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# Effect of IGF Gene Polymorphisms Alone or In Interaction with Diabetes on the Risk of Pancreatic Cancer

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## Abstract

Insulin-like growth factors (IGFs) have been associated with risk of common human cancers, but the association between IGFs and pancreatic cancer risk is unclear. To determine whether genetic variations of *IGF* modify pancreatic cancer risk, we compared the frequency of six single nucleotide polymorphisms (SNPs) of *IGF1* and *IGF2* in a large-scale case control study.

SNPs were investigated using the Taqman method in 892 patients with pancreatic ductal adenocarcinoma and 783 healthy controls who were recruited from M. D. Anderson Cancer Center from 2000−2007. Cases and controls were frequency matched by age (± 5 years), race, and sex. Risk factor information was collected using direct interviews. We estimated odds ratios (ORs) and 95% confidential intervals (CIs) using unconditional multivariate logistic regression models.

A haplotype of *IGF1* gene containing the *3'UTR Ex4* −177 G>C G allele had a significantly lower frequency in cases (0.027) than in controls (0.041), *P*=0.039. A statistically significant joint effect of the *IGF1 3'UTR Ex4* −177 G>C C allele and diabetes on pancreatic cancer risk was observed. The ORs (95% CI) were 1.07 (0.81−1.42), 2.12 (1.53−2.93), and 5.69 (2.63−12.3) for individuals who had the CC/CG genotype alone, diabetes alone, or both factors, respectively, compared with subjects without either of the two factors with adjustment for other risk factors. The *IGF2 3'UTR Ex4* −233C>T TT genotype was significantly associated with a reduced risk of pancreatic cancer (OR: 0.07, 95% CI: 0.01−0.57, *P*=0.013).

The polymorphic variants of the *IGF* genes may serve as a susceptibility factor for pancreatic cancer.

### Keywords

IGF; Diabetes; Pancreatic cancer

## Introduction

Pancreatic cancer is the fourth leading cause of death from cancer and causes more than 33,000 deaths per year in the United States (1). The known and suspected risk factors for pancreatic cancer include cigarette smoking, type II diabetes, obesity, family history of pancreatic cancer, and diet (2). Germline mutations that are associated with several hereditary syndromes account for less than 10% of the pancreatic cancer burden. Few studies have examined the role of polymorphic variations of carcinogen metabolic genes (3,4) and DNA repair genes (5-7), but

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the genetic susceptibility factors in the majority of sporadic pancreatic cancer cases are not well defined.

Accumulating evidence suggests that diet and related factors, such as physical activity and body size, may influence cancer risk through their effects on the serum concentration of insulin-like growth factor (IGF)1 and its binding proteins (8). IGF1 and IGF2 are structurally similar growth hormone-regulated polypeptides involved in both human development and the maintenance of normal function and homeostasis in most human cells. In addition to their classical role as endocrine hormones, IGFs regulate a wide range of biological functions, such as cell proliferation, differentiation, and apoptosis, through paracrine and autocrine mechanisms (9). Also, the IGF1 receptor (IGF1R)–mediated initiation of signal transduction activates important intracellular signal pathways, including the Ras/Raf/MAP kinase and phosphoinositide-3 kinase pathways (10). IGF1 and IGF1R are highly expressed in pancreatic cancer cells (11), and in pancreatic cancer cell lines, IGF1-mediated signaling transduction leads to increased proliferation and invasion, the expression of angiogenesis mediators, and decreased apoptosis (12-14). IGF2 is overexpressed and its imprinting is disrupted in many tumors (15,16). Furthermore, in vitro and in vivo studies have suggested that the over expression of IGF2 results in a more malignant phenotype and increased tumor formation in nude mice (17).

Although strong experimental evidence suggests that IGFs play a role in carcinogenesis, the results of epidemiological investigations are less persuasive. For example, polymorphic variants of the *IGF1* gene and elevated serum levels of IGF1 protein have been associated with an increased risk of common cancers like prostate, colorectal, and breast cancers in some studies but not in others (18-24). Information on *IGF2* polymorphisms and their association with cancer risk is scarce, and the results that have been published are similarly inconsistent (25,26). In addition, no study we are aware of has yet examined the association between *IGF* gene polymorphisms and risk of pancreatic cancer. When cohort studies were conducted to examine the association between pancreatic cancer risk and prediagnostic plasma levels of IGF1 and IGF2, no association was observed (27,28). However, in one of two case control studies we identified in our review of the literature, investigators found a slightly increased risk of pancreatic cancer associated with elevated plasma IGF1 in a limited number of samples (29,30).

To fill in the gap between IGF and pancreatic cancer, we tested the hypothesis that polymorphic variants of the *IGF1* and *IGF2* genes alone or through an interaction with diabetes modify the risk of pancreatic cancer. To do so, we examined the associations between pancreatic cancer risk and selected six single nucleotide polymorphisms (SNPs) of the *IGF1* and *IGF2* genes in 892 patients with pancreatic adenocarcinoma and in 793 healthy controls. Our results demonstrate for the first time that the *IGF1* gene alone or in concert with diabetes to increase the risk of pancreatic cancer.

## Materials and Methods

### Study Population

The study population and design of this hospital-based case control study have previously been described in detail (31). Briefly, consecutive patients with pathologically confirmed primary pancreatic ductal adenocarcinoma and controls were recruited at The University of Texas M. D. Anderson Cancer Center from 2000 to 2007. Controls were the healthy spouses, friends, or non–blood relatives of patients with various non-gastrointestinal and non–smoking related cancers. Controls were frequency-matched to cases by age at enrollment (± 5 years), sex, and race. Smoking and alcohol consumption history, medical history including diabetes, family history of cancer, and information on other risk factors were collected by personal interview.

Body mass index (BMI, kg/m$^2$) data were collected from study participants recruited in 2004 and later. All study subjects were U.S. residents and were able to communicate in English. Written informed consent was obtained from each study participant for the interviews and the collection of a blood sample. The study was approved by the Institutional Review Board of M. D. Anderson Cancer Center. A total of 1318 cases were consented from the 1635 cases approached with a response rate of 80.6% and 969 controls were recruited from 1260 individuals approached with a response rate of 76.9%. Eighty-nine cases and 167 controls were excluded from the current study because of missing or incomplete risk factor information; 171 cases and 19 controls were excluded because of missing or inadequate DNA samples. In addition, 166 cases were excluded because of final diagnosis of disease other than pancreatic adenocarcinoma. Therefore the total number of cases and controls involved in the current study is 892 and 783, respectively.

## DNA Extraction and Genotyping Assays

Peripheral blood mononuclear cells were collected from freshly drawn blood by Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscataway, NJ) density gradient centrifugation and stored at −80°C. DNA was extracted with the use of a FlexiGene DNA kit (Qiagen, Valencia, CA) and a Maxwell16 automated system (Promega, Madison, WI) and stored at 4 °C for immediate use. DNA concentration was determined by using a NanoDrop ND1000 spectrophotometer (NanoDrop, Wilmington, DE).

The *IGF* SNPs were selected from the NCI SNP500Cancer database. Neither of the two genes has nonsynonymous SNPs listed in this database. We selected SNPs from the 3'-UTR region and the intron regions with minor allele frequency greater than 5%. Genotypes were determined using the Taqman diallelic discrimination method (32). Details on the six SNPs examined in this study are given in Table 1. Probes and oligonucleotides were obtained from Applied Biosystems (Foster City, CA) using the Assay-by-Design product. The reactions were prepared by using 2x Taqman Universal Master Mix, 40x SNP Genotyping Assay Mix, DNase-free water, and 10 ng genomic DNA in a final volume of 5 μL per reaction. PCR amplification was completed using the ABI Prism 7900 HT sequence-detector under the following conditions: 10 min at 95 °C enzyme activation, followed by 45 cycles at 92 °C for 15 s and 60 °C for 1 min (annealing/extension). About 5% of the samples were analyzed in duplicate and 100% consistency was achieved.

## Statistical Analysis

The distribution of categorical variables and genotype frequencies between cases and controls was tested by using the $\chi^2$ test. The deviation of genotype distribution from the Hardy-Weinberg equilibrium was tested by using the goodness-of-fit $\chi^2$ test to compare the observed genotype frequencies with the expected genotype frequencies with one degree of freedom. Fisher's exact test was applied when any of the comparison groups had < 5 subjects. Risk of pancreatic cancer was estimated as odds ratios (ORs) and 95% confidence intervals (CIs) calculated using unconditional logistic regression analysis.

The statistical models on epidemiological factors were adjusted for gender, race (Non-Hispanic White, Hispanic, African American, Asian), age in years (< 50, 51−60, 61−70, > 70), smoking status (non-smoker, ≤ 20 pack-years, > 20 pack years), diabetes (yes or no), and family history of cancer among first-degree relatives (yes or no), whenever appropriate. Genotype evaluations were restricted to Non-Hispanic Whites because of the small sample size in the other groups and the known racial differences in genotype distribution. The association between genotype and risk of pancreatic cancer was adjusted for smoking and diabetes status and family history of cancer and in some analyses, was further adjusted for duration of diabetes and BMI.

To detect possible interactions of specific genotypes and diabetes, ORs were estimated using unconditional logistic regression for the following groups, with non-diabetics with the wild-type genotype as the reference group: non-diabetics with the at-risk genotype ($OR_{10}$), diabetics with the wild-type genotype ($OR_{01}$), and diabetics with the variant genotype ($OR_{11}$). An $OR_{11}$ greater than the sum of $OR_{10} + OR_{01}$ or greater than the product of $OR_{10} \times OR_{01}$ indicates a more than additive or more than multiplicative effect, respectively. To control for multiple testing, the multiplicity-adjusted *P*-value was calculated using the Bonferroni method (33). For any statistically significant association we observed, we also estimated the false-positive report probability (FPRP) using the methods described by Wacholder et al. (34). We considered that a prior probability of 25% might be appropriate when there is biologic plausibility and availability of previous epidemiologic evidence for such an association. The FPRP value for noteworthiness was set as 0.2.

Haplotype was inferred from the genotyping data and the frequencies were compared between cases and controls using Haploview 4.1 software. All other statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL) and STATA 9.0 (Stata Corp., College Station, TX) software. Values of $P < 0.05$ were considered statistically significant.

## Results

### Characteristics of the Study Subjects

Table 2 demonstrates the demographics and potential risk factors in patients and controls. The mean ages ± standard deviations (SDs) of cases and controls, in years, were 61.9 ± 10.2 and 61.0 ± 10.0, respectively ($P = 0.85$). Cases and controls were well matched by sex and age but the study enrolled slightly fewer controls in all groups except Non-Hispanic Whites. As reported previously (31,35), smoking status, diabetes status, BMI, and family history of cancer were all associated with a significantly increased risk of pancreatic cancer.

### Genotypes/Haplotypes and Risk of Pancreatic Cancer

The genotype frequencies of *IGF1* and *IGF2* are presented in Table 3. The six SNPs were successfully amplified in 97.6% to 100% of the patients and controls. The distributions of these genotypes were all in agreement with Hardy-Weinberg equilibrium (*P* value ranges: 0.556 −1.0). All three SNPs of each gene were in linkage disequilibrium with |D'| > 0.92. None of the *IGF1* genotypes was significantly associated with pancreatic cancer. Four haplotypes of the *IGF1* gene was inferred from the genotyping data. A haplotype containing the 16540 G, 1830 T and −177 G alleles had a significantly lower frequency in cases (0.27) than in controls (0.41), $P = 0.039$. Although the number is small, a significant difference was observed between the *IGF2−233* C>T TT genotype distributions of cases and controls. Eleven controls and only one case had the homozygous mutant TT genotype, which translates into a 93% reduction of pancreatic cancer risk ($P = 0.013$). The multiplicity-adjusted $P = 0.078$. The FPRP = 0.152 ($P = 0.01$), which indicates that the chance of this result being false-positive is small. Similarly, another *IGF2* SNP, −69 TT, also showed a protective effect against pancreatic cancer, although this result was at borderline significant ($P = 0.051$). None of the 3 major haplotypes of the *IGF2* gene was associated with pancreatic cancer. To avoid the reversal causation problem caused by pancreatic caner-associated diabetes, we performed risk analysis after excluding individuals with less than 2 years of diabetes duration. However, no significant change in the risk estimates was observed in these analyses compared to the results from the analyses of all study participants (Table 3).

### Interaction of *IGF* Genotypes with Diabetes and BMI

To investigate the influence of genotype on the associations between both diabetes and BMI with risk of pancreatic cancer, we compared genotype distributions in subgroup analyses.

Because the homozygous mutations occurred so infrequently in the study subgroups, they were combined with the heterozygous variants in this analysis. As shown in Table 4, a significant difference between the distributions of *IGF1* −177 GC/CC genotype in cases and controls was observed among diabetics but not among non-diabetics. The GC/CC genotype frequencies in cases and controls were 19.7% and 18.8% among non-diabetics and 25.0% and 11% among diabetics ($P = 0.013$, $\chi^2$ -test). The *IGF1* −177 GC/CC genotype frequencies tended to be lower among diabetic controls but higher among diabetic cases compared with the non-diabetic counterparts, but none of the differences was statistically significant (Table 4). The *IGF1* −177 GC/CC genotype frequencies tended to be higher among both cases (24.1%) and controls (21.0%) with BMI > 25 kg/m$^2$ compared to cases (17.7%) and controls (16.1%) with BMI ≤ 25 kg/m$^2$, but the differences were not statistically significant. The other two *IGF*1 genotypes and all *IGF2* genotypes were not distributed differently between cases and controls by BMI (data not shown).

Next we used two-by-four tables and conducted logistic regression analyses to examine the interaction between the *IGF1* genotype and diabetes and how it modifies the risk of pancreatic cancer. Using the non-diabetics with the wild-type genotype as the reference group, we observed a more than additive effect of the *IGF1*-177 C allele and diabetes on the risk of pancreatic cancer. When compared to subgroups with neither the C allele nor diabetes, the adjusted ORs (95% CIs) for individuals with the C allele alone, diabetes alone, and both factors were 1.07 (0.81−1.42), 2.12 (1.53−2.93), and 5.69 (2.63−12.3), respectively. When the same analysis was performed on data from individuals who had had diabetes for > 2 years, the OR for individuals with diabetes alone dropped from 2.12 to 1.38, which indicates that the association of diabetes and pancreatic cancer was overestimated when all study subjects were included in the analysis. However, the OR remained at 5.50 for individuals with both diabetes and the *IGF1* −177 GC/CC genotypes. Further adjusting for BMI reduced the magnitude of the ORs but all ORs for individuals with diabetes and carrying the −177 C allele remained statistically significant (Table 5). Assuming the OR for individuals with both diabetes and the IGF variant allele was 3.5, the FPRP for the observed ORs were < 5%.

## Discussion

In this large case-control study, we found an *IGF1* haplotype and the *IGF2* Ex4 −233 C>T TT genotype was significantly associated with reduced risk of pancreatic cancer. We also observed a synergistic effect of the *IGF1* Ex4 −177 G>C C allele and diabetes on the risk of pancreatic cancer. These observations support the hypothesis that polymorphic variants of the *IGF* genes modulate pancreatic carcinogenesis.

The relationship between diabetes and pancreatic cancer has been controversial (36). Even though diabetes frequently occurs as a consequence of pancreatic cancer (37), increasing epidemiological evidence supports the idea that long-standing diabetes has a role in causing pancreatic cancer (38,39). Overt type II diabetes is usually characterized by hyperglycemia, insulin resistance, and hyperinsulinemia. Even though insulin does not have direct carcinogenic effects on the pancreas, it may function in an integrated fashion with IGFs to promote tumor development (9). Specifically, it is known that more than 90% of the circulating IGF1 is bound to IGFBP-3 and insulin affects IGF1 bioavailability by regulating the growth hormone receptor and reducing the level of IGFBPs. Considering the role that insulin and IGF1 play in cell growth promotion; it is conceivable that individuals with a higher level of IGF1 activity as conferred either by genetic variation or by hyperinsulinemia would have much greater chance of tumor development.

Among the three *IGF1* SNPs examined in this study, two are located in the 3'UTR region of Exon 4 of the gene. Even though the 3'-UTR sequences do not translate into proteins, they

may contain sequence motifs crucial for the regulation of transcription, mRNA stability, and cellular location of the mRNA or the binding of microRNA (40). Due to the small number of the homozygous mutant allele carriers in the current study we could not directly explore the genotype and phenotype association. However, two previous studies have shown that the homozygous mutant allele of one of the two 3' UTR SNPs (rs6220, *IGF1* Ex4 +1830 C>T) was associated with elevated serum IGF1 level (41). Also, our finding that the SNP associated with an increased risk of pancreatic cancer among patients with diabetes (*IGF1* Ex4−177 G>C, rs5742714) is in linkage disequilibrium (D'=0.96) with SNP rs6220 indicates that the increased risk of pancreatic cancer associated with this SNP is most likely caused by the higher level of IGF1 activity conferred by the variant allele. The association of pancreatic cancer and *IGF1* −177 GC/CC genotype seemed to be related to a lower frequency of diabetes in controls and a higher frequency of diabetes in cases among the genotype carriers. The mechanism underlying this differential association is not understood. It could be related to the different cellular functions that IGF1 plays in normal versus cancer cells (9,10).

The current study has also observed a possible protective effect of the *IGF2* gene 3'UTR −233 C>T TT homozygous mutant against pancreatic cancer. However, the frequency of the variant genotype was extremely low (< 2%) and the *P* value for the risk association was not statistically significant after adjusting for multiple test, so the possibility that this observation was made by chance alone cannot be excluded. Compared to levels of IGF1, high concentrations of IGF2 were present in circulation. IGF2 is involved in cell growth and development and is assumed to act in an autocrine manner, primarily via IGF1R, where IGF2R functions as a "scavenger" receptor. *IGF2* is overexpressed, and its imprinting is disrupted in many primary tumors and cancer cell lines (15,16). Furthermore, in nude mice, the overexpression of IGF2 has been shown to promote more malignant tumor phenotypes and results in more efficient tumor formation (17). We presume that the reduced risk of pancreatic cancer associated with the *IGF2* genotype was via a reduced growth-promoting activity that the polymorphic variant confers. The *IGF2* Ex4−233 C>T SNP is located within a CpG island of the 3'UTR region; whether the C-to-T transition affected the methylation status as suggested in other studies (42-44) need further investigation. A number of studies have shown *IGF2* polymorphisms to be significantly associated with BMI and birth weight (45-48). However, we failed to detect any association of *IGF2* genotype and BMI among controls in this study. Because of the low frequency of the homozygous mutant variants we examined and incomplete BMI data, our study did not have sufficient power to examine the interaction of the *IGF2* genotype with BMI on modifying the risk of pancreatic cancer.

Our study has several limitations. First, the study was conducted in a single tertiary referral hospital; results from this study population may not be generalized to the U.S. population. Second, the association of genotype and risk of pancreatic cancer could be biased if our study missed a lot of patients that were succumbed to this fatal disease rapidly and if the SNPs in question were associated with patient survival. Third, the study has a lower power to detect the main genotype effect in low-frequency homozygous mutants. For example, at the current study size, the power for detecting the main effect of the IGF2−233 C>T SNP was only 60%. Even though the IGF1−177 G>C C allele frequency was >10%, the prevalence of diabetes was 10% among controls, and the synergistic effect of gene-diabetes was observed in only 44 cases and eight controls, which yields less precision than we desired in the risk estimate. Fourth, the number of SNPs and genes included in this study were limited, the functional significance of the SNPs studied was unknown, and no phenotypic markers were included. Because it is known that IGF function can be significantly affected by the status of IGF receptors and IGF binding proteins, a comprehensive analysis of genes involved in the IGF axis and a systematic selection of SNPs of these genes would be required to fully elucidate the role of these genetic variations in pancreatic cancer. Illustrating genotype-phenotype associations by continuing this line of

investigation may help reveal the mechanisms underlying the associations between genetic variations in IGF and the development of pancreatic cancer.

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## Abbreviations used

IGF, insulin-like growth factor; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction.

## References

1. American Cancer Society. Cancer Figures and Facts. American Cancer Society; Atlanta, GA: 2007.
2. Anderson, K.; Potter, JD.; Mack, TM. Pancreatic Cancer.. In: Schottenfeld, D.; Fraumeni, JF., Jr., editors. Cancer Epidemiology and Prevention. Vol. 725−771. Oxford University Press; New York, NY: 2006.
3. Duell EJ, Holly EA, Bracci PM, et al. A population-based, case–control study of polymorphisms in carcinogen-metabolizing genes, smoking, and pancreatic adenocarcinoma risk. J Natl Cancer Inst 2002;94:297–306. [PubMed: 11854392]
4. Li D, Jiao L, Li Y, et al. Polymorphisms of cytochrome P4501A2 and N-acetyltransferase genes, smoking, and risk of pancreatic cancer. Carcinogenesis 2006;27:103–111. [PubMed: 15987714]
5. Duell EJ, Holly EA, Bracci PM, Liu M, Wiencke JK, Kelsey KT. A population-based study of the Arg399Gln polymorphism in X-ray repair cross- complementing group 1 [XRCC1] and risk of pancreatic adenocarcinoma. Cancer Res 2002;62:4630–4636. [PubMed: 12183419]
6. Jiao L, Hassan MM, Bondy ML, et al. XRCC2 and XRCC3 Gene polymorphism and risk of pancreatic cancer. Am J Gastro 2008;103(2):360–367.
7. McWilliams RC, Bamlet WR, Cunningham JM, et al. Polymorphisms in DNA Repair Genes, Smoking, and Pancreatic Adenocarcinoma Risk. Cancer Res 2008;68:4928–4935. [PubMed: 18544627]
8. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363(9418):1346–1353. [PubMed: 15110491]
9. Fürstenberger G, Senn HJ. Insulin-like growth factors and cancer. Lancet Oncol 2002;3:298–302. [PubMed: 12067807]
10. Werner H, Le Roith D. New concepts in regulation and function of the insulin-like growth factors: implications for understanding normal growth and neoplasia. Cell Mol Life Sci 2000;57:932–42. [PubMed: 10950308]
11. Bergmann U, Funatomi H, Yokoyama M, Beger HG, Korc M. Insulin-like growth factor I overexpression in human pancreatic cancer: evidence for autocrine and paracrine roles. Cancer Res 1995;55:2007–2011. [PubMed: 7743492]
12. Ohmura E, Okada M, Onoda N, et al. Insulin-like growth factor I and transforming growth factor alpha as autocrine growth factors in human pancreatic cancer cell growth. Cancer Res 1990;50:103–107. [PubMed: 2152769]
13. Zeng H, Datta K, Neid M, Li J, Parangi S, Mukhopadhyay D. Requirement of different signaling pathways mediated by insulin-like growth factor-I receptor for proliferation, invasion, and VPF/VEGF expression in a pancreatic carcinoma cell line. Biochem Biophys Res Commun 2003;302:46–55. [PubMed: 12593846]
14. Stoeltzing O, Liu W, Reinmuth N, et al. Regulation of hypoxia-inducible factor-1α, vascular endothelial growth factor, and angiogenesis by an insulin-like growth factor-I receptor autocrine loop in human pancreatic cancer. Am J Pathol 2003;163:1001–1011. [PubMed: 12937141]
15. Cui H, Cruz-Correa M, Giardiello FM, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 2003;299:1753–1755. [PubMed: 12637750]

16. Nordqvist AC, Mathiesen T. Expression of *IGF*-II, IGFBP-2, -5, and -6 in meningiomas with different brain invasiveness. J Neurooncol 2002;57:19–26. [PubMed: 12125963]

17. Sakatani T, Kaneda A, Iacobuzio-Donahue CA, et al. Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. Science 2005;307:1976–1978. [PubMed: 15731405]

18. Cheng I, Stram DO, Penney KL, et al. Common genetic variation in *IGF1* and prostate cancer risk in the Multiethnic Cohort. J Natl Cancer Inst 2006;98:123–34. [PubMed: 16418515]

19. Ma J, Pollak MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 1999;91:620–625. [PubMed: 10203281]

20. Wong HL, Delellis K, Probst-Hensch N, et al. A new single nucleotide polymorphism in the insulin-like growth factor I regulatory region associates with colorectal cancer risk in Singapore Chinese. Cancer Epidemiol Biomarkers Prev 2005;14:144–151. [PubMed: 15668488]

21. Canzian F, McKay JD, Cleveland RJ, et al. Polymorphisms of genes coding for insulin-like growth factor 1 and its major binding proteins, circulating levels of IGF-I and IGFBP-3 and breast cancer risk: results from the EPIC study. Br J Cancer 2006;94:299–307. [PubMed: 16404426]

22. Al-Zahrani A, Sandhu MS, Luben RN, et al. *IGF1* and IGFBP3 tagging polymorphisms are associated with circulating levels of *IGF1*, IGFBP3 and risk of breast cancer. Hum Mol Genet 2006;15:1–10. [PubMed: 16306136]

23. Missmer SA, Haiman CA, Hunter DJ, et al. A sequence repeat in the insulin-like growth factor-1 gene and risk of breast cancer. Int J Cancer 2002;100:332–336. [PubMed: 12115549]

24. Hernandez W, Grenade C, Santos ER, Bonilla C, Ahaghotu C, Kittles RA. *IGF1* and IGFBP-3 gene variants influence on serum levels and prostate cancer risk in African-Americans. Carcinogenesis 2007;28:2154–2159. [PubMed: 17724372]

25. Lai MT, Chen RH, Tsai FJ, Wan L, Chen WC. Glutathione S-transferase M1 gene but not insulin-like growth factor-2 gene or epidermal growth factor gene is associated with prostate cancer. Urol Oncol 2005;23:225–229. [PubMed: 16018936]

26. Kim YJ, Yoon JH, Kim CY, et al. *IGF2* polymorphisms are associated with hepatitis B virus clearance and hepatocellular carcinoma. Biochem Biophys Res Commun 2006;346:38–44. [PubMed: 16750516]

27. Stolzenberg-Solomon RZ, Limburg P, Pollak M, Taylor PR, Virtamo J, Albanes D. Insulin-like growth factor (IGF)-1, IGF-binding protein-3, and pancreatic cancer in male smokers. Cancer Epidemiol Biomarkers Prev 2004;13:438–444. [PubMed: 15006921]

28. Wolpin BM, Michaud DS, Giovannucci EL, et al. Circulating insulin-like growth factor axis and the risk of pancreatic cancer in four prospective cohorts. Br J Cancer 2007;97:98–104. [PubMed: 17533398]

29. Karna E, Surazynski A, Orlowski K, et al. Serum and tissue level of insulin-like growth factor-I (IGF-I) and IGF-I binding proteins as an index of pancreatitis and pancreatic cancer. Int J Exp Pathol 2002;83:239–245. [PubMed: 12641820]

30. Evans JD, Eggo MC, Donovan IA, Bramhall SR, Neoptolemos JP. Serum levels of insulin-like growth factors (IGF-I and IGF-II) and their binding protein (IGFBP-3) are not elevated in pancreatic cancer. Int J Pancreatol 1997;22:95–100. [PubMed: 9387030]

31. Hassan MM, Wolff RA, Bondy ML, et al. Risk factors for pancreatic cancer: synergy of smoking with family history of pancreatic cancer and diabetes mellitus. Am J Gastr 2007;102:2696–2707.

32. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 1999;14:143–149. [PubMed: 10084106]

33. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310:170. [PubMed: 7833759]

34. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 2004;96:434–442. [PubMed: 15026468]

35. Li D, Hassan MM. Letter of response to comments on the article entitled “Passive smoking and use of noncigarette tobacco products and risk for pancreatic cancer: case-control study.”. Cancer 2008;112(3):672.

36. Wang F, Herrington M, Larsson J, et al. The relationship between diabetes and pancreatic cancer. Mol Cancer 2003;2:4. [PubMed: 12556242]
37. Permert J, Ihse I, Jorfeldt L, et al. Pancreatic cancer is associated with impaired glucose metabolism. Eur J Surg 1993;159:101–107. [PubMed: 8098623]
38. Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. JAMA 1995;273:1605–1609. [PubMed: 7745774]
39. Huxley R, Ansary-Moghaddam A, Berrington de González A, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. Br J Cancer 2005;92:2076–2083. [PubMed: 15886696]
40. Mazumder B, Seshadri V, Fox PL. Translational control by the 3'-UTR: the ends specify the means. Trends Biochem Sci 2003;28(2):91–98. [PubMed: 12575997]
41. Diorio C, Brisson J, Berube S, Pollak M. Genetic polymorphisms involved in insulin-like growth factor (IGF) pathway in relation to mammographic breast density and IGF levels. Cancer Epidemiol Biomarkers Prev 2008;17:880–888. [PubMed: 18398029]
42. Sandovici I, Leppert M, Hawk PR, Suarez A, Linares Y, Sapienza C. Familial aggregation of abnormal methylation of parental alleles at the IGF2/H19 and IGF2R differentially methylated regions. Hum Mol Genet 2003;12:1569–1578. [PubMed: 12812984]
43. Sakatani T, Wei M, Katoh M, et al. Epigenetic heterogeneity at imprinted loci in normal populations. Biochem Biophys Res Commun 2001;283:1124–1130. [PubMed: 11355889]
44. Savage SA, Woodson K, Walk E, et al. National Osteosarcoma Etiology Study Group. Analysis of genes critical for growth regulation identifies insulin-like growth factor 2 receptor variations with possible functional significance as risk factors for osteosarcoma. Cancer Epidemiol Biomarkers Prev 2007;16:1667–1674. [PubMed: 17684144]
45. Gaunt TR, Cooper JA, Miller GJ, Day IN, O'Dell SD. Positive associations between single nucleotide polymorphisms in the IGF2 gene region and body mass index in adult males. Hum Mol Genet 2001;10:1491–1501. [PubMed: 11448941]
46. Gomes MV, Soares MR, Pasqualim-Neto A, Marcondes CR, Lôbo RB, Ramos ES. Association between birth weight, body mass index and *IGF2*/ApaI polymorphism. Growth Horm IGF Res 2005;15:360–362. [PubMed: 16102992]
47. O'Dell SD, Miller GJ, Cooper JA, et al. Apal polymorphism in insulin-like growth factor II (*IGF2*) gene and weight in middle-aged males. Int J Obes Relat Metab Disord 1997;21:822–825. [PubMed: 9376897]
48. Heude KK, Ong R, Luben NJ, Wareham, Sandhu MS. Study of association between common variation in the insulin-like growth factor 2 gene and indices of obesity and body size in middle-aged men and women. J Clin Endocrinol Metab 2007;92(7):2734–2738. [PubMed: 17488802]

**Table 1**

Characteristics of examined SNPs

| Gene | Chromosome | SNP | Location | RS# | MAF | D' | D' |
|---|---|---|---|---|---|---|---|
| ***IGF1*** | 12q22-q23 | IVS2−16540 A>G | Intron | 2288378 | 0.75 | | |
| | | Ex4+1830 C>T | 3'UTR | 6220 | 0.603 | 0.96 | |
| | | Ex4−177 G>C | 3'UTR | 5742714 | 0.137 | 0.97 | 0.94 |
| ***IGF2*** | 11p15.5 | IVS1+1280 A>G | Intron | 3213216 | 0.325 | | |
| | | IVS2−69 C>T | Intron | 3213232 | 0.118 | −0.92 | |
| | | Ex4−233 C>T | 3'UTR | 2230949 | 0.051 | −0.97 | 0.98 |

SNP: single nucleotide polymorphism; RS#: SNP reference number; MAF: minor allele frequency, obtained from NCI SNP500 cancer database.

**Table 2**

Distribution of selected variables among patients and controls

| Variable | No. cases (%) | No. controls (%) | Odds ratio (95% confidence interval) |
|---|---|---|---|
| Total | 892 (100) | 783 (100) | |
| Age at recruitment, years | | | |
| < 50 | 123 (14) | 121 (15) | Matching factor |
| 51−60 | 262 (29) | 246 (31) | |
| 61−70 | 314 (35) | 270 (34) | |
| > 70 | 193 (22) | 146 (19) | |
| Sex | | | |
| Female | 375 (42) | 318 (40) | Matching factor |
| Male | 517 (58) | 465 (59) | |
| Race | | | |
| Non-Hispanic White | 795 (89) | 721 (92) | Matching factor |
| Hispanic | 51 (6) | 38 (5) | |
| African American | 46 (5) | 24 (3) | |
| Family history of cancer[a] | | | |
| No | 314 (35) | 357 (46) | 1.0 (reference) |
| Yes | 574 (65) | 423 (54) | 1.54 (1.27−1.88) |
| History of diabetes | | | |
| None | 671 (75) | 702 (90) | 1.0 (reference) |
| <2 years[b] | 95 (11) | 20 (3) | 4.97 (3.03−8.14) |
| ≥2 years | 126 (14) | 61 (8) | 2.16 (1.56−2.97) |
| Smoking status | | | |
| Non-smoker | 363 (41) | 390 (50) | 1.0 (reference) |
| ≤ 20 pack-years | 210 (24) | 200 (26) | 1.13 (0.89−1.44) |
| > 20 pack-years | 319 (36) | 193 (25) | 1.78 (1.41−2.23) |
| Body mass index (kg/m$^2$)[c] | | | |
| < 25.0 | 262 (52) | 300 (62) | 1.0 (reference) |
| 25.0−30.0 | 180 (35) | 155 (32) | 1.33 (1.01−1.74) |
| > 30.0 | 66 (13) | 26 (5) | 2.91 (1.79−4.71) |

[a] Four cases and three controls had been adopted; this information was not available.

[b] Years between diabetes and cancer diagnoses for cases or between diabetes diagnosis and study enrollment for controls.

[c] Information was available for only 508 cases and 481 controls.

**Table 3**

Genotype distribution and association with risk for pancreatic cancer

| Genotype | All subjects | | | Subjects with history of ≥ 2 years of diabetes mellitus | |
|---|---|---|---|---|---|
| | Cases No. (%) | Controls No. (%) | OR (95% CI)[a]P | Case/control No./No, | OR (95% CI)[a]P |
| ***IGF1*** 16540 A>G | | | | | |
| GG | 436 (55.2) | 389 (54.1) | 1.0 (reference) | 386/380 | 1.0 (reference) |
| AG | 296 (37.5) | 284 (39.5) | 0.93 (0.74−1.15) 0.490 | 268/276 | 0.95 (0.76−1.19) 0.655 |
| AA | 58 (7.3) | 46 (6.4) | 1.21 (0.80−1.85) 0.362 | 56/46 | 1.24 (0.82−1.90) 0.311 |
| A allele | 0.261 | 0.262 | | | |
| ***IGF1*** 1830 C>T | | | | | |
| TT | 380 (49.1) | 362 (51.3) | 1.0 (reference) | 339/354 | 1.0 (reference) |
| CT | 322 (41.6) | 286 (40.5) | 1.05 (0.84−1.30) 0.698 | 288/278 | 1.06 (0.85−1.34) 0.587 |
| CC | 72 (9.3) | 58 (8.2) | 1.28 (0.87−1.88) 0.212 | 68/58 | 1.28 (0.87−1.88) 0.218 |
| C allele | 0.291 | 0.285 | | | |
| ***IGF1*** −177 G>C | | | | | |
| GG | 629 (79.1) | 591 (82.0) | 1.0 (reference) | 565/577 | 1.0 (reference) |
| CG | 154 (19.4) | 124 (17.2) | 1.16 (0.89−1.52) 0.272 | 137/121 | 1.16 (0.88−1.52) 0.304 |
| CC | 12 (1.5) | 6 (0.8) | 2.28 (0.78−6.61) 0.131 | 12/6 | 2.41 (0.83−6.95) 0.105 |
| C allele | 0.112 | 0.094 | | | |
| ***IGF2*** 1280 A>G | | | | | |
| GG | 298 (38.2) | 280 (39.3) | 1.0 (reference) | 284/279 | 1.0 (reference) |
| AG | 373 (47.8) | 335 (47.1) | 1.07 (0.85−1.34) 0.556 | 351/328 | 1.10 (0.87−1.38) 0.428 |
| AA | 109 (14.0) | 97 (13.6) | 1.09 (0.79−1.51) 0.615 | 101/94 | 1.14 (0.81−1.59) 0.451 |
| G allele | 0.379 | 0.372 | | | |
| ***IGF2*** −69 C>T | | | | | |
| CC | 659 (83.4) | 595 (82.8) | 1.0 (reference) | 591/578 | 1.0 (reference) |
| CT | 127 (16.1) | 114 (15.9) | 1.00 (0.75−1.33) 0.997 | 116/114 | 0.97 (0.73−1.30) 0.859 |
| TT | 4 (0.5) | 10 (1.4) | 0.26 (0.07−1.01) 0.051 | 3/10 | 0.21 (0.04−0.97) 0.046 |
| T allele | 0.086 | 0.094 | | | |
| ***IGF2*** −233 C>T | | | | | |
| CC | 661 (84.1) | 597 (83.7) | 1.0 (reference) | 593/580 | 1.0 (reference) |
| CT | 124 (15.8) | 105 (14.7) | 1.07 (0.80−1.42) 0.670 | 114/105 | 1.04 (0.78−1.39) 0.797 |
| TT | 1 (0.1) | 11 (1.5) | 0.07 (0.01−0.57) 0.013[b] | 1/11 | 0.08 (0.01−0.65) 0.018 |

| Genotype | All subjects | | | Subjects with history of ≥ 2 years of diabetes mellitus | |
|---|---|---|---|---|---|
| | Cases No. (%) | Controls No. (%) | OR (95% CI)[a] P | Case/control No./No, | OR (95% CI)[a] P |
| T allele | 0.080 | 0.089 | | | |

[a] Adjusted for age, sex, diabetes status, smoking status, and family history of cancer.

[b] OR (95% CI) = 0.09 (0.01−0.89), $P$=0.024 with further adjustment for body mass index.

**Table 4**

Distribution of genotypes by diabetes status

| Genotype | No. subjects (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Without diabetes | | With diabetes | | | With diabetes > 2 years | | |
| | Cases | Controls | Cases | Controls | *P* | Cases | Controls | *P* |
| ***IGF1*** 16540 A>G | | | | | 0.107 | | | 0.062 |
| GG | 339 (55.1) | 343 (53.1) | 97 (55.4) | 46 (63.0) | | 47 (49.5) | 37 (66.1) | |
| AG & AA | 276 (44.9) | 303 (46.9) | 78 (46.6) | 27 (37.0) | | 48 (50.5) | 19 (33.9) | |
| ***IGF1*** 1830 C>T | | | | | 0.595 | | | 0.515 |
| TT | 297 (49.3) | 324 (50.9) | 83 (48.3) | 38 (54.3) | | 42 (45.2) | 30 (55.6) | |
| CT & CC | 305 (50.7) | 312 (49.1) | 89 (51.7) | 32 (45.7) | | 51 (54.8) | 24 (44.4) | |
| ***IGF1*** − 177 G>C | | | | | 0.097 | | | 0.065 |
| GG | 497 (80.3) | 526 (81.2) | 132 (75.0) | 65 (89.0) | | 68 (71.6) | 51 (91.1) | |
| CG & CC | 122 (19.7) | 122 (18.8) | 44 (25.0) | **8 (11.0)**[a] | | 27 (28.4) | **5 (8.9)** | |
| ***IGF2*** 1280 A>G | | | | | 0.363 | | | 0.901 |
| GG | 231 (37.7) | 256 (39.9) | 67 (40.1) | 24 (34.3) | | 38 (41.3) | 22 (40.7) | |
| AG & AA | 382 (62.3) | 386 (60.1) | 100 (59.9) | 46 (65.7) | | 54 (58.7) | 32 (59.3) | |
| ***IGF2*** −69 C>T | | | | | 0.847 | | | 0.441 |
| CC | 511 (83.1) | 534 (82.7) | 148 (84.6) | 61 (83.6) | | 80 (84.2) | 44 (78.6) | |
| CT & TT | 104 (16.9) | 112 (17.3) | 27 (15.4) | 12 (16.4) | | 15 (15.8) | 12 (21.4) | |
| ***IGF2*** −233 C>T | | | | | 0.769 | | | 0.533 |
| CC | 515 (83.7) | 535 (83.6) | 146 (85.4) | 62 (84.9) | | 78 (83.9) | 45 (80.4) | |
| CT & TT | 100 (16.3) | 105 (16.4) | 25 (14.6) | 11 (15.1) | | 15 (16.1) | 11 (19.6) | |

[b] *P* values are from $\chi^2$-test for comparison of genotype distributions between non-diabetics and diabetics with >2 years of duration among controls.

[c] *P* = 0.013 and [d] *P*=0.052 from $\chi^2$-test for comparison of genotype distributions between cases and controls. All other P values are >0.05.

[a] *P* values are from $\chi^2$-test for comparison of genotype distributions between diabetic and non-diabetic controls.

**Table 5**

Joint Effect of *IGF1* genotype with diabetes in on risk of pancreatic cancer

| Diabetes status | All subjects | | | | | Subjects with history of ≥ 2 years of diabetes mellitus | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. cases/no. controls | AOR (95% CI)[a] | *P* | AOR (95% CI)[b] | | No. cases/no. controls | AOR (95% CI)[a] | | AOR (95% CI)[b] | |
| No | 339/343 | 1.00 | | 1.00 | | 339/343 | 1.00 | | 1.00 | |
| No | 276/303 | 0.91 (0.73−1.15) | 0.433 | 1.02 (0.77−1.38) | 0.859 | 276/303 | 0.91 (0.73−1.15) | 0.438 | 1.03 (0.76−1.38) | 0.849 |
| Yes | 97/46 | 2.07 (1.41−3.05) | <0.001 | 2.13 (1.29−3.52) | 0.003 | 47/37 | 1.22 (0.77−1.94) | 0.397 | 1.00 (0.55−1.81) | 0.996 |
| Yes | 78/27 | 2.86 (1.79−4.57) | <0.001 | 2.44 (1.39−4.29) | 0.002 | 48/19 | 2.52 (1.44−4.41) | 0.001 | 1.86 (0.94−3.68) | 0.073 |
| No | 297/324 | 1.00 | | 1.00 | | 297/324 | 1.00 | | 1.00 | |
| No | 305/312 | 1.06 (0.84−1.33) | 0.627 | 1.17 (0.87−1.57) | 0.300 | 305/312 | 1.05 (0.84−1.32) | 0.625 | 1.17 (0.87−1.58) | 0.297 |
| Yes | 83/38 | 2.32 (1.52−3.53) | <0.001 | 2.22 (1.29−3.81) | 0.004 | 42/30 | 1.45 (0.88−2.39) | 0.148 | 1.09 (0.57−2.05) | 0.803 |
| Yes | 89/32 | 2.94 (1.88−4.48) | <0.001 | 2.52 (1.49−4.27) | 0.001 | 51/24 | 2.26 (1.35−3.80) | 0.002 | 1.71 (0.91−3.22) | 0.097 |
| No | 497/526 | 1.00[c] | | 1.00 | | 497/526 | 1.00 | | 1.00 | |
| No | 122/122 | 1.07 (0.81−1.42) | 0.628 | 1.19 (0.82−1.73) | 0.358 | 122/122 | 1.07 (0.81−1.42) | 0.627 | 1.19 (0.82−1.73) | 0.355 |
| Yes | 132/65 | 2.12 (1.53−2.93) | <0.001 | 2.00 (1.32−3.03) | 0.001 | 68/51 | 1.38 (0.93−2.03) | 0.109 | 1.10 (0.67−1.82) | 0.705 |
| Yes | 44/8 | 5.69 (2.63−12.3) | <0.001 | 4.77 (1.91−11.9) | 0.001 | 27/5 | 5.50 (2.09−14.5) | 0.001 | 3.05 (1.07−8.70) | 0.037 |

d 95% confidence interval adjusted for sex, age, family history of cancer, and smoking status.

d 95% confidence interval adjusted for sex, age, family history of cancer, smoking status, and BMI.

action term of genotype and diabetes is 0.037.

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