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Authors
Ho, JW
Choi, S-C
Lee, Y-F
et al.

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Replication study of SNP associations for colorectal cancer in Hong Kong Chinese

JW Ho1, S-c Choi2, Y-f Lee3, TC Hui2, SS Cherny3, M-M Garcia-Barcelo1, L Carvajal-Carmona1, R Liu4, S-h To5, T-k Yau6, CC Chung7, CC Yau8, SM Hu9, PY Lau10, C-h Yuen11, Y-w Wong12, S Ho1, SS Fung1, IP Tomlinson3, RS Houlston1, KK Cheng14 and PC Sham1,2

1Department of Surgery, The University of Hong Kong, Pokfulam, Hong Kong; 2Department of Psychiatry, The University of Hong Kong, Pokfulam, Hong Kong; 3Nuffield Department of Medicine, Molecular and Population Genetics, University of Oxford, Wellcome Trust Centre for Human Genetics, Oxford OX3 7BN, UK; 4Department of Clinical Oncology, Queen Mary Hospital, Pokfulam, Hong Kong; 5Department of Surgery, Ruttonjee Hospital, 266 Queen’s Road East, Wai Chai, Hong Kong; 6Department of Clinical Oncology, Pamela Youde Nethersole Eastern Hospital, 3 Lok Man Road, Chai Wan, Hong Kong; 7Department of Surgery, Pamela Youde Nethersole Eastern Hospital, 2 Lok Man Road, Chai Wan, Hong Kong; 8Department of Clinical Oncology, Princess Margaret Hospital, 2-10 Princess Margaret Hospital Road, Lai Chi Kok, Kowloon, Hong Kong; 9Department of Surgery, Princess Margaret Hospital, 2-10 Princess Margaret Hospital Road, Lai Chi Kok, Kowloon, Hong Kong; 10Department of Surgery, Kwong Wah Hospital, 25 Waterloo Road, Kowloon, Hong Kong; 11Department of Surgery, Tseung Kwan O Hospital, 2 Po Ning Lane, Hang Hau, Tseung Kwan O, New Territories, Hong Kong; 12Department of Surgery, Tuen Mun Hospital, Tsing Chung Koon Road, Tuen Mun, New Territories, Hong Kong; 13Section of Cancer Genetics, The Institute of Cancer Research, Brookes Lawley Building, Haddow Laboratories, Sutton, Surrey SM2 5NG, UK; 14Department of Public Health, Epidemiology and Biostatistics, The University of Birmingham, Public Health Building, Edgbaston, Birmingham B15 2TT, UK.

BACKGROUND: Recent genome-wide association studies of colorectal cancer (CRC) have identified common single-nucleotide polymorphisms (SNPs) mapping to 10 independent loci that confer modest increased risk. These studies have been conducted in European populations and it is unclear whether these observations generalise to populations with different ethnicities and rates of CRC.

METHODS: An association study was performed on 892 CRC cases and 890 controls recruited from the Hong Kong Chinese population, genotyping 32 SNPs, which were either associated with CRC in previous studies or are in close proximity to previously reported risk SNPs.

RESULTS: Twelve of the SNPs showed evidence of an association. The strongest associations were provided by rs10795668 on 10p14, rs4779584 on 15q14 and rs12953717 on 18q21.2. There was significant linear association between CRC risk and the number of independent risk variants possessed by an individual (P = 2.29 x 10^-5).

CONCLUSION: These results indicate that some previously reported SNP associations also impact on CRC risk in the Chinese population. Possible reasons for failure of replication for some loci include inadequate study power; differences in allele frequency, linkage disequilibrium structure or effect size between populations. Our results suggest that many associations for CRC are likely to generalise across populations.

Keywords: colorectal cancer; genetic; association; replication; Chinese

Colorectal cancer (CRC) affects over one million people each year worldwide (Tenesa and Dunlop, 2009). It is currently the third commonest malignancy and the fourth commonest cause of cancer-related mortality in the world (Stewart et al., 2003). The overall burden of the disease is set to increase further from the increasing incidence rates in Asian and African populations associated with the adoption of western diets (Tenesa and Dunlop, 2009). In Hong Kong, CRC is now the second commonest cancer (with 4084 cases in 2007) and the second commonest cause of cancer death (1690 deaths in 2007) (Hong Kong Cancer Registry, Hospital Authority, 2009).

Although dietary and lifestyle risk factors undoubtedly are major risk factors for CRC, twin studies have shown that ~30% of the variation in susceptibility to CRC involves inherited genetic differences (Lichtenstein et al., 2000). However, high-penetration susceptibility mutations account for <6% of CRC cases; the majority of inherited variance appearing to be a consequence of the co-inheritance of multiple low-risk variants (Lichtenstein et al., 2000; Bost et al., 2001).

Recent genome-wide association studies (GWAS) have provided statistically robust evidence for common susceptibility loci for CRC. These studies have so far identified common single-nucleotide polymorphisms (SNP) at 10 independent loci that confer modest increased risk to CRC (odds ratios (OR) ~1.1–1.3) at 8q23.3, 8q24.21, 10p14, 11q23.1, 14q22.3, 15q13.3, 16q22.1, 18q21.1, 19q13.11 and 20p12.3 (Easton and Eeles, 2008; Le Marchand, 2009). These GWAS have been performed almost
SNP associations for CRC in HK Chinese

JW Ho et al

exclusively in populations of European ancestry, and the effects of these risk alleles in other populations are as yet unknown.

Understanding the effects of these variants in different populations is extremely important in terms of inferring the causality and mechanisms of colorectal tumourigenesis, as well as for the translation of these results to risk prediction in different populations. Colorectal cancer is a disease with very different incidences rates between populations (Curado et al, 2007). The risk variants may confer different magnitudes of increased risk in different populations for a variety of reasons, including differences in allele frequency and linkage disequilibrium (LD) structure, and difference in genetic and environmental backgrounds that interact with the variants (Sawyer et al, 2005; Weir et al, 2005; Ireland et al, 2006; Ioannidis, 2007).

To further our knowledge of the role of common genetic predisposition to CRC, we have examined the impact of the 10 known low-penetrance CRC risk loci in the Han Chinese population in Hong Kong using a case–control study design. We first examined variants, which were previously reported to have reached genome-wide significance (Brodie et al, 2007; Tomlinson et al, 2007; Houlston et al, 2008; Jaeger et al, 2008; Tenesa et al, 2008) for association with CRC risk, in an initial case–control sample. We then examined in an extended case–control series 22 additional SNPs, which have been associated with CRC risk in unpublished studies on European populations. Some of these SNPs were located close to SNPs genotyped in the first part of the study. Phase 1 can be regarded as a replication study of established associations in European populations, whereas Phase 2 is a replication study of more tentative associations as well as a more comprehensive screening of the risk loci evaluated in Phase 1.

MATERIALS AND METHODS

Subjects

Since October 2006, subjects (CRC cases and controls) have been recruited from seven departments of surgery and three departments of oncology in seven public hospitals in Hong Kong. The CRC cases were adults with histologically proven adenocarcinoma of the colon or rectum (international diseases 9 codes 153 and 154) diagnosed either (1) within 18 months before recruitment commencement date (prevalent cases) or (2) within the recruitment period (incident cases), treated at the seven participating hospitals. The controls were sex- and age-matched hospital inpatients or outpatients without a personal history of cancer or a family history of CRC in first-degree relatives treated at the participating hospitals.

Informed consent was obtained from all participants and the study protocol was approved by the Institution Review Boards of the seven participating hospitals in accordance with the declaration of Helsinki.

Genotyping

Variation at 8q24.21, 10p14, 11q23.1, 14q22.3, 15q14, 16q22.1, 18q21.2, 19q12 and 20p12.3 loci was evaluated by genotyping cases and controls for rs6983267, rs7014346, rs706771, rs827401, rs7894531, rs7898455, rs4474353, rs10795668, rs3802842, rs11623717, rs17563, rs2071047, rs2761887, rs8014363, rs4444235, rs6949587, rs16969681, rs16970016, rs1554865, rs11632717, rs1406389, rs1919360, rs16970016, rs7165427, rs10318, rs4779584, rs9929218, rs12953717, rs4464148, rs4939827, rs10411210, rs961253 and rs355527.

DNA was extracted from EDTA-venen blood samples using standard methodology. The SNP genotyping was conducted using the Sequenom MassARRAY system (Sequenom, San Diego, CA, USA). Genotyping assays were designed using SpectroDESIGNER software version 2.0.0.17 (Sequenom). Quality control was monitored by including duplicate and four negative controls in each 384-well plate. Further quality control included the exclusion of SNPs with genotype call rates <95%, minor allele frequency (MAF) <5% and those that deviated significantly from Hardy–Weinberg equilibrium in the controls (P < 0.01).

Statistical and bioinformatic analysis

Haploview version 4.1 (Barrett et al, 2005) and HapMap CHB+JPT data (release 22; http://hmap.ncbi.nlm.nih.gov/) was used to generate LD plots. The PLINK (Purcell et al, 2007) and R (Version 2.8.1; http://www.r-project.org/) were used for association analyses. The Cochran–Armitage trend test was used to examine association between CRC and SNP genotype (Armitage, 1995). In addition, logistic regression analysis of CRC on allele dosage (0, 1, 2) was performed, with adjustment for sex as covariate. Statistical significance was assessed on the basis of two-sided P-values, and allowance for multiple testing was made by using Bonferroni’s correction and false discovery rates (FDR) methodology. Heterogeneity between the ORs in this study and those of previous studies was assessed by the Breslow–Day’s test. Association between clinico-pathological variables and SNP genotype was analysed by the Armitage trend test or by logistic regression with sex as covariate, on the cases only. A composite score of genetic susceptibility was created from nine independent SNPs in Part 1, choosing only one SNP (the most significant) from each group of tightly linked SNPs. The composite score in an individual was calculated as the total number of high-risk alleles present in the individual (possible range 0–18). The association between the composite score and CRC risk was assessed by χ² tests and by a Cochran–Armitage trend test.

RESULTS

In the first phase, we genotyped 716 CRC cases and 714 controls. The cases comprised 445 males and 271 females. In the second phase, an additional 176 cases and 180 controls were genotyped yielding a total of 892 cases and 890 controls. The clinical characteristics of the cases and controls are detailed in Table 1.

Table 1

| Case subject characteristics | Phase 1 | Phase 2 |
|-----------------------------|---------|---------|
| Number                      | 716     | 892     |
| Age at diagnosis (year)     |         |         |
| Median                      | 68 (58–76, 18) | 68 (58–76, 18) |
| (range, interquartile range)|         |         |
| Mean (range, s.d.)          | 66.75 (31–96, 12.25) | 66.43 (31–96, 12.21) |
| Sex (%)                     |         |         |
| Male                        | 445 (62.2) | 519 (58.2) |
| Female                      | 271 (37.8) | 373 (41.8) |
| Tumour site (%)             |         |         |
| Colon                       | 444 (62.0) | 549 (61.5) |
| Rectum                      | 265 (37.0) | 338 (37.9) |
| Both sites (synchronous)    | 7 (1.0)  | 5 (0.6) |
| AJCC cancer stage (%)       |         |         |
| Stage I                     | 90 (12.6) | 112 (12.6) |
| Stage II                    | 220 (30.7) | 276 (30.9) |
| Stage III                   | 227 (31.7) | 287 (32.2) |
| Stage IV                    | 171 (23.8) | 207 (23.2) |
| Not defined                 | 8 (1.1)  | 10 (1.1) |

Abbreviation: AJCC = American Joint Committee on Cancer.
The 14 SNPs included in the first phase (rs6983267, rs7014346, rs10795668, rs3802842, rs4444235, rs4779584, rs10318, rs9929218, rs4939827, rs12953717, rs4646148, rs10411210 and rs355527) had an average genotyping call rate of 99.9% (Supplementary Table 1). One SNP, rs16893766, was monomorphic in this cohort and was thus not analysed. For the 22 SNPs included in the second phase, the overall genotyping call rates were 95.3%. Three SNPs were excluded from analysis because they had genotyping call rates <95% (rs133344771) or MAF ≤5% (rs11986063 and rs10424333). A total of 32 SNPs (13 from Phase 1 and 19 from Phase 2) annotating nine distinct loci provided data for the full analysis. Ten SNPs were mapped to 15q, six SNPs each to 10p and 14q, three SNPs to 18q, two SNPs each to 8q and 20p and one SNP each to 1q, 11q and 9p.

Five of the 13 SNPs genotyped in Phase 1 were significantly associated with CRC risk (Table 2A). Although only the most significant SNP (rs10795668, P = 0.0018) would be significant after Bonferroni’s adjustment, all five nominally significant SNPs would be considered significant on a basis of an FDR of 0.1 (rs10795668, rs12953717, rs4779584 and rs4939827). For all five SNPs, the risk-increasing allele in this study is the same as in the original report of association. Two of the significant SNPs, rs4939827 and rs12953717 on Chromosome 18q21.2, are in strong LD with each other.

Seven of the 19 SNPs in Phase 2 were significant, but none were significant after Bonferroni’s adjustment (Table 2B). All seven nominally significant SNPs would be considered significant at an FDR of 0.1 (rs7898455, rs4444235, rs7894531, rs1554865, rs16970016, rs706771 and rs827401). However, these significant SNPs are all in strong LD with SNPs significant in Part 1: rs7898455, rs4444235, rs7894531 rs706771 and rs827401 are in LD with rs10795668 on Chromosome 10p14, whereas rs16970016 and rs1554865 are in LD with rs4779584 on Chromosome 15q14. In logistic regression analyses of SNPs within each LD region, the inclusion of additional SNPs to a model containing the most strongly associated SNP in each (i.e. rs10795668 on 10p14, rs4779584 on 15q14 and rs12953717 on 18q21.2) did not significantly improve the fit of the model, thus providing no evidence for more than one disease locus in each of these regions (Supplementary Table 2).

Collectively, these data are consistent with four independent CRC loci defined by SNPs rs10795668, rs12953717, rs4779584 and rs7014346.

In order to avoid bias, a composite index was calculated from all nine independent SNPs from Phase 1. This index was significantly associated with CRC risk ($P_{\text{trend}} = 2.29 \times 10^{-5}$) with an OR of 2.70 (95% CI 1.40 - 5.27; Table 3). The composite index was significantly associated with male gender (P = 0.03 and 0.01, respectively).

We assessed the association between SNP genotype and various clinico-pathological variables through case-only logistic regression analyses. There were no consistent associations with sex, age at diagnosis, site of cancer or a family history of CRC.

Five of the 14 SNPs associated with CRC risk, which provides support for the CRC association findings in European populations. Although we recommend caution in implementing genetic models for predicting individual risk, approaches incorporating multilocus genotypes could help identify high-risk subgroups within a population. This underscores the potential for future risk profiling, even without identification of the causative variant (Wray et al, 2007).

There are no proven protein-coding transcripts in the vicinity of the marker SNPs that we tested, and there is no predicted gene within 0.4 Mb of rs10795668. The nearest predicted genes are BC031880, located 0.4 Mb proximal to rs10795668, and LOC389935, located 0.7 Mb distally. Although loss of heterozygosity involving Chromosome 10p14 is seen in CRC (Shima et al, 2005), the underlying basis of the association identified at rs10795668 is presently unclear, but there is no evidence to implicate the predicted gene FLJ3802842 (Tomlinson et al, 2008). In the CEU population, there was some evidence that the effect of rs10795668 on CRC risk varied by the site of the tumour, with the susceptibility allele more common in rectal cancers (Tomlinson et al, 2008). This was not seen in the Han Chinese population we studied.

The SNP rs4779584 maps to Chr15:30 782 048, that is the CRAC (HMS) locus. Although the risk allele in our population is the same as the European population, T is a major allele (0.83) in our population, whereas it is a minor allele in the CEU population (0.19). A previous meta-analysis by Jaeger et al (2008) showed a very strong association of rs4779584 with CRC risk. Two out of nine additional SNPs tested in this region were also statistically significant (rs16970016 and rs1554865) in our population; rs10318, which maps 31 kb distal to rs4779584, was one of the two most strongly associated SNPs in the CEU population; yet, such finding

DISCUSSION

Although a number of CRC risk variants have now been identified, almost all have been through analyses based on European Caucasian populations. As the incidence of CRC and the allele frequencies of SNPs differ across populations, it is important to understand the effects of these markers in other populations. We, therefore, comprehensively examined the association between 32 SNPs and CRC risk and clinico-pathological variables in Chinese CRC patients recruited from hospitals across Hong Kong. Twelve SNPs from four independent susceptibility loci (at 8q24.21, 10p14, 15q14 and 18q21.2) were found to be significantly associated with CRC in the Han Chinese population in Hong Kong. A composite index of nine independent SNPs was significantly associated with CRC risk, which provides support for the CRC association findings in European populations. Although we recommend caution in implementing genetic models for predicting individual risk, approaches incorporating multilocus genotypes could help identify high-risk subgroups within a population. This underscores the potential for future risk profiling, even without identification of the causative variant (Wray et al, 2007). However, large multilocus cohort studies will be needed to validate such genetic risk predictive models.

The rs10795668 provided the strongest evidence for an association in the Han Chinese population. This SNP maps to an 82-kb block of LD (8.73 - 8.81 Mb) within 10p14. All five additional SNPs, rs706771, rs827401, rs7894531, rs7898455 and rs4474353, mapping to this LD block showed evidence of association with CRC risk. The inclusion of each of these additional SNPs did not significantly improve the fit of the model compared with rs10795668 alone, providing no evidence for more than one disease locus at 10p14.

There are no proven protein-coding transcripts in the vicinity of the marker SNPs that we tested, and there is no predicted gene within 0.4 Mb of rs10795668. The nearest predicted genes are BC031880, located 0.4 Mb proximal to rs10795668, and LOC389935, located 0.7 Mb distally. Although loss of heterozygosity involving Chromosome 10p14 is seen in CRC (Shima et al, 2005), the underlying basis of the association identified at rs10795668 is presently unclear, but there is no evidence to implicate the predicted gene FLJ3802842 (Tomlinson et al, 2008). In the CEU population, there was some evidence that the effect of rs10795668 on CRC risk varied by the site of the tumour, with the susceptibility allele more common in rectal cancers (Tomlinson et al, 2008). This was not seen in the Han Chinese population we studied.

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could not be replicated in our study. One of the possible reasons for this disparity is that there are differences at this locus between the CEU and CHB population in terms of LD structure (Supplementary Table 3a and b). For example, there are vast differences in the MAFs for rs10316 (CHB 0.49 and HK control 0.46). Differences in MAFs between the two populations and the nature of the minor alleles were also found for other SNPs tested in this study (MAF: rs6494587, rs1696968, rs11632715 and rs1406387; nature of minor alleles: rs1554865, rs16970016, rs1406389, rs1919360 and rs1714527).

Moreover, there was significant interaction between this SNP and different variables tested. In the Han Chinese population, the risk allele of rs4779584 was significantly associated with the male gender. It is possible that there are differences in the MAFs for rs10316 (CHB 0.49 and HK control 0.46). Differences in MAFs between the two populations and the nature of the minor alleles were also found for other SNPs tested in this study (MAF: rs6494587, rs1696968, rs11632715 and rs1406387; nature of minor alleles: rs1554865, rs16970016, rs1406389, rs1919360 and rs1714527).

In the European studies, no association was found between the genotypes of rs4779584 and any of the clinico-pathological variables tested. In the Han Chinese population, the risk allele of rs4779584 was significantly associated with the male gender. Moreover, there was significant interaction between this SNP and the gender. This SNP rs4779584 lies between GREM1 and
Table 4  Association of independent SNPs with various clinico-pathological variables

| SNP          | Genotype     | Sex: male (M), female (F) | Cancer stage: stage I/II, stage III/IV | Metastatic disease: not stage IV, stage IVs | Family history of CRC | Site of cancer (C) rectum (R) |
|--------------|--------------|----------------------------|--------------------------------------|----------------------------------------------|-----------------------|-------------------------------|
|              | M            | F                          | P                                    | I and II                                     | III and IV            | P                             |
| rs10795668   | AA           | 54                         | 55                                   | 68                                          | 68                    | 3                             | 106                           | 73                             | 33                             |
| Risk allele: A | AG           | 209                        | 161                                  | 165                                         | 169                   | 36                            | 334                           | 222                           | 145                            | 0.50                           |
|              | CC           | 244                        | 156                                  | 162                                         | 170                   | 36                            | 364                           | 247                           | 151                            |
| rs4779584    | CC           | 124                        | 172                                  | 162                                         | 170                   | 36                            | 364                           | 247                           | 151                            |
| Risk allele: T | AG           | 107                        | 187                                  | 187                                         | 187                   | 15                            | 15                            | 11                            | 107                            | 110                            |
|              | TT           | 324                        | 172                                  | 248                                         | 248                   | 15                            | 15                            | 11                            | 107                            | 110                            |
| rs1293717    | TT           | 60                         | 37                                   | 46                                          | 51                    | 5                             | 7                             | 7                             | 7                              | 25                             |
| Risk allele: T | CC           | 220                        | 123                                  | 168                                         | 175                   | 5                                     | 43                            | 300                           | 222                           | 125                            | 0.24                           |
|              | CC           | 165                        | 111                                  | 125                                         | 151                   | 15                            | 15                            | 11                            | 107                            | 110                            |
| rs7014346    | AA           | 50                         | 30                                   | 51                                          | 46                    | 5                             | 7                             | 7                             | 7                              | 25                             |
| Risk allele: A | AG           | 199                        | 132                                  | 162                                         | 169                   | 21                            | 5                             | 33                            | 298                           | 0.35                            |
|              | GG           | 196                        | 108                                  | 143                                         | 161                   | 60                            | 22                            | 282                           | 183                           | 117                            |

Abbreviations: CRC = colorectal cancer; SNP = single-nucleotide polymorphism. P – P-values obtained from logistic regression model.

Table 5  Heterogeneity of associations between Hong Kong Han Chinese population and other populations

| SNP          | chromosome | OR (95% CI), high-risk allele frequency in control subjects; ref allele: low-risk allele in this study | Breslow–Day’s test P heterogeneity with this study |
|--------------|------------|------------------------------------------------------------------------------------------------|--------------------------------------------------|
| rs7014346, 8q24.21 | HK/England/CEU, Japan/Scotland                                                                 | England/CEU, Japan/Scotland Reference |
| rs10795668, 10p14 | 1.23 (1.05 – 1.44), 30% | 1.29 (1.18 – 1.40), 36% | 0.85 (0.79 – 0.92), 77% | 1.23 (1.15 – 1.33), 37% | 0.60 | 3.67 × 10⁻³ | 0.95 |
| rs4779584, 15q14 | 1.28 (1.1 – 1.5), 62% | 1.12 (1.0 – 1.25), 67% | 1.23 (1.15 – 1.33), 37% | 0.60 | 3.67 × 10⁻³ | 0.95 |
| rs1293717, 18q21.2 | 1.62 (1.04 – 1.52), 79% | 1.21 (1.12 – 1.31), 99% | 1.62 (1.04 – 1.52), 79% | 1.21 (1.12 – 1.31), 99% | 1.21 (1.12 – 1.31), 99% | 1.62 (1.04 – 1.52), 79% | 1.21 (1.12 – 1.31), 99% | 1.62 (1.04 – 1.52), 79% | 1.21 (1.12 – 1.31), 99% |

Abbreviations: CEU = Caucasian European; CI = confidence interval; CRC = colorectal cancer; HK = Hong Kong; OR = odds ratio; SNP = single-nucleotide polymorphism. *High-risk allele different in this and reference study.

SGCS. Jaeger et al (2008) have previously reported no association between SGCS or GREM1 expression and the genotype of rs4779584. The GREM1 encodes a secreted bone morphogenetic protein (BMP) antagonist. The TGF-β/BMP pathway is known to have an important role in colorectal tumourigenesis. It is, therefore, plausible that GREM1 may increase tumour proliferation, for example, through its expression in the stroma (Sneddon et al, 2006). Although SGCS is genetically and functionally slightly worse candidate than GREM1, neuroendocrine signalling involving SGCS (Seidah and Chretien, 1999) could influence cellular proliferation in the large bowel through, for example, signalling of nutrient availability or systemic hormonal effect.

The SNP rs1293717 is located at intron 3 of the SMAD7 gene on 18q21. One of the other two SNPs (rs4939827) tested in this region was also statistically significant. Yet, the inclusion of rs4939827 did not improve the fit of the model compared with rs1293717 alone; such result was compatible with being there a single risk locus in the SMAD7 region. The risk allele, C, was a major allele in our study, whereas it was a minor allele in the CEU studies. Although 18q21.1 contains another protein-coding gene (CR621005) and a predicted gene of unknown function (KIA0427), the decay in LD away from SMAD7 intron 3 incorporating all three SNPs as shown by Broderick et al (2007) did not support these genes as the location of a causative variant.

Loss of chromosome 18q is very common in individuals with CRC. Broderick et al (2007) observed that lower median SMAD7 mRNA expression was associated with CRC risk allele at rs1293717. The SMAD7 acts as an intracellular antagonist of TGF-β signalling by binding stably to the receptor complex and blocking activation of downstream signalling events. Perturbation of SMAD7 expression has been documented to influence CRC progression (Levy and Hill, 2006) and SMAD7 has also been shown to induce hepatic metastasis in CRC (Haldet al, 2008). Our finding of significant association of rs1293717 with metastatic disease supports the observations that SMAD7 influences CRC progression and induces distant metastasis. In a recent study, Thompson et al (2009) had shown gender-specific association of SMAD7 with colon cancer risk (i.e. risk association in women only). However, in our study, stratified analysis revealed significant association of rs1293717 with CRC risk in men only, while the interaction between rs1293717 and gender was not significant. There is no obvious explanation for the disparity in these study findings.
has been previously reported to influence the risk of adenomas as well as CRC (Tomlinson et al., 2007), suggesting that the 8q24.21 locus was involved in tumour initiation rather than progression. In our study, we found an association of rs7014346 with aggressive-advanced cancer raising the possibility that the 8q24.21 locus is also involved in tumour progression.

Twenty previously identified risk SNPs were not associated with CRC risk in the Chinese population. Although rs3808242 was significantly associated with CRC risk in various Caucasian populations, this association has not been replicated in the Japanese and Hong Kong Han Chinese populations. Several reasons exist for a failure to replicate findings. First, it could be that this study had insufficient power to detect the modest effect sizes of these SNPs. Second, for some non-replicated SNPs, there are differences in terms of the allele frequencies and LD patterns between the CEU and HCB/HK data. Third, the magnitude of the effect of a risk allele may differ between populations because of gene–gene or gene–environment interactions.

The study provides replication of four independent SNPs and suggests that there is a great deal of commonality in the aetiology of CRC across populations. This may not be entirely surprising for such high-frequency variants as these are likely to have quite ancient origins before ethnic diversification.

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