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Ten recently identified associations between nsSNPs and colorectal cancer could not be replicated in German families

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Abstract

Ten non-synonymous single nucleotide polymorphisms (nsSNPs), which were recently associated with colorectal cancer risk in a comprehensive, array based study (AKAP9 M463I, DKK3 G335R, AMPD1 Q12X, LIPC L356F, PSMB9 V32I, THBS1 N700S, CA6 S90G, ASCC3 C1995S, DHX36 S416C and CPA4 G303C) were re-evaluated in the present study based on 626 German familial non-HNPCC colorectal cancer patients and 736 healthy controls. No associations of

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any of the 10 nsSNPs with colorectal cancer could be replicated. The combined analyses indicated that further research based on additional independent samples is required.

Keywords: Colorectal cancer risk; Non-synonymous single nucleotide polymorphisms; Replication studies

1. Introduction

According to twin studies, inherited genetic factors contribute around 35% to susceptibility to colorectal cancer (CRC), whereas less than 5% may be attributed to mutations in *APC* and the mismatch repair genes, *MYH*, *SMAD4*, *BMPR1A* and *STK11* [1,2]. Much of the remaining genetic risk is probably explained by combinations of common low-penetrance variants [2]. In a recent kin-cohort analysis, Webb and colleagues [3] evaluated the impact of 1467 non-synonymous single nucleotide polymorphisms (nsSNPs) on CRC risk, most of the nsSNPs directly affecting protein function. Forty-four SNPs showed a significant association in British Caucasians [3–5]. Among the associated SNPs, we selected the top ten regarding disease association (AKAP9 M463I, DKK3 G335R, AMPD1 Q12X, LIPC L356F, PSMB9 V32I, THBS1 N700S, CA6 S90G, ASCC3 C1995S, DHX36 S416C and CPA4 G303C, Table 1) and tried to reproduce the results using 626 familial CRC cases and 736 healthy control individuals.

2. Materials and methods

CRC cases comprised 626 German Caucasian index patients (age range 9–88 years, mean 42.1 years) recruited by the six German university hospitals of Bochum (BO), Bonn (BN), Dresden (DD), Düsseldorf (DÜ), Heidelberg (HD) and Munich/Regensburg (MR). Cases were collected as part of a large study on susceptibility to hereditary nonpolyposis CRC (HNPCC) [6]. Analysis for microsatellite instability was applied as a prescreening test prior to mutation analysis in the *MSH2* and *MLH1* genes. All cases were tested to be microsatellite stable, hence HNPCC-negative. Inclusion criteria for the cases were (i) a family history of CRC or (ii) CRC diagnosed under the age of 50. The study population consisted of 317 unrelated male (age range 9–79 years, mean 42.6 years) and 307 female patients (age range 16–88 years, mean 41.6 years); the sex of two individuals was unknown. The control series consisted of 736 healthy, unrelated and ethnicity-, sex- and age-matched blood donors (26–68 years, mean 45.9 years) which were recruited between 2004 and 2006 by the Institute of Transfusion Medicine and Immunology, Faculty of Mannheim, Germany. The matching intervals for age were 'younger than 30 years', five-year groups (30–34, 35–39,. . .,60–64) and 'older than 65 years'. Controls were healthy volunteers from the southwestern region of Germany. Blood sampling was performed during regular blood donation according to German guidelines. The study was approved by the appropriate local Ethics Committees, and written informed consent was obtained from all individuals.

Among the 44 nsSNPs which showed statistically significant associations in the study of Webb et al. [3], 10 SNPs were selected on the basis of (i) the strength of the association and (ii) data from the literature on biological and functional relevance [3–5].

Table 1
Description of SNPs tested for association on familial CRC risk

SNP ID	Gene symbol	Gene description	Substitution
rs6964587	*AKAP9*	A kinase (PRKA) anchor protein 9	M463I
rs3206824	*DKK3*	Dickkopf homolog 3	G335R
rs17602729	*AMPD1*	AMP deaminase 1	Q12X
rs3829462	*LIPC*	LIPH, lipase, hepatic	L356F
rs241419	*PSMB9*	Proteasome subunit beta type 9	V32I
rs17632786	*THBS1*	Thrombospondin 1	N700S
rs2274333	*CA6*	Carbonic anhydrase VI, GUSTIN	S90G
rs240780	*ASCC3*	Activating signal cointegrator 1 complex subunit 3, RNAH	C1995S
rs9438	*DHX36*	DEAH (Asp-Glu-Ala-His) box polypeptide 36	S416C
rs2171492	*CPA4*	Carboxypeptidase A4	G303C

The selected SNPs included AKAP9 M463I [7], DKK3 G335R [8], AMPD1 Q12X [9,10], LIPC L356F [11,12], PSMB9 V32I [13,14], THBS1 N700S [15–17], CA6 S90G [18], ASCC3 C1995S [19,20], DHX36 S416C [21] and PA4 G303C [22]. Genotyping for all nsSNPs was carried out by TaqMan allelic discrimination as previously described [23].

Odds ratios (ORs), 95% confidence intervals (95% CIs) and two-sided *P* values were estimated by unconditional logistic regression to examine the association between the selected nsSNPs and familial CRC risk. Deviations of the genotype frequencies in the controls from those expected under Hardy–Weinberg equilibrium (HWE) were assessed using Pearson's goodness-of-fit χ^2 test with one degree of freedom. All analyses were carried out using the Statistical Analysis System software (Version 9.1.; SAS Institute Inc., Cary, NC). Power calculations were carried out with the power and sample size software PS (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize).

The genotype distributions in controls from the two studies were compared by χ^2 tests. The heterogeneity of odds ratios between studies was evaluated by unconditional logistic regression, the model included genotype and study as main fixed effects, plus their interaction, and the hypothesis of interest was the absence of interaction. The combined odds ratios were also calculated by logistic regression, considering the study as a fixed effect. Likelihood ratio tests were used to compare the recessive and the dominant penetrance models.

Table 2
Genotype frequencies of the investigated SNPs in German familial CRC patients and in healthy unrelated control individuals

Gene	SNP	Genotype	German controls *N* (%)	Familial CRC cases *N* (%)
AKAP9	M463I	GG	264 (36.5)	232 (37.9)
		GT	334 (46.1)	284 (46.4)
		TT	126 (17.4)	96 (15.7)
		GT + TT	460 (63.5)	380 (62.1)
DKK3	G335R	GG	414 (57.2)	332 (55.1)
		GA	259 (35.8)	231 (38.4)
		AA	51 (7.0)	39 (6.5)
		GA + AA	310 (42.8)	270 (44.9)
AMPD1	Q12X	CC	527 (73.1)	451 (74.2)
		CT	179 (24.8)	146 (24.0)
		1 1	15 (2.1)	11 (1.8)
		CT + TT	194 (26.9)	157 (25.8)
LIPC	L356F	AA	697 (98.2)	568 (96.8)
		AC	13 (1.8)	19 (3.2)
		CC	0 (0)	0 (0)
PSMB9	V32I	GG	704 (97.0)	600 (97.6)
		GA	22 (3.0)	15 (2.4)
		AA	0 (0)	0 (0)
THBS1	N700S	AA	594 (80.8)	515 (83.1)
		AG	136 (18.5)	99 (16.0)
		GG	5 (0.7)	6 (1.0)
		AG + GG	141 (19.2)	105 (16.9)
CA6	S90G	AA	373 (52.5)	307 (51.8)
		AG	271 (38.1)	231 (39.0)
		GG	67 (9.4)	55 (9.3)
		AG + GG	338 (47.5)	286 (48.2)
ASCC3	C1995S	GG	243 (33.6)	207 (34.5)
		GC	335 (46.3)	296 (49.3)
		CC	145 (20.1)	97 (16.2)
		GC + CC	480 (66.4)	393 (65.5)
DHX36	S416C	CC	266 (36.7)	204 (34.3)
		CG	354 (48.9)	303 (51.0)
		GG	104 (14.4)	87 (14.6)
		CG + GG	458 (63.3)	390 (65.7)
CPA4	G303C	GG	259 (36.2)	211 (34.6)
		GT	345 (48.2)	293 (48.0)
		TT	112 (15.6)	106 (17.4)
		GT + TT	457 (63.8)	399 (65.4)

3. Results

The distribution of genotypes in controls and within familial/early onset cases is shown in Table 2. Genotype frequencies for the analyzed polymorphisms were in agreement with Hardy–Weinberg expectations. No significant differences in genotype frequencies between CRC cases and controls were observed (data not shown). The results of both studies were compared and combined in Table 3. Since adjustment for age made no significant difference to findings, we only present unadjusted ORs. The only association of borderline significance among Germans (ASCC3 C1995S variant [CG + GG] vs. [CC]: OR = 0.77, 95% CI = 0.58–1.02, *P*-val = 0.07) was in a contrary direction to the results from Webb et al. [3]. The genotype distributions of controls were strongly correlated in the two studies. Interestingly, the significant differences were found for the three rarest variants (LIPC L356F, PSMB9 V32I and THBS1 N700S). Regarding the heterogeneity of ORs between the two studies, the estimated OR for LIPC L356F in the study of Webb et al. (0.61, 95% CI 0.44–0.83) was statistically lower than the German OR (1.79, 95% CI 0.88–3.66, *P*-val = 0.007). A significant difference between the ORs was also observed for ASCC3 C1995S (*P*-val = 0.020). Concerning the penetrance model, similar inheritances were selected in the two studies for all but two variants (AKAP9 M463I and CA6 S90G). However, model selection in the study of Frank et al. relied on data where no significant association was identified. After combination of genotypes and phenotypes for the 10 investigated SNPs, eight associations remained statistically significant. After data aggregation, the highest reduction in the ORs from the British study was found for the ASCC3 C1995S variant (20% versus 9% risk excess, 55% decrease). The variants ASCC3 and CPA4 showed recessive penetrances in the two independent studies but dominant inheritance in the combined study, but the differences in goodness of fit between models were small and the association in the combined sample was not significant.

4. Discussion

The strengths of the present study were a sound sample size, a homogeneous study cohort of a single ethnic group and the selection of familial cases affected by microsatellite-stable CRC. With the present sample size, we had an overall power of 80% at a significance level of 0.05 to detect an OR of $\geqslant$1.45 for AKAP9 M463I, DKK3 G335R, AMPD1 Q12X, THBS1 N700S, CA6 S90G, ASCC3 C1995S, DHX36 S416C and CPA4 G303C, and higher than 2.68 for LIPC L356F and PSMB9 V32I. However, it should be noted here that investigation of cases with a fam-

Table 3
Results of the association studies from Webb et al. and Frank et al. (minor allele frequency in controls (MAF), odds ratio (OR) and best fitting model, D: dominant, R: recessive), *P* values for heterogeneous allele frequencies and odds ratios, and combined results from the two studies

Gene	Substitution	Webb et al.				Frank et al.				Heterogeneity between studies		Combined results			
		MAF	OR	95% CI	Model	MAF	OR	95% CI	Model	P_{valMAF}	P_{valOR}	MAF	OR	95% CI	Model
AKAP9	M463I	0.38	**1.28**	1.14-1.44	D	0.40	0.88	0.66-1.18	R	0.322	0.051	0.39	**1.20**	1.08-1.33	D
DKK3	G335R	0.23	**1.20**	1.07-1.33	D	0.25	1.09	0.87-1.35	D	0.173	0.422	0.24	**1.17**	1.06-1.29	D
AMPD1	Q12X	0.13	**0.81**	0.71-0.92	D	0.14	0.95	0.74-1.21	D	0.480	0.463	0.14	**0.84**	0.75-0.94	D
LIPC	L356F	0.02	**0.61**	0.44-0.83	D	0.01	1.79	0.88-3.66	D	**0.018**	**0.007**	0.02	**0.72**	0.55-0.96	D
PSMB9	V32I	0.03	**0.73**	0.58-0.92	D	0.02	0.80	0.41-1.56	D	**0.001**	0.792	0.03	**0.74**	0.59-0.92	D
THBS1	N700S	0.13	**0.83**	0.73-0.95	D	0.10	0.86	0.65-1.14	D	**0.002**	0.639	0.13	**0.84**	0.75-0.94	D
CA6	S90G	0.31	**0.78**	0.64-0.94	R	0.28	1.03	0.83-1.28	D	0.081	0.468	0.31	**0.82**	0.69-0.97	R
ASCC3	C1995S	0.42	**1.20**	1.05-1.38	R	0.43	0.77	0.58-1.02	R	0.152	**0.020**	0.42	1.09	0.90-1.21	D
DHX36	S416C	0.39	**1.15**	1.03-1.29	D	0.39	1.11	0.88-1.39	D	0.720	0.791	0.39	**1.14**	1.03-1.26	D
CPA4	G303C	0.40	**0.85**	0.73-0.99	R	0.40	1.14	0.85-1.52	R	0.989	0.151	0.40	0.92	0.84-1.02	D

MAF, minor allele frequency in controls; OR, odds ratio; CI, confidence interval; Model, best fitting penetrance model; bold type represents statistical significance at the 0.05 level.

ily history of the disease and/or early onset cases instead of unselected cases may increase the statistical power of association studies by a factor of around two [24], thus representing a substantial improvement that emphasizes the significance of our study. No associations of any of the 10 SNPs with colorectal cancer was identified in the German study. The present study underlines the relevance of risk heterogeneity in genetic association studies and the necessity of extensive replication using independent samples.

Conflict of interest

None of the authors has any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work.

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