Title: Pancreatic cancer risk in relation to lifetime smoking patterns, tobacco type, and dose-response relationships

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Abstract

BACKGROUND: Despite smoking being a well-established risk factor for pancreatic cancer (PC), there is a need to further characterize PC risk according to lifespan smoking patterns and other smoking features such as tobacco type. Our aim was to deeply investigate them within a large European case-control study.

METHODS: Tobacco smoking habits and other relevant information was obtained from 2,009 cases and 1,532 controls recruited in the PanGenEU study using standardized tools. Multivariate logistic regression analysis was performed to evaluate PC risk by smoking characteristics and interactions with other PC risk factors. Fractional polynomials and restricted cubic splines were used to test for non-linearity of the dose-response relationships and to analyse their shape.

RESULTS: Relative to never-smokers, current smokers (OR=1.72, 95%CI: 1.39-2.12), those inhaling into the throat (OR=1.48, 95%CI: 1.11-1.99), chest (OR=1.33, 95%CI: 1.12-1.58), or using non-filtered cigarettes (OR=1.69, 95%CI: 1.10-2.61), were all at an increased PC risk. PC risk was highest in current black tobacco smokers (OR=2.09, 95%CI: 1.31-3.41), followed by blond tobacco smokers (OR=1.43, 95%CI: 1.01-2.04). Childhood exposure to tobacco smoke relative to parental smoking was also associated with increased PC risk (OR=1.24, 95%CI: 1.03-1.49). Dose-response relationships for smoking duration, intensity, cumulative dose, and smoking cessation were non-linear and showed different shapes by tobacco type. Effect modification by family history of PC and diabetes was likely.

CONCLUSIONS: This study reveals differences in PC risk by tobacco type and other habit characteristics, as well as non-linear risk associations.

IMPACT: This characterization of smoking-related PC risk profiles may help in defining PC high-risk populations.

Introduction

Pancreatic cancer (PC) is one of the deadliest cancer types worldwide (5-year relative survival rate in the range 5-15%) (1). Disastrously, estimates of PC incidence are increasing both in USA and Europe (2). Despite the aetiology of PC is relatively unknown, it is estimated that 10-30% of all PC cases are caused by smoking (3). Prevention of smoking is therefore a strong measure to reduce the burden of PC in the population.

While the association between smoking and PC is well-established, a detailed characterization of tobacco smoking habits in relation to PC risk is still lacking. A meta-analysis including 10,490 cases and 526,813 controls, showed that being a current smoker, jointly with a longer smoking duration and a higher smoking intensity, were associated with an increase in PC risk (4). However, the authors assumed a linear trend for PC risk associated with increasing smoking exposure, a fact that was disputed by *Zou et al*. in an updated analysis combining 9,044 cases and 32,039 controls that showed a non-linear dose-response relationship between several smoking characteristics and PC risk (5). In addition, the pooled analysis within the Pancreatic Cancer Case-Control Consortium (PanC4), including 6,507 cases and 12,890 controls, indicated that after a certain amount of smoking exposure PC risk levelled-off (6), shedding a different perspective on the dose-response relationship of smoking in relation to PC risk. However, in the aforementioned studies, an exploration of the shape of the association between smoking measures and PC risk was not further pursued. The shape of the dose-response relationship between cigarette smoking and PC risk was investigated in a recent meta-analysis of 38 case-control and 40 cohort studies (7). Risk patterns of PC in current *versus* smokers were compared in this study for smoking intensity and duration, ignoring the contribution to risk of former smokers. To understand multi-dimensional aspects of smoking in PC aetiology, there is a need to provide consistent risk estimates for all smoking groups and to address the mutual influence of smoking intensity and duration.

Moreover, several aspects of tobacco smoking habits have not been considered until now. For instance, differences in PC risk by either black or blond tobacco use have not been explored despite the presumed differences in their chemical composition and damaging effects (8,9). In fact, several studies have shown that black tobacco is associated with higher risk of bladder (8), colorectal (10), oesophageal (11), and head-and-neck cancer (12,13), than blond tobacco.

Therefore, we set out to investigate the association and dose-response relationship between tobacco smoking and PC risk in a large European population, considering every aspect of the smoking habit including use of black *versus* blond tobacco.

Methods

Study design and participants

The PanGenEU is an ongoing multicentre case-control study initiated in 2007, recruiting participants from six European countries (Germany, Ireland, Italy, Spain, Sweden and United Kingdom) across 28 centres. Newly diagnosed PC patients >18 years old and controls matched by age (±10 years), gender, and geographical area were included if they had lived in the study area for at least 6 months. A rapid ascertainment approach was applied: PC cases with a suspicion of the disease were recruited and remained in the study if the diagnosis was verified by the treating physician. Controls, sex-, age-, and centre- individually matched to cases, were mostly hospital-based and eligible if principal diagnosis at admission was unrelated to known risk factors of PC. Conditions of admission of controls are reported in Supplementary Methods. Population-based controls (Sweden and Ireland) were eligible if history of PC was absent. Participation rates were 86.3% for cases and 77.8% for controls. The study was approved by the IRB of all participant centres and all subjects gave written informed consent. More details of the study are provided elsewhere (14,15).

157 *Variables*

158 Personal interviews to the study subjects were conducted by trained monitors using standardized
159 protocols and questionnaires to obtain detailed information on lifetime smoking habits, among other PC risk
160 factors. The smoking status of the participants was categorized into never-smokers if they smoked <100
161 cigarettes during their lifetime; occasional smokers if they smoked ≥1 cigarette/day for ≥6 months; former
162 smokers if they quitted smoking for >1 year; and current smokers otherwise (>100 cigarettes during lifetime
163 without permanent smoking cessation). Information on smoking habits by tobacco type (only black, blond
164 or both) was only collected in the Spanish centres. Smoking exposure was further assessed by the age at
165 smoking initiation (years), age when last smoked (years), cigarettes/cigar/pipe-use (yes, no), the amount of
166 cigarettes/cigars/pipes smoked in units of time (days, months, years), depth of inhalation (mouth, throat,
167 chest), filter-use (filtered cigarettes, non-filtered, both), and smoking status of the parents (never- or ever-
168 smoker). From these characteristics, data on smoking duration (years), smoking intensity for cigarettes (per
169 day) and cigars/pipes (per week), and time since cessation (years) was derived. Number of pack-years,
170 representing cumulative dose, was calculated as (cig*arettes per day/20)*smoking duration in years*.
171 Smoking variables by use of tobacco type were generated likewise. Environmental tobacco smoke (ETS)
172 exposure during childhood was categorized according to the smoking status of the parents (none, one or
173 both).

174 *Statistical analysis*

175 Imputation of missing values, assumed to be at random, was performed using the Random Forest
176 algorithm (R package missForest). Predictor variables such as centre, country, and case-control status
177 were kept in the imputation set. The performance of the imputation (Supplementary Table 1) was assessed
178 by calculating the out of bag mean square error (OOB), representing the mean of squared differences
179 between each observed value and its prediction, based on random forest trees (n=100 was applied). The
180 average OOB for all smoking variables was 5.27, with categorical variables presenting a markedly lower

estimate (OOB=0.04), indicating a better imputation performance of the latter. Use of unimputed data of all continuous variables, for which the proportion of missing values was relatively low (6.7%), was therefore deemed more appropriate for dose-response analyses. The performance of the imputation was also assessed with concordance rates between the observed and imputed data, considering a test dataset consisting of only subjects with complete data and missing values introduced by following the missingness rates of the original data. The concordance of all categorical variables was 94.4%.

Differences between cases and controls regarding smoking characteristics were evaluated by $\chi 2$ and Student's t-test (or Kruskal–Wallis test, where appropriate). Unconditional logistic regression analysis was performed to estimate odds ratios (OR) and 95% Confidence Intervals (95%CI). Never-smokers were chosen as the reference category, except for the variables "age when last smoked" and "time since smoking cessation", where current smokers were taken as the reference. Tertiles were created for the continuous variables based on the distribution of controls. A p-value for trend was calculated by assuming ordinal variables in linear regression models. Age (≤54, 55-64, 65-74, ≥75 years), gender and country-adjusted models (aOR) were considered (Model 1). For the tobacco type-specific analyses within the Spanish PanGenEU study population, the same model was applied, but replacing country by region (East, Central and Northern Spain). The attributable risk (AR) of smoking in relation to PC (population exposed: 59%) was calculated from the fitted multivariate adjusted logistic regression models (R packages attribrisk and epiR). Since heterogeneity by country ($p<0.05$) was evident for all smoking variables (for example, p-value for interaction by smoking status=0.007: Supplementary Figure 1), random effects for country were applied in mixed effects models. Due to absence of heterogeneity in the Spanish study population, logistic regression models without random effects were considered.

The influence of confounding factors or effect modification on the association was assessed for several variables: gender (female, male), age (<65 years, ≥ 65 years), obesity (body mass index >30: yes, no), diabetes (no, yes less than 2 years, yes more than 2 years), asthma (yes, no), chronic pancreatitis

(yes, no), alcohol status/consumption (never, former, current), presence of periodontitis (yes, no) and recession (yes, no), educational level as a proxy for socioeconomic status (low, medium, high), and family history of PC (yes, no). Variables changing estimates by more than 10% or having a significant influence in the model (diabetes and family history of PC in some smoking-related variables) were considered as potential confounders. The le Cessie-van Houwelingen-Copas-Hosmer unweighted sum of squares test indicated a high goodness of fit of the models (16). Effect modification was assessed in interaction and stratified analyses. Additive interaction by time-related variables such as smoking duration was also evaluated by the relative excess risk due to interaction (RERI) and Delta-method Cis (17,18).

To test for interaction, a likelihood ratio (LR) test was performed comparing models with and without an interaction term between the smoking variables and the covariates (e.g., age, gender, BMI and obesity, diabetes, asthma, alcohol, periodontitis, recession, educational level, and family history of PC). Effect modification was tested further via stratified analyses. To assess interaction by time-related variables we explored the combined effect of smoking duration and other smoking characteristics such as tobacco type on PC risk. Smoking duration was categorized into <20, 20-30, and ≥30 years of smoking and stratified further by tobacco type considering never-smokers as the reference category.

To assess the dose-response relationships, PC risk estimates were calculated per 1-unit of increase in continuous smoking exposure variables considering linear and non-linear models if so indicated by fractional polynomials (R package mfp) (19). In addition, restricted cubic splines were used to confirm non-linear associations and for modelling the shape of the dose-response relationships (R package splines)(20). Non-linearity of the models was tested via the likelihood-ratio test comparing the model with and without restricted cubic splines. Knots were set at the 10%, 50% and 90% percentile of the exposure distribution, as comparable results were obtained with five knots (21).

Sensitivity analyses were performed comparing the risk estimates in magnitude and trend regarding the unimputed and imputed data, and the PanGenEU study population with and without Italy

(since Italy provided cases only). As information bias could be induced by neglecting the quantity of smoking exposure, adjustment for cumulative dose (pack-years) was considered, thereby accounting for both smoking duration and smoking intensity. Additional adjustments were made also for smoking intensity and duration separately, to assess both the individual and joined effects of smoking characteristics independent of smoking duration and/or intensity. These adjustment variables were considered on the continuous scale and modelled as fractional polynomials to account for non-linear effects. To further assess the performance of the restricted cubic splines, additional smoothing was applied by varying the degrees of freedom, allowing more flexibility into the model (22).

The threshold for statistical significance in two-sided tests was set at p-value<0.05. Data was analysed with R-project (version 3.4.1) (23).

Results

Table 1 shows the characteristics of the 2,009 cases and 1,532 controls included in this analysis. The Spanish centres contributed the most to both cases (N=876) and controls (N=762). PC cases presented more frequently with a family history of PC and a diagnosis of diabetes or chronic pancreatitis.

Table 2 shows PC risk associated with smoking characteristics. The prevalence of smoking was higher in cases (27.4%) than in controls (17.6%), with a corresponding aOR of 1.72 (95%CI: 1.39-2.12) for current smokers compared to never-smokers. Furthermore, a statistically significant increased trend (p-value<0.001) in PC risk was observed for longer smoking duration, higher smoking intensity and higher cumulative dose. The use of non-filtered cigarettes increased risk of PC more prominently (aOR=1.69, 95%CI: 1.10-2.61), although use of filtered cigarettes was also associated with an increased PC risk (aOR=1.25, 95%CI: 1.06-1.48). Marked increases in PC risk were also observed for inhalation into the throat (aOR=1.48, 95%CI: 1.11-1.99) and chest (aOR=1.33, 95%CI: 1.12-1.58). Childhood exposure to ETS by smoking parents (*vs.* non-parental exposure) was also associated with a 24% (95%CI: 1.03-1.49)

253 increased PC risk. Risk for former smokers decreased progressively with longer time since smoking
254 cessation when compared to current smokers (aOR for 14-28 years after cessation=0.67, 95%CI: 0.51-
255 0.88). A negative trend of the risk was also observed if compared to never-smokers (PC risk was
256 diminished from 14 years of cessation), and when considering smoking cessation time at 5-year intervals
257 (Supplementary Table 2). No significant associations between PC risk and pipe/cigar-use or other smoking
258 variables were observed (Supplementary Table 3). Additional adjustment for diabetes and family history of
259 PC led to minimal differences in risk estimates (Supplementary Table 3). Effect modification was apparent
260 only for family history of PC and diabetes status (Supplementary Table 4), pointing towards a higher PC
261 risk among current smokers with family history of the disease (aOR=2.24, 95%CI: 0.66-7.61) and former
262 smokers with diabetes (aOR=1.44, 95%CI: 0.91-2.28) (p-value for interaction<0.001).

263 Table 3 shows PC risk estimates by tobacco type in PanGenEU-Spain. Compared to never-
264 smokers, PC risk was significantly increased for smokers of only black tobacco (aOR=1.55, 95%CI: 1.13-
265 2.12) and of both tobacco types (aOR=1.58, 95%CI: 1.14-2.17). Considering smokers of only blond
266 tobacco, PC risk tended to be increased (aOR=1.23, 95%CI: 0.94-1.62), though without reaching statistical
267 significance. When further stratifying by smoking status, a significant increase in risk was observed for
268 current smokers of only black tobacco (aOR=2.09, 95%CI 1.31 - 3.41) and blond tobacco (aOR=1.43, 95%
269 CI: 1.01-2.04). Former smokers of only black tobacco were at increased, though milder, PC risk
270 (aOR=1.40, 95%CI: 0.98-1.99).

271 Table 4 shows the combined effect of smoking duration and type of tobacco on PC risk. Compared
272 to never-smokers, smoking for ≥30 years of both tobacco types was associated with a higher PC risk than
273 smoking only black or blond tobacco (aOR=2.05, 95%CI: 1.25-3.36; RERI=0.206, 95%CI: -0.49-0.91).

274 Table 5 shows risk estimates for continuous smoking variables associated with PC. Non-linear
275 associations were evident for smoking duration and intensity, cumulative dose, time since cessation and
276 age at smoking initiation. Adjusted fractional polynomials models suggested a statistically significantly

277 higher PC risk per 1-unit increase in smoking duration, smoking intensity and cumulative dose, and
278 decreasing PC risks for age at smoking initiation and time since smoking cessation. Linear associations
279 were observed for other variables such as intensity of smoking cigars/pipes (data not shown). The
280 restricted cubic splines approximating the shape of the dose-response relationships confirmed these non-
281 linear associations. Compared to never-smokers, smoking for >25 years (Figure 1, A-B) and smoking >20
282 cigarettes/day (Figure 1, D-E) was associated with a statistically significant increase of PC risk. Similarly, a
283 cumulative dose of >14 pack-years was associated with increased PC risk (Figure 1, C). Visual inspection
284 for smoking intensity and cumulative dose was suggestive of plateauing of PC risk, at approximately 27
285 cigarettes/day or pack-years. Concerning time since smoking cessation (Figure 1, F-I), and relative to
286 current smokers, risk appeared to decrease between 8 and 11 years of cessation and after around 18
287 years of cessation regardless of cumulative dose. In between these periods, the significant effect
288 disappeared. By tobacco type, corresponding periods of significant decrease in PC risk were observed for
289 black tobacco (after about 14 years since cessation) and for blond tobacco (between 2 and 8 years and
290 after >20 years of cessation).

291 No relevant differences in the trend or magnitude of the estimates were found in sensitivity
292 analyses (Supplementary Tables 3, 5,to 7), including further smoothing of the splines fit (Supplementary
293 Figure 2). In analyses adjusting for smoking intensity, risk estimates decreased in magnitude but showed a
294 similar trend. By tobacco type, this adjustment did not affect either the associations nor the shapes of the
295 relationships despite black tobacco smokers smoked heavier and for a longer time (Supplementary Table
296 8). Importantly, adjustment for smoking duration led to statistically non-significant risk estimates and
297 change in the shape of the dose-response relationships (Supplementary Table 9). Joint effect analyses of
298 smoking intensity and duration showed that long-lasting smoking together with intense smoking increase
299 pancreatic cancer risk, whereas for less intense smoking the association weakened (Supplementary Table
300 10).

Discussion

The present study confirms that, in comparison to never-smokers, being a current smoker increases the risk of PC by 72%. In terms of attributable risk, this study also endorses that around 16% (95%CI: 9.24-22.47) of all PC diagnoses could be avoided through tobacco preventive measures. A more detailed examination of smoking characteristics showed that the use of non-filtered cigarettes, deep inhalation into the throat or chest, and exposure to tobacco smoke in the parental household were all associated with increased PC risk. PC risk in black tobacco smokers was significantly higher compared to never-smokers, with blond tobacco smokers showing a less prominent risk pattern. Analysis of dose-response relationships corraborated that a higher smoking intensity, longer smoking duration, and increased levels of cumulative dose were associated further with an increased PC risk, whereas smoking cessation led to a gradual decline in PC risk, all in a non-linear manner.

Our results are concordant with earlier studies on the same topic. Regarding the magnitude of PC risk associated with current versus never tobacco smoking, a meta-analysis and pooled analyses from the PanC4 and the Pancreatic Cancer Cohort Consortium showed similar estimates (RR=1.74, 95%CI: 1.61-1.87, OR=2.20, 95%CI: 1.71-2.83 and OR=1.77, 95%CI: 1.38-2.26, respectively) (4,6,24). Similarly, our study confirmed the trends and timing of tobacco smoking (4,6), the excess risk conferred by tobacco smoking (4,25,26), the non-linear tobacco-PC associations (5,7), and risk due to childhood ETS (27). Compared with studies restricting ETS exposure to never-smokers, we also did not observe significant risk estimates (aOR=1.24, 95%CI: 0.95-1.63) (28,29), suggesting that smokers, possibly more likely being exposed to childhood ETS, were driving this association in the overall analyses (aOR for current smokers exposed to parental smoking vs never smoking exposure =2.01; 95%CI: 1.50-2.69). In contrast to the positive association between current cigar/pipe smokers and PC risk reported before (4,30), we did not observe a significant associations in our study, probably due to low statistical power.

Effect-modification factors

The higher PC risk among smokers with family history of PC was previously described in our study population (14). Although statistical significance was not reached, former smoking diabetes patients tended to have a higher PC risk too. Were this true, lifestyle changes among diabetic patients including smoking cessation, which in turn may lead to weight gain and insulin resistance (31),(32), might explain this finding. Previous studies suggested differences in smoking effects on PC risk by gender (5,6), although they failed to demonstrate effect modification by this variable. Similarly, non-significant differences by gender were found in our study, which included a large female sample with a relatively high smoking prevalence.

Dose-response relationships

Non-linear relationships of the association between smoking variables and PC risk were supported by both fractional polynomials and restricted cubic splines approaches. Since fractional polynomials in regression models become imprecise with small sample sizes (22), we based the dose-response curves on results derived from restricted cubic splines, which allow a more flexible modelling (33). Concordant with the observation of non-linear associations for smoking duration, intensity, and cumulative dose, a plateuing in the dose-response relationship was apparent. This observed pattern was previously reported (5,7), and could be attributed to the saturation of the detoxification processes of tobacco carcinogens in the body (34), or to a presumably weaker inhalation of tobacco smoke but stronger DNA repair efficiency among heavy smokers (35,36), amongst other factors. Non-linear associations for smoking cessation, with decreased PC risk after 20 years of smoking cessation, were also suggested (5) and confirmed by other studies (7). However, in these earlier studies, consideration was not given to the influence of smoking intensity and duration on these associations. Patterns in PC risk in our study changed after adjusting for smoking duration mainly, whereby the magnitude of the risk estimates was affected (Supplementary Table 9).

Black versus blond tobacco-use

Compared to never-smokers, black tobacco smokers showed a significantly higher PC risk, this tobacco type appearing to be more harmful than blond tobacco. This result is consistent with the few studies that examined the association between smoking by tobacco type in bladder (8,37,38) and other cancers (10–13). Smoking both black and blond tobacco for a long time (≥30 years) tended to be related to higher PC risk, this also being shown in previous studies on tobacco smoking and bladder cancer (8).

The difference between the two tobacco types could be explained by their smoke composition: black tobacco mostly contains early-stage carcinogens, such as N-nitrosamines and aromatic amines including 4-amino-biphenyl and 2-naphthylamine (39), whereas blond tobacco may mostly consist of late-stage carcinogens (37). It is conceivable that the two tobacco types contribute to pancreas carcinogenesis through different mechanisms: black tobacco may predominantly cause DNA mutations whereas blond tobacco may preferentially act through epigenetic change, as has been shown for LINE-1 (9). As a consequence, an immediate and significantly higher increase in PC risk could be expected in black tobacco smokers, while blond tobacco might need a longer time to trigger PC development. This may also imply that following smoking cessation of blond tobacco PC risk can keep increasing for some time, slowing down after recovery of certain DNA methylation changes. In fact, methylation changes due to smoking seem to persist up to 22 years after smoking cessation (40). For black tobacco, instead, the PC risk reduction effects might not take place or might require longer since smoking cessation. Our results support these hypotheses to some extent. Compared to never-smokers, not only did black tobacco smoking have a more detrimental effect on PC risk, but also the risk tended to increase soon after smoking initiation, whereas downward risks were observed after smoking cessation for >10 years. A similar decreasing risk with long-term smoking cessation of black tobacco has been observed in bladder cancer in some (37,38), but not all (8), studies. Among blond tobacco smokers, the trend towards a reduction in PC risk became evident shortly after smoking cessation (Supplementary Table 9). The shape of dose-response curves

supported the aforementioned trends, specifically regarding smoking cessation. Thus, our study suggests that black tobacco consumption may play a role in several steps of the carcinogenic process with possibly both early and late-stage carcinogens being involved. For blond tobacco, our results point to a two-tier mechanism after smoking cessation driven by late-stage carcinogens, the first consisting of a sudden change in risk estimates with risk levels more akin to never-smokers likely due to desaturation of detoxification routes of tobacco-carcinogens (5,41), the second showing risks levelling-off after approximately 20 years of smoking cessation, once alteration of DNA methylation levels of key genes regain the state of normalcy.

Among the limitations of the study, stratifying by tobacco type might have underpowered the analyses to detect any differences. As in any other study, subgroup analyses and multiple statistical tests are prone to chance findings due to increased type I error. Also, we could not consider potential differences in the content of carcinogens because we lacked information on tobacco brands, likely to contain varying amounts of heavy metals (42) and other carcinogens (39). Residual confounding can be therefore expected, also due to lack of, or imprecise, information on other relevant data such as ETS in adulthood. Extensive efforts have been made to adjust for as much confounding as possible, thereby alleviating the bias to the highest extent possible. Moreover, differential misclassification of the exposure due to recall bias of smoking habits among either the cases or controls is possible, or because use of only black or blond tobacco smoking might not have been reliably reported. Therefore, mixed effects due to alternate use of both tobacco types cannot be ruled out. We considered only smokers of black or blond tobacco in order to keep the effects by tobacco type separate, and considered switching from one type to the other in the group of users of both tobacco types.

Major strengths of the study are the large number of PC cases representing a European-wide PC population and the degree of detail in the information collected about smoking habits. This allowed us to undertake exhaustive and solid analyses considering many aspects of the habit in relation to PC risk. In

fact, this is the first study assessing PC risk by black and blond tobacco. Also, as a novelty, the shapes of dose-response relationships have been fully characterized using different modelling strategies to account for non-linear effects of smoking on PC risk.

In conclusion, findings of this study support and add to the previous evidence that smoking increases PC risk and demonstrates, for the first time, that both blond and black tobacco smoke are key in PC aetiology, though probably acting through different genetic mechanisms. Considering these smoking-related PC risk profiles may help to refine the definition of high-risk PC population towards screening interventions implementation. Future studies should confirm our findings on type of tobacco and shed light on the mechanisms underlying their differential association with PC risk.

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Table 1: Baseline characteristics of the PanGenEU study population (2,009 cases and 1,532 controls).

	PanGenEU					PanGenEU - Spain				
	Cases (%)		Controls (%)		p-value[2]	Cases (%)		Controls (%)		p-value[2]
Country					<0.001					--
Spain	876	(43.6)	762	(49.7)		876	(100.0)	762	(100.0)	
England	126	(6.3)	22	(1.4)		-	-	-	-	
Germany	130	(6.5)	111	(7.3)		-	-	-	-	
Ireland	173	(8.6)	290	(18.9)		-	-	-	-	
Italy	533	(26.5)	0	(0.0)		-	-	-	-	
Sweden	171	(8.5)	347	(22.7)		-	-	-	-	
Gender					0.164					0.455
Female	871	(43.4)	701	(45.8)		384	(43.8)	349	(45.8)	
Male	1138	(56.6)	831	(54.2)		492	(56.2)	413	(54.2)	
Age					<0.001					0.086
≤54	413	(20.6)	262	(17.1)		157	(17.9)	155	(20.3)	
55-64	497	(24.7)	321	(21.0)		203	(23.2)	173	(22.7)	
65-74	699	(34.8)	495	(32.3)		285	(32.5)	208	(27.3)	
≥75	400	(19.9)	454	(29.6)		231	(26.4)	226	(29.7)	
BMI (kg/m^2)					0.997					0.900
< 25	769	(38.3)	588	(38.4)		303	(34.6)	271	(35.6)	
25-29.99	854	(42.5)	651	(42.5)		397	(45.3)	343	(45.0)	
≥ 30	386	(19.2)	293	(19.1)		176	(20.1)	148	(19.4)	
Alcohol status[3]					<0.001					0.412
Never-drinker	585	(29.1)	383	(25.0)		273	(31.2)	254	(33.3)	
Light drinker	805	(40.1)	756	(49.3)		377	(43.0)	338	(44.4)	
Moderate drinker	564	(28.1)	360	(23.5)		214	(24.4)	160	(21.0)	
Heavy drinker	55	(2.7)	33	(2.2)		12	(1.4)	10	(1.3)	
Family history of PC					<0.001					<0.001
No	1882	(93.7)	1492	(97.4)		815	(93.00)	739	(97.0)	
Yes	127	(6.3)	40	(2.6)		61	(7.0)	23	(3.0)	
Ever been diagnosed with asthma					<0.001					0.014
No	1878	(93.5)	1374	(89.7)		817	(93.3)	684	(89.8)	
Yes	131	(6.5)	158	(10.3)		59	(6.7)	78	(10.2)	
Ever been diagnosed with diabetes					<0.001					<0.001
No	1515	(75.4)	1349	(88.1)		604	(68.9)	630	(82.7)	
Yes, ≤ 2 years	214	(10.7)	27	(1.7)		112	(12.8)	20	(2.6)	
Yes, >2 years	280	(13.9)	156	(10.2)		160	(18.3)	112	(14.7)	
Ever been diagnosed with chronic pancreatitis					0.004					0.460
No	1990	(99.1)	1530	(99.9)		871	(99.4)	760	(99.7)	
Yes	19	(0.9)	2	(0.1)		5	(0.6)	2	(0.3)	

521 PC: pancreatic cancer; BMI: body mass index.
522 Descriptives are shown for the imputed baseline characteristics. Descriptives of the unimputed baseline characteristics can be found in Supplementary Table 9
523 [1] Chi-square test applied to evaluate differences between the groups. Significance was set at p-value<0.05
524 [2] Light drinker: 0-1 drink/day for men and women; Moderate drinker: men: 1-5drinks/day, women: 1-2.5 drinks/day; Heavy drinker: men: ≥5drinks/day, women:
525 ≥2.5 drinks/day

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Table 2: Association between smoking variables and pancreatic cancer risk in the PanGenEU study population (2,009 cases and 1,532 controls).

	Cases (%)		Controls (%)		p-value[1]	Unadjusted OR	(95%CI)	Adjusted aOR	(95%CI)
Smoking status (cigarettes)					<0.001				
Never-smoker	759	(37.8)	690	(45.0)		1.00		1.00	
Occasional	33	(1.6)	42	(2.7)		0.72	(0.44 - 1.14)	1.00	(0.61 - 1.67)
Former	667	(33.2)	530	(34.6)		1.14	(0.98 - 1.33)	1.14	(0.95 - 1.37)
Current	550	(27.4)	270	(17.6)		1.85	(1.55 - 2.21)	1.72	(1.39 - 2.12)
								p-trend	<0.001
Smoking intensity in tertiles (cigarettes per day)					<0.001				
Never-smoker	759	(37.8)	690	(45.0)		1.00		1.00	
< 10	389	(19.4)	343	(22.5)		1.03	(0.86 - 1.23)	1.02	(0.83 - 1.25)
10 - 20	533	(26.5)	287	(18.7)		1.69	(1.42 - 2.02)	1.64	(1.34 - 2.02)
≥ 20	328	(16.3)	212	(13.8)		1.41	(1.15 - 1.72)	1.41	(1.12 - 1.78)
								p-trend	<0.001
Smoking duration in tertiles (years)					<0.001				
Never-smoker	759	(37.8)	690	(45.0)		1.00		1.00	
< 23	292	(14.5)	284	(18.5)		0.93	(0.77 - 1.13)	0.91	(0.72 - 1.14)
23 - 35	477	(23.8)	281	(18.4)		1.54	(1.29 - 1.85)	1.52	(1.23 - 1.87)
≥ 35	481	(23.9)	277	(18.1)		1.58	(1.32 - 1.89)	1.51	(1.23 - 1.86)
								p-trend	<0.001
Cumulative dose in tertiles (pack-years)					<0.001				
Never-smoker	759	(37.8)	690	(45.0)		1.00		1.00	
< 14	279	(13.9)	281	(18.3)		0.90	(0.74 - 1.10)	0.90	(0.72 - 1.13)
14 - 32	494	(24.6)	275	(18.0)		1.63	(1.36 - 1.96)	1.57	(1.27 - 1.94)
≥ 32	477	(23.7)	286	(18.7)		1.52	(1.27 - 1.81)	1.50	(1.21 - 1.84)
								p-trend	<0.001
Age at smoking initiation in tertiles (years)					<0.001				
Never-smoker	759	(37.8)	690	(45.0)		1.00		1.00	
< 15	423	(21.1)	278	(18.1)		1.38	(1.15 - 1.66)	1.36	(1.10 - 1.70)
15 - 18	455	(22.6)	299	(19.6)		1.38	(1.16 - 1.65)	1.31	(1.06 - 1.61)
≥ 18	372	(18.5)	265	(17.3)		1.28	(1.06 - 1.54)	1.29	(1.04 - 1.59)
								p-trend	= 0.010
Inhalation					<0.001				
Never-smoker	759	(37.8)	690	(45.0)		1.00		1.00	
Mouth only	125	(6.2)	99	(6.5)		1.15	(0.86 - 1.53)	1.10	(0.80 - 1.50)
Throat	158	(7.9)	108	(7.1)		1.33	(1.02 - 1.74)	1.48	(1.11 - 1.99)
Chest	967	(48.1)	635	(41.4)		1.38	(1.20 - 1.60)	1.33	(1.12 - 1.58)
								p-trend	<0.001
Filter-use					<0.001				
Never-smoker	759	(37.8)	690	(45.0)		1.00		1.00	
Filtered only	1042	(51.9)	706	(46.1)		1.34	(1.17 - 1.54)	1.25	(1.06 - 1.48)
Non-filtered only	65	(3.2)	49	(3.2)		1.20	(0.82 - 1.78)	1.69	(1.10 - 2.61)
Both	143	(7.1)	87	(5.7)		1.49	(1.12 - 1.99)	1.65	(1.21 - 2.27)
								p-trend	<0.001
ETS exposure during childhood					<0.001				
Both parents were never-smokers	420	(20.9)	391	(25.6)		1.00		1.00	
One of the parents smoked	1378	(68.6)	952	(62.1)		1.35	(1.15 - 1.58)	1.24	(1.03 - 1.49)
Both parents smoked	211	(10.5)	189	(12.3)		1.04	(0.82 - 1.32)	1.07	(0.81 - 1.42)
								p-trend	= 0.610
Time since cessation in tertiles (years)					<0.001				
Current smoker	551	(27.4)	274	(17.9)		1.00		1.00	
< 14	320	(15.9)	191	(12.5)		0.83	(0.66 - 1.05)	0.81	(0.62 - 1.04)
14 - 28	229	(11.4)	185	(12.1)		0.62	(0.48 - 0.78)	0.67	(0.51 - 0.88)
≥ 28	150	(7.5)	192	(12.5)		0.39	(0.30 - 0.50)	0.49	(0.36 - 0.66)
Never-smoker	759	(37.8)	690	(45.0)		-	-	-	-
								p-trend	<0.001

ETS: environmental tobacco smoke

Risk estimates are shown for the imputed smoking variables. Risk estimates of the unimputed smoking variables can be found in Supplementary Table 5

Adjusted model for age (≤ 54, 55-64, 65-74, ≥75 years), gender (male, female) and country (Spain, England, Germany, Ireland, Italy, Sweden). Random effects model applied for country

[1] Chi-square test applied to evaluate differences between the groups. Significance was set at p-value<0.05

Table 3: Association between smoking variables and pancreatic cancer risk by tobacco type and smoking status in the PanGenEU-Spain study population (876 cases and 762 controls).

	Cases (%)		Controls (%)		p-value[1]	Unadjusted OR	(95%CI)	Adjusted aOR	(95%CI)
Tobacco type					0.012				
Never-smoker	355	(40.5)	360	(47.2)		1.00		1.00	
Smoker of black tobacco only	165	(18.8)	114	(15.0)		1.47	(1.11 - 1.94)	1.55	(1.13 - 2.12)
Smoker of blond tobacco only	204	(23.3)	182	(23.9)		1.14	(0.89 - 1.46)	1.23	(0.94 - 1.62)
Smoker of both tobacco types	152	(17.4)	106	(13.9)		1.45	(1.09 - 1.94)	1.58	(1.14 - 2.17)
Tobacco type by smoking status					0.028				
Never-smoker	369	(42.0)	377	(49. 5)		1.00		1.00	
Former									
Black tobacco	104	(11.9)	79	(10.4)		1.34	(0.97 - 1.87)	1.40	(0.98 - 1.99)
Blond tobacco	90	(10.3)	88	(11.5)		1.04	(0.75 - 1.45)	1.12	(0.79 - 1.57)
Both	76	(8.7)	58	(7.6)		1.34	(0.92 - 1.94)	1.44	(0.97 - 2.14)
Current									
Black tobacco	60	(6.8)	31	(4.1)		1.98	(1.26 - 3.16)	2.09	(1.31 - 3.41)
Blond tobacco	103	(11.8)	83	(10.9)		1.27	(0.92 - 1.75)	1.43	(1.01 - 2.04)
Both	74	(8.5)	46	(6.0)		1.64	(1.11 - 2.45)	1.81	(1.19 - 2.76)

Risk estimates are shown for the imputed smoking variables

Adjusted model for age (≤ 54, 55-64, 65-74, ≥75 years), gender (male, female) and region (East, Central and Northern Spain)

[1] Chi-square test applied to evaluate differences between the groups. Significance was set at p-value<0.05

Table 4: Combined effects of smoking duration and tobacco type on pancreatic cancer risk in the PanGenEU-Spain study population (876 cases and 762 controls).

		Smoking duration of blond tobacco (years)							
		Never-smoker		<20 years		20-30 years		≥ 30 years	
		aOR (95%CI)	Case/ Controls	aOR (95%CI)	Case/ Controls	aOR (95%CI)	Case/ Controls	aOR (95%CI)	Case/ Controls
tobacco (years)	Never-smoker	1.00	355/360	1.03 (0.65 - 1.64)	42/47	1.27 (0.81 - 2.00)	51/45	1.33 (0.95 - 1.84)	112/90
	<20 years	1.37 (0.71 - 2.64)	25/17	0.84 (0.66 - 1.07)	25/32	0.93 (0.52 - 1.67)	10/8	1.43 (0.54 - 3.76)	6/5
	20-30 years	1.68 (0.92 - 3.09)	31/21	1.28 (0.38 - 4.28)	7/2	3.91 (0.79 - 19.33)	13/6	2.61 (0.96 - 7.09)	1/1
	≥ 30 years	1.58 (1.11 - 2.27)	109/76	1.86 (0.84 - 4.13)	18/11	1.83 (0.79 - 4.26)	15/10	2.05 (1.25 - 3.36)	56/31

Risk estimates are shown for the imputed smoking variables
Adjusted OR for age (≤ 54, 55-64, 65-74, ≥75 years), gender (male, female) and region (East, Central and Northern Spain)
Relative excess risk due to interaction = RERI=0.206, 95%CI: -0.49 - 0.91

Table 5: Non-linear association between continuous smoking variables and pancreatic cancer risk per 1-unit increase in the variables for the PanGenEU study population (2,009 cases and 1,532 controls)

	Restricted Cubic splines	**Fractional polynomials**	**aOR (95% CI) per 1-unit increase**		
			Model 1	Model 2	Model 3
	LR test[1] p-value	Formula resulting from the fractional polynomials[2]	aOR (95%CI)	aOR (95%CI)	aOR (95%CI)
Age at smoking initiation (years)	0.031	$\left(\frac{\text{smoke first}+1}{10}\right)^{-2}$	1.11 (1.04 - 1.20)	1.00 (0.89 - 1.11)	0.96 (0.87 - 1.07)
Age last smoked (years)	0.008	$\left(\frac{\text{smoke last}+1}{10}\right)^{0.5} + \left(\left(\frac{\text{smoke last}+0.1}{10}\right)^{0.5} * \log\left(\frac{\text{smoke last}+0.1}{10}\right)\right)$	1.07 (1.04 - 1.10)	1.05 (1.00 - 1.11)	0.99 (0.93 - 1.06)
Smoking duration (years)	0.020	$\left(\frac{\text{duration}+0.1}{10}\right)^{3} + \left(\left(\frac{\text{duration}+0.1}{10}\right)^{3} * \log\left(\frac{\text{duration}+0.1}{10}\right)\right)$	1.04 (1.02 - 1.05)	1.03 (1.02 - 1.05)	N.A.
Smoking intensity (cigarettes per day)	0.001	$\left(\frac{\text{intensity}+0.2}{10}\right)^{0.5}$	1.29 (1.18 - 1.45)	N.A.	1.04 (0.88 - 1.23)
Cumulative dose (pack-years)	0.000	$\left(\frac{\text{pack-years}+0.1}{10}\right)^{0.5}$	1.24 (1.16 - 1.35)	N.A.	N.A.
Time since cessation (years)[3]	0.016	$\left(\frac{\text{cessation}+1}{10}\right)^{1} + \left(\frac{\text{cessation}+1}{10}\right)^{3}$	0.81 (0.74 - 0.88)	0.80 (0.72 - 0.87)	0.89 (0.71 - 1.05)
Time since cessation (years) for PanGenEU – Spain[3,4]	0.073	$\left(\frac{\text{cessation}+1}{10}\right)^{1} + \left(\frac{\text{cessation}+1}{10}\right)^{3}$	0.85 (0.74 - 0.96)	0.85 (0.73 - 0.96)	0.88 (0.66 - 1.11)

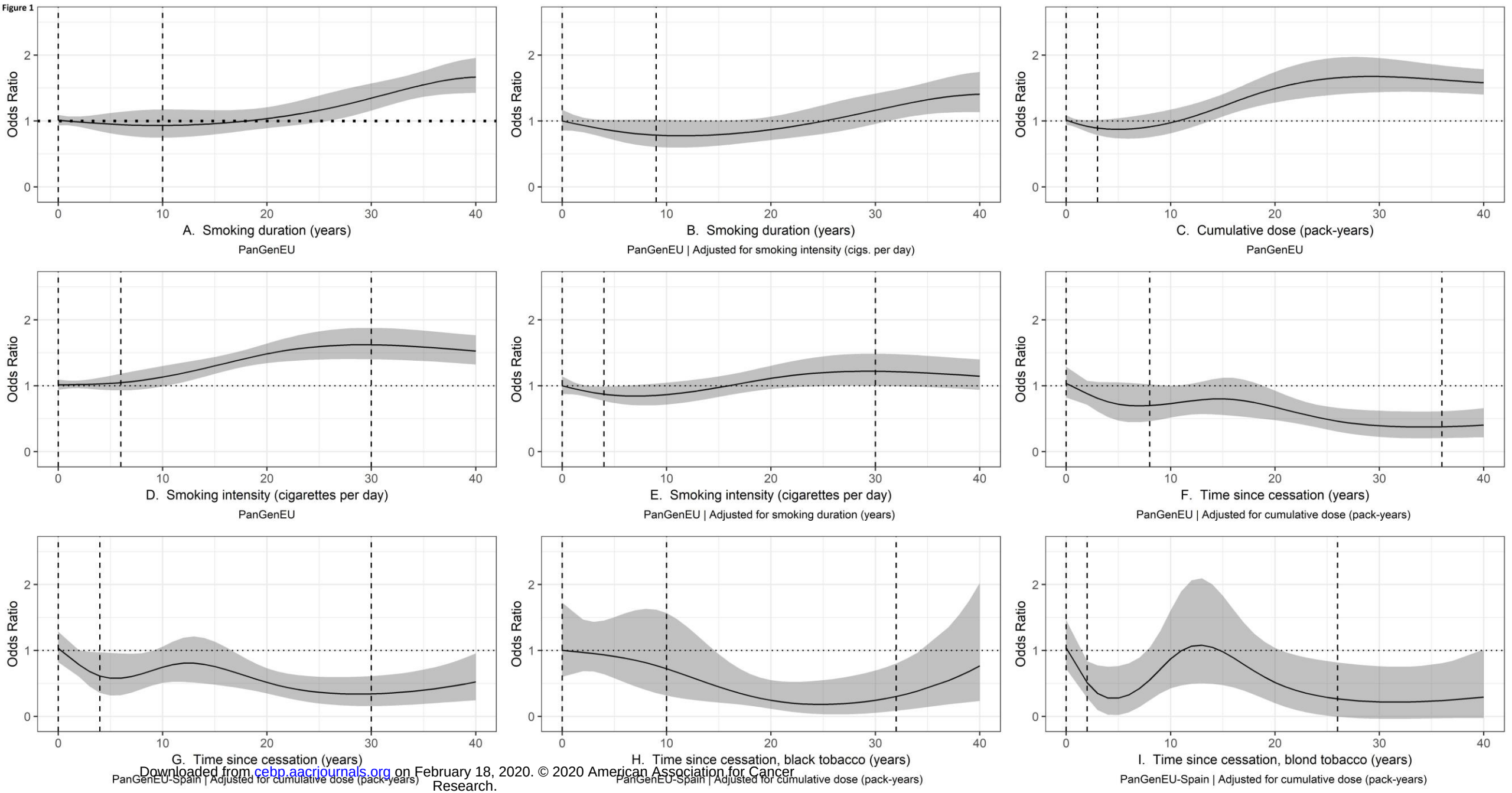
PC: pancreatic cancer; N.A.: not applicable
Risk estimates are shown for the unimputed continuous smoking variables
Model 1: adjusted for age (≤ 54, 55-64, 65-74, ≥75 years), gender (male, female) and country (Spain, England, Germany, Ireland, Italy, Sweden);
Model 2: Model 1 plus additional adjustment for smoking intensity (cigarettes per day, continuous, non-linear);
Model 3: Model 1 plus additional adjustment for smoking duration (years, continuous, non-linear)

[1] Likelihood ratio test (LR test) comparing two models, adjusted for age (≤ 54, 55-64, 65-74, ≥75 years), gender (male, female), and country (Spain, England, Germany, Ireland, Italy, Sweden), with and without restricted cubic splines applied (knots at 10, 50 and 90%)
[2] Fractional polynomials adjusted for age (≤ 54, 55-64, 65-74, ≥75 years), gender (male, female), and country (Spain, England, Germany, Ireland, Italy, Sweden)
[3] Never-smokers were removed from time since cessation variables
[4] The PanGenEU-Spain study population consists of 876 cases and 762 controls. The model was adjusted for age (≤ 54, 55-64, 65-74, ≥75 years), gender (male, female) and region (East, Central and Northern Spain)

Figures

Figure 1 (A-I): Dose-response relationships between several smoking variables and the risk of PC, depicted by restricted cubic splines with knots at 10%, 50% and 90%, represented as dashed, vertical lines. Adjusted for age, gender and country (for the PanGenEU study population), or region (for the PanGenEU-Spain study population). Restricted cubic splines are shown for the unimputed smoking variables, and additional adjustment variables were modelled as fractional plolynomials to account for non-linear effects. The spline curve is shown as a black trend line and 95% confidence intervals are shadowed in grey. The dotted horizontal black line represents the reference odds ratio of 1. A: Smoking duration in years (PanGenEU); B: Smoking duration in years (PanGenEU), adjusted for smoking intensity (cigarettes per day); C: Cumulative dose in pack-years (PanGenEU); D: Smoking intensity in cigarettes per day (PanGenEU); E: Smoking intensity in cigarettes per day (PanGenEU), adjusted for smoking duration (years); F: Time since cessation in years (PanGenEU), adjusted for cumulative dose (pack-years); G: Time since cessation in years (PanGenEU-Spain), adjusted for cumulative dose (pack-years); H: Time since cessation in years for smokers of only black tobacco (PanGenEU-Spain), adjusted for cumulative dose (pack-years); I: Time since cessation in years for smokers of only blond tobacco (PanGenEU-Spain), adjusted for cumulative dose (pack-years). PC: pancreatic cancer; RCS: restricted cubic splines

Figure 1



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Pancreatic cancer risk in relation to lifetime smoking patterns, tobacco type, and dose-response relationships

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