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Polymorphisms in the *adenomatous polyposis coli* (APC) gene and advanced colorectal adenoma risk

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ABSTRACT

While germline mutations in the *adenomatous polyposis coli* (*APC*) gene cause the hereditary colon cancer syndrome (familial adenomatous polyposis (FAP)), the role of common germline *APC* variants in sporadic adenomatous polyposis remains unclear. We studied the association of eight *APC* single nucleotide polymorphisms (SNPs), possibly associated with functional consequences, and previously identified gene–environment (dietary fat intake and hormone replacement therapy (HRT) use) interactions, in relation to advanced colorectal adenoma in 758 cases and 767 sex- and race-matched controls, randomly selected from the screening arm of the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Cases had at least one verified advanced adenoma of the distal colon; controls, a negative sigmoidoscopy. We did not observe an association between genotypes for any of the eight *APC* SNPs and advanced distal adenoma risk ($P_{global\ gene\text{-}based} = 0.92$). Frequencies of identified common haplotypes did not differ between cases and controls ($P_{global\ haplotype\ test} = 0.97$). However, the risk for advanced distal adenoma was threefold higher for one rare haplotype (cases: 2.7%; controls: 1.6%) (odds ratio (OR) = 3.27; 95% confidence interval (CI) = 1.08–9.88). The genetic association between D1822V and advanced distal adenoma was confined to persons consuming a high-fat diet ($P_{interaction} = 0.03$). Similar interactions were not observed with HRT use. In our large, nested case-control study of advanced distal adenoma and clinically verified adenoma-free controls, we observed no association between specific *APC* SNPs and advanced adenoma. Fat intake modified the *APC* D1822V-adenoma association, but further studies are warranted.

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1. Background

Colorectal cancer (CRC) is the leading cause of cancer-related death, second only to lung cancer in developed countries.[1,2] Though fewer than 10% of colorectal adenomas are thought to progress to adenocarcinomas,[3] more than 70% of colonic carcinomas are thought to arise within pre-existing sporadic precursors of malignant lesions, adenomatous polyp (adenoma), of the colorectal epithelium.[4] Advanced adenoma, associated with the greatest increased risk of CRC, is characterised as large (>1 cm) adenomatous polyps and polyps with villous or tubulovillus histology, specifically the presence of multiple adenomas, or high-grade dysplasia.[5] Other well-documented risk factors for colorectal cancer include positive family history of colorectal cancer, age, a personal history of inflammatory bowel disease, race/ethnicity, level of education, smoking, calcium intake, folate intake and non-steroidal anti-inflammatory drugs intake.[6,7] Apart from age and calcium intake, risk factors for advanced colorectal adenoma (as compared to low-grade polyps or polyp-free controls) are less understood with inconsistent findings implicating smoking, obesity, physical activity among men and hormone replacement therapy use in women, folic acid intake and non-steroidal anti-inflammatory drugs.[8–12] Adenomas are very prevalent among asymptomatic persons (12–43%)[13–16] as compared to CRC. Defining the risk factors for advanced adenomas, an intermediate marker in CRC development, will facilitate colorectal cancer prevention.

A model of CRC development is an autosomal-dominantly inherited CRC predisposition syndrome, familial adenomatous polyposis coli (FAP; MIM#175100).[17–19] The genetic cause of FAP is germline loss-of-function mutations in the tumour suppressor gene, *adenomatous polyposis coli* (*APC*).[17–19] These *APC* mutation carriers are predisposed to develop thousands of colorectal adenomas a subset of which will subsequently progress to invasive colorectal tumours.[20] The *APC* gene spans 108,352 base pairs on chromosome 5q21 and has 21 exons encoding a protein with multiple functional domains that interact with regulators of proliferation and apoptosis.[21] *APC*-inactivating mutations in somatic cells lead to constitutive stimulation of a crucial pathway, the Wnt/β-catenin signalling pathway,[22] where activating Wnt/β-catenin mutations are found in approximately 90% of CRC.[23] Loss-of-function *APC* mutations in CRC predominate in the β-catenin control domain,[24,25] resulting in truncated APC proteins and inappropriate stabilisation of β-catenin that leads to activation of target genes in carcinogenesis, e.g. oncogenes cyclin D1 and *c*-MYC.[26]

Although somatic mutations in the *APC* gene clearly play a role in the formation of colorectal adenomas,[27] inactivating germline, APC mutations are only observed in the context of FAP and a less severe subtype, attenuated FAP. As most (>95%) of CRC occurs in individuals without a family history of FAP,[28] it is possible that common variants in the APC gene contribute to CRC risk. We hypothesise that relatively common (>2%) single nucleotide polymorphisms (SNPs) in the *APC* gene predispose to the continuum of colorectal adenomatous phenotype and underlie risk of colonic adenoma. Individuals with these germline, low penetrance variants may possess APC alleles with reduced, but not fully inactivated APC activity.[29,30] Reduced APC activity may render these individuals more susceptible to polyp formation and CRC, especially in conjunction with other environmental or genetic exposures.

There is a paucity of data on common genetic variants in the *APC* gene and colorectal adenoma risk. Previous studies[31–43] have focused on four germline missense variants, i.e. *APC* I1307K, E1317Q, D1822V and G2502S, of which, *APC* I1307K[32,44] and *APC* E1317Q[31] are founder mutations in Ashkenazi Jews and have a rare prevalence (minor allele frequency (MAF) of <1%) in non-Hispanic Whites.[33–36] In Askenazim, *APC* I1307K (MAF = 6%) is associated with risk of colon cancer without the corresponding polyposis seen in FAP patients[32,44] and *APC* E1317Q[31] is associated with colorectal tumours in some but not all studies.[38,39] In non-Hispanic White populations, the remaining two reported *APC* SNPs, D1822V and G2502S, have an MAF of 22.5% and 2.5%, respectively (120 HapMap representative European ancestry panel).

Potential interactions between common genetic variants in the APC gene and lifestyle factors with colorectal adenoma have been even less well studied. In CRC, the common *APC* D1822V variant has previously been inconsistently reported to interact with dietary fat intake[41–43] and post-menopausal hormone therapy (HRT) use.[43] Dietary fat intake is a risk factor for colorectal adenoma in some[45–47] but not all studies.[48] The possible effect of dietary fats on colorectal adenoma risk may differ with *APC* allelic variation, but in the only study that examined this association in colorectal adenomas using unscreened controls, no interaction was observed.[43] On the other hand, high intake of fats and red meat was shown to increase the risk of sporadic colorectal adenomas that tested negative for truncating somatic *APC* mutations.[49] HRT use in women was associated with reduced risk of advanced adenomas in some[8,50] but not all[51,52] studies. Only one study examined the interaction between HRT use and *APC* D1822V and reported an interaction for CRC risk but not for colorectal adenoma.[43]

To address if common genetic variants in the *APC* gene, alone or in combination with previously reported lifestyle factors, are associated with risk of colorectal adenomatous polyp, we examined eight *APC* SNPs in 758 cases with at least one verified advanced adenoma of the distal colon compared with a control group of 769 controls clinically verified free from colonic adenoma selected from a United States-based multi-institutional screening trial. The *APC* SNPs we examined include *APC* D1822V, G2502S and six other SNPs selected because they may be associated with functional consequences. We also examined associations between haplotypes defined by the eight *APC* SNPs with advanced adenoma of the distal colon. Last, we explored gene–environment interactions on the common non-synonymous SNP, *APC* D1822V, previously inconsistently reported to interact with the lifestyle factors, dietary fat intake[41–43] and HRT use[43] in colorectal cancer risk.

2. Materials and methods

This case-control study was nested within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, which was designed to evaluate selected methods for the early detection

Table 1 – Characteristics of the study population, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial.

Characteristics	Controls (N = 769)	Cases (N = 758)	P-value
	N(%) or median (range)	N(%) or median (range)	
Sex			
Male	532 (69.2)	530 (69.9)	0.78[a]
Female	237 (30.8)	228 (30.1)	
Age (years)			
55–59	360 (46.8)	252 (33.3)	<0.001[a]
60–64	198 (25.8)	238 (31.4)	
65–69	138 (17.9)	170 (22.4)	
70–74	73 (9.5)	98 (12.9)	
Race			
Non-Hispanic White	721 (93.8)	711 (93.8)	1.0[a]
Non-Hispanic black	23 (3.0)	22 (2.9)	
Others	25 (3.2)	25 (3.3)	
Education			
<12 years	49 (6.4)	69 (9.1)	0.001[a]
12 years/high school equivalent	174 (22.7)	189 (24.9)	
Some college	244 (31.7)	274 (36.2)	
College and above	301 (39.2)	226 (29.8)	
Body mass index			
<18.5	2 (0.3)	5 (0.6)	0.30[a]
18.5–24.9	211 (27.8)	193 (25.6)	
25–29.9	356 (46.8)	342 (45.3)	
>30	191 (25.1)	215 (28.5)	
Smoking exposure (pack-years)	1 (0.7)	2 (0.7)	0.001[b]
NSAIDs usage, >1 times/week (1 year before randomisation)			
Never	305 (39.7)	314 (41.5)	
Ever	464 (60.3)	443 (58.50)	0.50[a]
Total calories from diet (kcal/d)	2031.1 (518.5, 7548.5)	1979.2 (338.7, 5593.9)	0.26[b]
Total dietary fat intake (g/d)	66.2 (94.0, 3146.2)	64.0 (71.8, 2984.4)	0.26[b]
Dietary folate and supplements (μg/d)	549.4 (94.0, 3146.2)	501.5 (71.8, 2984.0)	0.01[b]
Dietary calcium and supplements (mcg/d)	1109 (148.4, 5135.5)	1041.8 (132.4, 3874.7)	0.006[b]
Postmenopausal hormone use			
Never	76 (32.2)	78 (34.2)	0.70[a]
Ever	160 (67.8)	150 (65.8)	
Family history of colorectal cancer (sibling, mother, father)			
No	699 (90.9)	662 (87.3)	0.03[a]
Yes	70 (9.1)	96 (12.7)	

[a] Fisher exact test.

[b] Kruskal–Wallis: 758 cases and 769 controls have at least one genotype available for eight *APC* single nucleotide polymorphisms listed in Table 2. *Note*: mean ± SD for smoking (pack-years) controls versus cases: 1.97 ± 2.27 versus 2.38 ± 2.32.

of these cancers and to investigate aetiologic factors and early markers of cancer.[53,54] Participants in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, ages 55–74 years, were recruited at 10 centres in the United States (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St Louis, MO and Washington, DC). Participants in the screening arm of the trial received sigmoidoscopic exam at baseline. If the sigmoidoscopy identified polyps or other suspect lesions, participants were advised to get further follow-up examination through their own medical care providers, which usually resulted in a full colonoscopy with polypectomy or surgical procedures, if indicated. All medical and pathologic reports of the follow-up examinations were obtained and coded by trained medical record abstractors. Written informed consent was obtained from participants, and the trial received approval from the institutional review boards of the US National Cancer Institute and the 10 study centres.

2.1. *Study population*

All cases and controls for this study were selected from 42,037 participants in the screening group who underwent a successful sigmoidoscopic examination at baseline (insertion to at least 50 cm with >90% of mucosa visible or a suspect lesion identified). Baseline flexible sigmoidoscopy (60 cm) screening of selected cases and controls was conducted between September 1993 and September 1999. Participants provided information on risk factors and donated a blood sample for use in aetiologic studies. After exclusion of 4834 participants with a self-reported history of cancer (except basal cell skin cancer),

ulcerative colitis, Crohn's disease, familial polyposis, colorectal polyps or Gardner's syndrome, 1234 cases with advanced distal adenoma (adenoma ⩾1 cm or containing villous elements or high-grade dysplasia) were available for study. We randomly selected 772 of these cases for budgetary reasons for inclusion in our analyses. A total of 777 sex- and race-matched participants with a negative screening sigmoidoscopy (i.e. no polyp or other suspect lesion; $n = 26{,}651$) were randomly selected as controls. The distributions by gender (about 31% female) and race/ethnic origin (about 94% White, non-Hispanic) were similar for cases and controls because we matched on these two variables.

2.2. Questionnaire-derived information

At initial screening, all participants completed a questionnaire covering sociodemographic factors, medical history and other risk factors for cancer including body mass index and family history of cancer. Information on hormone replacement therapy in the questionnaire was on current and former use (ever and current use of tablets, pills, creams) and duration of use; the formulation and dose of hormones were not ascertained. A 137-item food frequency questionnaire was administered to assess dietary intake including total dietary fat, folate and calcium intake.

2.3. SNP selection and genotyping

Initial selection of SNPs were focused on those within exonic gene regions and exon–intron junctions with priority given to non-synonymous change, those linked with FAP in previous studies, previously described effect on APC function, located in either functionally defined domains of the APC protein (e.g. β-catenin binding sites or armadillo repeat) and/or located within regions of shared identity across the mouse, rat and frog orthologs of APC. This led to the selection of nine SNPs that were verified in the NCI Core Genotyping Facility SNP500 panel of 102 individuals of self-described White ($n = 31$), African-American ($n = 24$), Hispanic ($n = 23$) and Pacific Rim ($n = 24$) race/ethnicity[21] by re-sequencing approximately 300 base pairs of DNA on either side of the putatively polymorphic locus (Table 2). One SNP (APC P870S) was removed from further analysis because it was monomorphic.

DNA was extracted with standard methods from the blood samples (buffy coat or whole blood samples) collected at study entry from 772 cases and 777 controls. Genotyping of the SNPs was performed at the Core Genotyping Facility of the National Cancer Institute using TaqMan (Applied Biosystems, Foster City, CA). Protocols for each specific assay are documented at <http://snp500cancer.nci.nih.gov>.[55] For validation purposes, TaqMan assays were initially applied to the 102 individuals with sequence information and were subsequently applied to the PLCO samples, only if sequencing and TaqMan results were 100% concordant, otherwise a new TaqMan assay was designed. Interassay concordance for all assays using blinded quality control samples (40 participants assayed two to four times, total 136 results) was 100%. All SNPs did not deviate from Hardy–Weinberg expectations among White controls ($P > 0.05$, exact test). Depending on the batch, 0.5–8.3% of the subjects had insufficient DNA for genotyping and a small percentage of participants (<1%) were found to have discrepancies on repeated DNA fingerprint analysis. Of those with sufficient DNA, genotyping was successfully completed for 96.3–99.5% of the participants, depending on the genotype. At least one genotype was available for 758 cases and 769 controls (Table 3).

2.4. Statistical methods

2.4.1. Single locus analyses

To assess the strength of association between genotypes and cancer risk, unconditional logistic regression models[56] were used to estimate odds ratio (ORs) and their corresponding 95% confidence intervals (CIs), adjusting for age (55–59, 60–64, 65–69 and 70–74 years), sex and screening centre. We also conducted analyses adjusting for race/ethnicity (non-Hispanic White, Black, Others) and subsetting analyses to non-Hispanic Whites given they comprised 94% of the sample. The genotype-specific risks of homozygotes for the rare

Table 2 – Description of the interrogated APC single nucleotide polymorphisms.

Position in gene	Amino acid location	dbSNP identifier build 127	Protein effect/functional domain[a]	Conserved regions[b]	Distance to next SNP, base pairs	Minor allele frequency, Whites, %[c]
Intron 2	53 bp upstream of Exon 3	rs2304793	–	Yes	–	2.2
Exon 12	Y486Y	rs2229992	Silent/armadillo repeats	Yes	60,884	40.2
Intron 14		rs548710	–	Yes	4733	48.4
Intron 14		rs2909786	–		478	44.0
Exon 16	T1493T	rs41115	Silent/CRC mutational cluster	Yes	1871	37.1
Exon 16	G1678G	rs42427	Silent/β-cateninbinding		555	37.2
Exon 16	D1822V	rs459552	Missense/β-cateninbinding	No	431	22.4
Exon 16	G2502S	rs2229995	Missense		2039	2.3

[a] Armadillo: β-catenin D. melanogaster homologue; CRC mutational cluster: hotspot for germline and somatic mutations in CRC; β-catenin binding: domain that controls β-catenin downregulation (codons 1324–2075).
[b] Evolutionarily conserved regions: at least 100 base pairs with >80 % nucleotide identity.
[c] Minor allele frequency calculated in non-Hispanic Whites (94% of the study population).

Table 3 – Association between polymorphisms in APC and advanced distal colorectal adenoma risk in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial.

dbSNP identifier (gene location)	Controls/cases	OR (95% CI)[a]	P for trend
rs2304793 (Intron 2)			
TT	673/647	1.0 (reference)	0.14
TC	30/38	1.48 (0.88, 2.51)	
CC	–	N/A	
rs2229992 (Ex12, Y486Y)			
CC	251/246	1.0 (reference)	0.72
CT	340/333	1.02 (0.80, 1.29)	
TT	114/116	1.06 (0.77, 1.47)	
rs548710 (Intron 14)			
CC	177/161	1.0 (reference)	0.78
CT	332/348	1.12 (0.85, 1.47)	
TT	195/179	0.97 (0.71, 1.32)	
rs2909786 (Intron 14)			
AA	223/215	1.0 (reference)	0.76
AG	331/343	1.08 (0.85, 1.39)	
GG	144/124	0.92 (0.67, 1.27)	
rs41115 (Ex16, T1493T)			
AA	272/275	1.0 (reference)	0.77
AG	339/324	0.99 (0.71, 1.38)	
GG	95/90	1.03 (0.73, 1.45)	
rs42427 (Ex16, G1678G)			
AA	296/296	1.0 (reference)	0.94
AG	366/360	0.97 (0.70, 1.34)	
GG	95/95	0.97 (0.70, 1.39)	
rs459552 (Ex16, D1822V)			
AA	455/462	1.0 (reference)	0.65
AT	271/251	0.92 (0.74, 1.15)	
TT	31/33	1.03 (0.61, 1.74)	
rs2229995 (Ex16, G2502S)			
AA	669/634	1.0 (reference)	0.54
AG	26/30	1.18 (0.68, 2.05)	
GG	–	N/A	

[a] OR: odds ratio, adjusted for age, sex, screening centre and race. CI: confidence interval.

allele and heterozygotes were contrasted with the homozygote common allele genotype. Tests for linear trend were conducted by including a single variable for each SNP, coded as the number of variant alleles in the regression model and evaluated with the Wald test statistic.

An omnibus test for association was conducted by comparing nested models, with and without the eight SNPs in the saturated and reduced model, respectively, using a likelihood ratio χ^2 statistic.

To assess interaction between the *APC* D1822V and lifestyle factors previously reported in the literature (dietary fat intake and HRT use), as well as to explore associations with other lifestyle factors associated with advanced adenoma in this population (gender, education level, folate intake, total calcium intake, family history of CRC), respective multiplicative interaction terms were included in regression models and Wald test statistics were conducted. All single locus analyses were performed with adjustment for race/ethnicity in multivariate models and separately among non-Hispanic Whites only given they comprised 94% of the study population. Since results were similar, race/ethnicity-adjusted results were presented for single locus analyses and gene–environment interactions.

2.4.2. *Multilocus analyses*

Linkage disequilibrium (LD) among the eight *APC* SNPs was assessed by Lewontin's D' and pairwise R^2.[57] To estimate haplotype frequencies from genotype information within our population of unrelated individuals, the expectation–maximisation (EM) algorithm implemented in the Haploview software[58] was used to resolve phase uncertainties in non-Hispanic Whites (94% of the study population). Haplotype blocks were defined using the four-gamete test for recombination[59] implemented in Haploview.[58] Haplotype analyses, adjusting for age, sex and clinical centre, were performed using the haplo.stats package.[60] As a global test of heterogeneity between the haplotype frequencies of cases and controls, a global score test, adjusting for age, sex and clinical centre, was conducted. All haplotype analyses are presented for non-Hispanic Whites only; results did not materially change when the other race/ethnic subgroups were included in analyses.

3. Results

In our nested case-control study from the PLCO cohort (previously described[61]), cases with advanced distal adenoma tend to be older, less educated, smoke more, have a family history of CRC and consume less folate and calcium (Table 1). The minor allele frequencies of the eight *APC* SNPs ranged from 2.2% to 48.4% among the non-Hispanic Whites that comprised 94% of the cases and controls (Table 2).

We examined the association between each of the *APC* SNPs on advanced distal adenoma risk (Table 3). Overall, we did not observe an association between advanced distal adenoma risk and any of the eight *APC* SNPs at each individual locus. Results for the eight *APC* SNPS did not differ by anatomic site (colon versus rectal adenomas; data not shown) or by number of adenomas (one versus multiple; data not shown). Using a gene-based test, we examined whether at least one of the SNPs had an independent association with advanced distal adenoma, adjusting for the others, to take into account LD among SNPs; there was no statistical evidence of an effect of at least one *APC* SNP on risk of advanced distal adenoma for all race/ethnicities, adjusting for race (global $P = 0.92$). Results were similar when restricted to non-Hispanic Whites (global $P = 0.90$).

We further investigated if multiple disease-causing APC alleles underlie sporadic adenoma susceptibility. High pairwise LD between adjacent SNPs, measured by the LD metric D' (range: 0.95–0.99), indicated little evidence for recombination spots within the *APC* genomic region represented by our selected eight SNPs. Using the four-gamete rule[59], all eight *APC* SNPs were represented in two haplotype blocks that captured at least 99.5% of the haplotype diversity in our population (Table 4). Overall frequencies of the common haplotypes did not statistically significantly differ between cases and controls (P for global haplotype test > 0.05). One rare haplotype was marginally associated with advanced distal colorectal adenoma risk (odds ratio (OR) = 3.27; 95% confidence interval (CI) = 1.08–9.88). Results did not differ when restricted to non-Hispanic Whites (OR = 3.96; 95% CI = 1.10–14.32). The 'at-risk' haplotype is defined by the rs2304793 C allele, rs2229992 T allele and rs548710 T allele (rs2304793_C-rs2229992_T-rs548710_T).

Results were similar when haplotype blocks were defined using the solid spline algorithm, which assigned the first two SNPs (rs2304793 and rs2229992) in one block and the remaining six SNPs in the second block. In the first block, the haplotype rs2304793_C-rs2229992_T was marginally significantly over-represented among cases as compared to the most common haplotype (2.2% versus 1.2% in controls), rs2304793_T-rs2229992_C (non-Hispanic Whites: OR = 4.14; 95% CI: 1.03–16.58). When haplotype blocks were defined by the 95% confidence-bound SNP pair rule no association was observed (data not shown). However, this is not surprising as this algorithm did not assign the first two SNPs (rs2304793, rs2229992) and the last SNP (rs2229995) to a haplotype block.

3.1. *Gene–environmental interactions*

We explored effect modification of *APC* D1822V by total dietary fat intake[42] and HRT use,[43] based on previously reported statistically significant interactions with CRC identified in the literature (Table 5). The genetic association between D1822V and advanced distal adenoma was confined to persons consuming a high-fat diet (P for interaction = 0.03); compared to persons not carrying a copy of the T allele (Asp/Asp variant), those carrying at least one copy (Asp/Val or Val/Val variants) had approximately 30% decreased risk for advanced adenoma (OR: 0.74; 95% CI: 0.55–1.00). This association was not observed in the low fat intake group (OR: 1.18, 95% CI: 0.86–1.60). No significant interaction was observed with *APC* D1822V and HRT use (Table 5). We also examined potential interactions between *APC* D1822V and risk factors associated with advanced distal adenomas in this population (Table 1, gender (male, female), level of education (low, high), total folate intake (<median in controls, >median in controls), total calcium intake (<median in controls, >median in controls) and family history of CRC (no, yes)). We observed no statistically significant interactions between *APC* D1822V and any of these risk factors (data not shown).

Table 4 – Association between four-gamete defined haplotypes of APC and advanced distal colorectal adenoma risk in non-Hispanic Whites in the Prostate, Lung, Colorectal and Ovarian Screening Trial.

Haplotype	Cases (%)	Controls (%)	OR[a]	95% CI[a]	P[b]
Block 1: rs2304793, rs2229992, rs548710, rs2909786					
T-C-C-A	50.9	51.3	1.00	Ref.	
C-T-T-A	2.7	1.6	3.27	1.08-9.88	0.04
T-C-T-G	8.0	8.3	1.00	0.56–1.76	0.98
T-T-T-A	2.7	2.5	1.10	0.43–2.81	0.84
T-T-T-G	35.2	35.7	1.01	0.73–1.41	0.95
Block 2: rs41115, rs42427, rs459552, rs2229995					
A-A-A-T	60.6	61.0	1.00	Ref.	
A-A-A-C	2.2	1.9	1.37	0.45–4.16	0.58
G-G-A-T	15.1	14.7	1.08	0.70–1.67	0.72
G-G-T-T	21.5	21.9	0.96	0.66–1.38	0.81

[a] OR: odds ratio, adjusted for age, sex, screening centre and race/ethnic groups. CI: confidence interval.
[b] Global haplotype test (exact test) $P = 0.97$ for all race/ethnic groups and $P = 0.94$ for non-Hispanic Whites only.

Table 5 – APC D1822V and advanced distal colorectal adenoma risk by dietary fats and post-menopausal use, Prostate, Lung, Colon, Ovarian Screening Trial.

Genotype	Control/case	OR (95% CI)	Control/case	OR (95% CI)[a]	$P_{interaction}$
Dietary fats[b]	Low (<66.2 g/d)		High (⩾66.2 g/d)		
rs459552 (Ex16, D1822V)					
AA	232/214	–	223/248	–	0.03
AT	118/138	1.22 (0.88, 1.68)	153/113	0.68 (0.50, 0.94)	
TT	16/18	1.15 (0.55, 2.37)	15/15	1.01 (0.47, 2.17)	
AA versus AT/TT		1.17 (0.86, 1.60)		0.74 (0.55, 1.00)	
Postmenopausal hormone use	Never		Ever (past and current)		
rs459552 (Ex16, D1822V)					
AA	40/47	–	95/88	–	0.43
AT	27/26	0.76 (0.35, 1.65)	58/57	1.07 (0.65, 1.76)	
TT	4th June	0.67 (0.16, 2.84)	5th May	1.09 (0.29, 4.04)	
AA versus AT/TT		0.70 (0.34, 1.42)		1.04 (0.64, 1.70)	

[a] OR:odds ratio, adjusted for age, sex, screening centre and race.CI:confidence interval.
[b] Low and high dietary intake as determined by above and below median intake in persons without adenoma.

4. Discussion

In the largest study to date of APC common variants and advanced colorectal adenoma risk, our single locus and haplotype data do not support the role of common polymorphisms in *APC* in colorectal development. No statistically significant associations were observed between any of the eight *APC* SNPs examined and advanced distal adenomas. One rare 'at-risk' haplotype was over-represented in advanced distal adenoma cases as compared to controls (2.7% in cases versus 1.6% in controls), no other identified haplotypes were associated with increased risk. While our initial SNP selection utilised the candidate SNP strategy, the chosen SNPs are in $R^2 > 0.8$ for some of the SNPs that cover the APC genomic region and thus are informative in haplotype analyses. We did not find evidence for gene–lifestyle interactions.

Of the eight SNPs examined, two missense variants previously examined in Whites (D1822V and G2502S) and six SNPs selected for possible functional effects all had similar genotype frequencies among both cases with advanced distal adenoma and controls. This finding is consistent with the four previous studies that examined associations between D1822V,[40–43] G2502S[43] and CRC[40–43] and one with colorectal adenoma[43] where no single locus associations were observed.

We next asked if cis-combination effects of 'potential *APC* causal variants' underlie advanced adenoma susceptibility. One rare 'at-risk' haplotype is over-represented in advanced distal adenoma cases as compared to controls (2.7% in cases versus 1.6% in controls). Given that the global haplotype test did not support that overall haplotype frequencies in cases differ from controls, larger studies are required to confirm this finding. If this is not a spurious finding, one biological explanation for this haplotype association is that multiple rare *APC* variants account for adenoma susceptibility and the biological effect is captured by the potentially more powerful haplotype-based test as compared to individuals SNPs.[62] Alternatively, since none of the eight SNPs examined, including the previously reported *APC* D1822V, was independently associated with colorectal adenoma risk, the observed 'at-risk' haplotype may be linked to an as yet unidentified 'causal variant'.

It has been proposed that low penetrance, germline variants in the genes *APC/WNT* signalling pathway contribute to CRC risk in the general population.[63] Our finding that common *APC* variants may not contribute to sporadic colorectal adenomatous polyp development, does not exclude the possibility that rare variants in the APC gene do contribute to CRC risk in humans. In a previous study, multiple rare germline *APC* non-synonymous variants (MAF < 2%) were over-represented in patients with colorectal adenomas relative to healthy controls.[30]

Our findings in APC are consistent with what has been observed in studies of breast and ovarian cancer susceptibility genes, *BRCA1* and *BRCA2*.[64–67] Like APC, these genes were identified in families with autosomal dominant transmission of cancer risk. These families segregate rare, high penetrance, germline mutations, but studied common polymorphisms in *BRCA1* and *BRCA2* have not been associated with disease risk.[64–66] A weakly significant and modest increased risk of 20% with a *BRCA1* haplotype and sporadic breast cancer was reported, but the global test of haplotype frequency difference between cases and controls was not significant.[67] Our observations and the well-documented loss of heterozygosity observed in tumours suggest that a complete loss of APC function may be required to produce adenoma risk.

The association between *APC* D1822V has been examined in several previous studies and no association was observed between this SNP and either CRC[40–43] or colorectal adenomas.[43] Associations have been reported, albeit, inconsistently, between this SNP and CRC or colorectal adenomas when study subjects were stratified by dietary fat intake[42] or HRT use.[43] To shed light on these previously reported inconsistencies, we explored gene–environment interactions between APC D18V22V and dietary fat intake and HRT use. We observed that *APC* D1822V influenced the risk of advanced distal adenoma in individuals with the highest fat intake (376 cases and 391 controls), in contrast to a previous report of an association between *APC* D1822V and CRC in individuals with the lowest fat intake (lowest tertile versus highest tertile of fat intake, 1585 colon cancer cases and 1945 controls).[42] No interaction between APC D18V22V and dietary fat intake, however, was observed in subsequent smaller CRC studies[40,41,43] or in

the only other study of colorectal adenomas (556 cases and 557 controls, Nurses Health Study; 197 cases and 490 controls, Physician's Health Study).[43] For HRT use, we did not observe any interaction between the *APC* D1822V and advanced distal adenoma risk in women (231 cases and 227 controls). The Nurses' Health Study did report an interaction between HRT use and *APC* D1822V for CRC (197 CRC cases, 490 controls) but consistent with our findings did not observe an association for adenomas (556 adenoma cases, 557 controls).[43]

Our study has several strengths and limitations important to consider in evaluating common *APC* variants, lifestyle factors and advanced distal adenoma risk. The prospective design of the PLCO study makes it less susceptible to selection bias or population stratification. Our cases and controls were all drawn from members of the PLCO cohort and thus well-matched. In addition, misclassification of controls in the PLCO is limited due to undetected distal adenoma in our comparison group of sigmoidoscopy-screened controls. Dietary data are also prospectively collected and therefore not subject to differential recall bias in report of total dietary fat intake and HRT use. One limitation is the lack of information on the presence of proximal adenoma in the controls; however, the prevalence of advanced proximal neoplasia among patients with no distal adenoma is less than 3% (1.5% and 2.7%).[14,16] Another limitation is the lack of a comprehensive definition of HRT use in this study. Though the largest APC-advanced adenoma study to date, our study was also insufficiently powered to robustly investigate previous reports of gene–environment interactions; we examined crude subgroups of environmental factors, i.e. dichotomous categories of total dietary fat intake rather than more refined quantiles of intake.

In conclusion, the data presented here represent one of the most comprehensive studies of the association between SNPs in the *APC* gene and the risk of colorectal adenoma. We did not observe strong evidence for the role of eight examined *APC* SNPs in advanced colorectal adenoma susceptibility. A marginally significant association was observed between a rare 'at-risk' haplotype that is defined by an intron 2 SNP (rs2304793), an Exon 12 silent SNP (rs2229992) and an Intron 14 SNP (rs2909786) with advanced distal adenomas. Interactions between low- and high-fat intake and D1822V with advanced distal adenoma risk were also noted, but results were inconsistent with previous findings. Further studies are warranted to confirm both observed haplotype and potential D1822V–dietary fat interaction associations with advanced distal adenomas.

Conflict of interest statement

None declared.

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