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Polymorphisms in the Insulin-Like Growth Factor Axis Are Associated with Gastrointestinal Cancer

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Abstract

Introduction: Numerous factors influence the development of gastrointestinal (GI) cancer. The insulin-like growth factor (IGF) axis plays a role in embryonic and postnatal growth and tissue repair. Elevated levels of IGFs, low levels of IGF binding proteins (IGFBPs) and over-expression of IGF receptor (IGFR-I) were associated with several stages of cancer. Here, the prevalence of the single nucleotide polymorphisms (SNPs) rs6214 in the IGF type I (IGF-I) gene and rs6898743 in the growth hormone receptor (GHR) gene in patients with GI cancer and controls was studied.

Materials & Methods: In this Dutch case-control study, DNA isolated from blood of 1,457 GI cancer patients; 438 patients with head and neck cancer (HNC), 475 with esophageal cancer (EC) and 544 with colorectal cancer (CRC) and 1,457 matched controls, was used to determine the rs6214 and rs6898743 genotypes by polymerase chain reaction. The association between these SNPs and GI cancer, HNC, esophageal adenocarcinoma (EAC), esophageal squamous-cell carcinoma (ESCC) and proximal or distal CRC was studied. Odds ratios (ORs) with 95% confidence interval (95% CI) were calculated via unconditional logistic regression.

Results: Overall for GI cancer, the ORs for SNPs rs6214 and rs6898743 were approximately 1.0 (p-value>0.05), using the most common genotypes GG as reference. An OR of 1.54 (95% CI, 1.05–2.27) was found for EC for genotype AA of rs6214. The ORs for EAC were 1.45 (95% CI, 1.04–2.01) and 1.71 (95% CI, 1.10–2.68), for genotypes GA and AA, respectively. Genotype GC of rs6898743 showed an OR of 0.47 (95% CI, 0.26–0.86) for ESCC.

Conclusion: The A allele of SNP rs6214 in the IGF-I gene was associated with EAC, and with HNC in women. The GC genotype of rs6898743 in the GHR gene was negatively associated with ESCC.

Introduction

Worldwide, cancer is one of the most prevalent causes of death [1]. In 2011, the overall incidence of cancer in the Netherlands was 100,577 cases, approximately 20% did develop gastrointestinal (GI) cancer [2]. The number of new patients with GI cancer is increasing in the Netherlands over the past 20 years [2].

Ten to twenty percent of the GI cancers are hereditary [3]. There are several other factors that play a role in the development of GI cancer, including age, gender, smoking, physical activity and obesity [4,5]. Also dietary factors, such as intake of alcohol, consumption of low amounts of vegetables and fruits, excessive consumption of red meat, and many other dietary factors may have an impact on the development of GI cancer [5]. In addition, risk modulating genetic factors with low penetrance, such as numerous single nucleotide polymorphisms (SNPs), may be involved in the development of GI cancer.

In this respect the insulin-like growth factor (IGF) axis may be of interest, since the evidence that it is involved in several stages of cancer is increasing [6–8]. The IGF axis plays a role in embryonic and postnatal growth, in tissue repair and many other important processes [6,9,10]. Elevated levels of IGFs, low levels of IGF binding proteins (IGFBPs) in the circulation and over-expression of IGF receptor type I (IGFR-I) were associated with cancer [7,11]. In vitro studies indicated that several components of the IGF axis induce mitosis and prevents apoptosis in cancer cells [12,13].

IGF is mainly produced by the liver and it is mediated by the growth hormone (GH) family. The predominant isoform of this somatotropin family is growth hormone 1 (GH1). The growth hormone releasing hormone (GHRH), secreted by the hypothalamus, regulates the production of GH in the adenohypophysis of the pituitary gland [14]. The GH binds to the growth hormone receptor (GHR) in the liver and other target tissues. This interaction promotes the synthesis of IGF-I and IGF-II [15].

IGF is able to bind to the members of the IGFBP family [16]. Most IGFs circulating in the blood stream are bound to IGFBPs with a high binding affinity, resulting in an increased half-life of the IGFs. The majority of IGFs in the circulation bind to IGFBP-
alleles are 55% and 45%, respectively [24].

mRNA [21–23]. In Europe, the allele frequencies of the G and A

important role in the translation, localization and stability of

23.2 and consists of six exons [20]. The SNP rs6214 is located in

This gene is located on the long arm of chromosome 12 at position

IGF-2 and eventually degradation of

cell signaling pathways. The main function of this receptor is

probably the sequestering of IGF-II and eventually degradation of

this protein [9].

The function of IGFR-II, a cation-independent mannose-6-

phosphate receptor, is less clear. This receptor interacts with IGF-I

as well as with IGF-II. The binding of this receptor with IGF-I is

weak, whereas IGFR-II has a high binding affinity for IGF-II. In

contrast to IGFR-I, these interactions do not lead to activation of

cell signaling pathways. The main function of this receptor is

probably the sequestering of IGF-II and eventually degradation of

this protein [9].

Several genetic variants in the components of the IGF axis were

already investigated in association with cancer risk. McElholm et al. analyzed 102 SNPs in the IGF axis [17] and characterized three genetic variants that appeared to be associated with esophageal adenocarcinoma: rs6214 in the IGF-I gene, rs6898743 in the GHR gene and a CA-repeat in the promoter region (5′-UTR) of the IGF-I gene, the latter variant however being very rare. The association between the SNPs rs6214 and rs6898743 and other GI cancers was not investigated yet.

The SNP rs6214 (c.*2716G>A) resides on the IGF-I gene [19].

This gene is located on the long arm of chromosome 12 at position

23.2 and consists of six exons [20]. The SNP rs6214 is located in

the three prime untranslated region (3′-UTR), which has an important role in the translation, localization and stability of

mRNA [21–23]. In Europe, the allele frequencies of the G and A alleles are 55% and 45%, respectively [24].

The SNP rs6898743 (c.71-26648G>C) is an intronic SNP in the GHR gene, localized on chromosome 5 [17]. In the European population, approximately 75% and 25% are G and C alleles, respectively [25].

In this study, the prevalence of the SNPs rs6214 and rs6898743 in patients with GI cancer and controls was investigated in a

Dutch population.
Genotyping

The rs6214 and rs6898743 polymorphisms were established by means of real-time polymerase chain reaction (RT-PCR) techniques. A specific set of primers, which flank the region of the SNPs, was used to amplify the DNA. In the TaqMan assay, two labeled probes with a fluorophore at the 5' end and a quencher at the 3' end, were added to the PCR mixture. Primers and probes were designed for each SNP, using the Beacon Designer 7.0 software (PREMIER Biosoft, Palo Alto, California, USA). Primers and TaqMan probes were prepared by Sigma-Aldrich Chemie BV. (Zwijndrecht, the Netherlands) and were checked for polymorphisms in their binding sites using SNPCheck version 3 (https://ngrl.manchester.ac.uk/SNPCheckV3/snpcheck.htm). The sequences of the primers and probes are given in Table 1.

Table 2. Characteristics of the study population.

<table>
<thead>
<tr>
<th>Cancer subtype</th>
<th>Characteristics</th>
<th>Patients with GI cancer</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and neck</td>
<td>n</td>
<td>438</td>
<td>438</td>
</tr>
<tr>
<td></td>
<td>Male (%)</td>
<td>345 (78.8)</td>
<td>345 (78.8)</td>
</tr>
<tr>
<td></td>
<td>Female (%)</td>
<td>93 (21.2)</td>
<td>93 (21.2)</td>
</tr>
<tr>
<td></td>
<td>Mean age ± SD (years)</td>
<td>60.9±11.3</td>
<td>56.5±6.7</td>
</tr>
<tr>
<td>Esophageal</td>
<td>n</td>
<td>475</td>
<td>475</td>
</tr>
<tr>
<td></td>
<td>Male (%)</td>
<td>384 (80.8)</td>
<td>384 (80.8)</td>
</tr>
<tr>
<td></td>
<td>Female (%)</td>
<td>81 (19.2)</td>
<td>81 (19.2)</td>
</tr>
<tr>
<td></td>
<td>Mean age ± SD (years)</td>
<td>65.1±11.0</td>
<td>65.1±11.3</td>
</tr>
<tr>
<td></td>
<td>Histological subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EAC</td>
<td>355</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>ESCC</td>
<td>112</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>Colorectal</td>
<td>n</td>
<td>544</td>
<td>544</td>
</tr>
<tr>
<td></td>
<td>Male (%)</td>
<td>325 (59.7)</td>
<td>325 (59.7)</td>
</tr>
<tr>
<td></td>
<td>Female (%)</td>
<td>219 (40.3)</td>
<td>219 (40.3)</td>
</tr>
<tr>
<td></td>
<td>Mean age ± SD (years)</td>
<td>65.0±11.7</td>
<td>64.7±12.5</td>
</tr>
<tr>
<td></td>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proximal</td>
<td>156</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>366</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>21</td>
<td>–</td>
</tr>
<tr>
<td>Total GI cancer</td>
<td>n</td>
<td>1,457</td>
<td>1,457</td>
</tr>
<tr>
<td></td>
<td>Male (%)</td>
<td>1,054 (72.3)</td>
<td>1,054 (72.3)</td>
</tr>
<tr>
<td></td>
<td>Female (%)</td>
<td>403 (27.7)</td>
<td>403 (27.7)</td>
</tr>
<tr>
<td></td>
<td>Mean age ± SD (years)</td>
<td>63.8±11.5</td>
<td>62.4±11.3</td>
</tr>
</tbody>
</table>

n = number of patients or controls; GI, gastrointestinal; SD = standard deviation; EAC = esophageal adenocarcinoma; ESCC = esophageal squamous cell carcinoma; 
1p-value mean age = 0.000;  
2p-value mean age = 0.001.  
doi:10.1371/journal.pone.0090916.t002

Table 3. Genotype distribution in patients with gastrointestinal cancer and controls.

<table>
<thead>
<tr>
<th>SNP (Gene)</th>
<th>Genotype</th>
<th>Patients with GI cancer</th>
<th>Controls</th>
<th>OR (95% CI) **</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)*</td>
<td>n (%)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs6214 (IGF-I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>491 (34.0)</td>
<td>528 (36.3)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>709 (49.1)</td>
<td>706 (48.5)</td>
<td>1.08 (0.92–1.27)</td>
<td>0.339</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>245 (17.0)</td>
<td>221 (15.3)</td>
<td>1.20 (0.96–1.49)</td>
<td>0.111</td>
<td></td>
</tr>
<tr>
<td>rs6898743 (GHR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>844 (58.5)</td>
<td>859 (59.0)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>518 (35.9)</td>
<td>522 (35.9)</td>
<td>1.01 (0.87–1.18)</td>
<td>0.896</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>81 (5.6)</td>
<td>75 (5.2)</td>
<td>1.10 (0.79–1.53)</td>
<td>0.558</td>
<td></td>
</tr>
</tbody>
</table>

*Note that some patients or controls are missing because of failure of the genotyping.  
**Adjusted for age.  
GI, gastrointestinal; OR, odds ratio; CI, confidence interval.  
doi:10.1371/journal.pone.0090916.t003
The 6-fluorescein amidite (6-FAM) and hexachloro-fluorescein amidite (HEX) were covalently bound to the probes for the most common alleles and the less common alleles, respectively. The Black Hole Quencher-1 (BHQ1), was used which was bound to the 3’-end of the TaqMan probe. The RT-PCRs were performed with the CFX96 Real-Time PCR detection system (Bio-Rad Laboratories Inc, Hercules, California, USA) and the results were analyzed with the data analysis software Bio-Rad CFX Manager 2.0 (Bio-Rad Laboratories Inc).

### Statistical Analyses

The results from the RT-PCRs, genotype and allele frequency distributions, were analyzed by using IBM SPSS Statistics software version 20 (International Business Machines Corp., Armonk, New York, USA).

The equality of the mean age was tested using the independent-samples t-test. In the analysis, age and gender were considered as confounders. Odds ratios (ORs) with their 95% confidence intervals (95% CIs) were calculated for the genotypes and for the alleles frequencies by performing unconditional as well as conditional logistic regression analyses. Homozygosity for the most common allele was taken as reference.

When the mean age between the patient (sub)group and the control group was significantly different, the ORs calculated with the unconditional logistic regression were corrected for this confounder. Odds ratios (ORs) with their 95% confidence intervals (95% CIs) were calculated for the genotypes and for the alleles frequencies by performing unconditional as well as conditional logistic regression analyses. Homozygosity for the most common allele was taken as reference.

Results with a p-value <0.05 were considered statistically significant.

### Results

The characteristics of the study population and the number of subjects per GI cancer subtype are summarized in Table 2. In this study, 1,457 patients with GI cancer and 1,457 controls were included, aged between 16 and 95 years. The mean age of the GI cancer patient and control groups was 63.8±11.5 and 62.4±11.3 years, respectively (p-value = 0.001).

In the sub-analyses, in which the association between the SNPs and the development of each cancer subtype was investigated, the mean age between the HNC patients and the corresponding control group was significantly different (p-value = 0.0001).

The genotype distribution for SNPs rs6214 and rs6898743 in patients with GI cancer and controls are presented in Table 3. For the SNPs rs6214 and rs6898743, the distribution of the genotypes in the overall GI cancer patient group, in the GI cancer sub-groups as well as in the control groups did not deviate from the Hardy-Weinberg equilibrium (all p-values >0.05), except for the ESCC patient sub-group, which showed a p-value of 0.003 for SNP rs6898743.

For both SNPs, no associations with GI cancer were seen (ORs between 1.01 and 1.20, p-values >0.05), except for a significant over-expression of the GA genotype of rs6214 in female patients (OR 1.38, 95% CI 1.01–1.87).

Table 4 summarizes the genotype distribution and the ORs (95% CI) for SNPs rs6214 and rs6898743 per cancer subtype. In this sub-analysis, a significant association with EC (OR 1.54, 95% CI, 1.05–2.27) was found for genotype AA of rs6214 (p-value = 0.029), whereas the GA genotype showed a non-significant OR of 1.27 (95% CI 0.96–1.69). The A-allele was significantly more present in the patient group (p-value = 0.021). Analyzed according to gender, genotype AA of rs6214 in men showed an even higher OR of 1.68 (95% CI, 1.09–2.59) with a p-value of 0.019.

No significant associations were found between patients with HNC or CRC and controls for the genotypes of both SNPs. However, for HNC in women an OR of 2.19 (95% CI, 1.09–4.39) was found for the GA genotype of rs6214, whereas an OR of 0.06,
95% CI, 0.01–0.65 was found for the CC genotype of SNP rs6898743. The ORs calculated for the rs6214 and rs6898743 genotypes, per histological subtype of EC are shown in Table 5. An OR of 1.45 (95% CI, 1.04–2.01) was found for GA heterozygotes of rs6214 in EAC (p-value = 0.028), whereas homozygotes for the variant allele AA showed an OR of 1.71 (95% CI, 1.10–2.68; p-value = 0.018). In men with genotype AA of rs6214, the association was even stronger (OR 1.87, 95% CI, 1.14–3.07; p = 0.013). The A-allele was significantly more present in the EAC patient group as well as in the male sub-group (p-values 0.008 and 0.009, respectively).

For SNP rs6898743, an inverse association with ESCC was noticed for genotypes GC (OR 0.47, 95% CI, 0.26–0.86) with a p-value of 0.014. In contrast, the homozygote variant CC genotype revealed an OR of 2.54 (95% CI, 0.66–9.81). However in these CC subgroups the number of individuals are very small. Likewise, a significant inverse association with ESCC was found in men with genotype GC (OR 0.31, 95% CI, 0.15–0.66; p = 0.002). No significant associations were found when CRC patients were analyzed according to tumor localization, either proximal or distal (Table 5).

The main results of this study are visualized in Figure 1.

### Discussion

This population-based case-control study was performed to investigate the role of the IGF associated SNPs rs6214 and rs6898743 in the development of gastrointestinal cancer. The SNPs rs6214 and rs6898743 were not found associated with GI cancer, however in GI cancer subgroups, some interesting associations were noticed.

Genotype AA of rs6214 in the IGFI gene was associated with EC, but this risk modifying effect of the rs6214 A allele was mainly the result of an association with the histological subgroup of EAC; individuals bearing the GA or AA genotypes showed relative risks of 1.45 and 1.71, respectively. The SNP rs6214 has been investigated in several previous studies on GI cancer risk. Feik et al. reported a significantly higher risk of CRC for carriers of genotype AA [26]. A similar trend was seen in our study but no significance was reached. This difference could be explained by the fact that the study population of Feik et al. only contained 178 CRC patients. Moreover, their ORs were adjusted for gender, age and body mass index (BMI), whereas BMI was not involved in our study.

McElholm et al. reported a significant decreased risk of Barrett’s esophagus (BE) for the AA genotype of rs6214 in an Irish study population (OR = 0.43, 95% CI 0.24–0.75) [17]. BE is an abnormal replacement in the esophagus of squamous esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; CRC, colorectal cancer.

doi:10.1371/journal.pone.0090916.t005

### Table 5. Genotype distribution and ORs (95% CI) for the genotypes of SNPs rs6214 and rs6898743 in patients with EAC, ESCC, proximal CRC or distal CRC.

<table>
<thead>
<tr>
<th>Cancer subtype</th>
<th>SNP (Gene)</th>
<th>Genotype</th>
<th>Patients n (%)</th>
<th>Controls n (%)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAC rs6214 (IGF-I)</td>
<td>GG</td>
<td>115 (32.9)</td>
<td>147 (42.1)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>171 (48.9)</td>
<td>154 (44.1)</td>
<td>1.45 (1.04–2.01)</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>64 (18.3)</td>
<td>48 (13.8)</td>
<td>1.71 (1.10–2.68)</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>rs6898743 (GHR)</td>
<td>GG</td>
<td>189 (54.2)</td>
<td>199 (57.0)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>137 (39.3)</td>
<td>133 (38.1)</td>
<td>1.10 (0.81–1.51)</td>
<td>0.540</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>23 (6.6)</td>
<td>17 (4.9)</td>
<td>1.43 (0.74–2.78)</td>
<td>0.289</td>
<td></td>
</tr>
<tr>
<td>EAC rs6214 (IGF-I)</td>
<td>GG</td>
<td>39 (34.8)</td>
<td>35 (31.3)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>54 (48.2)</td>
<td>60 (53.6)</td>
<td>0.80 (0.44–1.45)</td>
<td>0.450</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>19 (17.0)</td>
<td>17 (15.2)</td>
<td>1.20 (0.53–2.74)</td>
<td>0.661</td>
<td></td>
</tr>
<tr>
<td>rs6898743 (GHR)</td>
<td>GG</td>
<td>78 (69.6)</td>
<td>66 (58.9)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>25 (22.3)</td>
<td>43 (38.4)</td>
<td>0.47 (0.26–0.86)</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>9 (8.0)</td>
<td>3 (2.7)</td>
<td>2.54 (0.66–9.81)</td>
<td>0.175</td>
<td></td>
</tr>
<tr>
<td>Proximal CRC rs6214 (IGF-I)</td>
<td>GG</td>
<td>51 (33.6)</td>
<td>55 (35.3)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>82 (52.6)</td>
<td>74 (47.4)</td>
<td>1.20 (0.73–1.96)</td>
<td>0.481</td>
<td></td>
</tr>
<tr>
<td>rs6898743 (GHR)</td>
<td>GG</td>
<td>99 (63.5)</td>
<td>90 (57.7)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>54 (34.6)</td>
<td>59 (37.8)</td>
<td>0.84 (0.53–1.34)</td>
<td>0.470</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>3 (1.9)</td>
<td>7 (4.5)</td>
<td>0.39 (0.10–1.58)</td>
<td>0.188</td>
<td></td>
</tr>
<tr>
<td>Distal CRC rs6214 (IGF-I)</td>
<td>GG</td>
<td>123 (33.6)</td>
<td>131 (35.8)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>180 (49.2)</td>
<td>186 (50.8)</td>
<td>1.03 (0.74–1.41)</td>
<td>0.879</td>
<td></td>
</tr>
<tr>
<td>rs6898743 (GHR)</td>
<td>GG</td>
<td>217 (59.3)</td>
<td>220 (60.1)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>126 (34.4)</td>
<td>119 (32.5)</td>
<td>1.09 (0.80–1.50)</td>
<td>0.581</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>23 (6.3)</td>
<td>27 (7.4)</td>
<td>0.90 (0.50–1.62)</td>
<td>0.721</td>
<td></td>
</tr>
</tbody>
</table>
epithelium by specialized columnar-lined epithelium, and is one of
the main risk factors for EAC [27]. Therefore it is surprising that
we found a significant inverse association with EAC for the rs6214
GA and AA genotypes.

Two main explanations may be given: first, the patient group
with BE and the control group in the study of McElholm et al.
were relatively small (n = 207 and n = 244, respectively). More
important however, is the large difference in allele frequencies,
being 48% and 52% for the rs6214 G and A alleles as reported by
McElholm et al. in their controls, whereas values of 57% and 43%
were reported for Europeans in the HAPMAP database [24]. The
latter values are in good agreement with our control allele
frequency data (60.5% G and 39.5% A allele).

The role of the genetic variant of rs6214 in pancreatic cancer in
a Japanese population was assessed by Nakao et al. [28]. Using AA
as the reference genotype, no association was found between
rs6214 and pancreatic cancer in the overall analysis, whereas 20
years old carriers of the GA and GG genotypes with a BMI ≥
25 kg/m² showed an increased risk for pancreatic cancer.
However, the number of patients included in this sub-category
was very low and thus reliability is limited.

High plasma levels of IGF-I were suggested to be a risk for
various cancers, including CRC [29,30]. Therefore, Canzian
et al., Al-Zahrani et al. and D’Aloisio et al. investigated the role of
the rs6214 SNP on the circulating IGF-I levels in Caucasians [20,31,32]. However, inconsistent results were obtained. In the
study of D’Aloisio et al., the AA genotype appeared to be
associated with high mean IGF-I plasma levels, whereas the other
two studies did not find an association between this SNP and
circulating IGF-I levels.

The SNP rs6898743 has not been extensively investigated so
far. Carriers of the CC genotypes were found inversely associated
with EAC by McElholm et al. [17], whereas our results showed a
tendency for the opposite. It was also remarkable that the allele
frequencies in the Irish study population as reported by McElholm
et al. [17] (19% G and 81% C for controls) were completely
different from the HAPMAP frequencies in the European
population [25] (77.9% G and 22.1% C). Our data of 76.9% G
allele and 23.1% C allele in controls completely fit with the
reported HAPMAP data for Europeans [25].

Some interesting gender related differences were found; in
women, genotype GA of SNP rs6214 was significantly associated
with GI- and HNC, whereas homozygosity for the variant C allele
of rs6898743 was inversely associated with HNC. In men, exactly
opposite effects were noticed, however results here are not
significant. It should be noticed however that the number of
female patients in the sub-group of HNC patients was small
(n = 93), which made the significance disputable.

The strength of the present study is that the patients and
controls were successfully matched by sex, race, recruiting area
and also largely by age. This enabled an assessment of association
between the SNPs and the outcomes, without being influenced by

Figure 1. Associations of the SNPs rs6214 and rs6898743 with gastrointestinal cancer. Only significant associations are visualized.
Associations are given as follows: rs number of the SNP, genotype, odds ratio with corresponding 95% confidence interval. The homozygous most common genotype is taken as reference. EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma.
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these confounders. Moreover, the overall study population was large.

This study also has its limitations. First of all, the matching by age was not succeeded completely, as the mean ages between the GI- and HNC patient and control groups differed significantly. Therefore, unconditional logistic regression was used which theoretically creates a lower power compared to conditional logistic regression. Secondly, the ORs calculated in the sub-analyses were not adjusted for multiple testing. This might be advisable since multiple testing may increase the change to find a significant association and thus creates bias. Thirdly, in this study no information on smoking habits, alcohol consumption, diet and other lifestyle factors is available. Furthermore, obesity of which BMI and hip-to-waist ratio are suitable indicators, is an important confounder for GI cancer [33]. Obesity may influence the risk of colorectal cancer, and excess of body fat and fat distribution also appeared to be related to EAC [33]. Co-morbidity, especially early diagnosed diabetes mellitus, is associated with various cancers, including cancers of the colorectum [34]. Moreover, in analyzing the relationship between polymorphisms in the components of the IGF axis and cancer in women, the use of birth control pills needs to be taken into consideration, since the sex hormone estradiol has a direct, as well as an indirect influence on the production of IGF-I in estrogen-responsive tissues and the liver [35]. Other limitation of this study is that the study populations in the sub-analyses were relatively small, and that only one control was matched with one patient.

In conclusion, the A allele of SNP rs6214 of the IGF-I gene is associated with esophageal cancer. This association might be mainly the result of effects on the histological subtype esophageal adenocarcinoma in men. Furthermore, women with the A allele of SNP rs6214 may have an increased risk for developing GI cancer, more in particular HNC. These SNPs could be used as markers to assess the cancer risk for these (histological) subtypes.

### Author Contributions

Conceived and designed the experiments: JO JS RHMTM HMJR PD WHMP. Performed the experiments: JO JS RHMTM HMJR. Analyzed the data: JO JS RHMTM HMJR WHMP. Contributed reagents/materials/analysis tools: JO JS RHMTM HMJR BJMW PD ML. Wrote the paper: JO JS RHMTM HMJR BJMW PD ML WHMP.

**References**