Original Article

Insulin receptor substrate-2 (IRS-2) rs1805097 G>A polymorphism is associated with colorectal cancer susceptibility: a meta-analysis involving 11,234 subjects

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Abstract: To assess the role of the insulin receptor substrate-2 (IRS-2) rs1805097 G>A polymorphism in colorectal cancer (CRC) susceptibility, we conducted a comprehensive meta-analysis, which included 5,220 CRC cases and 6,014 controls in six case-control studies published up to September 30, 2015. We used the crude odds ratios (ORs) with their 95% confidence intervals (95% CIs) to assess the relationship of IRS-2 rs1805097 G>A variants with CRC risk. Overall, IRS-2 rs1805097 G>A polymorphism was associated with the decreased risk of overall CRC risk (AA+GA vs. GG: OR, 0.91; 95% CI, 0.84-0.99; P=0.022). In a subgroup analysis by the region of CRC, IRS-2 rs1805097 G>A polymorphism was associated with a significantly decreased risk of colon cancer (AA+GA vs. GG: OR, 0.84; 95% CI, 0.76-0.94; P=0.002), but not mixed colorectal cancer and rectal cancer. In a subgroup analysis by ethnicity, a significantly decreased CRC risk was identified among American populations (AA+GA vs. GG: OR, 0.88; 95% CI, 0.80-0.97; P=0.007), but not Caucasians. Our meta-analysis suggested the IRS-2 rs1805097 G>A polymorphism might act as a CRC protective factor, especially in colon cancer and American populations subgroups.

Keywords: Polymorphism, IRS-2, colorectal cancer, susceptibility, meta-analysis

Introduction

Colorectal cancer (CRC) is the third most common malignancy in males and the second in females worldwide, with an estimated 1.4 million CRC patients and 693,900 cancer-related mortality occurring in 2012 [1]. Accumulating evidences demonstrate that insulin resistance (IR) and hyperinsulinemia are correlated with the pathogenesis of CRC, and high insulin secretion has been considered as a putative mechanism which links obesity, a vital susceptibility factor for a number of common diseases including cancer, with CRC [2, 3]. Numerous epidemiologic investigations have highlighted that individuals with type 2 diabetes mellitus which is correlated with hyperinsulinemia and IR are at increased susceptibility of CRC [4]. In addition, preclinical and experimental studies have shown that CRC patients have higher serum levels of insulin [4], and insulin therapy may increase the susceptibility and development of CRC by increasing cell proliferation and decreasing apoptosis [4-6].

The insulin receptor substrate-2 (IRS-2) gene is located on chromosome 13q34. IRS-2, a member of IRS family (IRS1-6), shares some important structure with IRS-1, in that both IRS-1 and IRS-2 proteins possess a N-terminal pleckstrin homology domain, several phosphotyrosine binding domains as well as a number of tyrosine and serine phosphorylation sites in a C-terminal tail [7, 8]. IRS-2 null mouse model showed metabolic defects in liver, muscle and adipose tissue, and then developed diabetes
IRS-2 polymorphisms and CRC risk

IRS1 and IRS2, two types of cytoplasmic proteins, almost express in various cells and mediate the function of metabolism, proliferation, and anti-apoptosis [10, 11]. Some prior studies demonstrated that IRS-2 played important roles in tumorigenesis and progression, specifically by facilitating cancer cell motility, invasion and metastasis [11, 12]. Studies linking obesity, IR and CRC demonstrated that the insulin pathway might play a vital role in the etiology of CRC [6, 13, 14].

IRS-2 gene is polymorphic. More than one thousand single nucleotide polymorphisms (SNPs) in IRS2 gene have been found (http://www.ncbi.nlm.nih.gov/SNP). Numerous SNPs have been studied, such as rs1805097 G>A, rs2289046 A>G, rs9515116 C>T, rs11069806 G>T, rs12429603 C>T, rs4773094 A>G, rs95-21508 A>T and rs9559654 A>G polymorphisms etc. Among them, the IRS-2 rs1805097 G>A, a non-synonymous SNP, was the most widely studied for its implication in the development of cancer. Several epidemiologic studies suggested that this SNP were involved in the etiology of CRC [15-19]. Although these studies have focused on the relationship of the IRS-2 rs1805097 G>A variants and the risk of CRC, all available results remain conflicting rather than conclusive. Recently, a meta-analysis have reported that IRS-2 rs1805097 G>A variants may not contribute to the susceptibility of CRC [20]. However, only three publications were included in that study. Now, additional studies were conducted on the association between IRS-2 rs1805097 G>A polymorphism and the susceptibility of CRC. To obtain a more precise assessment, an updated meta-analysis was carried out.

Materials and methods

Inclusion and exclusion criteria

Studies were selected according to the major inclusion criteria: (1) case-control studies; (2) evaluating the association between IRS-2 rs1805097 G>A SNP and CRC risks; (3) CRC was confirmed by histopathology; (4) providing data on genotype frequencies. Accordingly, studies without detail genotype frequencies, not case-control study design, duplicated data, reviews and comments were excluded.

Data extraction

For each recruited study, data was collected by two authors (J. Lin and Y. Wang) independently in duplicate. The following original data were extracted: name of first author, publication year, country where the study was performed, source of control, adjusted factors, genotyping methods, ethnicity, the region of CRC, Hardy-Weinberg equilibrium (HWE), number of cases/controls and genotype frequency. In addition, different ethnicity descents were defined as Caucasian and American populations. The regions of CRC were classified as mixed colorectal cancer, colon cancer and rectal cancer. An online chi-square test program (http://ihg.gsf.de/cgi-bin/hw/hwa1.p) was used to calculate the HWE in controls [21]. When came to duplicated data, these studies with larger sample sizes or according with HWE in controls were included. Two authors (J. Lin and Y. Wang) reached consensus on each item after a discussion.

Methodological quality assessment

The quality of included papers was independently assessed by two reviewers (J. Lin and Y. Wang) according to a ‘methodological quality assessment scale’ [22-24]. The quality scores ≥ 6, papers were defined as ‘high quality’; otherwise, papers were defined as ‘low quality’ [24].

Statistical analysis

The correlation strength between IRS-2 rs-1805097 G>A polymorphism and CRC susceptibility was assessed by odds ratio (OR) with its 95% confidence intervals (95% CI). The pooled ORs and CIs were calculated for homozygote comparison (AA versus GG), allele comparison (A versus G), dominant (GA/AA versus GG) and recessive (AA versus GA/GG) genetic models, respectively. Subgroup analyses were carried...
out to check the effects of confounding factors: CRC region and ethnicities. Sensitivity analysis was performed to examine the reliability of our findings. Chi-square based Q test and $I^2$ test were used to assess the statistical heterogeneity across the included studies, and the heterogeneity was considered significant when $I^2 > 50\%$ or $P < 0.10$ [25]. The fixed-effects model was applied when there was no significant heterogeneity [26]; otherwise, the random-effects model was harnessed [27]. Publication bias was assessed by Begg's funnel plot and the Egger's linear regression test [28], and a $P < 0.10$ was considered significant. All statistical analyses were conducted with STATA software (version 12.0; Stata Corporation, College Station, Texas USA). And all $P$ values were defined as two-side.

**Results**

**Characteristics**

A total of thirty-one relevant publications were retrieved. Figure 1 showed the major selecting process. Finally, there were five publications [15-19] (including six case-control studies) focused on the association between the IRS-2 rs1805097 G>A polymorphism and CRC risk. Of these articles, three investigated mixed colorectal cancer [15-17], two investigated colon cancer [18, 19] and one investigated rectal cancer [18]. Among these case-control studi-
Table 1. Characteristics of the individual studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Source of control</th>
<th>Region</th>
<th>No. of cases/controls</th>
<th>Adjusted factors</th>
<th>Quality score</th>
<th>Genotype Method</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahmoudi et al</td>
<td>2014</td>
<td>Iran</td>
<td>Caucasians</td>
<td>Hospital-based</td>
<td>Colorectal cancer</td>
<td>261/339</td>
<td>Sex, ethnic background, and geographic origin</td>
<td>6.5</td>
<td>PCR-RFLP</td>
<td>0.638</td>
</tr>
<tr>
<td>Yukseloglu et al</td>
<td>2014</td>
<td>Turkey</td>
<td>Caucasians</td>
<td>Hospital-based</td>
<td>Colorectal cancer</td>
<td>161/197</td>
<td>Age, sex, ethnic background, and geographic origin</td>
<td>7</td>
<td>PCR-RFLP</td>
<td>0.621</td>
</tr>
<tr>
<td>Pechlivanis et al</td>
<td>2007</td>
<td>Czech Republic</td>
<td>Caucasians</td>
<td>Hospital-based</td>
<td>Colorectal cancer</td>
<td>712/748</td>
<td>Sex, ethnic background, and geographic origin</td>
<td>7.5</td>
<td>TaqMan</td>
<td>0.281</td>
</tr>
<tr>
<td>Samowitz et al</td>
<td>2006</td>
<td>American</td>
<td>American population</td>
<td>Hospital-based</td>
<td>Colon cancer</td>
<td>1788/1981</td>
<td>Age, sex and geographic origin</td>
<td>8</td>
<td>PCR-RFLP</td>
<td>0.436</td>
</tr>
<tr>
<td>Slattery et al</td>
<td>2004</td>
<td>American</td>
<td>American population</td>
<td>Mixed</td>
<td>Colon cancer</td>
<td>1346/1544</td>
<td>Sex, ethnic background, and geographic origin</td>
<td>6.5</td>
<td>TaqMan</td>
<td>0.197</td>
</tr>
<tr>
<td>Slattery et al</td>
<td>2004</td>
<td>American</td>
<td>American population</td>
<td>Mixed</td>
<td>Rectal cancer</td>
<td>952/1205</td>
<td>Sex, ethnic background, and geographic origin</td>
<td>6.5</td>
<td>TaqMan</td>
<td>0.051</td>
</tr>
</tbody>
</table>

*PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism.*

Table 2. Distribution of IRS-2 rs1805097 G>A polymorphism genotype and allele

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>No. of cases/control</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahmoudi et al</td>
<td>2014</td>
<td>109/118 34/139 135/147 186/336</td>
<td>GG GA AA</td>
</tr>
<tr>
<td>Yukseloglu et al</td>
<td>2014</td>
<td>79/58 24/88 85/24 106/216</td>
<td>GG GA AA</td>
</tr>
<tr>
<td>Pechlivanis et al</td>
<td>2007</td>
<td>211/277 81/268 309/106 439/699</td>
<td>GG GA AA</td>
</tr>
<tr>
<td>Samowitz et al</td>
<td>2006</td>
<td>718/657 197/829 229/1051 2093/1364</td>
<td>GG GA AA</td>
</tr>
<tr>
<td>Slattery et al</td>
<td>2004</td>
<td>467/409 128/481 552/134 665/1343</td>
<td>GG GA AA</td>
</tr>
<tr>
<td>Slattery et al</td>
<td>2004</td>
<td>325/343 98/421 423/139 539/993</td>
<td>GG GA AA</td>
</tr>
</tbody>
</table>

HWE: Hardy-Weinberg equilibrium.

ies, three were from Caucasians [15-17] and three were from American populations [18, 19]. The characteristics of the included studies and the distribution of IRS-2 rs1805097 G>A variants as well as their alleles are summarized in Tables 1 and 2, respectively. Results of quality scores were presented in Table 1.

Quantitative synthesis

There were five papers met the inclusion criteria with 5,220 CRC cases and 6,014 controls, one article (Slattery et al.) provided two independent groups, thus, we treated them separately [18]. A total of six case-control studies were eligible in the present meta-analysis. Overall, IRS-2 rs1805097 G>A polymorphism was associated with the decreased risk of overall CRC risk in dominant genetic model (OR, 0.91; 95% CI, 0.84-0.97; P=0.007, *Table 3 and Figure 2*). In a subgroup analysis by the region of CRC, IRS-2 rs1805097 G>A polymorphism was associated with a significantly decreased risk of colon cancer in dominant genetic model (OR, 0.84; 95% CI, 0.76-0.94; P=0.002, *Table 3 and Figure 3*), but not mixed colorectal cancer and rectal cancer. In a subgroup analysis by ethnicity, a significant decreased CRC risk was identified among American populations in dominant genetic model (OR, 0.88; 95% CI, 0.80-0.97; P=0.007, *Table 3 and Figure 2*), but not Caucasians. Other results of comparison are listed in *Table 3*.

Tests for publication bias, sensitivity analyses, and heterogeneity

Begg’s funnel plot used to check potential publication biases indicated nearly symmetrical pattern, suggesting that there was no significant bias (A vs. G: Begg’s test P=0.707; AA vs. GG: Begg’s test P=1.000; AA+GA vs. GG: Begg’s test P=0.707; AA vs. GA+GG: Begg’s test P=1.000; *Figure 4*). In addition, Egger’s test harnessed to quantitatively examine the publication bias, also found no evidence of bias (A vs. G: Egger’s test P=0.467; AA vs. GG: Egger’s test P=0.835; AA+GA vs. GG: Egger’s test P=0.540; AA vs. GA+GG: Egger’s test P=0.841).

Sensitivity analyses were performed by eliding an individual study at a time for each case-con-
**Table 3. Meta-analysis of the IRS-2 rs1805097 G>A polymorphism and colorectal cancer risk**

<table>
<thead>
<tr>
<th>No. of study</th>
<th>A vs. G</th>
<th>A vs. GG</th>
<th>AA+GA vs. GG</th>
<th>AA vs. GA+GG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P (Q-test)</td>
<td>OR (95% CI)</td>
<td>P (Q-test)</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>0.96 (0.90-1.01)</td>
<td>0.132 0.939</td>
<td>0.97 (0.86-1.10)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td>3</td>
<td>1.00 (0.88-1.13)</td>
<td>0.943 0.913</td>
<td>0.98 (0.76-1.27)</td>
</tr>
<tr>
<td>American populations</td>
<td>3</td>
<td>0.94 (0.88-1.01)</td>
<td>0.093 0.772</td>
<td>0.97 (0.84-1.12)</td>
</tr>
<tr>
<td>Cancer type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>3</td>
<td>1.00 (0.88-1.13)</td>
<td>0.943 0.913</td>
<td>0.98 (0.76-1.27)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>2</td>
<td>0.93 (0.86-1.00)</td>
<td>0.078 0.696</td>
<td>0.99 (0.84-1.17)</td>
</tr>
<tr>
<td>Rectal cancer</td>
<td>1</td>
<td>0.98 (0.85-1.13)</td>
<td>0.772 N/A</td>
<td>0.91 (0.68-1.23)</td>
</tr>
</tbody>
</table>
IRS-2 polymorphisms and CRC risk

Figure 2. Meta-analysis with a fixed-effects model in different ethnicities for the association between IRS-2 rs1805097 G>A polymorphism and colorectal cancer risk (AA+GA vs. GG compare genetic model).

Figure 3. Meta-analysis with a fixed-effects model in the different CRC region for the association between IRS-2 rs1805097 G>A polymorphism and colorectal cancer risk (AA+GA vs. GG compare genetic model).
IRS2 polymorphisms and CRC risk

IRS2 express in various cells and modulate the function of metabolism, proliferation, and apoptosis [10, 11]. IRS-2, one of the typical signaling adaptors, was involved in several pathways, such as the extracellular signal-regulated kinase pathways and the phosphatidylinositol 3'-kinase pathways, despite the fact it has no intrinsic kinase activity and requires upstream activators [11]. Owing to its implication in multiple cancer-related pathways, IRS-2 was considered to play an important role in accelerating the progression and metastasis of malignancy [11, 29-31].

Of late, several epidemiologic investigations focused on the relationship of polymorphisms in IRS-2 gene with CRC risk. The most prevalent IRS-2 gene variants, rs1805097 G>A, has been most extensively studied. An amino acid substitution (Gly to Asp) at codon 1057 in IRS2 gene by transversion of rs1805097 G>A polymorphism was located close to two putative tyrosine phosphorylation sites (positions 1042 and 1072), and might alter the tertiary structure and function of IRS2 [32]. Recently, Slattery et al. and Samowitz et al. reported that a nonsynonymous mutation (G→A) in IRS-2 (rs1805097 G>A polymorphism) decreased the susceptibility of colon cancer [18, 19]. Nevertheless, these studies may have insufficient power to obtain a conclusive result, probably due to certain limitations such as small sample size. Meta-analysis is considered a powerful way for pooling the conflicting findings from different studies with more statistical power; thus, it can get more reliable results than a single study [33]. In this pooled-analysis, we found that IRS-2 rs1805097 G>A polymorphism was correlated with the decreased susceptibility of CRC (Table 3). Our findings suggest the presence of the A allele, which is correlated with affecting IRS2 structure and activ-

Figure 4. Begg’s funnel plot of meta-analysis of the association between the IRS-2 rs1805097 G>A polymorphism and the risk of colorectal cancer (AA+GA vs. GG compare genetic model).

Figure 5. Sensitivity analysis of the influence of AA+GA vs. GG compare genetic model in overall colorectal cancer meta-analysis (random-effects estimates for IRS-2 rs1805097 G>A polymorphism).

Table 3. Heterogeneity was not significant in all genetic comparison models, suggesting the stability of our findings.

Discussion

IRS2 express in various cells and modulate the function of metabolism, proliferation, and anti-
IRS-2 polymorphisms and CRC risk

...might decrease the risk of CRC. A stratified analysis was also performed regarding different ethnicities and the region of CRC for this SNP. This polymorphism may be associated with the decreased susceptibility of CRC among American populations, but not Caucasians. We also found that IRS-2 rs1805097 G>A polymorphism was associated with a significantly decreased risk of colon cancer, but not mixed colorectal cancer and rectal cancer. This pooled analysis indicated the influence of IRS-2 rs1805097 G>A variants and diversity in different populations and different region to the risk of CRC. To the best of our knowledge, numerous genetic and environmental factors can influence the susceptibility of CRC on different levels. Due to lack of sufficient data of environmental factors and life style, these important factors were not considered. Future studies are needed to validate our results, particularly with regard to environmental factors and the interactions of gene-gene and gene-environment.

Certain merits in our study should be addressed. Firstly, the present meta-analysis was the most extensive synthesis exploring the relationship of IRS-2 rs1805097 G>A polymorphism with CRC susceptibility. Secondly, our findings suggested the correlation between IRS-2 rs1805097 G>A polymorphism and CRC risk. Thirdly, all included studies were high quality (the quality score ≥ 6), suggesting our results were relatively reliable.

However, in the present meta-analysis, there are some limitations inherited from the published studies. First of all, only six published case-control studies were recruited, and certain negative and/or non-significant studies maybe remain unpublished, therefore the bias might inevitably occur. Secondly, the findings were based on crude ORs and CIs. Although the participants were matched on sex, age and residence in all included studies, these factors might only slightly affect the effective evaluations and further precise assessment should be adjusted by other potentially suspected factors, such as alcohol consumption, smoking status, glucose level and body mass index et al. Due to lack of these detailed background data in the included studies, an adjusted estimates was not conducted. Finally, the IRS-2 rs1805097 G>A polymorphism and other SNPs may locate on the same exon, given this polymorphism might involve in the development of CRC by altering the spatial structure and function of IRS-2 protein, thus, other important SNPs in IRS-2 gene should not be ignored.

In conclusion, our findings indicate that the IRS-2 rs1805097 G>A polymorphism is correlated with the decreased susceptibility of CRC, especially in American populations and colon cancer subgroups. Nevertheless, for practical reasons, larger epidemiologic studies assessing different populations (e.g., Asians and Africans) and incorporating with detailed functional comprehensive assessment are warranted to validate these findings.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[5] Wang L, Cai S, Teng Z, Zhao X, Chen X and Bai X. Insulin therapy contributes to the increased...
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[29] de Blaquiere GE, May FE and Westley BR. Increased expression of both insulin receptor substrates 1 and 2 confers increased sensitivity to IGF-1 stimulated cell migration. Endocr Relat Cancer 2009; 16: 635-647.

[30] Pankratz SL, Tan EY, Fine Y, Mercurio AM and Shaw LM. Insulin receptor substrate-2 regulates aerobic glycolysis in mouse mammary...

