Insulin-like growth factor genetic variation, colorectal cancer and diabetes

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Abstract
Insulin-like growth factor 2 (IGF2) has been shown to be involved in proliferation and apoptosis, playing a significant role in some diseases, like cancer and diabetes. The goal of this study was to analyze the association between IGF2 ApaI polymorphism (rs680) and susceptibility to colorectal cancer (CRC) and type 2 diabetes mellitus (T2DM) in Romanian patients.

Blood samples were obtained, after written consent, from 400 subjects (patients and sex- and age-matched controls). Genomic DNA was extracted from peripheral blood leucocytes using commercial kits and the IGF2 ApaI polymorphism was genotyped by PCR-RFLP. Statistical analysis was performed using the \( \chi^2 \) test.

The GG genotype had a higher frequency in the T2DM control group compared with CRC control group (38% vs. 29%), but the differences are not statistically significant (\( \chi^2_{GG vs AA} = 0.03; p = 0.86 \)). We didn’t find any statistically association relationship between IGF2 ApaI polymorphism (genotypes or alleles) and CRC (OR_{GG} = 0.81, p = 0.52, respectively OR_{G} = 0.81, p = 0.31) or T2DM (OR_{GG} = 0.54, p = 0.07, respectively OR_{G} = 0.66, p = 0.054).

These findings suggest that the IGF2 ApaI polymorphism do not affect the susceptibility to CRC and T2DM in Romanian patients.

Keywords: IGF2 Apal polymorphism, loss of imprinting, risk disease

Introduction
The environmental risk factors [1], diet and associated factors, like physical activity [2] and body size [3] have an important role in colorectal cancer (CRC) etiology. There are evidences that increased risk of CRC results from the influences of these factors on hyperinsulinemia [4], hyperglycemia [5] and regulation of IGF1 and IGF2 levels that can modulate tumorigenesis [6].

Also, recent studies show that CRC [7] and colorectal adenoma [8] are positively associated with type 2 diabetes mellitus (T2DM), hyperinsulinemia representing the link between T2DM and CRC [5, 9].

IGF2 gene located 11p15.5 encodes for insulin-like growth factor 2 that is a mitogenic peptide with important autocrine and paracrine signalling actions [10]. It plays an important...
role in fetal and adult development processes (like cellular proliferation, differentiation) as well in apoptosis and tumor growth [11, 12]. IGF2 gene is part of the IGF2 - H19 locus, a cluster of imprinted genes. In normal tissues, IGF2 is expressed only from the paternally inherited copy [13]. Loss of imprinting (LOI) of IGF2 gene was associated with diabetes [14, 15] as well with many types of tumors: breast [16], esophagus [17], hepatic [18], gastric [19], Wilms [20], prostate [21] and colorectal [22 - 24] cancers.

IGF2 expression is modulated by some polymorphisms in other genes or in its self sequence. Thus, it has been showed that the shorter alleles (class I) of variable number of tandem repeats (VNTR) of insulin (INS) gene are associated with increased steady-state IGF2 mRNA levels in human placenta [25]. Also, the Apal restriction fragment length polymorphism (RFLP) in the 3'-untranslated region (3'-UTR) of IGF2 was associated with an increased mRNA level for IGF2 gene [26].

IGF2 Apal (rs680) polymorphism was intensively studied in relationship with diabetes [27, 28], obesity [29], breast [30] and prostate [31] cancers. To our knowledge, there are no other reports referring to the association between this polymorphism and CRC risk.

The goal of this study was to analyze the relation between IGF2 Apal polymorphism, CRC and T2DM in Romanian patients.

Materials and Methods

Subjects

Between January 2010 and December 2012, blood samples from 400 unrelated individuals were collected at Coltea Hospital (Bucharest) and N. Paulescu Institute (Bucharest). The subjects were included in four groups: CRC patients (M:F = 59:41; age 63.3±4.5; with normal values of glycemia); T2DM patients (M:F = 59:41; age 55.7±8.1; duration of T2DM 13±8.4 years); CRC control (M:F = 59:41; fasting glycemia 90±8 mg/dl), and T2DM control (M:F = 59:41; fasting glycemia 92±6 mg/dl), both control groups without known family history of malignancies and clinical signs of CRC or diabetes. The each control group was matched with CRC and respectively T2DM group for both age and gender. Diagnostic of CRC was confirmed after histopathological examination. The colorectal tumours were localized in colon and sigmoid (64.2% of cases) and in rectum (35.8% of cases).

The subjects were enrolled in the study after written consent was obtained, according to the Declaration of Helsinki. This study was approved by Research Ethics Committee of Biology Faculty, University of Bucharest.

DNA isolation and genotyping

Five ml of blood were collected in tube containing EDTA from all participants. DNA was extracted from peripheral blood leukocytes using the Genomic DNA Purification Kit (Fermentas, Lithuania) according to the manufacturer’s protocol.

The IGF2 Apal gene polymorphism was detected by PCR-RFLP [32]. Briefly, about 60 ng DNA were amplified in a final volume of 10 μL, containing 1×PCR buffer, 1.5 mmol/L MgCl2, 1 unit Taq DNA polymerase, 100 μmol/L dNTP, and 0.5 μmol/L of each primer (F 5’- CTT GGA CTT TGA GTC AAA TTG G -3’ and R 5’- CCT CCT TTG GTC TTA CTG GG -3’) [32]. PCR was performed in a Corbett research thermocycler (Corbett Research Pty Ltd, Sydney, Australia) and the program consists of: an initial melting step of 1 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; a final elongation step of 3 min at 72°C.
The digestion reaction was performed with 5U *ApaI* restriction enzyme (Fermentas, Lithuania) at 30°C for 3 hours. The restriction products were electrophoresed (PAGE 8%) and were visualized using Bio-Imaging System after ethidium bromide staining. The genotypes were determined by examining gel fragments: the 236 bp fragment revealed the AA genotype; three fragments of 236, 175 and 61 bp indicated the AG genotype and two fragments of 175 and 61 bp indicated the GG genotype.

**Statistical analysis**

Statistical analysis was performed using *SISA* software [33]. Alleles and genotypes distribution was tested for Hardy–Weinberg equilibrium condition. Chi-square test ($\chi^2$) was used to compare the distribution of genotypes and alleles in patients and control groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the relevant $2 \times 2$ contingency table. A $p$ value < 0.05 was considered statistically significant.

**Results and Discussions**

We genotyped the *IGF2 ApaI* polymorphism in 100 CRC patients, 100 T2DM patients and 200 healthy controls (number, age and gender matched for each lot of patients). The frequencies of genotypes are shown in Table 1 and Table 2. The *IGF2 ApaI* genotype distribution for patients and controls was in accordance to Hardy-Weinberg equilibrium expectation (verified by $\chi^2$ test) in all groups: CRC patients ($\chi^2 = 0.16, p = 0.68$), T2DM patients ($\chi^2 = 1.54, p = 0.21$), CRC control ($\chi^2 = 0.92, p = 0.34$) and T2DM control ($\chi^2 = 0.52, p = 0.47$).

As it can be observed, the GG genotype had a higher frequency in the T2DM control group compared with CRC control group (38% vs. 29%), but the differences are not statistically significant ($\chi^2_{GG \ vs. \ AA} = 0.03; p = 0.86$). These differences can be explained by mean age distribution into the control groups or by selection criteria of the investigated populations.

For CRC, no statistically significant differences in the distribution of GG genotype (25% vs. 29%) and G allele (51% vs. 56%) between patients and controls have been identified (OR=0.816, $p=0.52$ for GG genotype, respectively OR=0.817, $p=0.316$ for G allele) (Table 1).

**Table 1.** Genotype distribution of *IGF2* gene polymorphism for CRC

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype</th>
<th>Gender</th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRC patients</td>
<td>M</td>
<td>14</td>
<td>33</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>11</td>
<td>19</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>25</td>
<td>52</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>CRC control</td>
<td>M</td>
<td>15</td>
<td>35</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>14</td>
<td>19</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>29</td>
<td>54</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

CRC, colorectal cancer; M, males; F, females

For T2DM (Table 2), we found a possible association relationship between the GG genotype and T2DM (OR=0.54; $p=0.048$), but the $p$-value obtained with the Yates correction (0.068) showed that the impact of GG genotype on T2DM is not statistically significant.
Table 2. Genotype distribution of IGF2 gene polymorphism for diabetes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype</th>
<th>Gender</th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>15 (25.4%)</td>
<td>33 (56.0%)</td>
<td>11 (18.6%)</td>
</tr>
<tr>
<td>T2DM patients</td>
<td></td>
<td>F</td>
<td>10 (24.4%)</td>
<td>23 (56.1%)</td>
<td>8 (19.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>25</td>
<td>56</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>20 (33.9%)</td>
<td>32 (54.2%)</td>
<td>7 (11.9%)</td>
</tr>
<tr>
<td>T2DM control</td>
<td></td>
<td>F</td>
<td>18 (43.9%)</td>
<td>18 (43.9%)</td>
<td>5 (12.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>38</td>
<td>50</td>
<td>12</td>
</tr>
</tbody>
</table>

T2DM, type 2 diabetes mellitus; M, males; F, females

Also, the alleles distribution revealed an association relationship between G allele and T2DM (OR=0.66; p=0.043), but not statistically significant when Yates correction is applied (p=0.054). The comparison of genotypes and alleles distribution (patients vs. controls) according to gender and age (5 years group) showed no statistically significant differences in either CRC or T2DM group.

IGF2 Apal is one of the most common polymorphisms found in this gene, analyzed for its contribution in different pathologies and complications. To our knowledge, this is the first research focused on the involvement of IGF2 Apal polymorphism in the CRC pathology. Also, is the first report regarding the relationship between this genetic variant and CRC and T2DM risk in Romanian population.

Our study suggests that there is no association relationship between IGF2 Apal polymorphism and CRC or T2DM in Romanian population. Even we didn't find an association between this polymorphism and CRC or T2DM, we need to take into consideration that IGF2 expression is a complex process that involves also H19 factor and imprinting silencing phenomenon [12, 34, 35].

Loss of imprinting (LOI) for IGF2 gene was associated with poor prognosis in patients with stage IV CRC [36] and with proximal localization of colonic tumors [37]. To note, LOI for IGF2 was detected in DNA extracted from surrounding peritumoral normal colonic mucosa of about 30% of colorectal cancer (CRC) patients, as well in 10% of healthy individuals [22]. Also, LOI for IGF2 was identified in a total of 33% of breast tumor samples, heterozygous for the Apal IGF2 polymorphism, but in any of the normal breast samples [38]. Therefore, as LOI for IGF2 gene allows the expression of maternal allele in tumor tissues, it is obvious that this phenomenon may modify the risk disease.

Also, when we analyze the contribution of an IGF2 gene polymorphism to a risk disease, we need to take into consideration the parental origin of the alleles. For IGF2, in the absence of LOI phenomenon, only paternal allele can affect the risk, being the one that is expressed. A recent study show that paternally transmitted fetal alleles were associated with maternal glucose concentration in the third trimester of pregnancy and placental IGF2 contents at birth [28].

In this context, our results may be influenced by the dilution of the statistical risk by the mixture of paternal and maternal origins. Thus, despite the lack of a statistical significant association as a result of our study, we cannot exclude a potential involvement of IGF2 Apal polymorphism in the predisposition for CRC or T2DM risk.

To overcome these difficulties in assessing the role of IGF2 polymorphisms in cancer or diabetes, familial studies (which allow analysis of the risk allele transmission’s rate from parents to affected offspring) are required. In this way, the possible bias due to the population stratification can be avoided.
Conclusions

Our results indicated that IGF2 ApaI polymorphism does not confer a risk for colorectal cancer and type 2 diabetes mellitus in Romanian patients. However, additional researches and replicate studies in other populations are required to confirm our results.

Acknowledgements

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References


