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1 **Risk and Protective Factors for the Occurrence of Sporadic Pancreatic Endocrine**
2 **Neoplasms**

3

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27

28 **Key Words:** Pancreas, Neuroendocrine Neoplasms, Insulinomas, Gastrinomas, Risk factors

29 **Short Title:** Risk Factors for the Occurrence of PNEN

30 **Word count:** 3858

31 **Abstract**

32

33 BACKGROUND: Pancreatic neuroendocrine neoplasms (PNENs) represent 10% of all
34 pancreatic tumors by prevalence. Their incidence has reportedly increased over recent
35 decades in parallel with that of pancreatic adenocarcinoma. PNENs are relatively rare and of
36 the few institutions that have published potential risk factors, findings have been
37 heterogenous.

38 AIM: To investigate the association between potential risk and protective factors for the
39 occurrence of sporadic PNENs across a European population from several institutions.

40 METHODS: A multinational European case-control study was conducted to examine the
41 association of selected environmental, family and medical exposure factors using a
42 standardized questionnaire in face-to-face interviews. A ratio of 1:3 cases to controls were sex
43 and age matched at each study site. Adjusted univariate and multivariate logistic regression
44 analysis were performed for statistically significant factors. RESULTS: In 201 cases and 603
45 controls non-recent onset diabetes (OR 2.09, CI 1.27-3.46) was associated with an increased
46 occurrence of PNENs. The prevalence of non-recent onset diabetes was higher both in cases
47 with metastatic disease (TNM stage III-IV) or advanced grade (G3) at the time of diagnosis.
48 The use of metformin in combination with insulin was also associated with a more aggressive
49 phenotype. Drinking coffee was more frequent in cases with localized disease at diagnosis.

50 CONCLUSIONS: Non-recent onset diabetes was associated with an increased occurrence of
51 PNENs and the combination of metformin and insulin was consistent with a more aggressive
52 PNEN phenotype. In contrast to previous studies, smoking, alcohol and first-degree family
53 history of cancer were not associated with PNEN occurrence.

54

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56

57

58 Introduction

59 Pancreatic neuroendocrine neoplasms (PNENs) are a group of tumors which originate
60 from endocrine cells within the pancreas gland. PNENs have heterogeneous clinical behavior
61 owing to their hormone functional status, cellular characteristics and the extent of metastatic
62 disease. Whilst representing only 1-2% of pancreatic neoplasms by incidence (Fitzgerald, et
63 al. 2008; Yao, et al. 2008), PNENs may account for as much as 10% by prevalence (Yao et al.
64 2008). Such discrepancy is due in part to the relatively indolent clinical course of many PNENs
65 compared to pancreatic adenocarcinoma. PNENs are rare tumors but their incidence has
66 reportedly increased in recent decades, particularly that of non-functioning tumors
67 (Fitzgerald et al. 2008; Lepage, et al. 2004; Yao et al. 2008). To a lesser degree investigators
68 have also identified a modest increase in the incidence of pancreatic adenocarcinoma
69 (Fitzgerald et al. 2008).

70 The increased reported incidence of both endocrine and exocrine pancreatic tumors is
71 likely due to rising population lifespan and the wider availability of high resolution cross-
72 sectional imaging (Del Chiaro, et al. 2013; Ellison, et al. 2014), but additionally raises the
73 possibility of changing exposure to factors which may alter the risk of pancreatic neoplasia.

74 There are a small number of case-control studies that have investigated potential risk
75 factors for the occurrence of PNENs (Ben, et al. 2016; Capurso, et al. 2009; Halfdanarson, et al.
76 2014; Hassan, et al. 2008b; Zhan, et al. 2013). These studies recruited participants from a
77 single institution or geographical region, and were recently summarized in a meta-analysis
78 that found personal history of diabetes mellitus and family history of cancer to be associated
79 with an increased risk of PNEN (Haugvik, et al. 2015). The association of PNENs with smoking
80 and alcohol drinking was less clear, and only heavy smoking and heavy alcohol consumption

81 reached statistical significance. The included studies differed in their design and population
82 definitions, with a considerable heterogeneity limiting the significance of the meta-analysis.
83 Moreover, these studies had some specific methodological limitations, such as the absence of
84 a power calculation and exposures were often recorded at the time of diagnosis or treatment,
85 rather than considering the exposure history prior to diagnosis. The latter raises the
86 possibility of a bias due to cancer symptoms (e.g. weight loss, new onset diabetes) or lifestyle
87 modifications such as changes in smoking behaviour and alcohol consumption.

88 It appears that there is overlap in risk factors, such as smoking and alcohol, for the
89 occurrence of PNENs and pancreatic adenocarcinoma, however, a number of other factors
90 that have been associated with the occurrence of pancreatic cancer have not, to our
91 knowledge, been investigated for PNENs. For example, the use of medications such as aspirin,
92 and the association with allergy and atopy that have been reported to be protective against
93 pancreatic adenocarcinoma occurrence (Gomez-Rubio, et al. 2015; Streicher, et al. 2014).

94 For these reasons, we conducted a multi-center European study aimed at assessing the
95 association between a large number of potential risk or protective factors for the
96 development of sporadic PNENs.

97

98 **Materials and Methods**

99

100 *Study Design and population*

101 A collaborative multicentre hospital based case-control study was conducted in six European
102 countries: Italy, Norway, Sweden, Slovenia, United Kingdom and Germany as part of the
103 “Pancreas 2000” educational project (www.pancreas2000.org), upon local hospital ethical
104 committee approval.

105 A standardized questionnaire, including questions about demographics and potential
106 risk factors, such as family history of cancer, environmental factors, previous use of drugs and
107 other medical history features was administered to patients by a trained medical doctor. Each
108 questionnaire took ~15 minutes to be completed during a face-to-face interview, after gaining
109 participant consent.

110 The cases were prevalent sporadic PNEN patients diagnosed within 24 months from
111 the beginning of the study (January 2013) and new incident cases of sporadic PNENs
112 diagnosed from January 2013 to December 2015 that were recruited at the participating
113 centers.

114 The inclusion criterion was to have a histological or cytological diagnosis of PNEN. The
115 date of the confirmatory pathological report was accepted as the date of diagnosis.

116 Exclusion criteria were the presence of an inherited form of PNEN such as those
117 associated with multiple endocrine neoplasia-type 1 (MEN1), von Hippel-Lindau disease
118 (VHL), neurofibromatosis type 1 (NF-1), tuberous sclerosis (TSC), or an inability to
119 participate, such as dementia.

120 According to the absence or presence of a clinical syndrome due to hormonal
121 hypersecretion, cases were classified as non-functioning or functioning tumors (gastrinoma,
122 insulinoma, glucagonoma). For example the Zollinger Ellison Syndrome was clinically
123 suspected in the presence of a PNEN associated with peptic disease and its complications and
124 diarrhea, insulinomas in the presence of severe hypoglycemia with associated neurological
125 symptoms (varying from confusion to coma), glucagonoma in the presence of rash, glucose
126 intolerance and weight loss. In any case the syndrome was confirmed with a specific
127 laboratory work-up according to guidelines.(Falconi, et al. 2016; Jensen, et al. 2012)

128 Cases were classified according with the European Neuroendocrine Tumor Society
129 (ENETs) and the 2010 World Health Organization classifications (Falconi et al. 2016; Rindi G ;
130 Solcia E 2002).

131 Eligible controls were either individuals seen in the participating hospitals' outpatient
132 clinic for a non-specific, non-organic gastrointestinal disorder (bloating, aspecific dyspeptic
133 symptoms, eructation) or visitors attending the same network of referring hospitals, matched
134 by country, sex and age (+/- 5 years). Visitors and hospital outpatients' clinic belonged to the
135 same catchment area of cases. Specific exclusion criteria for controls were: 1) the presence of
136 any genetic syndrome associated with the occurrence of PNENs; 2) a history of active cancer
137 (diagnosed within 5 years); 3) any biological relation of a participating PNEN case in this
138 study; 4) a history of any chronic inflammatory condition (e.g. chronic obstructive pulmonary
139 disease, liver cirrhosis, inflammatory bowel disease, end stage kidney disease); 5) undergoing
140 evaluation of a possible familial cancer syndrome. Controls were included in the same country
141 and interviewed within 6 months of the inclusion of the matched corresponding case.

142

143 *Exposure definitions*

144 Subjects were questioned about risk factors that were present at least 12 months before
145 diagnosis or presentation of symptoms, in order to avoid potential bias due to lifestyle
146 modifications, cancer symptoms or cancer treatments.

147 Subjects were considered ever smokers if they reported a cumulative lifetime smoking
148 history greater than 6 months or 100 cigarettes smoked. A quantification of the smoking habit
149 for cases and controls was performed considering the number of pack-years (pack-year =
150 number of packs per day x years of smoking), with 20 pack-years being the lower limit to
151 qualify a participant a heavy smoker.

152 A daily intake of at least 12.5 g of alcohol, equivalent to one glass of wine, one pint of
153 beer or one shot of hard liquor, for at least one year, was considered the cut-off to be a regular
154 ever alcohol drinker. Because of possible different drinking habits within different European
155 countries, the weekly alcohol amount was also sub-analyzed according to low (1-7 weekly
156 units assumption), medium (8-14 weekly units), medium-high (14-20 weekly units) and
157 heavy alcohol consumption (≥ 21 weekly units). Coffee drinking was also recorded as ever
158 drinking (at least one cup per day) or heavy coffee drinking (>5 cups per day).

159 Height and weight were recorded from which body mass index (BMI) (kg/m^2) was
160 calculated. A history of chronic pancreatitis, acute pancreatitis, peptic ulcer disease, biliary
161 stones and previous surgery were specifically recorded. Additionally, a diagnosis of diabetes
162 was documented and subdivided for type and onset. For cases, recent onset diabetes was
163 defined as that which was diagnosed in the 12 months prior to the PNEN diagnosis, and for
164 controls a diagnosis of diabetes 12 months prior to the date of recruitment was required.
165 Sensitivity analysis were also performed for different intervals of the onset of diabetes,
166 (respectively inferior to 1 year, between 1 and 3 years, between 3 and 5 years and above 5
167 years). Another sensitivity analysis was also conducted considering incident/prevalent cases
168 and hospitals controls/visitors controls compared to respective cases.

169 As atopy and allergy have been associated with a reduced risk of pancreatic cancer
170 (Gomez-Rubio et al. 2015), cases and controls were interrogated about a history of allergy,
171 with specific enquiry for eczema, hay fever and asthma. The use of aspirin, proton pump
172 inhibitors, metformin and insulin were recorded. Subjects were interrogated about 1st and 2nd
173 degree family history of cancer, and the total number of siblings and children was recorded.

174

175 *Statistical analysis*

176 An a priori power calculation was performed. We estimated sample size based on the
177 differences reported in the frequencies of exposure in cases and controls according to a
178 previous study (Capurso et al. 2009), and considering a ratio 1:3, with a statistical power
179 equal to 80% and an alpha error equal to 0.05.

180 According to Capurso and colleagues (Capurso et al. 2009) the reported prevalence in cases
181 and controls was respectively 53% vs. 32% for 1st degree family history of cancer, 10% vs. 2%
182 for diabetes and 14% vs. 3% for heavy alcohol consumption. Therefore, the sample size's
183 estimate of cases and controls, to show true differences whether existing, were respectively
184 64 cases and 191 controls for 1st degree family history of cancer, 117 cases and 350 controls
185 for diabetes, 85 cases and 253 controls for heavy alcohol consumption.

186 We therefore estimated that a total of 200 cases and 600 controls across participating
187 centers, to be an adequate sample size to reveal differences in the prevalence of most risk
188 factors analyzed among cases and controls, where these exist.

189 Characteristics of cases and controls were compared by chi-square test for categorical
190 variables or Student's t test for continuous variables. Significant variables were analyzed by
191 logistic regression analysis adjusted for sex, age and enrolment center. A multivariable logistic
192 regression analysis, adjusted for sex, age and enrolment center was also performed with an
193 enter selection procedure for statistically significant risk factors. A dedicated statistical
194 software package (MedCald Mariakerke, Belgium) was used for data analysis. The 95 %
195 confidence interval (CI 95) was calculated where possible. All p-values were two-sided and a
196 $p < 0.05$ was considered statistically significant .

197

198 **RESULTS**

199 *Characteristics of cases and controls*

200 A total of 201 cases and 603 sex and age matched controls were enrolled among the six
201 centers (Italy, Norway, Sweden, United Kingdom, Slovenia and Germany) as shown in table 1.
202 The mean age was 59.6 years in cases (CI 57.7-61.4) and 59.5 years in controls (CI 58.4-
203 60.55), and 51% of cases and controls were male.

204 Amongst the 201 PNEN cases, 154 (76.6%) had a non-functioning tumor. Of the 47
205 functioning PNENs, 26 (55.3%) had an insulinoma, 9 (19.1%) a gastrinoma, 7 (14.9%) a
206 glucagonoma and 5 (10.6%) other functioning tumors. The majority were G1 (44.8%) or G2
207 (43.3%) PNENs, and were equally distributed among different disease stages (**Table 1**). Of the
208 201 PNEN cases, 80 (38%) cases were incident and 121 (62%) prevalent. Of the 603 controls,
209 422 (70%) were hospital outpatients controls and 181 (30%) visitors.

210

211 *Risk factors for the occurrence of PNEN*

212

213 *Family history of cancer*

214 The proportion of subjects who had a 1st degree family history of cancer was similar in cases
215 and in controls (respectively 51.1% vs. 45.3% $p=0.17$), while 2nd degree family history was
216 slightly more prevalent in cases (36.8% vs. 30.2% $p=0.09$). A 1st degree family history of
217 specific cancer sites was also not significantly different (**Table 2**). No cases or controls
218 reported a family history of neuro-endocrine tumor (NET). At multiple regression analysis 2nd
219 degree family history of any cancer was, however associated with an increased risk of PNEN
220 (**see Table 2 and 3**).

221

222 *Body Mass Index*

223 Mean BMI was not significantly different amongst cases and controls (26.8 kg/m² (CI 26.0-
224 27.5) and 26.4 kg/m² (CI 26.4-26.8), p=0.10. Similarly, whilst the overall prevalence of obesity
225 was more frequent in cases than controls (25.5% vs. 18.2%) this was not significant (p=0.44).
226 At regression analysis after adjustment for matching variables, there remained no significant
227 association with obesity (OR 1.36, 95% CI 0.88-2.08, p=0.15) (**Table 3**).

228

229 *Cigarette smoking, alcohol intake and coffee drinking*

230 The proportion of smokers (55.5% vs. 53.5%, p=0.59), heavy smokers (25.5% vs. 24.0%,
231 p=0.59), alcohol drinkers (75.6% vs. 70.8%, p=0.33), heavy alcohol drinkers (3.4% vs. 4.2%,
232 p=0.33), coffee drinkers (84.8% vs. 87.8%, p=0.24) and heavy coffee drinkers (18.8% vs.
233 19.2%, p=0.24) did not significantly differ between cases and controls (**Table 2**).

234

235 *History of diabetes mellitus*

236 A history of diabetes mellitus was more prevalent in cases than in controls (18.4% vs. 12.3%,
237 p=0.03). This difference was greater on analysis of non-recent onset diabetes, defined as
238 diabetes diagnosed more than 12 months before the diagnosis of PNEN in cases, or 12 months
239 prior to the interview for controls (17.4% vs. 9.7%, p=0.004) (**Table 2**). After adjustment for
240 the matching variables, non-recent onset diabetes was confirmed to be consistent with the
241 occurrence of PNEN (OR 1.89, 95% CI 1.17-3.05, p=0.008). At multivariable analysis the
242 association with non-recent onset diabetes remained statistically significant (OR 2.09, 95% CI
243 1.27-3.45, p=0.003) (**Table3**). At sensitivity analysis, a history of diabetes with an onset
244 between 1-3 year and between 3- 5 years, was increasingly prevalent in PNEN compared to
245 controls (respectively 3.0% vs 1.1% p=0.07 and 4% vs 0.9% p=0.004). At univariable logistic
246 regression analysis this difference remained significant (respectively OR 2.56, 95% C.I 0.83-
247 7.91 and OR 4.31, 95% C.I 1.43-12.98). For intervals of occurrence of diabetes superior to 5

248 years no statistically significant difference was found between cases and controls
249 (respectively 10.7% vs 7.4%, $p=0.16$) (**Table 2**). We also performed a separate analysis for
250 “late onset diabetes” using as controls either only the 422 hospital controls or only the 181
251 visitors controls. In the first case the OR resulted to be 2.52 (95%CI 1.08-5.86; $p=0.03$), while
252 in the second one the OR was 1.7 (95%CI 0.95-3; $p=0.07$), most likely due to the lower
253 number of controls reducing the power of the analysis.

254 Neither the use of metformin (7.3% vs. 5.1%, $p=0.3$), nor insulin (4.1% vs. 1.6%, $p=0.1$), or
255 their association together (5.2% vs 3.4%, $p=0.4$) were statistically different between cases
256 and controls.

257 *Past medical history*

258 With regards to past medical history, the prevalence of acute pancreatitis (3.5% vs. 2.4%,
259 $p=0.60$), peptic ulcer disease (12.3% vs. 10.6%, $p=0.59$), cholecystectomy (9.0% vs. 8.5%,
260 $p=0.92$) and gastrectomy (1.0% vs. 0.7%, $p=0.99$) were similar in cases and controls. None of
261 the participants reported a medical history of chronic pancreatitis (**Table 2**). A higher
262 proportion of cases reported a history of gallstone disease than controls (19.2% vs. 13.3%)
263 but this did not reach the significance threshold ($p=0.06$). After adjustment for age, sex and
264 enrolment center at multivariable analysis, this latter association remained borderline
265 significant (OR 1.52, 95% CI 0.95-2.44, $p=0.08$) (**Table 3**).

266

267 *Non-diabetic medications*

268 The use of proton pump inhibitors (PPI) (39.2% vs. 39.6%, $p=0.97$) and aspirin (22.5% vs.
269 26.5%, $p=0.29$) did not differ among cases and controls respectively (**Table 2**).

270

271 *Allergies*

272 A history of allergies was not different in cases and in controls (28.9% vs. 25.0%, $p=0.32$).
273 Specifically, neither asthma (12.1% vs. 8.6%, $p=0.19$), eczema (11.1% vs. 7.6%, $p=0.17$) nor
274 hay fever (15.6% vs. 14.5%, $p=0.79$) were more prevalent in PNEN patients than in controls
275 **(Table 2)**.

276

277 *Risk factors for the advanced grades and stages of PNEN*

278 When stratifying cases for the TNM stage at diagnosis and for the tumor grade, diabetes
279 mellitus was statistically more prevalent in patients with G3 tumors (pancreatic neuro-
280 endocrine carcinoma; PNEC) than with G1 or G2 tumors (40.9% vs. 15.8%, $p=0.01$). Amongst
281 cases, non-recent onset diabetes was associated with a more advanced stage at diagnosis
282 (TNM III-IV vs TNM I-II respectively 23.3% vs. 11.8%, $p=0.05$) and with a G3 vs G1-2 tumor
283 (respectively 40.9% vs. 14.9%, $p=0.006$). The use of metformin in combination with insulin
284 was more prevalent in patients with G3 than G1-G2 tumors (respectively 23.5% vs. 3.2%,
285 $p=0.003$) **(Table 4)**. Asthma was more prevalent in G3 cases than in G1-2 (30.0% vs. 10.2%
286 $p=0.02$), and eczema was also more prevalent in G3 cases than in G1-2 but without reaching
287 statistical significance (25.0% vs. 9.6%, $p=0.08$). Coffee drinking was more prevalent in
288 localized disease (TNM 1-2) at diagnosis compared with advanced stage (TNM 3-4) (92.3% vs.
289 75.9%, $p=0.003$).

290

291

292 **Discussion**

293 The present study was designed to recruit cases of PNEN from multiple sites across Europe
294 using a standardised questionnaire in a face-to-face interview setting. To further strengthen
295 our method, we incorporated a preliminary power calculation based upon results from a
296 similar previous study. To the best of our knowledge, there have been six published studies to
297 have investigated risk factors for PNEN. Due to the low incidence of such tumors, which would
298 make a longitudinal cohort study highly problematic, it is not unexpected that these were all
299 case-control studies. Three were conducted in the USA, one in Europe and two in China (Ben
300 et al. 2016; Capurso et al. 2009; Halfdanarson et al. 2014; Hassan, et al. 2008a; Hassan et al.
301 2008b; Zhan et al. 2013). Collective analysis of these studies has been limited by the variety
302 data collection methods employed, the selection of investigated exposures (and differing
303 definitions), in addition to the disparate population pools, all of which lead to substantial
304 heterogeneity (Haugvik et al. 2015). One study from China exclusively investigated a cohort of
305 insulinomas, with the exclusion of non-functioning endocrine tumors; the latter represent the
306 majority of PNEN cases, accounting from 60 to 90% of cases (Falconi et al. 2016). Another
307 study from China investigated a cohort of PNEN cases in which 63% had functioning tumors,
308 and 84% had early stage disease (Ben et al. 2016). The results of this study may have limited
309 applicability to other populations, as the majority of PNENs arising in Western populations
310 are non-functioning and would typically present with more advanced disease (Panzuto, et al.
311 2011).

312 The two publications from the USA reported different risk factors in the same population,
313 from a retrospective analysis of a large hospital database of neuroendocrine tumors (Hassan
314 et al. 2008a, b). None of the reported studies sought to investigate potentially protective
315 factors against the occurrence of PNENs. Two meta-analyses have summarized the results of
316 the previous primary studies and reached similar conclusions: Diabetes mellitus and family

317 history of cancer are risk factors for the occurrence of PNENs, whilst the role of
318 environmental factors was unclear and warranted further investigation (Haugvik et al. 2015;
319 Leoncini, et al. 2016).

320 Our study affirms an increased risk of PNEN occurrence with diabetes mellitus,
321 however it is noteworthy that we identify the significance of non-recent onset diabetes as a
322 risk factor. Four studies previously identified an association between PNEN and diabetes (Ben
323 et al. 2016; Capurso et al. 2009; Halfdanarson et al. 2014; Hassan et al. 2008b), however in
324 contrast to the current study, this association was for recent onset diabetes, which can
325 represent an epiphenomenon of the disease as suggested elsewhere for pancreatic
326 adenocarcinoma (Pannala, et al. 2008).

327 Given that beta cells typically express low levels of cytoprotective antioxidant
328 enzymes (Tiedge, et al. 1997), and because oxidative stress contributes to both the
329 pathogenesis of diabetes (Rolo and Palmeira 2006) and can potentiate somatic mutations, it
330 would not be unexpected for long standing diabetes to have an association with oncogenic
331 transformation of islet cells. Indeed, PNEN proliferation, tumor invasion and disease stage
332 have been found to be associated with expression of mTOR (mechanistic/mammalian target
333 of rapamycin) and its effectors (Capurso, et al. 2015), a cytoplasmic kinase that is activated by
334 both glucose and insulin (Blagosklonny 2013).

335 As the relation between diabetes and carcinogenesis is complex and still not clear, we sought
336 to specifically investigate the timing of onset of diabetes in respect to the clinical presentation
337 of the cancer. We therefore analyzed risk factors present at least 12 months before diagnosis,
338 minimizing the overlap between risk factors and cancer-related symptoms, which could
339 include cancer-induced endocrine insufficiency. With such premises, our results support the
340 view that long standing diabetes is a risk factor for PNEN rather than sign of disease. In order
341 to further analyze possible overlaps between diabetes as a risk factors and diabetes as a

342 cancer-related symptom, we performed a further sensitivity analysis, investigating several
343 different intervals of time between the onset of diabetes and the diagnosis of cancer. Non
344 recent onset diabetes was confirmed to be increasingly consistent with the occurrence of
345 PNEN for intervals superior to 1 year and up to 5 years. For intervals of onset of diabetes
346 superior to 5 years this association was not anymore statistically significant. This might be
347 interpreted on the base of a lack of power of the study when considering small subgroups or,
348 alternatively, it could be biologically explained by the trophic influence that diabetes plays on
349 cancer. On the other hand one should also take into account that PNEN display a slower
350 growing rate compared to PDAC and therefore it might justify a major latency of occurrence of
351 symptom diabetes.

352 As the potential role of anti-diabetic drugs (metformin and insulin) in influencing pancreatic
353 carcinogenesis has been reported (De Souza, et al. 2016), we also specifically investigated the
354 role of such drugs in our multi-national cohort. Although the prevalent use insulin alone was
355 more frequent among cases than in controls (4.1% vs 1.6%), this difference was not significant
356 as the study was underpowered to assess it.

357 Another noteworthy finding of the present study was the increased proportion of
358 gallstone disease amongst PNEN cases compared to controls. However, this did not reach
359 statistical significance and we therefore cannot conclude that this was anything more than a
360 chance observation. The apparent proportional increase amongst cases, however, may reflect
361 the universal use of abdominal imaging in those diagnosed with PNEN, as compared to occult
362 gallstones in controls. It may be that our study was underpowered to detect a true association
363 between PNEN and biliary calculi, as the latter have been found to be associated with
364 'malignant neoplasm of the pancreas' (ICD-Oncology C25.0-C25.9) as a single entity, using a
365 large combined US cancer registry with population-based controls (Nogueira, et al. 2014).

366 In the present study the rate of family history of cancer was not different between

367 cases and controls. This finding was in contrast with previous studies on this topic. However,
368 study design issues and/or selection bias in previous studies may account for this difference.
369 For example, three of these reports (Hassan et al. 2008a, b; Zhan et al. 2013) did not exclude
370 PNEN cases with genetic syndromes (ie. MEN1 or VHL). Of particular note, 25% of PNEN cases
371 had a genetic syndrome in the study by Zhan and colleagues, and therefore a higher
372 proportion of family history of any cancer would be expected (Zhan et al. 2013). Halfdanarson
373 and colleagues studied only sporadic cases, but excluded insulinomas and poorly differentiated
374 PNECs which may have affected results in this regard (Halfdanarson et al. 2014). In the
375 present study, controls had a significantly higher number of siblings compared with cases,
376 potentially biasing the probability of cancer family history in the control study arm. On the
377 other hand, we found an association between 2nd degree family history of cancer and risk of
378 PNEN, thus suggesting that some kind of hereditary component might exist in this patients.

379 Environmental factors such as smoking and alcohol, even in high doses, did not
380 increase the risk of developing a PNEN in our study. This result is in keeping with the study by
381 Hassan and colleagues (Hassan et al. 2008b) but in contrast with others (Capurso 2009;
382 Halfdanarson et al. 2012; Zhan et al. 2013). A recent meta-analysis highlighted that the role of
383 smoking and alcohol might be less relevant in PNENs than in pancreatic adenocarcinoma
384 (Haugvik et al. 2015). To explore potential environmental factors which might alter the risk of
385 PNEN occurrence, we investigated for the first time a number of factors associated with a
386 lower incidence of pancreatic adenocarcinoma, such as the use of aspirin and a personal
387 history of allergies. No statistically significant differences were detected, possibly reflecting
388 intrinsic biological differences between endocrine and exocrine neoplasia of the pancreas.
389 Furthermore, as the power of the present study was based on risk factors for which there
390 were previous data, and this was not the case for previously uninvestigated exposures, a type
391 II error may have occurred.

392 Finally, we investigated the possible prognostic relevance of the investigated factors,
393 analyzing their distribution in PNEN patients according to their stage of disease at diagnosis
394 (TNM stages III or IV compared with I and II) or with their grade assessed by proliferative
395 activity (G1 and G2 compared to G3). Interestingly, of the prevalence of non-recent onset
396 diabetes was higher both in cases with metastatic disease (TNM stage III-IV) or advanced
397 grade (G3) at the time of diagnosis (**Table 5**). Drinking coffee was more frequent in cases with
398 localized disease at diagnosis. The use of metformin in combination with insulin, was also
399 associated with a more aggressive phenotype. Therefore, diabetes and use of insulin might
400 also exert a proliferative effect on tumor progression, as reported for other cancer types
401 (Vigneri, et al. 2016).

402 The present study displays several strengths as well as limitations. The strengths of the study
403 are represented by the relatively large sample size keeping in mind the low incidence of this
404 tumor type, the European multicentre setting (6 countries involved), the preliminary power
405 calculation, the investigation for the first time of a large set of factors possibly associated with
406 the risk of PNENs and the conduct of the study by face-to-face interview with a standardised
407 questionnaire. The inclusion criteria were clearly defined, controls were well matched for age
408 and gender with a 1:3 ratio and all questionnaires were administered by trained medical
409 doctors fluent in the local language, who evaluated exposures present 12 months before
410 diagnosis, to minimize bias due to cancer symptoms. Inherent with a multi-national case-
411 control design, our study displays some limitations such as potential recall bias and
412 heterogeneity in data from different countries, although the analysis was corrected for centre
413 of enrollment. Furthermore, the analysis might have been underpowered for some of the
414 investigated factors and additional studies might be important to confirm the lack of
415 significant association.

416 Another important matter of concern, as for any case-control study, regards the choice of the

417 control population. We opted for a mixed control group that we believed to represent the
418 same population as the case group, as living in the same catchment area of the corresponding
419 cases, to limit possible bias that could have been specific of either hospital controls or visitors.
420 Interestingly, “late onset diabetes” seemed to be associated with an increased risk of PNEN
421 with both kind of controls used in separate analyses.

422 In conclusion, the findings of this large multicentre case-control study suggest that
423 non-recent onset diabetes was associated with an increased risk of PNENs occurrence. Our
424 results do not support the view of a strict similarity with factors affecting the risk of
425 pancreatic adenocarcinoma.

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427

428 **Declaration of interest**

429 Potential competing interests: None.

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434 Wrote the paper: Roberto Valente, Gabriele Capurso, Alastair Hayes.

435 Analyzed the data: Roberto Valente, Gabriele Capurso, Patrick Maisonneuve.

436 All authors contributed to the design, data collection, data interpretation, and writing of the
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438

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