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REVIEW

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Gene-by-Environment Interactions in Pancreatic Cancer: Implications for Prevention

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Pancreatic cancer (PC†) has been estimated to have higher incidence and correspondingly higher mortality rates in more developed regions worldwide. Overall, the age-adjusted incidence rate is $4.9/10^5$ and age-adjusted mortality rate is at $4.8/10^5$. We review here our current knowledge of modifiable risk factors (cigarette smoking, obesity, diet, and alcohol) for PC, genetic variants implicated by genome-wide association studies, possible genetic interactions with risk factors, and prevention strategies to provide future research directions that may further our understanding of this complex disease. Cigarette smoking is consistently associated with a two-fold increased PC risk. PC associations with dietary intake have been largely inconsistent, with the potential exception of certain unsaturated fatty acids decreasing risk and well-done red meat or meat mutagens increasing risk. There is strong evidence to support that obesity (and related measures) increase risk of PC. Only the heaviest alcohol drinkers seem to be at an increased risk of PC. Currently, key prevention strategies include avoiding tobacco and excessive alcohol consumption and adopting a healthy lifestyle. Screening technologies and PC chemoprevention are likely to become more sophisticated, but may only apply to those at high risk. Risk stratification may be improved by taking into account gene environment interactions. Research on these modifiable risk factors is key to reducing the incidence of PC and understanding who in the population can be considered high risk.

INTRODUCTION

The International Agency for Research on Cancer [1] estimated higher incidence and correspondingly higher mortality rates for pancreatic cancer (PC) in more developed regions worldwide. Overall, the age-adjusted incidence rate is $4.9/10^5$ and age-adjusted mortality rate is $4.8/10^5$ with little change over the past decades, reflecting lack of effective treatment and low survivorship from this cancer. The American Cancer Society estimates that there will be 48,960 new cases of PC and 40,560 deaths due to PC in the United States in 2015 [2]. Although incidence rates have risen only slightly, the absolute number of new cases is increasing. This is due to the "baby boomer" phenomenon of an aging demographic stratum of the population [3]. Older age is associated with increased risk of PC, with median age at diagnosis of 71 [4]. By the year 2030, it is projected that

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†Abbreviations: PC, pancreatic cancer; BMI, body mass index; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research; CI, confidence interval; OR, odds ratio; HR, hazard ratio; CT, computed tomography; MRI, magnetic resonance imaging; EUS, endoscopic luminal ultrasound; ERCP, endoscopic retrograde cholangiopancreatography; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; NSAID, non-steroidal anti-inflammatory drug; No-ASA, nitric oxide-donating aspirin; GWAS, genome-wide association study; PanC4, Pancreatic Cancer Case-Control Consortium.

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PC will be the second leading cause of cancer death after lung cancer among the major cancers [5]. Generally, PC is diagnosed at a late stage, which contributes to low (20 percent) resection rates [6,7]. One-year survival rate is 28 percent, and 5-year survival rate is around 7 percent, indicating poor prognosis [2]. Attempts to identify early stage PC are hindered by lack of understanding of its natural history [6], and although current imaging may be able to detect some precursor lesions [6,8], the infrequency of disease within the population makes general population screening unfeasible. An important strategy at present is to focus on modifiable risk factor identification and prevention of PC.

We review here our current knowledge of modifiable risk factors for PC, possible genetic interactions with these risk factors, and prevention strategies to provide future research directions that may further our understanding of this complex disease.

MODIFIABLE RISK FACTORS FOR PC

Tobacco Smoking

The relationship between smoking and PC has been studied extensively [9]. In the majority of published studies, smoking increases risk of PC about two-fold, with variation in estimates due to specific populations studied, sample sizes, and ways of measuring smoking exposure. A recent pooled analysis of 12 PC case-control studies reported that current smokers had an odds ratio (OR) of 2.2, compared with never smokers [10]. The risk was dependent on duration of smoking and current status with about a 10- to 20-year period required for ex-smokers to eliminate excess risk [9,10]. In addition, smoking oftentimes has a multiplicative increase in risk of PC when combined with other risk factors such as alcohol [11] and recent-onset diabetes and family history [12]. Cigarette smoke contains many known carcinogens, including N-nitrosamines, benzo(a)pyrene, polycyclic aromatic hydrocarbons, A-napthylamine, methylfluoranthenes, and arylamines [13,14], which reach the pancreas through the bloodstream. These carcinogens are capable of forming DNA adducts that increase the risk of somatic mutations and pancreatic cancer. Even among non-smokers, exposure to environmental tobacco smoke increases the risk of PC in a dose-dependent manner, with childhood exposure doubling risk of PC [14].

Obesity

The World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) reported that there is convincing evidence for an increased risk of PC for those with high body weight [15]. Based on pooled analyses, the estimated increased risk (shown here as range of relative risk (RR)) associated with specific measures is as follows: 1.02-1.14 per 5 unit increase in body mass index (BMI (kg.m2)) and 1.26 comparing BMI > 35 to 18.5-24.9; 1.38 [1.14-1.66] increase in BMI from adolescence (< 25) to study enrollment (> 30); 1.04-1.23 for high versus low waist circumference; 1.34-1.71 for high versus low waist to hip ratio. Since September 2011, two cohort studies and a pooled analysis of 14 cohort studies provide additional evidence for obesity as a risk factor for PC. Stolzenberg-Solomon et al. [16] reported that an increased risk of PC was associated with BMI > 25, increased duration of being overweight, and significant weight gain (> 10) after age 50. Levi et al. [17] reported that overweight adolescents were at an increased risk of PC (hazard ratio (HR) = 2.09; 95% confidence interval (CI): 1.26-3.50, p = 0.005). In the pooled analysis of 14 prospective studies, Genkinger et al. [18] focused on a comparison of BMI between present and early life and reported that being overweight in early adulthood and obese at time of study enrollment increased risk of PC along with BMI gains of 10 between these periods. In addition, those with the highest compared to lowest quartile of waist-to-hip ratio had an increased risk of PC (MVRR=1.35). A pooled analysis of the National Cancer Institute Pancreatic Cancer Cohort Consortium (PanScan) by Arslan et al. [19] observed a significant increased risk among those with the highest compared to lowest quartile of waist-to-hip ratio using various adjustment factors, but not with waist circumference. Hypothesized mechanisms to explain why obese individuals are at a higher risk of PC include the fact that adipocytes affect levels of circulating hormones and create chronic inflammation, making the local environment more conducive to carcinogenesis and cancer progression [20,21]. This may be a key feature in development of PC, as fat is often preferentially stored in the abdominal region in close proximity to the pancreas.

Diet

Based on an extensive literature review for PC, the WCRF/AICR reported in 2011 that evidence was limited that fruits provide protection and inconsistent regarding vegetables, suggestive for an increased risk associated with red and processed meat, food and beverages containing fructose, and saturated fatty acids [15]. Since that report was published, three cohort and four case-control studies have investigated various dietary components and PC. The focus of the more recent literature has largely been on dietary components (e.g., specific fatty acids) rather than food categories (e.g., citrus fruit). Most studies have generally suggested that numerous components of fruits and vegetables (including β-carotene, zeaxanthin and α-tocopherol, flavonoids) and whole grains provide a protective effect [22-24]. Unsaturated fatty acids appear also to provide a protective effect while fats found in dairy increased associations with risk [25,26]. Compared to cohort studies, the case-control studies tended to report a higher number of significant results. For meat mutagens and meat preparation/doneness preferences, the evidence from two cohort and two case-control studies has generally shown positive associations between PC and increas-

ing intake of well-done grilled/barbecued meat, heterocyclic amines, and a mutagenicity activity index (revertants/grams of daily meat intake), based on mutagenicity in the Salmonella-based Ames Assay [27-29]. A newer case-control study has reported no association [30]. Proposed explanations for the inconsistencies between case-control and cohort studies include information and reporting bias with respect to dietary ascertainment, variability in histologically verified tumor types, and heterogeneous intake [31]. Case-control study participant selection could explain the observed inverse associations since the majority of cohort studies report null results, which are not affected by possible diet changes after cancer diagnosis. Another suggested explanation for these diet-cancer inconsistencies is variation in underlying gene polymorphisms involved in metabolizing components of the diet or antioxidant defense.

Alcohol

The WCRF/AICR reported that there is suggestive evidence of increased risk associated with heavy alcohol use [15]. Across epidemiological studies, there are often variations in measuring and reporting alcohol exposure, leading to difficulty in direct study result comparisons. For pooled analyses, when comparing the highest versus lowest intake categories, the RR ranged from 1.22 to 1.38. Since 2009, two pooled data analyses and one meta-analysis have been performed [32-34]. There were several different control groups for these studies (0 g ethanol/day or > 0-4.99 g ethanol/day or < 1 drink/day), with a wide range of definitions of heavy drinking (> 30 gram/day or > 45 grams/day or > 9 drinks/day or > 3 drinks/day). All studies showed a significantly increased risk for PC among heavy drinkers. In individual epidemiological studies, this association is difficult to detect since they typically are limited by sample size, potential recall bias, or possible selection bias. Additionally, power issues arise when alcohol is split based on type of alcohol consumed (i.e., beer, wine, or liquor).

KEY GENETIC ASSOCIATIONS WITH PC IN HUMANS IDENTIFIED BY GWAS

Based upon the hypothesis that common genetic variants contribute to susceptibility of common diseases such as cancer [35], the genome-wide association study (GWAS) design was proposed [36,37]. Briefly, single nucleotide polymorphisms (SNPs) across the genome are agnostically compared for associations between cases with the disease of interest to healthy controls. All cases and controls are genotyped for thousands of SNPs. Using statistical criteria that account for multiple comparisons, SNPs may implicate novel predisposition genetic loci in the disease. Two major PC research teams, the NCI Cohort Consortium of Pancreatic Cancer (PanScan) [17] and the Pancreatic Cancer Case-Control Consortium (PanC4), performed three GWAS analyses. In the first report by Amundadottir et al. [38], ABO blood group variants were the major discovery from an analysis of 500,000 SNPs genotyped in 1,896 PC cases and 1,939 controls and replicated by analysis of 2,457 PC cases and 2,654 controls. The OR for this association was 1.20 (95% CI 1.12-1.28). In the second report from these teams by Petersen et al. [39], an additional 1,955 PC cases and 1,995 controls were genotyped for 620,000 SNPs, and a combined analysis with this additional statistical power identified variants of the NR5A2 gene on chromosome 1q32.1, OR = 0.77 (95% CI 0.71-0.84); the *CLPTM1L-TERT* region on chromosome 5p15.33, OR = 1.19 (95% CI 1.11-1.27) and a nongenic region on chromosome 13q22.1, OR = 1.26 (95% CI 1.15-1.35) and OR = 1.21 (95% CI 1.13-1.30). A study team in China performed GWAS on 981 PC cases and 1,991 controls using a panel of over 660,000 SNPs and was able to replicate the PanScan/PanC4 study's finding of the nongenic region SNPs on 13q22.1 in the Chinese population and identified an additional five noncoding SNPs in genic regions: *BACH1* on chromosome 21, *DAB2* on chromosome 5, *PRLHR* on chromosome 10, *TFF1* on chromosome 21, and *FAM19A5* on chromosome 22 [40]. A study on a Japanese population [41] of 991 PC cases and 5,209 controls using a panel of over 420,000 SNPs identified *FOXQ1*, *BICD1*, and *DPP6* SNPs on chromosomes 6q25.3, 12p11.1, and 7q36.2, respectively. The 13q22.1 locus association reported by PanScan was also modestly supported. Interestingly, a European consortium, PANDORA [42], was unable to replicate the SNPs reported in either the Chinese or Japanese samples. Most recently, the PanScan group reported a third GWAS analysis based on 7,683 PC cases and 14,397 controls, including a combination of new genotyped cases plus those previously studied [43]. They found four new loci: *LINC-PINT* on chromosome 7q32.2, *BCAR1/CTRB1/CTRB2* on chromosome 16q23.1, *PDX1* on chromosome 13q12.2, and *ZNRF3* on chromosome 22q12.1. Across these studies, the magnitude of the effect size was generally modest, similar in range to those reported in the first GWAS by the PanScan and PanC4 groups. The GWAS databases are publicly available and serve as a valuable resource for exploring hundreds of candidate genes or pathways either alone, in gene-by-gene interactions, or gene-by-environment interactions, as described below.

INTERACTIVE EFFECTS BETWEEN RISK FACTORS AND GENETIC VARIANTS ON PC RISK

Statistical Approaches to Detecting Gene-by-Environment Interactions

Logistic regression is the most common way to evaluate associations between potential risk factors and cancer. Most researchers, especially in earlier studies, would add SNP, environmental factor (E), and an interaction term (SNP x E) into the model and compare cases to controls with a list of potential confounders and perform a similar

test for each SNP of interest. It has since been recognized that this approach has low power to detect associations and has high false positive rates, leading to potential misrepresentation of results. Variations on simple logistic regression also have been proposed [44,45]. These approaches include case-only (gene-environment independence conditional on S), profile likelihood [46] using case-control data, empirical Bayes [47], model averaging [48], two-step [49], and permutation and parametric bootstrap tests [50]. Each of these variations provides better performance than the simple logistic model, but each provides optimal power and type 1 error rates under different conditions. In Table 1, we summarize published reports of possible genetic interactions with modifiable risk factors for PC and describe the limitations and strengths of each study.

Smoking and Genes

Smoking has been hypothesized to interact with genes that play a role in carcinogen metabolism, DNA repair, nicotine dependence, oxidative stress, hormone metabolism, inflammation, insulin secretion, and chromatin-remodeling and risk of PC [51-56]. Twelve PC case-control studies have investigated potential interaction between smoking and polymorphisms in targeted genes. An increased risk has been reported between smoking status and those with minor allele for *XRCC2* ($p = 0.02$) [57], *CAPN10* [58], *EPHX1* ($p = 0.04$), and *NAT2* ($p = 0.03$) [59]. The pancreas is reported to have highest expression of the CAPN10 protein among organs of the body [60]. Variants in the *CYP1A2* and *NAT1* genes interact with heavy smoking among women [61]. Both genes are involved in detoxifying and bioactivation of aromatic amines. There are *NAT1* rapid acetylator genotypes and *NAT2* slow acetylator genotypes [61,62,63]. Gender-specific results support a role of hormones or other factors. Higher level of dietary mutagen exposure or higher iron levels in men may provide a suggested explanation. There is an observed interaction between *XPD* and smoking, in which having a polymorphism in *XPD* Asn312Asn and being an ever smoker (current and former) reduced the risk of PC (OR = 0.42 [0.21-.083]; $p = 0.01$) [64]. Functionally, the Asn312Asn polymorphism may change the folding pattern of the resulting protein and corresponding function [65]. There is a significant interaction between smoking and cytotoxic T lymphocyte-associated protein (CTLA-4) on risk of PC, in which smokers with at least one A allele have an increased risk of PC (p for interaction = 0.037) [66].

Obesity and Genes

Two studies have investigated the potential interaction for PC risk between obesity and genes responsible for regulating balance of energy and tumor development and progression. Nakao et al. [67] studied the interaction with the insulin-like growth factor-1 (*IGF-1*) gene in a Japanese hospital-based case-control study. Alcohol was reported as daily consumption in grams, and weight was self-reported at baseline and recalled for 20 years of age. Those with minor allele for rs574214 and BMI ≥ 25 were at an increased risk of PC. In a previous study [67,68], this polymorphism was found to be associated with risk of PC and diabetes mellitus, but not BMI. Genetic variation in *FTO* has been associated with obesity [69,70,71] and is regulated by fasting and feeding status [72] and negatively regulates lipid metabolism [73]. Those with the *FTO* polymorphism and BMI < 25 have a reduced risk of PC, and those with BMI ≥ 25 have an increased risk [74]. The mechanistic relationship with BMI is currently not known. *ADIPOQ* codes for adipocyte-secreted hormone and has a low frequency of the homozygous variant in the study population. However, a significant interaction with BMI < 25 was observed ($p = 0.005$) [74].

Diet and Genes

Dietary intake has been proposed to interact with genes involved with metabolism, antioxidant defense, and DNA repair. Catalase (*CAT*) is involved in antioxidant defenses and glucosidase, alpha; acid (*GAA*) is required for the glycogen to glucose conversion. The *CAT* polymorphism, rs12807961, interacts with total grain intake, and the *GAA* polymorphism, rs3816257, interacts with deep-yellow vegetables to affect PC risk [75]. Superoxide dismutase 2 (*SOD2*) catalyzes the dismutation of superoxides, and its overexpression suppresses growth and reverses PC phenotype [75-77]. The product of *SOD2* catalysis is hydrogen peroxide, which is either further reduced by catalase or forms reactive hydroxyl radicals that initiate lipid peroxidation chain reactions that vitamin E can break [78,79,80]. The AA genotype of the *SOD2* variant, 1221G>A, increases risk of PC with low vitamin E intake but decreases risk among those with high vitamin E intake ($p = 0.002$) [81]. There is a protective effect of *SOD2* variation among participants with low dietary intake of lutein/zeaxanthin, lycopene, alpha-carotene, and alpha-tocopherol [82]. These are carotenoids that have antioxidant properties. An increased risk has been associated with *NAT1* slow metabolizers and high dietary mutagen intake of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and benzo[a]pyrene among men [61].

Alcohol and Genes

Alcohol has been hypothesized to interact with genes that play a role in tumor development and progression. Alcohol and its major metabolite, acetaldehyde, are categorized as carcinogens [83]. The pancreas has the ability to metabolize alcohol through both oxidative and non-oxidative routes [84]. The oxidative route involves alcohol dehydrogenase (*ADH*) and cytochrome P450 producing acetaldehyde and reactive oxygen species leading to oxidative stress and tissue damage [84,85,86]. The non-oxidative route generates fatty acid ethyl esters (*FAEE*) and *FAEE* synthases resulting in acinar cell injury [87]. *ADH1B*1* are slow metabolizers. This is associated with an increased risk of PC among those who drink. However,

no interactions were observed between *CYP2A13*, *ADH1B*, and *ADH1C* and alcohol intake [88]. Genes in the *IGF* axis regulate cell differentiation, proliferation, and migration and play an important part in initiating carcinogenesis [88-91,92]. Two genes that encode components of the *IGF*-axis, *IGF2R* and *IRS1*, interact with alcohol consumption, but the mechanisms are unknown [93]. Cytotoxic T lymphocyte-associated protein (*CTLA-4*) is involved in regulating T cell function, proliferation, and apoptosis [93,94,95]. Among drinkers with at least one A allele for *CTLA-4* 49G>A, risk of PC is increased with interaction ($p = 0.042$) [66].

PREVENTION STRATEGIES FOR PC

Primary Prevention: Risk Stratification and Behavior Modification

Primary prevention involves the identification and eradication of carcinogenic factors. Currently, key primary prevention strategies for PC focus on the elimination of direct environment risk factors (e.g., tobacco smoking) and indirect factors that promote chronic pancreatitis, principally excess alcohol consumption. Cigarette smoking is the most consistent risk factor for PC, so public health programs, among others, to discourage smoking are vital to prevent PC. Additionally, approximately 70 percent of cases of chronic pancreatitis are attributable to alcohol [96], and an increased risk of PC is also seen in patients with chronic pancreatitis [97]. The role of high fat and meat diet remains debatable. Data continue to accumulate that eating fruit and vegetables is protective, although confirmatory evidence is required from large prospective trials. Moreover, tools to predict individual risk for PC is limited, and any prediction model needs to take into account genes and environmental factors and their interaction in predicting PC risk.

Secondary Prevention: Early Detection and Screening of PC

Secondary prevention involves the early detection and eradication of premalignant lesions or the detection of early stage cancer by screening. At present, there are no effective screening tests for PC routinely available to the general population. Somatic mutation of the *K-ras* oncogene, an early and probably essential event in the pathogenesis of PC, has been extensively investigated, and specific *K-ras* mutations were detected in pancreatic juice, peripheral blood, and stools of patients with the disease [98]. However, this can be affected by both the model of collection and the assay method, and *K-ras* mutations also can be detected in patients with chronic pancreatitis, limiting its sensitivity and specificity as primary screening test. *P53* gene mutations with a greater specificity for PC appear to occur relatively late in the molecular pathogenesis of PC and may therefore limit its use in detecting early lesions. Deletions in both *P16* and *SMAD4* have been detected in pancreatic secretions, but at this time, they do not appear to confer any additional diagnostic power in the detection of early PC [99].

Besides screening using molecular markers, both multislice computed tomography (CT) and magnetic resonance imaging (MRI) can be used to image the pancreas. However, they are limited by parenchymal pathology secondary to diseases such as chronic pancreatitis, and this precludes their use as screening investigations. Endoscopic luminal ultrasound (EUS) and a more invasive approach, an endoscopic retrograde cholangiopancreatography (ERCP), may have a role in diagnostic examinations; however, in the presence of background pathology, the power of these modalities to identify early pancreatic neoplasia remains to be established [100]. Therefore, the current emphasis is on primary prevention and developing public health measures based on consistent epidemiological evidence.

PC Chemoprevention

Cancer chemoprevention is defined as the use of natural, synthetic, or biologic chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer. There are several natural, diet-derived bioactive compounds that have been evaluated as PC chemopreventive agents. The use of chemopreventive agents, such as metformin and aspirin, for PC prevention has promise, but this is still in its early phases of investigation. Several epidemiological studies have linked the administration of metformin with a reduced risk of PC in patients with type 2 diabetes mellitus. For example, Li et al. reported that use of metformin was associated with a 62 percent lower risk of developing PC compared with metformin nonuse (OR 0.38, 95% CI 0.22-0.69, $p = 0.001$) [101]. Additionally, metformin has been shown to prevent the promotional effect of high-fat diet on N-nitrosobis(2-oxopropyl)amine (BOP)-induced pancreatic carcinogenesis in Syrian hamsters [102] and inhibit the growth of PC cells (MIAPaca2 and PANC1) in xenograft models in athymic nude mice [103]. A recent study reported that metformin prevents the progression of pancreatic intraepithelial neoplasia (PanIN) to pancreatic ductal adenocarcinoma (PDAC) by targeting cancer stem cells and mTOR signaling in p48Cre/+.LSL-KrasG12D/+ transgenic mice [104]. Tan et al. also recently showed that metformin treatment may inhibit pancreatic tumorigenesis in the LSL-$Kras^{G12D/+}$;$Trp53^{F2\text{-}10}$ mice by modulating multiple molecular targets in signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa B (NFκB) inflammatory pathways [105].

Findings from observational/ epidemiological studies of aspirin and NSAID use in relation to PC risk have been inconsistent. Using systematic meta-analyses, two studies summarized the available epidemiologic evidence on the relationship between aspirin or non-aspirin NSAID exposure and risk of PC, and both studies indicated null associations [105,106,107]. In a pooled analysis of 25,570 patients in eight trials, Rothwell et al. recently reported that daily aspirin use reduced deaths from several com-

Table 1. Published studies on possible genetic interactions with modifiable risk factors of pancreatic cancer (PC).

Study	Cases	Controls	Analysis	Genes	Significant Interactions	Limitations/ Strengths*
Obesity						
Nakao et al., 2011 [67]	176	1402	Logistic regression adjusting for age, sex, smoking pack-years (<5/<20/<40/≥41), alcohol intake (<23/<46/≥46 g/day), BMI at age 20 and current BMI (<18.5/<22.5/<25/<30/≥30 kg/m2), history of diabetes, family history of PC	*IGF-1*	Current BMI ≥25 and rs5742714 (p=0.029) increased PC risk	Small sample size
Tang et al., 2011 [74]	904	805	Logistic regression adjusting for age, sex, race (white/Hispanic/Black/other), education (<bachlelor's degree/advanced degree), smoking pack-years (non-smoker/≤20/>20), alcohol intake (non-drinker/≤420/>420 g/week), BMI at 30 years old (<25/25-30/≥30 kg/m2), history of diabetes, family history of PC	*PPARG, PRKAA2, PRKAB2, NR5A2, ADIPOQ, FTO*	Increased risk of PC for those with BMI≥25 and rs822393 (p=0.03), rs8050136 (p=0.0001), rs9939609 (p=0.0015)	Large sample size
Li et al., 2009 [124]	452	464	Logistic regression adjusting for age, sex, smoking pack-years (non-smoker/≤20/>20), alcohol intake (non-drinker/≤60/>60 ml/day), history of diabetes, family history of PC	*LIG3, LIG4, OGG1, ATM, RAD54L, POLB, RECQL*	None	Referral hospital population; functional status of many SNPs unknown
Alcohol						
Dong et al., 2012 [93]	680	703	Logistic regression adjusting for age, sex, race (white/Hispanic/Black/other), smoking pack-years (non-smoker/≤20/>20), alcohol intake (non-drinker/≤420/>420 g/week), BMI at 30 years old (<25/25-30/≥30 kg/m2), history of diabetes, family history of PC	*IGF1, IGF2, IGF1R, IGF2R, IGFBP1, IGFBP3, IGFBP5, IRS1, IRS2, IRS4*	Increased risk of PC for those with IGF2r and IRS1 genotypes and alcohol consumption	Hypothesis driven selection of genes, function status of many SNPs unknown
Li et al., 2009 [124]	734	780	Logistic regression adjusting for age, sex, smoking pack-years (non-smoker/≤20/>20), alcohol intake (non-drinker/≤60/>60 ml/day), history of diabetes, family history of PC	*LIG3, LIG4, OGG1, ATM, RAD54L, POLB, RECQL*	None	Large sample size
Mohelnikova-Duchonova et al., 2010 [88]	187	256	Logistic regression adjusting for age, sex, weight, pancreatitis, smoking (non-smoker/former ≤10years/former >10 years/current), alcohol intake (non-drinker/former/regular), history of diabetes	*CYP2A13, ADH1B, ADH1C*	None	Small sample size
Diet						
Suzuki et al., 2008 [61]	755	636	Logistic regression adjusting for age, smoking status, alcohol intake (non-drinker/≤420/>420 g/week), history of diabetes, family history of PC	*CYP1A2, SULT1A1*		Missing adjustment factors that may be important
Tang et al., 2010 [81]	575	648	Logistic regression adjusting for age, sex, race, education, smoking, alcohol, history of diabetes, and history of cancer	*SOD2, CAT, GPX, GSTA4*	SOD2 and low dietary vitamin E are at increased risk	Low FFQ response rate, possible disease associated diet change
Zhang et al., 2011 [82]	189	486	Logistic regression adjusted for age, sex, race, education, smoking, drinking, physical activity, energy intake	*CAT, SOD2, hOGG1, XRCC1*	Reduced risk of PC with rs4880 and low dietary intake of lutein/zeaxanthin, lycopene, alpha-carotene, and alpha-tocopherol	Misreporting of food intake, disease may have affected diet, small sample size
Jansen et al., 2013 [75]	251	970	Logistic regression adjusting for age, sex, smoking status, BMI, family history of pancreas cancer, energy intake, number of drinks per week	*CAT, GAA, GCK, GSTA1, GSTP1, MT1E, SOD2, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B4, UGT2B7*	Increased risk of PC for rs3816257 minor allele and low deep yellow vegetable intake and rs12807961 no minor allele and high total grain intake	Rapidly enroll cases, small sample size

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Table 1. Published studies on possible genetic interactions with modifiable risk factors of pancreatic cancer (PC). Continued from previous page.

Study	Cases	Controls	Analysis	Genes	Significant Interactions	Limitations/ Strengths*
Smoking						
Li et al., 2009 [124]	734	780	Logistic regression adjusting for age, sex, smoking pack-years (non-smoker/≤20/>20), alcohol intake (non-drinker/≤60/>60 ml/day), history of diabetes, family history of PC	*LIG3, LIG4, OGG1, ATM, RAD54L, POLB, RECQL*	None	Large sample size
Mohelnikova-Duchonova et al., 2010 [88]	187	256	Logistic regression adjusting for age, sex, weight, pancreatitis, smoking (non-smoker/former ≤10years/former >10 years/current), alcohol intake (non-drinker/former/regular), history of diabetes	*CYP2A13, ADH1B, ADH1C*	None	Use of different analysis techniques
Duell et al., 2008 [125]	308	964	Multifactor Dimensionality Reduction Analysis, Focused Interaction Testing Framework Analysis, Logistic Regression	*APE1, hOGG1, XRCC1, XPD, XPA, XPC,ERCC1, XRCC3, GSTM1, GSTT1, GSTP1, UGT1A7, SOD2, CYP1A1, CYP1B1, CCK, TNF-A, RANTES, CCR5, MMP3*	Increased risk associated with rs861539 and smoking	
Jang et al., 2012 [59]	438	887	Logistic regression adjusting for age, sex, race (white/Hispanic/Black/other), education (<bachlelor's degree/advanced degree), smoking pack-years (non-smoker/≤20/>20), alcohol intake (non-drinker/≤420/>420 g/week), BMI at 30 years old (<25/25-30/≥30 kg/m2), history of diabetes, family history of PC	*AHR, COMT, CYP1A1, CYP1A2, CYP1B1, CYP2C9, CYP2E1, GSTM3, GSTP1, EPHX1, NAT1, NAT2, UGT1A7, GSTT1, GSTM1*	Increased risk of PC associated with interaction between smoking and with each rs2234922 and rs1799931	Underpowered for analyses of low prevalence SNPs, no multiple testing adjustment
Jiao et al., 2007 [64]	344	386	Logistic regression adjusted for age and gender	*XPD*	Reduced risk of PC with interaction between smoking and Asn312Asn	No functional information
Suzuki et al., 2008 [61]	755	636	Logistic regression adjusting for age, alcohol intake (non-drinker/≤420/>420 g/week), history of diabetes, family history of PC	*CYP1A2, SULT1A1, NAT1, NAT2*		Missing adjustment factors that may be important
Yang et al., 2012 [66]	368	926	Logistic regression adjusted for age, sex, smoking, drinking, and history of diabetes	*CTLA-4*	Increased risk associated with having minor allele and smoking	
Zhu et al., 2014 [126]	310	457	FDR and logistic regression adjusted for age, sex, smoking and drinking	*SMARCA4, SMCRB1, PBRM1, BRD7, ARID1, ARID2*	Smoking and each of rs2073389 and rs11085754	Did eQTL and top SNPs where suggested to play functional role
Jiao et al., 2008 [57]	408	449	Logistic regression	*XRCC2, XRCC3*	XRCC2 Arg188His and smoker at increased risk of PC	Underpowered for analyses of g x e
Fong et al., 2010 [58]	83	166	Logistic regression	*CAPN10*	Population all smokers: rs3792267 increased risk of PC	Small sample size
Nakao et al., 2012 [127]	185	1456	Logistic regression, age, sex, current BMI, BMI at age 20, smoking status, drinking habit, history of diabetes mellitus, family history of PC	*OGG1, XRCC1, APE1, PARP1*	None	Data collected before diagnosis, small number of cases

* beyond those normally identified for case-control studies (e.g., cases may have different assessment of past exposures than controls in a differential way)

mon cancers, including significant reductions in colorectal and PC deaths, with most benefit seen after 5 years of the scheduled trial treatment [108]. In a clinic-based case-control study, we showed that aspirin use, but not non-aspirin NSAID use, is associated with lowered risk of developing PC [109]. In addition, aspirin has been shown to suppress pancreatic cancer growth both *in vitro* and *in vivo* [110]. A derivative of aspirin, nitric oxide-donating aspirin (NO-ASA), also showed chemopreventive effect in pancreatic cancer cell lines [111] and transgenic mice models [112]. In the future, prevention strategies for PC may be improved with the identification of more genetic alterations responsible for developing an increased risk to PC, and chemoprevention may be of particular value in high-risk PC populations.

EPIGENETICS AND PC RISK

In recent years, there has been an increasing amount of research regarding dynamic epigenetic processes and how they affect gene regulation. In 2014, van Kampen et al. [113], summarized currently identified epigenetic modifications, including histone modifications, methylation, and microRNAs, and their associations with PC. They then discussed potential targeted epigenetic-based therapeutic approaches for PC. Low expression of *TGFBR2* by *HDAC1* and *HDAC2/SIN3a* [113-115,116] and *CDH1* [117] leads to increased risk or progression of PC. Overexpression of *HDAC* [117,118,119] and *EZH2* [120] leads to increased risk or progression of PC. Hypermethylation (silencing) of *CDKN2A* is associated with PC [120,121,122]. MicroRNAs including miR-21 are associated with PC. The authors mention several epigenetic therapies, including those targeting short-chain fatty acids, HMT inhibitors, DNA methylation, and miRNA expression; however, most of these therapies are still ongoing or have produced poor or limited results. In 2012, Heichman and Warren [123] reviewed DNA methylation biomarkers for several solid cancers including PC, and the methylation of 99 genes have shown an association with PC, including hypermethylation of 21 of those genes being unique to PC among solid cancers.

CURRENT STUDY LIMITATIONS

All epidemiologic study designs are subject to limitations and biases that affect the interpretation and generalizability of reported results. Many of the exposures described in the epidemiologic studies are subject to various biases, including recall bias, social desirability bias, and selection bias. For example, differential misclassification and recall of dietary patterns between cases and controls could contribute to biased risk estimates. Co-morbidities associated with smoking, obesity, and alcohol intake affect selection of cases with these exposures. For each of the four exposures discussed here, there are social stigmas associated with high levels of consumption that may influence how a participant completes survey questions. In retrospective population-based studies of rapidly fatal disease, bias can occur due to demise of eligible cases with a higher proportion of later stage disease, possibly resulting in non-random non-response. In prospective studies, the rarity of PC limits the number of potential cases seen during follow-up. Both of these situations lead to a reduced power to detect associations. Moreover, GxE studies are often criticized for being underpowered, and it has been suggested that the associations seen are often false positives and cannot be replicated.

CONCLUSION AND OUTLOOK

With the increasing obesity epidemic, especially among youth, and the strong association between obesity and PC, it can be expected that obesity-related PC rates will increase over the coming decades. Dietary results regarding PC risk have largely been inconsistent with the potential exception of certain fatty acids and well-done red meat. Dietary data has been fraught with measurement error and oftentimes a large percentage of the data is missing for participants. Technology provides a potential solution as it may lead to ascertaining a more accurate record of what is eaten, how much, and in what combination. As smoking rates continue to decrease, cigarette smoking related PC also will decrease. The role that e-cigarettes may play in PC has yet to be determined; the effect of environmental exposure, especially in early childhood, needs further exploration. Alcohol seems to be a risk for PC only among those in the heaviest consumption category. Methods for identifying and targeting these individuals for early detection may prove useful. Genetic data can assist in identifying individuals at high risk of developing PC, but new statistical and epidemiological methods or processes are needed to pinpoint the responsible genetic variants and their interaction with modifiable risk factors.

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