Common variation at the adiponectin locus is not associated with colorectal cancer risk in the UK

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A recent study examined common genetic variants at the adiponectin locus (ADIPOQ) in two case–control colorectal cancer (CRC) series from the USA and reported a positive association between a single nucleotide polymorphism (SNP) in the 5′ region of the gene (rs266729) and decreased disease risk. In an attempt to replicate the previously reported association, we examined data from two CRC genome-wide association studies based on the UK population. The first cohort comprised 931 familial colorectal tumour cases and 929 cancer-free controls. The second included 1216 individuals with Dukes stage B or C CRCs from two clinical trials and 1436 controls from the 1958 Birth Cohort. We tested associations between CRC risk and 82 SNPs in a region of 250 kb around the ADIPOQ gene; nine of these SNPs were located in the coding and promoter regions. None of the markers tested was significantly associated with CRC risk after correction for multiple testing under any of the models in any of the two cohorts. A meta-analysis of the data also failed to detect any association. We, therefore, failed to replicate an association between common variants at ADIPOQ and CRC risk in the UK, and suggest that the previous report is either population-specific or a false-positive result.

INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer morbidity and mortality in the western world. Studies have suggested that genetic factors can explain about a third of the trait variance (1). An important fraction of this genetic variation is probably explained by either rare or common low-penetrance loci. Recently, we have used genome-wide association studies (GWASs) to demonstrate the existence of common low-penetrance CRC susceptibility alleles, resulting in the discovery of 10 low-penetrance CRC risk loci to date (2–9). It is highly likely that there are more low-penetrance CRC loci to be found using GWAS and other approaches.

Candidate gene studies have been used extensively to investigate the role of low-penetrance alleles in CRC risk (10). Unlike GWASs, candidate gene studies have rarely detected reliable and consistent associations. This has been largely due to intrinsic difficulties in selecting candidate single nucleotide polymorphisms (SNPs) and/or the use of relatively small sample sizes. Although the use of larger studies will increase the chances of finding associations between common alleles at candidate loci, the selection of the candidates to be investigated, most of which are SNPs in coding regions, remains inherently difficult. In this respect, our GWASs have highlighted the importance of regulatory regions in CRC susceptibility and do not suggest that common, non-synonymous changes are heavily involved in disease risk (6,7).

Using a candidate gene approach, Kaklamani et al. (11) recently investigated the relationship between genetic variation at the adiponectin (ADIPOQ) locus and CRC in North American populations. ADIPOQ protein levels are inversely associated with obesity and hyperinsulinaemia (12), and ADIPOQ promoter polymorphisms have been shown to affect the protein’s plasma levels (13). ADIPOQ is an interesting and plausible candidate CRC gene, because there is evidence of an association between obesity and the disease and because a previous study reported increased ADIPOQ...
plasma levels in men with CRC (14,15). Kaklamani et al. reported that the minor allele at rs266729, a SNP located in the 5’ region of the ADIPOQ gene, was associated with decreased CRC risk under a dominant disease model. The odds ratio associated with the protective ADIPOQ genotypes was 0.64, making it a stronger effect than any other CRC SNP and suggesting an effect independent of obesity. Owing to the potential importance of ADIPOQ, we examined associations between tagging SNPs around the ADIPOQ locus and CRC risk using the data of our two large case–control series from the UK.

**RESULTS**

We examined 82 tagging SNPs spanning ~250 kb around the ADIPOQ locus in 921 familial cases of colorectal tumour [CRC or ‘advanced’ adenoma(s)] and 929 cancer-free controls, all from the UK. These samples were recruited through the COloRectal Gene Identification Consortium (CORGI). In order to assess statistical significance, we used a global threshold of $P = 0.05$, resulting in a nominal threshold of $P = 6 \times 10^{-4}$ after correction for multiple testing using the conservative Bonferroni method. None of the 82 SNPs showed any significant association with colorectal tumour risk under any of the disease models tested (see Supplementary Material, Table S1). A minimum $P$-value of 0.002 was found using the recessive model at rs6807774, 96 kb proximal to ADIPOQ. The minimum $P$-value for the dominant model, the one used by Kaklamani et al. to find their association (11), was 0.016 at rs6767800, 58 kb distal to ADIPOQ (see Fig. 1).

Nine of the 82 tagging SNPs were located in the promoter and coding region of ADIPOQ. The $P$-values under the dominant model for these SNPs ranged from 0.46 (rs10937273) to 0.76 (rs3821799). rs266729, the associated SNP reported by Kaklamani et al. (11), is not included in the arrays typed in our samples, but was in moderate/strong linkage disequilibrium with the one used by Kaklamani et al. to find their association.

![Figure 1. Association between SNPs around the ADIPOQ locus and CRC risk in the white population from the UK. SNP physical position (in megabases, x-axis) and negative log of uncorrected $P$-values (y-axis) for the dominant model are shown. The arrow depicts the approximate physical location (chromosome 3:188 043 157–188 058 946 bases) of the ADIPOQ gene.](image)

We then examined ADIPOQ SNP data from our VQ58 case–control series of 1216 CRC patients and 1436 control individuals. We focused on three SNPs (rs10993273, rs1648707 and rs822387) located in the ADIPOQ 5’ region where rs266729 lies. We found no significant association between two of these SNPs (rs10993273 or rs822387) and CRC under any of the disease models (specifically, dominant $P = 0.67$ and dominant $P = 0.23$, respectively; see Supplementary Material, Table S2). A nominally significant $P$-value ($P = 0.01$) was found for the third SNP, rs1648707, under the dominant model. However, the minor allele at this locus (rs1648707C) was associated with increased CRC risk (OR = 1.225; 95% CI: 1.049–1.43), whereas—on the basis of the known strong LD relationship between rs1648707 and rs266729 and the results of Kaklamani et al.—a protective effect was expected. Imputation showed that genotypes at rs266729 were not associated with CRC risk under any model in the VQ58 study (for example, dominant model $P = 0.70$, Table 1).

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A meta-analysis of the CORGI and VQ58 case–control series for rs1648707 and rs266729 did not detect any association between CRC risk and genotypes at either of these two loci (rs1648707: dominant model, $P = 0.24$, OR = 1.06; rs266729: dominant model, $P = 0.91$, OR = 1.00). We, therefore, failed to replicate an association between common ADIPOQ variants and CRC risk in the UK.

**DISCUSSION**

We tested association between common variation around the adiponectin locus and CRC risk in two large case–control
samples from the UK. Our study failed to replicate the previously reported association in North American populations (11). Our inability to replicate the study carried out by Kaklamani et al. on ADIPOQ variation and CRC risk may result from differences between ethnic groups: Kaklamani et al.’s first case–control series was of Ashkenazi origin and their second group was of mixed origin. In addition, differential environmental effects may have been important. However, chance may also be involved. Genotype frequencies at rs266729 deviate from Hardy–Weinberg equilibrium (HWE) in the controls of Kaklamani et al. ($\chi^2 = 7.63, P = 0.006$). Although this is not formally significant given the number of SNPs typed by Kaklamani et al., it raises some concerns that a larger control series might have produced a weaker association. Furthermore, the overall association between rs266729 and CRC risk in the study of Kaklamani et al. is only significant at nominal $P = 0.04$, some way below the threshold for global significance. We note that our study alone had estimated $>90\%$ power at $P = 0.05$ to detect an OR of 0.75 at rs266729, similar to that reported by Kaklamani et al.

ADIPOQ is an interesting candidate for CRC risk due to its association with obesity and because the alleged association between CRC and obesity (15,17). However, the hypothesis of increased CRC risk in obese individuals remains controversial (15,17). Furthermore, a role of genetic variation at ADIPOQ in obesity and other metabolic disorders such as type 2 diabetes (18) has not been fully proven. For example, no ADIPOQ alleles have been associated with the disease in recent large and highly empowered obesity GWASs (19–24); even though there is good coverage of this locus in most commercial arrays. Thus, although ADIPOQ could be a good CRC candidate, it would be first necessary to conclusively establish a consistent relationship between its variants and obesity and between obesity and CRC risk.

In conclusion, we have used data from two large (2147 patients and 2365 controls) and well-characterized case–control studies to demonstrate that there is no detectable association between common ADIPOQ variation and CRC risk in the UK population. Future studies aiming to replicate or disprove our findings should be based on well-designed studies that use large CRC cohorts.

**MATERIALS AND METHODS**

**Study samples**

This study used two independent case–control series of white British ethnicity. The first series was ascertained through the CORGI Consortium. This series included 921 cases with either CRC ($n = 620$) or advanced/multiple adenomas ($n = 311$). All these individuals had at least one first-degree relative with CRC and had no mutations in known, highly penetrant CRC genes. Controls ($n = 929$) included spouses or partners unaffected by cancer and without family history of colorectal tumours. The second case–control sample series comprised 1216 CRC patients from a randomized trial of VIOXX (rofecnidoxib) in Dukes stages B and C CRC patients (VICTOR trial, $n = 920$) and from the QUASAR2 trial that compares capecitabine against capecitabine plus bevacizumab ($n = 296$) (http://www.octo-oxford.org.uk/alltrials/infollowup/vic.html) and http://www.octo-oxford.org.uk/alltrials/trials/q2.html). We used publicly available data from 1436 individuals from the 1958 Birth Cohort as controls. We refer to the first series as the CORGI study and to the second series as the VQ58 study. DNA samples were isolated from blood samples using standard methods and quantified with picogreen. Full informed consent was obtained from all individuals. Ethical approval to collect these samples was granted by the Southampton and South West Hampshire Research Ethics committee.

**Genotyping**

SNPs were typed using Illumina Hap 300/370/550 arrays. Duplicate samples were used to check genotyping quality. General quality control assessment was as previously described, and all SNPs described herein passed the required thresholds (2).

**Statistical analysis**

Genotype frequencies at each SNP were used to test for deviations from HWE. $\chi^2$ or Fisher’s exact tests were used to evaluate allelic, genotypic, recessive and dominant disease risk models. Association and HWE $P$-values were calculated using PLINK (25). We used genotype data from the CEPH Hapmap samples and the IMPUTE software (16) to generate genotypes at rs266729. Meta-analysis of association data was carried out with the R software package (http://www.r-project.org/). In previous studies, we used Q–Q plots and the STRUCTURE (26) program to demonstrate that there is no evidence of population stratification or of differential genotyping between cases and controls in our study samples (2).

**SUPPLEMENTARY MATERIAL**

Supplementary Material is available at HMG online.

**ACKNOWLEDGEMENTS**

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**Conflict of Interest statement.** None declared.

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**REFERENCES**


