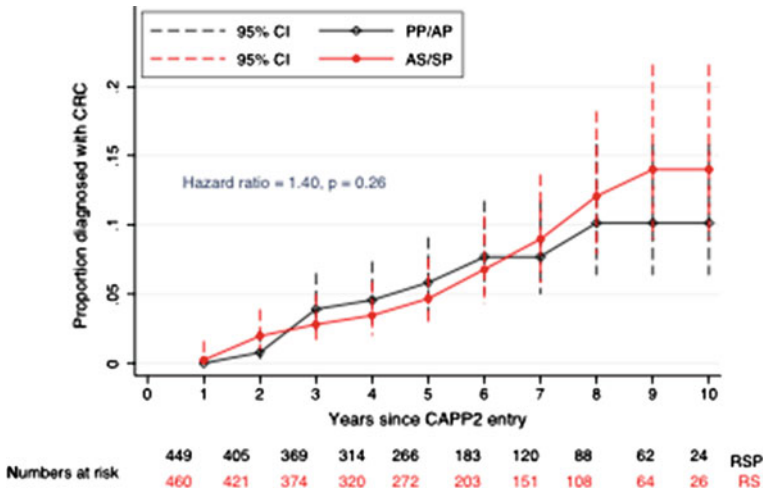


**Fig. 4** Time to first colorectal cancer in those randomized to RS compared with those randomized to RSP. Kaplan–Meier analysis was adjusted for sex. Each point on the plot shows the estimated cumulative incidence by years of follow-up together with the corresponding 95 % confidence interval [15]

On intention to treat analysis, the HR for "all Lynch syndrome cancers" was 1.02 (95 % CI 0.66–1.57, $p = 0.94$) and IRR was 0.93 (95 % CI 0.62–1.39, $p = 0.72$) while the per protocol analysis HR was 0.83 (95 % CI 0.49–1.40, $p = 0.48$) and IRR was 0.77 (95 % CI 0.47–1.26, $p = 0.30$) for those taking RS for ≥2 years indicating a non significant protective effect of RS. Cox proportional hazards models analysis by cumulative RS consumption found no evidence of a significant dose-response effect for CRC ($p = 0.56$), non-CRC LS cancers ($p = 0.21$) or LS cancers overall ($p = 0.56$) (Tables 5, 6, 7).

Where possible, details of adenoma development were also collected by blinded observers in the post-intervention period. Whilst incomplete, these data on 1,068 colonoscopy reports revealed no apparent effect of RS on numbers of participants who developed adenomas subsequent to the intervention phase i.e. 114 reports of adenomas in each of the RS and RSP groups from 558 and 488 colonoscopies respectively ($p = 0.25$). There was no difference in number of colonoscopies in those randomized to RS and to the corresponding placebo ($p = 0.24$). In addition, there was no evidence of an effect of RS treatment on adenomas when the analysis was restricted to those on starch treatment for ≥2 years ($p = 0.25$).

Given the suggestion that the RS fermentation end product butyrate may have more potent antineoplastic effects on colon cancer cells with dysfunction of the DNA MMR gene *MLH1* [16], the data were analyzed according to the underlying MMR gene defect and no effect was apparent.

Twenty (32.8 %) of 61 multiple CRC diagnosed in RS or RSP arms were Dukes stage A, 25 (41.0 %) Dukes B, 12 (19.7 %) had Dukes C and D, and 4 (6.5 %) were unknown Thirty-three (54.0 %) tumours were located in the ascending colon, transverse colon and splenic flexure, 6 (9.8 %) in the descending colon, 13 (21.4 %) in the

**Table 6** Cox proportional hazards analysis and Poisson regression for non-CRC Lynch syndrome cancers (adjusted for sex and duration of aspirin taken) based only on those randomized to RS or RSP

| Estimate of effect of | Non-CRC Lynch cancer | | Non-CRC LS cancer | |
|---|---|---|---|---|
| | HR (95 % CI)[a] | *p* value | IRR[c] (95 % CI) | *p* value |
| Intention to treat | | | | |
| RS versus RSP | 0.72 (0.38–1.35) | 0.30 | 0.71 (0.38–1.32) | 0.28 |
| Per protocol analysis | | | | |
| RSP | 1.00 | | 1.00 | |
| <2 years RS ** | 0.87 (0.34–2.22) | 0.77 | 1.02 (0.42–2.51) | 0.96 |
| ≥2 years RS ** | 0.63 (0.28–1.40) | 0.26 | 0.56 (0.26–1.23) | 0.15 |
| Cumulative starch dose | | | | |
| Units of 100 RS | 0.98 (0.94–1.01) | 0.21 | 0.98 (0.94–1.01) | 0.17 |

[a] Cox proportional hazards analysis based on 43 participants with non-CRC lynch syndrome cancers involving a total of 46 cancer diagnoses

[b] The threshold for 2 years intervention was consumption of more than 1,400 starch packs; rounded down from a 2 year total of 1,461 packs to allow for early scheduling of the exit colonoscopy and/or occasional missed dosage

[c] Incidence rate ratio (IRR) from Poisson regression

**Table 7** Cox proportional hazards analysis and Poisson regression for all LS cancers (adjusted for sex and duration of aspirin taken) based only on those randomized to RS or RSP [15]

| Estimate of effect of | All LS cancers | | All LS cancers | |
|---|---|---|---|---|
| | HR (95 % CI)[a] | *p* value | IRR[c] (95 % CI) | *p* value |
| Intention to treat | | | | |
| RS versus RSP | 1.02 (0.66-1.57) | 0.94 | 0.93 (0.62-1.39) | 0.72 |
| Per protocol analysis | | | | |
| RSP | 1.00 | | 1.00 | |
| <2 years RS[b] | 1.45 (0.77-2.73) | 0.26 | 1.29 (0.70-2.36) | 0.41 |
| ≥2 years RS[b] | 0.83 (0.49-1.40) | 0.48 | 0.77 (0.47-1.26) | 0.30 |
| Cumulative starch dose | | | | |
| Units of 100 RS | 0.99 (0.97-1.02) | 0.56 | 0.99 (0.97-1.01) | 0.52 |

[a] Cox proportional hazards analysis based on 94 participants with LS cancers involving a total of 107 cancer diagnoses

[b] The threshold for 2 years intervention was consumption of more than 1,400 starch packs; rounded down from a 2 year total of 1,461 packs to allow for early scheduling of the exit colonoscopy and/or occasional missed dosage

[c] Incidence rate ratio (IRR) from Poisson regression

sigmoid and rectum, and 9 (14.8 %) were unknown. There were no significant differences in tumour staging (Chi squared [3] = 6.73, $p = 0.08$) and tumour location (Chi squared [3] = 0.54, $p = 0.91$) between RS and RSP groups.

## CaPP3

The CAPP2 study has demonstrated that aspirin can prevent over half of the cancers in LS with a time delay from commencement of intervention of about 4 years. It is clear that this low cost and relatively low risk intervention is suitable for people at such high cancer risk and further placebo controlled clinical trials are neither feasible nor ethical. On the other hand, there is still much to learn; what mechanisms underlie the beneficial effect of aspirin, can we stratify the population on the basis of metabolic variants such that we can, perhaps, achieve a greater benefit with fewer side effects and can we assume from the other recent studies in the literature that 75–100 mg aspirin is as effective as the 600 mg dose used in CAPP2?

The international community involved in the care of families with hereditary colorectal cancer has endorsed, via the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) (www.insightgroup.org) the idea of a large scale dose inferiority study which will be known as CaPP3. The CaPP programme, now converted to stand for Cancer Prevention Programme, will lead on this study with support from Cancer Research UK and Bayer Pharma who will supply active and placebo enteric coated aspirin in 100 and 300 mg doses so as to construct a three category study which will supply in double blind fashion, one small and two standard "aspirin tablets" delivering either 100, 300 or 600 mg daily.

CaPP3 will be active from 2013 until at least 2021. In the meantime the CaPP group consider the evidence is now sufficient to recommend aspirin to all gene carriers with the clear statement that we still do not know what dose is ideal. The trial used 2 standard tablets daily whereas the Rothwell studies suggest that low dose aspirin taken long term may be just as effective in the low risk population. The recent case control study from Scotland has also supported the beneficial effects of low dose aspirin [17].

It is hoped that all clinicians will encourage their patients who carry a MMR gene defect to sign up to the dose inferiority study. Having a history of having taken aspirin will not preclude their involvement. We will simply need to collect careful data on the quantity of aspirin consumed prior to randomisation. Since the minimum dose we will distribute will be 100 mg enteric coated aspirin (equivalent to 75 mg of soluble aspirin), a reasonable suggestion would be that all gene carriers should discuss with their doctor commencing on the usual regime employed in patients at increased vascular risk, namely 75–100 mg "low dose" aspirin having first followed the usual preparation of checking a blood count and considering H pylori eradication.

There is no doubt that low dose aspirin has fewer side effects that a 600 mg daily dose so commencing with a low dose regime will minimise adverse events while establishing individual tolerance of aspirin. Those who are not able to join the trial for any reason may discuss using a higher dose with their primary care team or gastroenterologist, possibly in conjunction with a proton pump inhibitor.

CaPP3 will provide a blinded daily aspirin dose over a minimum 2 year period followed by 100 mg for all, with regular surveillance continued as usual and collection of detailed information on adverse events and gastrointestinal tumours.

Power analysis indicates a need to recruit 3,000 gene carriers in total and follow the three dose groups for 5–10 years in order to test whether 100 mg is inferior to 300 or 600 mg doses. These figures are based on the data gathered to date which suggests a 20 % cancer reduction in those on 100 mg and a 50 % reduction in those on the 600 mg dose. Based on the number of cancers seen in CAPP2, we should see sufficient difference at 7 years to differentiate the three doses.

The lack of a discernible difference in the number of polyps recorded in the aspirin and placebo groups in CAPP2 mean that adenoma counts are not a reliable biomarker in LS. It may be that aspirin is having effects on adenoma progression, influencing, for example, the development of new vessels [18] or it may be a quite different effect such as encouraging apoptosis of aberrant crypt cells preventing the development of adenomas with major malignant potential. This would fit with the effect of natural salicylates in plants where they induce apoptosis to help prevent the spread of disease [19].

Whatever the influence of the aspirin, a presumed consequence is that there will be fewer cells with loss of both alleles of a MMR gene. This in turn means that, if aspirin works, there will be fewer cells accumulating the frameshift mutations which result from a breakdown in mismatch repair. This molecular defect leads to new gene sequences because loss or gain of one or two bases from a coding sequence results in a different reading frame and therefore a predictably different string of amino acids in the new peptides formed. The Heidelberg group under Magnus von Knebel Doeberitz [20] have demonstrated that individuals with microsatellite unstable CRCs develop antibodies to these neopeptides, which may contribute to the improved prognosis. Antibodies are also detectable in carriers of MMR gene defects who have not been known to have a previous cancer. In CaPP3, we will collect regular samples to monitor the development of these neopeptide antibodies as a biomarker of the benefits of aspirin intervention.

## Discussion

The CAPP2 study was the first double blind RCT of aspirin and resistant starch chemoprevention with colorectal cancer as the primary endpoint. The outcome in the aspirin limb was consistent with over 2 decades of observational data showing CRC risk is halved in regular aspirin consumers [21] and long term follow-up of aspirin trials for cardiovascular disease (CVD) prevention which found that dosing with ≥75 mg aspirin/day for several years resulted in fewer deaths from gastrointestinal cancers, particularly involving the proximal colon [22, 23]. This concept of delayed cancer chemoprevention was apparent in observational studies, where protection against cancer among regular aspirin users took approximately 10 years to emerge [21, 24]. It was presumed that this effect was dependent on continued aspirin exposure but in the CVD trials trial medication ended at mean 6 years. Analysis of cancer related death in 8 trials [25] revealed significant protection in those allocated aspirin for ≥4 years but only when followed for a further 5 years. Our observations support this hypothesis of a delayed effect of aspirin on CRC by showing that aspirin reduced CRC incidence with the effect becoming apparent after 3–4 years from beginning aspirin intervention, a difference consistent with faster cancer development in those with LS [26, 27].

We also performed Poisson regression analysis on the "intention to treat" data and as expected, we saw a greater level of significance. This approach is justified as it is more useful to consider all primary cancers rather than only the first. The per protocol analysis showed a similar effect.

In keeping with our observed impact of aspirin on non-colonic LS cancers (endometrial cancer, ovarian cancer, pancreatic cancer, and cancer of the brain, small bowel, gall bladder, ureter, stomach and kidney) (Table 3), Rothwell et al. [25] reported that aspirin treatment reduced risk of death from several non-colonic solid cancers including oesophageal, pancreatic, brain, lung, stomach and prostate. It is not clear whether LS cancers are more responsive to aspirin therapy though it is noteworthy that in CAPP2 "non-LS" extra-colonic cancers appeared unaffected by aspirin intervention (Table 1). A weakness of our international study was the inability to collect a comprehensive series of tumour blocks in which to confirm that tumour development was related to the germline MMR mutation.

Our discovery of substantial protection by aspirin against CRC and other LS cancers is in striking contrast with our earlier report [12] of no effect of aspirin on large bowel neoplasia. Taken together, these findings may help explain the marked disparity between the 50 % cancer reduction reported in observational studies and the outcomes of randomized adenoma prevention trials which have demonstrated, at best, a modest reduction effect; meta-analysis revealed a pooled risk ratio of any adenoma for any dose of aspirin versus placebo of 0.83 (95 % CI 0.72–0.96) [28]. In the light of the CAPP2 findings it will be interesting to revisit the CAPP1 participants to see if aspirin has long term effects on their disease progression.

Important questions include (1) does aspirin target the minority of adenomas with the greatest malignant potential, (2) do some LS CRCs arise from lesions other than

adenomas [29] and, (3) why are some tumours aspirin "resistant"?

The mechanism by which aspirin suppresses cancer development long after cessation of exposure to the drug remains unclear. The assumption that the primary action of anti-inflammatories is on COX2 in colonic tumours [30] is unlikely to be the primary mechanism. The rapid progression from adenoma to carcinoma in LS [27] makes it likely that many screen-detected cancers would have begun to develop after aspirin intervention ended. Aspirin may be pro-apoptotic at early stages of CRC development, perhaps preceding adenoma formation. Ruschoff et al. [31, 32] reported reduced microsatellite instability and enhanced apoptosis in MMR-deficient cells exposed to aspirin and argued that aspirin may induce genetic selection for microsatellite stability in a subset of MMR-deficient cells. Aspirin may delete those aberrant stem cells most likely to progress rapidly to cancer. Analysis of the conditional MSH2 knockout mouse, reported recently to survive significantly longer when exposed to aspirin [33], might shed light on the mechanism.

Despite regular colonoscopy, 1 in 14 of those not taking aspirin in CAPP2 developed CRC in under 5 years, emphasizing the need for additional prevention strategies. Our results, taken in conjunction with recent literature, provide a basis for recommending aspirin chemoprevention in LS as standard of care.

The evidence is now sufficient to recommend aspirin to all gene carriers with the clear statement that we still do not know what dose is ideal. It is hoped that all clinicians will encourage their patients who carry a MMR gene defect to sign up to the CAPP3 dose inferiority study.

CAPP3 will aim to provide a blinded daily aspirin dose over a 5 year period with regular surveillance continued as usual and collection of detailed information on adverse events and gastrointestinal tumours.

CaPP3 will provide a daily blinded dose for the first 2 years then follow to at least 5 years with a particular focus on new tumours and adverse event rate. We will need 1,000 gene carriers in each of the treatment groups receiving 100, 300 or 600 mg daily. Antibodies to neopetides will be measured throughout to assess their role as a biomarker. Their appearance in MMR gene defect carriers despite a lack of recorded malignancy [20] suggests this feature could increase the power of the trial to demonstrate a clinically significant difference between the different doses of aspirin, even in the younger recruits where overt cancers are more rare.

## Conclusion

Chemoprevention for gastrointestinal cancer is desirable but the many logistical challenges make it difficult to get effective agents into clinical practice. After 25 years we now have sufficient evidence to recommend aspirin in people at high risk of colorectal cancer, particularly those with LS. We must continue to explore the underlying mechanisms and combine this with efforts to refine the dose. The failure to demonstrate an effect of a daily supplement of resistant starch was disappointing. It may be that we must accept that dietary components do not lend themselves to the formal randomised controlled trial approach. Nevertheless, even though the study did not prove an effect, it demonstrated the resilience of people with LS and their willingness to be active partners in the search for effective methods of prevention.

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